ISSN 1007-9327
CN 14-1219/R

WJG

World Journal of Gastroenterology®

Indexed and Abstracted in:

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *Index Medicus*, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, PubMed Central, Digital Object Identifier, CAB Abstracts and Global Health.

ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

Volume 15 Number 23
June 21, 2009

World J Gastroenterol
2009 June 21; 15(23): 2817-2944

Online Submissions
wjg.wjgnet.com
www.wjgnet.com

Printed on Acid-free Paper
世界胃肠病学杂志

A Weekly Journal of Gastroenterology and Hepatology

World Journal of Gastroenterology ®

Editorial Board

2007-2009

Editorial Office: *World Journal of Gastroenterology*
Room 903, Building D, Ocean International Center
No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com http://www.wjgnet.com Telephone: 0086-10-5908-0039 Fax: 0086-10-8538-1893

The World Journal of Gastroenterology Editorial Board consists of 1212 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 60 countries, including Albania (1), Argentina (4), Australia (39), Austria (10), Belarus (1), Belgium (15), Brazil (2), Bulgaria (1), Canada (29), Chile (1), China (60), Croatia (2), Cuba (1), Czech (2), Denmark (7), Egypt (4), Estonia (1), Finland (4), France (44), Germany (108), Greece (9), Hungary (2), Iceland (1), India (12), Iran (4), Ireland (3), Israel (8), Italy (97), Japan (177), Lebanon (3), Lithuania (1), Macedonia (1), Malaysia (3), Mexico (6), Monaco (1), Morocco (1), The Netherlands (26), New Zealand (1), Nigeria (1), Norway (3), Pakistan (2), Peru (1), Poland (6), Portugal (1), Russia (3), Saudi Arabia (2), Serbia (1), Singapore (4), Slovakia (2), Slovenia (1), South Africa (2), South Korea (15), Spain (38), Sweden (15), Switzerland (13), Turkey (8), United Arab Emirates (1), United Kingdom (83), United States (315) and Uruguay (2).

Andrew V Biankin, *Sydney*
Filip Braet, *Sydney*
Andrew D Clouston, *Sydney*
Graham Cooksley, *Queensland*
Darrell HG Crawford, *Brisbane*
Adrian G Cummins, *Woodville South*
Guy D Eslick, *Sydney*
Michael A Fink, *Melbourne*
Robert JL Fraser, *Daw Park*
Peter Raymond Gibson, *Victoria*
Jacob George, *Westmead*
Mark D Gorrell, *Sydney*
Yik-Hong Ho, *Townsville*
Gerald J Holtmann, *Adelaide*
Michael Horowitz, *Adelaide*
John E Kellow, *Sydney*
Rupert Leong, *Concord*
Geoffrey W McCaughan, *Sydney*
Finlay A Macrae, *Victoria*
Daniel Markovich, *Brisbane*
Phillip S Oates, *Perth*
Jacqui Richmond, *Victoria*
Stephen M Riordan, *Sydney*
Ian C Roberts-Thomson, *Adelaide*
Devanshi Seth, *Camperdown*
Arthur Shulkes, *Melbourne*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Huy A Tran, *New South Wales*
Debbie Trinder, *Fremantle*
Martin J Veysey, *Gosford*
Daniel L Worthley, *Bedford*

Austria

Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Alfred Gangl, *Vienna*
Christoph Gasche, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Rudolf E Stauber, *Auenbruggerplatz*
Herbert Tilg, *Innsbruck*
Michael Trauner, *Graz*
Harald Vogelsang, *Vienna*
Guenter Weiss, *Innsbruck*

Belarus

Yury K Marakhouski, *Minsk*

Belgium

Rudi Beyaert, *Gent*
Bart Rik De Geest, *Leuven*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Benedicte Y De Winter, *Antwerp*
Karel Geboes, *Leuven*
Thierry Gustot, *Brussels*
Yves J Horsmans, *Brussels*
Geert G Leroux-Roels, *Ghent*
Louis Libbrecht, *Leuven*
Etienne M Sokal, *Brussels*
Marc Peeters, *De Pintelaan*
Gert A Van Assche, *Leuven*
Yvan Vandenplas, *Brussels*
Eddie Wisse, *Keerbergen*

Brazil

Heitor Rosa, *Goiania*
Ana Cristina Simões e Silva, *Belo Horizonte*

Bulgaria

Zahariy Krastev, *Sofia*

Canada

Fernando Alvarez, *Québec*
David Armstrong, *Ontario*
Jeffrey P Baker, *Toronto*
Olivier Barbier, *Québec*
Nancy Baxter, *Toronto*
Matthew Bjerknes, *Toronto*
Frank J Burczynski, *Manitoba*
Michael F Byrne, *Vancouver*
Wang-Xue Chen, *Ottawa*
Chantal Guillemette, *Québec*
Samuel S Lee, *Calgary*
Gary A Levy, *Toronto*
Andrew L Mason, *Alberta*
John K Marshall, *Ontario*
Donna-Marie McCafferty, *Calgary*
Thomas I Michalak, *St. John's*
Gerald Y Minuk, *Manitoba*
Paul Moayyedi, *Hamilton*
Kostas Pantopoulos, *Québec*
William G Paterson, *Kingston*
Eldon Shaffer, *Calgary*
Morris Sherman, *Toronto*
Martin Storr, *Calgary*
Elena F Verdu, *Ontario*
Waliul Khan, *Ontario*
John L Wallace, *Calgary*
Eric M Yoshida, *Vancouver*

Chile

Silvana Zanlungo, *Santiago*

China

Henry LY Chan, *Hong Kong*
Xiao-Ping Chen, *Wuhan*
Zong-Jie Cui, *Beijing*
Da-Jun Deng, *Beijing*
Er-Dan Dong, *Beijing*
Sheung-Tat Fan, *Hong Kong*
Jin Gu, *Beijing*
Xin-Yuan Guan, *Pokfulam*
De-Wu Han, *Taiyuan*
Ming-Liang He, *Hong Kong*
Wayne HC Hu, *Hong Kong*
Chee-Kin Hui, *Hong Kong*
Ching-Lung Lai, *Hong Kong*
Kam Chuen Lai, *Hong Kong*
James YW Lau, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
Suet-Yi Leung, *Hong Kong*
Wai-Keung Leung, *Hong Kong*
John M Luk, *Pokfulam*
Chung-Mau Lo, *Hong Kong*
Jing-Yun Ma, *Beijing*
Ronnie Tung Ping Poon, *Hong Kong*
Lun-Xiu Qin, *Shanghai*
Yu-Gang Song, *Guangzhou*
Qin Su, *Beijing*
Wai-Man Wong, *Hong Kong*
Hong Xiao, *Shanghai*
Dong-Liang Yang, *Wuhan*
Winnie Yeo, *Hong Kong*
Yuan Yuan, *Shenyang*
Man-Fung Yuen, *Hong Kong*
Jian-Zhong Zhang, *Beijing*
Xin-Xin Zhang, *Shanghai*
Bo-Jian Zheng, *Hong Kong*
Shu Zheng, *Hangzhou*

Croatia

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*

Cuba

Damian C Rodriguez, *Havana*

Czech

Milan Jirsa, *Praha*
Pavel Trunečka, *Prague*

Denmark

Peter Bytzer, *Copenhagen*
Asbjørn M Drewes, *Aalborg*
Hans Gregersen, *Aalborg*
Jens H Henriksen, *Hvidovre*
Claus P Hovendal, *Odense*
Fin S Larsen, *Copenhagen*
Søren Møller, *Hvidovre*

Egypt

Abdel-Rahman El-Zayadi, *Giza*
Amr M Helmy, *Cairo*
Ayman Yosry, *Cairo*

Estonia

Riina Salupere, *Tartu*

Finland

Irma E Jarvela, *Helsinki*
Katri M Kaukinen, *Tampere*
Minna Nyström, *Helsinki*
Pentti Sipponen, *Espoo*

France

Bettaieb Ali, *Dijon*
Corlu Anne, *Rennes*
Denis Ardid, *Clermont-Ferrand*
Charles P Balabaud, *Bordeaux*
Soumeya Bekri, *Rouen*
Jacques Belghiti, *Clichy*
Jacques Bernuau, *Clichy Cedex*
Pierre Brissot, *Rennes*
Patrice P Cacoub, *Paris*
Franck Carbonnel, *Besancon*
Laurent Castera, *Pessac*
Bruno Clément, *Rennes*
Benoit Coffin, *Colombes*
Jacques Cosnes, *Paris*
Thomas Decaens, *Cedex*

Francoise L Fabiani, *Angers*
Gérard Feldmann, *Paris*
Jean Fioramonti, *Toulouse*
Jean-Noël Freund, *Strasbourg*
Jean-Paul Galmiche, *Nantes*
Catherine Guettier, *Villejuif*
Chantal Housset, *Paris*
Juan L Iovanna, *Marseille*
Rene Lambert, *Lyon*
Patrick Marcellin, *Paris*
Philippe Mathurin, *Lille*
Tamara Matysiak-Budnik, *Paris*
Francis Mégraud, *Bordeaux*
Richard Moreau, *Clichy*
Thierry Piche, *Nice*
Raoul Poupon, *Paris*
Jean Rosenbaum, *Bordeaux*
Dominique Marie Roulot, *Bobigny*
Thierry Poynard, *Paris*
Jean-Philippe Salier, *Rouen*
Didier Samuel, *Villejuif*
Jean-Yves Scoazec, *Lyon*
Alain L Servin, *Châtenay-Malabry*
Khalid A Tazi, *Clichy*
Emmanuel Tiret, *Paris*
Baumert F Thomas, *Strasbourg*
Jean-Pierre H Zarski, *Grenoble*
Jessica Zucman-Rossi, *Paris*

Germany

Hans-Dieter Allescher, *G-Partenkirchen*
Martin Anlauf, *Kiel*
Rudolf Arnold, *Marburg*
Max G Bachem, *Ulm*
Thomas F Baumert, *Freiburg*
Daniel C Baumgart, *Berlin*
Hubert Blum, *Freiburg*
Thomas Bock, *Tuebingen*
Katja Breitkopf, *Mannheim*
Dunja Bruder, *Braunschweig*
Markus W Büchler, *Heidelberg*
Christa Buechler, *Regensburg*
Reinhard Buettner, *Bonn*
Elke Cario, *Essen*
Uta Dahmen, *Essen*
Christoph F Dietrich, *Bad Mergentheim*
Arno J Dormann, *Koeln*
Rainer J Duchmann, *Berlin*
Volker F Eckardt, *Wiesbaden*
Paul Enck, *Tuebingen*
Fred Fändrich, *Kiel*
Ulrich R Fölsch, *Kiel*
Helmut Friess, *Heidelberg*
Peter R Galle, *Mainz*
Nikolaus Gassler, *Aachen*
Andreas Geier, *Aachen*
Markus Gerhard, *Munich*
Wolfram H Gerlich, *Giessen*
Dieter Glebe, *Giessen*
Burkhard Göke, *Munich*
Florian Graepler, *Tuebingen*
Axel M Gressner, *Aachen*
Veit Gülberg, *Munich*
Rainer Haas, *Munich*
Eckhart G Hahn, *Erlangen*
Stephan Hellmig, *Kiel*
Martin Hennenberg, *Bonn*
Johannes Herkel, *Hamburg*
Klaus R Herrlinger, *Stuttgart*
Eva Herrmann, *Homburg/Saar*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Berlin*
Ferdinand Hofstaedter, *Regensburg*
Werner Hohenberger, *Erlangen*
Jörg C Kalff, *Bonn*
Ralf Jakobs, *Ludwigshafen*
Jutta Keller, *Hamburg*
Andrej Khandoga, *Munich*
Sibylle Koletzko, *München*
Stefan Kubicka, *Hannover*
Joachim Labenz, *Siegen*
Frank Lammert, *Bonn*
Thomas Langmann, *Regensburg*
Christian Liedtke, *Aachen*
Matthias Löhr, *Mannheim*
Christian Maaser, *Muenster*
Ahmed Madisch, *Dresden*
Peter Malfertheiner, *Magdeburg*
Michael P Manns, *Hannover*
Helmut Messmann, *Augsburg*
Stephan Miehlke, *Dresden*
Sabine Mihm, *Göttingen*
Silvio Nadalin, *Essen*
Markus F Neurath, *Mainz*
Johann Ockenga, *Berlin*
Florian Obermeier, *Regensburg*
Gustav Paumgartner, *Munich*
Ulrich KS Peitz, *Magdeburg*
Markus Reiser, *Bochum*
Emil C Reisinger, *Rostock*
Steffen Rickes, *Magdeburg*
Tilman Sauerbruch, *Bonn*
Dieter Saur, *Munich*
Hans Scherubl, *Berlin*
Joerg Schirra, *Munich*
Roland M Schmid, *München*
Volker Schmitz, *Bonn*
Andreas G Schreyer, *Regensburg*
Tobias Schroeder, *Essen*
Henning Schulze-Bergkamen, *Mainz*
Hans Seifert, *Oldenburg*
Norbert Senninger, *Muenster*
Manfred V Singer, *Mannheim*
Gisela Sparmann, *Rostock*
Christian J Steib, *München*
Jurgen M Stein, *Frankfurt*
Ulrike S Stein, *Berlin*
Manfred Stolte, *Bayreuth*
Christian P Strassburg, *Hannover*
Wolfgang R Stremmel, *Heidelberg*
Harald F Teutsch, *Ulm*
Robert Thimme, *Freiburg*
Hans L Tillmann, *Leipzig*
Tung-Yu Tsui, *Regensburg*
Axel Ulsenheimer, *Munich*
Patrick Veit-Haibach, *Essen*
Claudia Veltkamp, *Heidelberg*
Siegfried Wagner, *Deggendorf*
Henning Walczak, *Heidelberg*
Heiner Wedemeyer, *Hannover*
Fritz von Weizsacker, *Berlin*
Jens Werner, *Heidelberg*
Bertram Wiedenmann, *Berlin*
Reiner Wiest, *Regensburg*
Stefan Wirth, *Wuppertal*
Stefan JP Zeuzem, *Homburg*

Greece

Alexandra A Alexopoulou, *Athens*
George N Dalekos, *Larissa*
Christos Dervenis, *Athens*
Melanie Maria Deutsch, *Athens*
Tsianos Epameinondas, *Ioannina*
Elias A Kouroumalis, *Heraklion*
George Papatheodoridis, *Athens*
Spiros Sgouros, *Athens*

Hungary

Peter L Lakatos, *Budapest*
Zsuzsa Szondy, *Debrecen*

Iceland

Hallgrimur Gudjonsson, *Reykjavik*

India

Philip Abraham, *Mumbai*
Rakesh Aggarwal, *Lucknow*
Kunissery A Balasubramanian, *Vellore*
Deepak Kumar Bhasin, *Chandigarh*
Sujit K Bhattacharya, *Kolkata*
Yogesh K Chawla, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Sri Prakash Misra, *Allahabad*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*

Iran

Mohammad Abdollahi, *Tehran*
Seyed-Moayed Alavian, *Tehran*
Reza Malekzadeh, *Tehran*
Seyed A Taghavi, *Shiraz*

Ireland

Billy Bourke, *Dublin*
Ronan A Cahill, *Cork*
Anthony P Moran, *Galway*

Israel

Simon Bar-Meir, *Hashomer*
Abraham R Eliakim, *Haifa*
Zvi Fireman, *Hadera*
Yaron Ilan, *Jerusalem*
Avidan U Neumann, *Ramat-Gan*
Yaron Niv, *Pardesia*
Ran Oren, *Tel Aviv*
Ami D Sperber, *Beer-Sheva*

Italy

Giovanni Addolorato, *Roma*
Luigi E Adinolfi, *Naples*
Domenico Alvaro, *Rome*
Mario Angelico, *Rome*
Vito Annese, *San Giovanni Rotond*
Filippo Ansaldi, *Genoa*
Adolfo F Attili, *Roma*
Giovanni Barbara, *Bologna*
Claudio Bassi, *Verona*
Gabrio Bassotti, *Perugia*
Pier M Battezzati, *Milan*
Stefano Bellentani, *Carpi*
Antonio Benedetti, *Ancona*
Mauro Bernardi, *Bologna*
Livia Biancone, *Rome*
Luigi Bonavina, *Milano*
Flavia Bortolotti, *Padova*
Giuseppe Brisinda, *Rome*
Elisabetta Buscarini, *Crema*
Giovanni Cammarota, *Roma*

Antonino Cavallari, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Massimo Colombo, *Milan*
Amedeo Columbano, *Cagliari*
Massimo Conio, *Sanremo*
Dario Conte, *Milano*
Gino R Corazza, *Pavia*
Francesco Costa, *Pisa*
Antonio Craxi, *Palermo*
Silvio Danese, *Milan*
Roberto de Franchis, *Milano*
Roberto De Giorgio, *Bologna*
Maria Stella De Mitri, *Bologna*
Giovanni D De Palma, *Naples*
Fabio Farinati, *Padua*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Fiorucci Stefano, *Perugia*
Andrea Galli, *Firenze*
Valeria Ghisett, *Turin*
Gianluigi Giannelli, *Bari*
Edoardo G Giannini, *Genoa*
Paolo Gionchetti, *Bologna*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Mario Guslandi, *Milano*
Pietro Invernizzi, *Milan*
Ezio Laconi, *Cagliari*
Giacomo Laffi, *Firenze*
Giovanni Maconi, *Milan*
Lucia Malaguarnera, *Catania*
Emanuele D Mangoni, *Napoli*
Paolo Manzoni, *Torino*
Giulio Marchesini, *Bologna*
Fabio Marra, *Florence*
Marco Marzioni, *Ancona*
Roberto Mazzanti, *Florence*
Giuseppe Mazzella, *Bologna*
Mario U Mondelli, *Pavia*
Giuseppe Montalto, *Palermo*
Giovanni Monteleone, *Rome*
Giovanni Musso, *Torino*
Gerardo Nardone, *Napoli*
Valerio Nobili, *Rome*
Fabio Pace, *Milano*
Luisi Pagliaro, *Palermo*
Francesco Pallone, *Rome*
Fabrizio R Parente, *Milan*
Maurizio Parola, *Torino*
Francesco Perri, *San Giovanni Rotondo*
Raffaele Pezzilli, *Bologna*
Alberto Pilotto, *San Giovanni Rotondo*
Alberto Piperno, *Monza*
Mario Pirisi, *Novara*
Anna C Piscaglia, *Roma*
Paolo Del Poggio, *Treviglio*
Gabriele B Porro, *Milano*
Piero Portincasa, *Bari*
Cosimo Prantera, *Roma*
Bernardino Rampone, *Siena*
Oliviero Riggio, *Rome*
Claudio Romano, *Messina*
Marco Romano, *Napoli*
Gerardo Rosati, *Potenza*
Mario Del Tacca, *Pisa*
Gloria Taliani, *Rome*
Pier A Testoni, *Milan*
Enrico Roda, *Bologna*
Domenico Sansonno, *Bari*
Vincenzo Savarino, *Genova*
Vincenzo Stanghellini, *Bologna*
Giovanni Tarantino, *Naples*
Roberto Testa, *Genoa*
Dino Vaira, *Bologna*
Anna Linda Zignego, *Florence*

Japan

Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Taiji Akamatsu, *Matsumoto*
Sk Md Fazle Akbar, *Ehime*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Taku Aoki, *Tokyo*
Masahiro Arai, *Tokyo*
Tetsuo Arakawa, *Osaka*
Yasuji Arase, *Tokyo*
Masahiro Asaka, *Sapporo*
Hitoshi Asakura, *Tokyo*
Takeshi Azuma, *Fukui*
Yoichi Chida, *Fukuoka*
Takahiro Fujimori, *Tochigi*
Jiro Fujimoto, *Hyogo*
Kazuma Fujimoto, *Saga*
Mitsuhiro Fujishiro, *Tokyo*
Yoshihide Fujiyama, *Otsu*
Hiroyuki Fukui, *Tochigi*
Hiroyuki Hanai, *Hamamatsu*
Naohiko Harada, *Fukuoka*
Makoto Hashizume, *Fukuoka*
Tetsuo Hayakawa, *Nagoya*
Toru Hiyama, *Higashihiroshima*
Kazuhide Higuchi, *Osaka*
Keisuke Hino, *Ube*
Keiji Hirata, *Kitakyushu*
Yuji Iimuro, *Nishinomiya*
Kenji Ikeda, *Tokyo*
Toru Ikegami, *Fukuoka*
Kenichi Ikejima, *Bunkyo-ku*
Fumio Imazeki, *Chiba*
Yutaka Inagaki, *Kanagawa*
Yasuhiro Inokuchi, *Yokohama*
Haruhiro Inoue, *Yokohama*
Masayasu Inoue, *Osaka*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Kei Ito, *Sendai*
Masayoshi Ito, *Tokyo*
Hiroaki Itoh, *Akita*
Ryuichi Iwakiri, *Saga*
Yoshiaki Iwasaki, *Okayama*
Terumi Kamisawa, *Tokyo*
Hiroshi Kaneko, *Aichi-Gun*
Shuichi Kaneko, *Kanazawa*
Takashi Kanematsu, *Nagasaki*
Mitsuo Katano, *Fukuoka*
Junji Kato, *Sapporo*
Mototsugu Kato, *Sapporo*
Shinzo Kato, *Tokyo*
Norifumi Kawada, *Osaka*
Sunao Kawano, *Osaka*
Mitsuhiro Kida, *Kanagawa*
Yoshikazu Kinoshita, *Izumo*
Tsuneo Kitamura, *Chiba*
Seigo Kitano, *Oita*
Kazuhiko Koike, *Tokyo*
Norihiro Kokudo, *Tokyo*
Satoshi Kondo, *Sapporo*
Shoji Kubo, *Osaka*
Masatoshi Kudo, *Osaka*
Shigeki Kuriyama, *Kagawa*[2]
Masato Kusunoki, *Tsu Mie*
Katsunori Iijima, *Sendai*
Shin Maeda, *Tokyo*
Shigeru Marubashi, *Suita*
Masatoshi Makuuchi, *Tokyo*
Osamu Matsui, *Kanazawa*
Yasuhiro Matsumura, *Chiba*
Yasushi Matsuzaki, *Tsukuba*
Kiyoshi Migita, *Omura*
Kenji Miki, *Tokyo*
Tetsuya Mine, *Kanagawa*
Hiroto Miwa, *Hyogo*
Masashi Mizokami, *Nagoya*
Yoshiaki Mizuguchi, *Tokyo*
Motowo Mizuno, *Hiroshima*
Morito Monden, *Suita*
Hisataka S Moriwaki, *Gifu*
Yasuaki Motomura, *Iizuka*
Yoshiharu Motoo, *Kanazawa*
Naofumi Mukaida, *Kanazawa*
Kazunari Murakami, *Oita*
Kunihiko Murase, *Tusima*
Hiroaki Nagano, *Suita*
Masahito Nagaki, *Gifu*
Masaki Nagaya, *Kawasaki*
Yujl Naito, *Kyoto*
Atsushi Nakajima, *Yokohama*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Shuji Nomoto, *Nagoya*
Susumu Ohmada, *Maebashi*
Hirohide Ohnishi, *Akita*
Masayuki Ohta, *Oita*
Tetsuo Ohta, *Kanazawa*
Kazuichi Okazaki, *Osaka*
Katsuhisa Omagari, *Nagasaki*
Saburo Onishi, *Nankoku*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Masanobu Oshima, *Kanazawa*
Hiromitsu Saisho, *Chiba*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Isao Sakaida, *Yamaguchi*
Michiie Sakamoto, *Tokyo*
Yasushi Sano, *Chiba*
Hiroki Sasaki, *Tokyo*
Iwao Sasaki, *Sendai*
Motoko Sasaki, *Kanazawa*
Chifumi Sato, *Tokyo*
Shuichi Seki, *Osaka*
Hiroshi Shimada, *Yokohama*
Mitsuo Shimada, *Tokushima*
Tomohiko Shimatan, *Hiroshima*
Hiroaki Shimizu, *Chiba*
Ichiro Shimizu, *Tokushima*
Yukihiro Shimizu, *Kyoto*
Shinji Shimoda, *Fukuoka*
Tooru Shimosegawa, *Sendai*
Tadashi Shimoyama, *Hirosaki*
Ken Shirabe, *Iizuka City*
Yoshio Shirai, *Niigata*
Katsuya Shiraki, *Mie*
Yasushi Shiratori, *Okayama*
Masayuki Sho, *Nara*
Yasuhiko Sugawara, *Tokyo*
Hidekazu Suzuki, *Tokyo*
Minoru Tada, *Tokyo*
Tadatoshi Takayama, *Tokyo*
Tadashi Takeda, *Osaka*
Koji Takeuchi, *Kyoto*
Kiichi Tamada, *Tochigi*
Akira Tanaka, *Kyoto*
Eiji Tanaka, *Matsumoto*
Noriaki Tanaka, *Okayama*
Shinji Tanaka, *Hiroshima*
Hideki Taniguchi, *Yokohama*
Kyuichi Tanikawa, *Kurume*
Akira Terano, *Shimotsugagun*
Hitoshi Togash, *Yamagata*
Shinji Togo, *Yokohama*
Kazunari Tominaga, *Osaka*
Takuji Torimura, *Fukuoka*
Minoru Toyota, *Sapporo*

Akihito Tsubota, *Chiba*
Takato Ueno, *Kurume*
Naomi Uemura, *Tokyo*
Shinichi Wada, *Tochigi*
Hiroyuki Watanabe, *Kanazawa*
Toshio Watanabe, *Osaka*
Yuji Watanabe, *Ehime*
Toshiaki Watanabe, *Tokyo*
Chun-Yang Wen, *Nagasaki*
Satoshi Yamagiwa, *Niigata*
Koji Yamaguchi, *Fukuoka*
Takayuki Yamamoto, *Yokkaichi*
Takashi Yao, *Fukuoka*
Masashi Yoneda, *Tochigi*
Hiroshi Yoshida, *Tokyo*
Masashi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Nara*
Kentaro Yoshika, *Toyoake*
Yasunobu Yoshikai, *Fukuoka*
Masahide Yoshikawa, *Kashihara*
Katsutoshi Yoshizato, *Higashihiroshima*

Lebanon
Bassam N Abboud, *Beirut*
Ala I Sharara, *Beirut*
Joseph D Boujaoude, *Beirut*

Lithuania
Limas Kupcinskas, *Kaunas*

Macedonia
Vladimir C Serafimoski, *Skopje*

Malaysia
Andrew Seng Boon Chua, *Ipoh*
Khean-Lee Goh, Kuala *Lumpur*
Jayaram Menon, *Sabah*

Mexico
Diego Garcia-Compean, *Monterrey*
Eduardo R Marin-Lopez, *Jesús García*
Nahum Méndez-Sánchez, *Mexico*
Saúl Villa-Treviño, *México*

Monaco
Patrick Rampal, *Monaco*

Morocco
Abdellah Essaid, *Rabat*

The Netherlands
Ulrich Beuers, *Amsterdam*
Gerd Bouma, *Amsterdam*
Lee Bouwman, *Leiden*
J Bart A Crusius, *Amsterdam*
NKH de Boer, *Amsterdam*
Koert P de Jong, *Groningen*
Henrike Hamer, *Maastricht*
Frank Hoentjen, *Haarlem*
Janine K Kruit, *Groningen*
Ernst J Kuipers, *Rotterdam*
CBHW Lamers, *Leiden*
Ton Lisman, *Utrecht*
Yi Liu, *Amsterdam*
Jeroen Maljaars, *Maastricht*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Michael Müller, *Wageningen*
Amado S Peña, *Amsterdam*
Robert J Porte, *Groningen*
Ingrid B Renes, *Rotterdam*
Andreas Smout, *Utrecht*
Paul E Sijens, *Groningen*
Reinhold W Stockbrugger, *Maastricht*
Luc JW van der Laan, *Rotterdam*
Karel van Erpecum, *Utrecht*
Gerard P VanBerge-Henegouwen,*Utrecht*

New Zealand
Ian D Wallace, *Auckland*

Nigeria
Samuel B Olaleye, *Ibadan*

Norway
Trond Berg, *Oslo*
Tom H Karlsen, *Oslo*
Helge L Waldum, *Trondheim*

Pakistan
Muhammad S Khokhar, *Lahore*
Syed MW Jafri, *Karachi*

Peru
Hector H Garcia, *Lima*

Poland
Tomasz Brzozowski, *Cracow*
Robert Flisiak, *Bialystok*
Hanna Gregorek, *Warsaw*
Dariusz M Lebensztejn, *Bialystok*
Wojciech G Polak, *Wroclaw*
Marek Hartleb, *Katowice*

Portugal
Miguel C De Moura, *Lisbon*

Russia
Vladimir T Ivashkin, *Moscow*
Leonid Lazebnik, *Moscow*
Vasiliy I Reshetnyak, *Moscow*

Saudi Arabia
Ibrahim A Al Mofleh, *Riyadh*
Ahmed Helmy, *Riyadh*

Serbia
Dusan M Jovanovic, *Sremska Kamenica*

Singapore
Bow Ho, *Singapore*
Khek-Yu Ho, *Singapore*
Fock Kwong Ming, *Singapore*
Francis Seow-Choen, *Singapore*

Slovakia
Silvia Pastorekova, *Bratislava*
Anton Vavrecka, *Bratislava*

Slovenia
Sasa Markovic, *Ljubljana*

South Africa
Rosemar Joyce Burnett, *Pretoria*
Michael C Kew, *Parktown*

South Korea
Byung Ihn Choi, *Seoul*
Ho Soon Choi, *Seoul*
Marie Yeo, *Suwon*
Sun Pyo Hong, *Gyeonggi-do*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Myung-Hwan Kim, *Seoul*
Chang Hong Lee, *Seoul*
Jeong Min Lee, *Seoul*
Jong Kyun Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Jae-Gahb Park, *Seoul*
Dong Wan Seo, *Seoul*
Dong Jin Suh, *Seoul*
Byung Chul Yoo, *Seoul*

Spain
Juan G Abraldes, *Barcelona*
Agustin Albillos, *Madrid*
Raul J Andrade, *Málaga*
Luis Aparisi, *Valencia*
Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Josep M Bordas, *Barcelona*
Xavier Calvet, *Sabadell*
Jordi Camps, *Catalunya*
Andres Cardenas, *Barcelona*
Vicente Carreño, *Madrid*
Jose Castellote, *Barcelona*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Juan C Garcia-Pagán, *Barcelona*
Jaime B Genover, *Barcelona*
Javier P Gisbert, *Madrid*
Jaime Guardia, *Barcelona*
Isabel Fabregat, *Barcelona*
Mercedes Fernandez, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
Laura Lladóa, *Barcelona*
María IT López, *Jaén*
Juan R Malagelada, *Barcelona*
José M Mato, *Derio*
Juan F Medina, *Pamplona*
Miguel A Muñoz-Navas, *Pamplona*
Julian Panes, *Barcelona*
Miguel M Perez, *Valencia*
Miguel Perez-Mateo, *Alicante*

Josep M Pique, *Barcelona*
Jesús M Prieto, *Pamplona*
Sabino Riestra, *Pola De Siero*
Luis Rodrigo, *Oviedo*
Manuel Romero-Gómez, *Sevilla*
Joan Roselló-Catafau, *Barcelona*

Sweden

Einar S Björnsson, *Gothenburg*
Curt Einarsson, *Huddinge*
Per M Hellström, *Stockholm*
Ulf Hindorf, *Lund*
Elisabeth Hultgren-Hörnquist, *Örebro*
Anders E Lehmann, *Mölndal*
Hanns-Ulrich Marschall, *Stockholm*
Lars C Olbe, *Molndal*
Lars A Pahlman, *Uppsala*
Matti Sallberg, *Stockholm*
Magnus Simrén, *Göteborg*
Xiao-Feng Sun, *Linköping*
Ervin Tóth, *Malmö*
Weimin Ye, *Stockholm*
Christer S von Holstein, *Lund*

Switzerland

Chrish Beglinger, *Basel*
Pierre A Clavien, *Zurich*
Jean-Francois Dufour, *Bern*
Franco Fortunato, *Zurich*
Jean L Frossard, *Geneva*
Gerd A Kullak-Ublick, *Zurich*
Pierre Michetti, *Lausanne*
Francesco Negro, *Genève*
Bruno Stieger, *Zurich*
Radu Tutuian, *Zurich*
Stephan R Vavricka, *Zurich*
Gerhard Rogler, *Zurich*
Arthur Zimmermann, *Berne*

Turkey

Yusuf Bayraktar, *Ankara*
Figen Gurakan, *Ankara*
Aydin Karabacakoglu, *Konya*
Serdar Karakose, *Konya*
Hizir Kurtel, *Istanbul*
Osman C Ozdogan, *Istanbul*
Özlem Yilmaz, *Izmir*
Cihan Yurdaydin, *Ankara*

United Arab Emirates

Sherif M Karam, *Al-Ain*

United Kingdom

David H Adams, *Birmingham*
Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Ahmed Alzaraa, *Manchester*
Lesley A Anderson, *Belfast*
Charalambos G Antoniades, *London*
Anthony TR Axon, *Leeds*
Qasim Aziz, *Manchester*
Nicholas M Barnes, *Birmingham*
Jim D Bell, *London*
Mairi Brittan, *London*
Alastair D Burt, *Newcastle*
Simon S Campbell, *Manchester*
Simon R Carding, *Leeds*
Paul J Ciclitira, *London*
Eithne Costello, *Liverpool*
Tatjana Crnogorac-Jurcevic, *London*
Harry Dalton, *Truro*
Amar P Dhillon, *London*
William Dickey, *Londonderry*
James E East, *London*
Emad M El-Omar, *Aberdeen*
Ahmed M Elsharkawy, *Newcastle Upon Tyne*
Annette Fristscher-Ravens, *London*
Elizabeth Furrie, *Dundee*
Daniel R Gaya, *Edinburgh*
Subrata Ghosh, *London*
William Greenhalf, *Liverpool*
Indra N Guha, *Southampton*
Peter C Hayes, *Edinburgh*
Gwo-Tzer Ho, *Edinburgh*
Anthony R Hobson, *Salford*
Lesley A Houghton, *Manchester*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
David P Hurlstone, *Sheffield*
Rajiv Jalan, *London*
Janusz AZ Jankowski, *Oxford*
Brian T Johnston, *Belfast*
David EJ Jones, *Newcastle*
Roger Jones, *London*
Michael A Kamm, *Harrow*
Peter Karayiannis, *London*
Laurens Kruidenier, *Harlow*
Patricia F Lalor, *Birmingham*
Chee Hooi Lim, *Midlands*
Hong-Xiang Liu, *Cambridge*
Yun Ma, *London*
Kenneth E L McColl, *Glasgow*
Stuart AC McDonald, *London*
Dermot P Mcgovern, *Oxford*
Giorgina Mieli-Vergani, *London*
Nikolai V Naoumov, *London*
John P Neoptolemos, *Liverpool*
James Neuberger, *Birmingham*
Philip Noel Newsome, *Birmingham*
Mark S Pearce, *Newcastle Upon Tyne*
Stephen P Pereira, *London*
D Mark Pritchard, *Liverpool*
Sakhawat Rahman, *London*
Stephen E Roberts, *Swansea*
Marco Senzolo, *Padova*
Soraya Shirazi-Beechey, *Liverpool*
Robert Sutton, *Liverpool*
Simon D Taylor-Robinson, *London*
Paris P Tekkis, *London*
Ulrich Thalheimer, *London*
David G Thompson, *Salford*
Nick P Thompson, *Newcastle*
David Tosh, *Bath*
Frank I Tovey, *London*
Chris Tselepis, *Birmingham*
Diego Vergani, *London*
Geoffrey Warhurst, *Salford*
Alastair John Watson, *Liverpool*
Peter J Whorwell, *Manchester*
Roger Williams, *London*
Karen L Wright, *Bath*
Min Zhao, *Foresterhill*

United States

Manal F Abdelmalek, *Durham*
Gary A Abrams, *Birmingham*
Maria T Abreu, *New York*
Reid B Adams, *Virginia*
Golo Ahlenstiel, *Bethesda*
BS Anand, *Houston*
Frank A Anania, *Atlanta*
M Ananthanarayanan, *New York*
Gavin E Arteel, *Louisville*
Jasmohan S Bajaj, *Milwaukee*
Subhas Banerjee, *Palo Alto*
Peter A Banks, *Boston*
Jamie S Barkin, *Miami Beach*
Kim E Barrett, *San Diego*
Marc D Basson, *Detroit*
Anthony J Bauer, *Pittsburgh*
Wallace F Berman, *Durham*
Timothy R Billiar, *Pittsburgh*
Edmund J Bini, *New York*
David G Binion, *Milwaukee*
Jennifer D Black, *Buffalo*
Herbert L Bonkovsky, *Charlotte*
Carla W Brady, *Durham*
Andrea D Branch, *New York*
Robert S Bresalier, *Houston*
Alan L Buchman, *Chicago*
Ronald W Busuttil, *Los Angeles*
Alan Cahill, *Philadelphia*
John M Carethers, *San Diego*
David L Carr-Locke, *Boston*
Maurice A Cerulli, *New York*
Ravi S Chari, *Nashville*
Jiande Chen, *Galveston*
Xian-Ming Chen, *Omaha*
Xin Chen, *San Francisco*
Ramsey Chi-man Cheung, *Palo Alto*
William D Chey, *Ann Arbor*
John Y Chiang, *Rootstown*
Parimal Chowdhury, *Arkansas*
Raymond T Chung, *Boston*
James M Church, *Cleveland*
Ram Chuttani, *Boston*
Mark G Clemens, *Charlotte*
Ana J Coito, *Los Angeles*
Vincent Coghlan, *Beaverton*
David Cronin II, *New Haven*
John Cuppoletti, *Cincinnati*
Mark J Czaja, *New York*
Peter V Danenberg, *Los Angeles*
Kiron M Das, New *Brunswick*
Conor P Delaney, *Cleveland*
Jose L del Pozo, *Rochester*
Sharon DeMorrow, *Temple*
Deborah L Diamond, *Seattle*
Douglas A Drossman, *Chapel Hill*
Katerina Dvorak, *Tucson*
Bijan Eghtesad, *Cleveland*
Hala El-Zimaity, *Houston*
Michelle Embree-Ku, *Providence*
Sukru Emre, New *Haven*
Douglas G Farmer, *Los Angeles*
Alessio Fasano, *Baltimore*
Mark A Feitelson, *Philadelphia*
Ariel E Feldstein, *Cleveland*
Alessandro Fichera, *Chicago*
Robert L Fine, *New York*
Chris E Forsmark, *Gainesville*
Glenn T Furuta, *Aurora*
Chandrashekhar R Gandhi, *Pittsburgh*
Susan L Gearhart, *Baltimore*
Xupeng Ge, *Boston*
Xin Geng, *New Brunswick*
M Eric Gershwin, *Suite*
Jean-Francois Geschwind, *Baltimore*
Ignacio Gil-Bazo, *New York*
Shannon S Glaser, *Temple*
Ajay Goel, *Dallas*
Richard M Green, *Chicago*
Julia B Greer, *Pittsburgh*
James H Grendell, *New York*

David R Gretch, *Seattle*
Stefano Guandalini, *Chicago*
Anna S Gukovskaya, *Los Angeles*
Sanjeev Gupta, *Bronx*
David J Hackam, *Pittsburgh*
Stephen B Hanauer, *Chicago*
Gavin Harewood, *Rochester*
Margaret M Heitkemper, *Washington*
Alan W Hemming, *Gainesville*
Samuel B Ho, *San Diego*
Peter R Holt, *New York*
Colin W Howden, *Chicago*
Hongjin Huang, *Alameda*
Jamal A Ibdah, *Columbia*
Atif Iqbal, *Omaha*
Hajime Isomoto, *Rochester*
Hartmut Jaeschke, *Tucson*
Dennis M Jensen, *Los Angeles*
Cheng Ji, *Los Angeles*
Leonard R Johnson, *Memphis*
Michael P Jones, *Chicago*
Peter J Kahrilas, *Chicago*
Anthony N Kalloo, *Baltimore*
Marshall M Kaplan, *Boston*
Neil Kaplowitz, *Los Angeles*
Serhan Karvar, *Los Angeles*
Rashmi Kaul, *Tulsa*
Jonathan D Kaunitz, *Los Angeles*
Ali Keshavarzian, *Chicago*
Miran Kim, *Providence*
Joseph B Kirsner, *Chicago*
Leonidas G Koniaris, *Miami*
Burton I Korelitz, *New York*
Robert J Korst, *New York*
Richard A Kozarek, *Seattle*
Alyssa M Krasinskas, *Pittsburgh*
Michael Kremer, *Chapel Hill*
Shiu-Ming Kuo, *Buffalo*
Paul Y Kwo, *Indianapolis*
Daryl Tan Yeung Lau, *Galvesto*
Stephen J Lanspa, *Omaha*
Joel E Lavine, *San Diego*
Bret Lashner, *Cleveland*
Dirk J van Leeuwen, *Lebanon*
Glen A Lehman, *Indianapolis*
Alex B Lentsch, *Cincinnati*
Andreas Leodolter, *La Jolla*
Gene LeSage, *Houston*
Josh Levitsky, *Chicago*
Cynthia Levy, *Gainesville*
Ming Li, *New Orleans*
Zhiping Li, *Baltimore*
Zhe-Xiong Lian, *Davis*
Lenard M Lichtenberger, *Houston*
Gary R Lichtenstein, *Philadelphia*
Otto Schiueh-Tzang Lin, *Seattle*
Martin Lipkin, *New York*
Chen Liu, *Gainesville*
Edward V Loftus, *Rocheste*
Robin G Lorenz, *Birmingham*
Michael R Lucey, *Madison*
James D Luketich, *Pittsburgh*
Guangbin Luo, *Cheveland*
Henry T Lynch, *Omaha*
Patrick M Lynch, *Houston*
John S Macdonald, *New York*
Bruce V MacFadyen, *Augusta*
Willis C Maddrey, *Dallas*
Ashok Malani, *Los Angeles*
Mercedes Susan Mandell, *Aurora*
Peter J Mannon, *Bethesda*
Charles M Mansbach, *Tennessee*
John F Di Mari, *Texas*
John M Mariadason, *Bronx*
Jorge A Marrero, *Ann Arbor*
Paul Martin, *New York*
Paulo Ney Aguiar Martins, *Boston*
Wendy M Mars, *Pittsburgh*
Laura E Matarese, *Pittsburgh*
Richard W McCallum, *Kansas*
Beth A McCormick, *Charlestown*
Lynne V McFarland, *Washington*
Kevin McGrath, *Pittsburgh*
Harihara Mehendale, *Monroe*
Ali Mencin, *New York*
Fanyin Meng, *Ohio*
Stephan Menne, *New York*
Didier Merlin, *Atlanta*
Howard Mertz, *Nashville*
George W Meyer, *Sacramento*
George Michalopoulos, *Pittsburgh*
James M Millis, *Chicago*
Fabrizio Michelassi, *New York*
Albert D Min, *New York*
Pramod K Mistry, *New Haven*
Emiko Mizoguchi, *Boston*
Smruti R Mohanty, *Chicago*
Satdarshan S Monga, *Pittsburgh*
Timothy H Moran, *Baltimore*
Peter L Moses, *Burlington*
Steven F Moss, *Providence*
Andrew J Muir, *Durham*
Milton G Mutchnick, *Detroit*
Masaki Nagaya, *Boston*
Victor Navarro, *Philadelphia*
Laura E Nagy, *Cleveland*
Hiroshi Nakagawa, *Philadelphia*
Douglas B Nelson, *Minneapolis*
Justin H Nguyen, *Florida*
Patrick G Northup, *Charlottesville*
Christopher O'Brien, *Miami*
Robert D Odze, *Boston*
Brant K Oelschlager, *Washington*
Curtis T Okamoto, *Los Angeles*
Stephen JD O'Keefe, *Pittsburgh*
Dimitry Oleynikov, *Omaha*
Stephen J Pandol, *Los Angeles*
Georgios Papachristou, *Pittsburgh*
Pankaj J Pasricha, *Galveston*
Zhiheng Pei, *New York*
Michael A Pezzone, *Pittsburgh*
CS Pitchumoni, *New Brunswiuc*
Paul J Pockros, *La Jolla*
Jay Pravda, *Gainesville*
Massimo Raimondo, *Jacksonville*
GS Raju, *Galveston*
Raymund R Razonable, *Minnesota*
Murray B Resnick, *Providence*
Adrian Reuben, *Charleston*
Douglas K Rex, *Indianapolis*
Victor E Reyes, *Galveston*
Basil Rigas, *New York*
Yehuda Ringel, *Chapel Hill*
Richard A Rippe, *Chapel Hill*
Maribel Rodriguez-Torres, *Santurce*
Marcos Rojkind, *Washington*
Philip Rosenthal, *San Francisco*
Barry Rosser, *Jacksonville Florida*
Hemant K Roy, *Evanston*
Sammy Saab, *Los Angeles*
Shawn D Safford, *Norfolk*
Dushyant V Sahani, *Boston*
Bruce E Sands, *Boston*
James M Scheiman, *Ann Arbor*
Eugene R Schiff, *Miami*
Nicholas J Shaheen, *Chapel Hill*
Vanessa M Shami, *Charlottesville*
Prateek Sharma, *Kansas City*
Harvey L Sharp, *Minneapolis*
Stuart Sherman, *Indianapolis*
Shivendra Shukla, *Columbia*
Alphonse E Sirica, *Virginia*
Shanthi V Sitaraman, *Atlanta*
Stuart J Spechler, *Dallas*
Subbaramiah Sridhar, *Augusta*
Shanthi Srinivasan, *Atlanta*
Michael Steer, *Boston*
Peter D Stevens, *New York*
Charmaine A Stewart, *Rochester*
Christian D Stone, *Saint Louis*
Gary D Stoner, *Columbus*
R Todd Stravitz, *Richmond*
Liping Su, *Chicago*
Christina Surawicz, *Seattle*
Robert W Summers, *Iowa City*
Wing-Kin Syn, *Durham*
Gyongyi Szabo, *Worcester*
Yvette Taché, *Los Angeles*
Toku Takahashi, *Milwaukee*
Seng-Lai Tan, *Seattle*
Andrzej S Tarnawski, *Orange*
K-M Tchou-Wong, *New York*
Jonathan P Terdiman, *San Francisco*
Neil D Theise, *New York*
Christopher C Thompson, *Boston*
Swan N Thung, *New York*
Michael Torbenson, *Baltimore*
Natalie J Torok, *Sacramento*
RA Travagli, *Baton Rouge*
George Triadafilopoulos, *Stanford*
Chung-Jyi Tsai, *Lexington*
Janet Elizabeth Tuttle-Newhall, *Durham*
Andrew Ukleja, *Florida*
Michael F Vaezi, *Nashville*
Hugo E Vargas, *Scottsdale*
Arnold Wald, *Wisconsin*
Scott A Waldman, *Philadelphia*
Jian-Ying Wang, *Baltimore*
Timothy C Wang, *New York*
Irving Waxman, *Chicago*
Steven A Weinman, *Galveston*
Steven D Wexner, *Weston*
Keith T Wilson, *Baltimore*
Jacqueline L Wolf, *Boston*
Jackie Wood, *Ohio*
George Y Wu, *Farmington*
Jian Wu, *Sacramento*
Samuel Wyllie, *Houston*
Wen Xie, *Pittsburgh*
Vijay Yajnik, *Boston*
Vincent W Yang, *Atlanta*
Francis Y Yao, *San Francisco*
Hal F Yee, *San Francisco*
Xiao-Ming Yin, *Pittsburgh*
Min You, *Tampa*
Zobair M Younossi, *Virginia*
Liqing Yu, *Winston-Salem*
David Yule, *Rochester*
Ruben Zamora, *Pittsburgh*
Michael E Zenilman, *New York*
Zhi Zhong, *Chapel Hill*
Michael A Zimmerman, *Colorado*
Stephen D Zucker, *Cincinnati*

Uruguay

Henry Cohen, *Montevideo*

[1]Passed away on October 20, 2007
[2]Passed away on June 11, 2007
[3]Passed away on June 14, 2008

World Journal of
Gastroenterology®
Weekly Established in October 1995
Volume 15 Number 23
June 21, 2009

Contents

Contents

World Journal of Gastroenterology
Volume 15 **Number** 23 **June** 21, 2009

Contents

World Journal of Gastroenterology
Volume 15 **Number** 23 **June** 21, 2009

INTRODUCTION

World Journal of Gastroenterology is an international, open-access, peer-reviewed, and multidisciplinary weekly journal that serves gastroenterologists and hepatologists. The biggest advantage of the open access model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the values of the readers, the authors and the society.

Maximization of the value of the readers can be comprehended in two ways. First, the journal publishes articles that can be directly read or downloaded free of charge at any time, which attracts more readers. Second, the readers can apply the knowledge in clinical practice without delay after reading and understanding the information in their fields. In addition, the readers are encouraged to propose new ideas based on those of the authors, or to provide viewpoints that are different from those of the authors. Such discussions or debates among different schools of thought will definitely boost advancements and developments in the fields. Maximization of the value of the authors refers to the fact that these journals provide a platform that promotes the speed of propagation and communication to a maximum extent. This is also what the authors really need. Maximization of the value of the society refers to the maximal extent of the social influences and impacts produced by the high quality original articles published in the journal. This is also the main purpose of many journals around the world.

NAME OF JOURNAL
World Journal of Gastroenterology

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, 77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology,* Room 903, Building D, Ocean International Center, No.62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHING
The WJG Press and Beijing Baishideng BioMed Scientific Co., Ltd.. Room 903, Building D, Ocean International Center, No.62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PRINTING
Beijing Kexin Printing House

OVERSEAS DISTRIBUTOR
Beijing Bureau for Distribution of Newspapers and Journals
(Code No. 82-261)
China International Book Trading Corporation PO Box 399, Beijing, China (Code No. M4481)

PUBLICATION DATE
June 21, 2009

EDITOR-IN-CHIEF
Lian-Sheng Ma, *Beijing*

SUBSCRIPTION
RMB 50 Yuan for each issue, RMB 2400 Yuan for one year

CSSN
ISSN 1007-9327
CN 14-1219/R

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/wjg/help/instructions.jsp. If you do not have web access please contact the editorial office.

ONLINE SUBMISSION
http://wjg.wjgnet.com

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2817

World J Gastroenterol 2009 June 21; 15(23): 2817-2833
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Drug-induced liver injury: Is it somehow foreseeable?

Giovanni Tarantino, Matteo Nicola Dario Di Minno, Domenico Capone

Giovanni Tarantino, Matteo Nicola Dario Di Minno, Department of Clinical and Experimental Medicine, Section of Hepatology in Internal Medicine, Federico II University, Medical School of Naples, 5 80131 Napoli, Italy
Domenico Capone, Department of Neurosciences, Unit of Clinical Pharmacology, Federico II University, Medical School of Naples, 5 80131 Napoli, Italy
Author contributions: All the authors equally contributed towards writing and editing the manuscript; All authors approved the final version of the manuscript.
Correspondence to: Giovanni Tarantino, MD, Professor, Department of Clinical and Experimental Medicine, Section of Hepatology in Internal Medicine, Federico II University, Medical School of Naples, Via S. Pansini, 5 80131 Napoli, Italy. tarantin@unina.it
Telephone: +39-81-7462024 Fax: +39-81-5466152
Received: April 22, 2009 Revised: May 13, 2009
Accepted: May 20, 2009
Published online: June 21, 2009

Abstract

The classic view on the pathogenesis of drug-induced liver injury is that the so-called parent compounds are made hepatotoxic by metabolism (formation of neo-substances that react abnormally), mainly by cytochromes P-450 (CYP), with further pathways, such as mitochondrial dysfunction and apoptosis, also playing a role. Risk factors for drug-induced liver injury include concomitant hepatic diseases, age and genetic polymorphisms of CYP. However, some susceptibility can today be predicted before drug administration, working on the common substrate, by phenotyping and genotyping studies and by taking in consideration patients' health status. Physicians should always think of this adverse effect in the absence of other clear hepatic disease. Ethical and legal problems towards operators in the health care system are always matters to consider.

Key words: Drug-induced liver injury; Cytochrome P-450; Drug metabolism; Pharmacogenomics; Herbal remedies

Peer reviewer: Dr. Yukihiro Shimizu, Kyoto Katsura Hospital, 17 Yamada-Hirao, Nishikyo, Kyoto 615-8256, Japan

Tarantino G, Di Minno MND, Capone D. Drug-induced liver injury: Is it somehow foreseeable? *World J Gastroenterol* 2009; 15(23): 2817-2833 Available from: URL: http://www.wjgnet.com/1007-9327/15/2817.asp DOI: http://dx.doi.org/10.3748/wjg.15.2817

INTRODUCTION

Drug metabolism is the major determinant of drug clearance and the factor most frequently responsible for inter-individual differences in drug pharmacokinetics. Most adverse hepatic reactions require metabolism of the drug to form reactive metabolites and free radicals (indirect toxicity), that subsequently lead to fatal insults, sensitization to the lethal effects of the innate immune system, or haptenization eliciting an immunoallergic response of the adaptive immune system. Besides licensed drugs, herbal and natural supplements are recognized as causing hepatotoxicity with increasing frequency as patients turn more and more to alternative medicine[1].

Many environmental and developmental factors can interact with each other and with genetic factors to affect drug response and the metabolic activation or inactivation of drugs generally used in medical practice.

Oxidation, reduction and hydrolysis are the main pathways along drug metabolism before excretion. The most important enzyme system of phase I metabolism is the cytochrome P-450 (CYP) system, a microsomal superfamily of isozymes that catalyze the oxidation of many drugs. The electrons are supplied by NADPH/CYP reductase, a flavoprotein that transfers electrons from NADPH (the reduced form of nicotinamideadenine dinucleotide phosphate) to CYP. Multiple forms of CYP enzymes play important roles in the oxidation of structurally diverse xenobiotics. CYP3A (about 30% of total CYP) and CYP2C (about 20% of total CYP) enzymes are major forms. Human cytochrome CYP3A subfamily members (mainly CYP3A4) mediate the metabolism of many marketed drugs (amiodarone, amlodipine, clarithromycin, cyclosporine, erythromycin, lovastatin, nifedipine, tamoxifen, terfenadine, verapamil, R-warfarin) and thus play a critical role in drug metabolism. Furthermore, P-glycoprotein and CYP3A are frequently co-expressed in the same cells and share a large number of substrates and modulators[2].

Other important human drug-metabolizing enzymes are CYP1A2 (caffeine, estradiol, lidocaine, tacrine, theophylline, verapamil, R-warfarin), CYP2C9 (diclofenac, phenytoin, piroxicam, tetrahydrocannabinol, tolbuta-

mide, S-warfarin), CYP2C19 (diazepam, hexobarbital, S-mephenytoin, omeprazole, pentamidine, propranolol, R-warfarin), and CYP2D6 (codeine, debrisoquine, dextromethorphan, encainide, haloperidol, metoprolol, mexiletine, paroxetine, phenothiazines, propanolol, risperidone, sertraline, tricyclic antidepressants, venlafaxine) as highlighted by commonly used probe cocktails[3,4]. The expression of drug metabolizing enzymes, mainly CYP, shows significant interspecies differences and variability among human individuals (polymorphic or inducible enzymes) which makes the accurate prediction of the metabolism of various compounds in humans difficult. For example, patients who metabolize certain drugs rapidly may require higher, more frequent doses to achieve therapeutic concentrations; patients who metabolize certain drugs slowly may need lower, less frequent doses to avoid toxicity, particularly for drugs with a narrow interval of safety.

HEPATOTOXICITY: GENERAL CONCEPTS

Several key issues need to be addressed to study drug induced liver injury (DILI), that is, what metabolites will be formed (metabolic profile); which enzymes are involved and to what extent; whether drug metabolism will be affected directly (drug-drug interactions) or indirectly (enzyme induction) by the administered compound. Drug metabolism studies are routinely performed in laboratory animals, but they are not sufficiently accurate to predict the metabolic profiles of drugs in humans. In fact, hepatotoxicity due to idiosyncratic reactions cannot be detected by conventional animal toxicity studies. Furthermore, predisposing factors in humans such as ethanol-induced metabolic sensitivity to acetaminophen hepatotoxicity do not exist in the animal model. Interspecies differences in bioavailability, distribution and metabolism may also explain a number of false positives and false negatives. There is clearly a need to better understand the drug metabolism enzyme profile of the most commonly used non-rodent species, i.e. the dog and the monkey. Some false negative results may be related to insufficient (not in the pharmaceutical industry) exposure of the animals either because the doses tested were too low or because intestinal absorption was poor. Many of these issues can now be addressed by the use of relevant human *in vitro* models, which may speed up the understanding of drug toxicity. Human hepatocytes are the closest *in vitro* model to the human liver and they are one of the few models which can produce a metabolic profile of a drug which is very similar to that found *in vivo*. However, the use of human hepatocytes is restricted, because limited access to suitable tissue samples prevents their use in high-throughput chemical and genetic screens[5]. Comparative studies on liver microsomes and cells from animal species, including humans, are very useful for demonstrating species differences in the metabolic profile of a given drug and are of great value in the selection of animal species for later pharmacokinetic and toxicological studies[6].

CYP-engineered cells (or microsomes from CYP-engineered cells, for example, Supersomes) have made the identification of CYPs involved in the metabolism of a drug candidate more straightforward and much easier[7]. However, the screening of substances acting as potential CYP inducers can be conducted only in cellular systems fully capable of transcribing and translating CYP genes.

EPIDEMIOLOGY AND CLINICAL ASPECTS OF DRUG INDUCED LIVER INJURY

Drug hepatotoxicity has been evaluated in case histories, surveys based on retrospective record reviews, and spontaneous adverse drug reactions reported to national pharmacovigilance systems, but in relatively few epidemiologic studies. Approximately 1 in 100 patients develops DILI during hospitalisation. DILI is frequently missed and, therefore, DILI detection by diagnosis will result in misleadingly low incidence rates[8]. Unfortunately, patients with drug-induced hepatocellular jaundice have an 11.7% chance of progressing to death or transplantation[9].

DILI cases have been reported to constitute approximately 6% of all out-patients and 3% of referrals and to occur more often in women[10]. The incidence of drug-induced hepatitis is higher in patients over 40 years of age[11]. Acute liver failure or injury not clearly attributable to other known causes occurred in the order of 1 per 10 000 person -years among diabetic patients treated with oral hypoglycemic drugs or insulin[12].

The long term outcome of drug-related liver disease is unknown. To study the natural history of histologically proved drug-induced hepatotoxicity, 44 patients with liver biopsies coded as drug-related liver disease were identified from hospital records. Initial histology showed acute hepatitis in 6, chronic hepatitis in 20, and cholestasis in 18. At a median of 5 years follow-up, one third of patients had persistent significant abnormalities in their liver blood tests. Factors predicting persistence or development of chronic liver disease were fibrosis and continued exposure to the drug[13]. To identify and quantify the risk of acute liver injury associated with individual drugs, authors reviewed and integrated all the published epidemiologic research on the subject. Participants were selected according to their use of selected agents [nonsteroidal antiinflammatory drugs (NSAIDs), antibiotics, acid-suppressing drugs, other drugs suspected of being hepatotoxic] during the study period. Among the agents, authors found a group of important hepatotoxic drugs, including chlorpromazine and isoniazid, with an associated incidence rate of acute liver injury greater than 100/100 000 users. Agents with less risk but greater than 10/100 000 users were amoxicillin-clavulanic acid and cimetidine[14].

DILI with an incidence rate of near 1 per 100 000 encloses a spectrum of clinical disease ranging from no symptoms with mild biochemical abnormalities to fatal, fulminant hepatitis. The majority of adverse liver reactions are idiosyncratic in nature; in fact, about 10% of all acute liver failure cases are attributed to this type of reaction. They can occur in some instances up to three months after the causative medication was last taken.

The diagnosis of DILI is prevalent clinically, and based primarily on history, that is, exclusion of other hepatic diseases (hepatitis A, B, C, Epstein-Barr virus, cytomegalovirus, ischemia, and biliary tract disease), high likelihood of suspicion based on a strict cause-effect sequence, the duration of latency to symptomatic presentation, the presence of immune-mediated hypersensitivity (hypereosinophilia, fever and rush) and the response to drug withdrawal. Re-challenge is not advised in cases with a hypersensitivity basis, although this is the most definitive means of diagnosis. Some of the hypersensitivity cases are associated with autoantibodies to CYP, which can be used to confirm the diagnosis; for example, halothane is associated with anti-CYP2E1, anticonvulsants are associated with CYP3A4, dihydralazine hepatitis is associated with anti-CYP1A2, and tienilic acid (a diuretic drug withdrawn from the market because of hepatic failure) is associated with anti-CYP2C9. In the cases of metabolic idiosyncrasy, careful reintroduction of the offending drug may be accomplished without recurrence of the liver disease but should be done only when the drug is absolutely necessary and with careful monitoring. In some instances, liver biopsy can be of help, showing characteristic features, but it usually is not necessary and tells more about prognosis than etiology.

DILI can be of hepatocellular (increase of both the transaminases, alanine-aminotransferase/aspartate-aminotransferase), cholestatic (predominant rise in serum alkaline phosphatase and/or γ-glutamyl-transferase, GGT) or mixed type. Negative prognostic factors are an elevated serum bilirubin level and high ammonia levels with a mortality of approximately 10%[15]. A further less favourable index is a marked hypoprothrombinemia. Overall, chronic liver injury may occur in up to 5%-6% of the patients on some drugs, even though the putative offending substance is withdrawn[16].

LIVER HISTOLOGY

As previously emphasized, the pathogenesis of drug- or toxin- induced liver injury usually involves the participation of "toxic metabolites" that either elicit an immune response or directly affect the biochemical processes or functions of the cell[17]. Although the same basic process determines the DILI appearance, the histopathological liver changes in these cases vary, including: (a) necrosis, which commonly occurs in acinar zone 3, (b) abundant neutrophil and/or eosinophil infiltration, (c) hepatocytic and/or canalicular cholestasis with little or no inflammation, (d) microvesicular steatosis mixed with macrovesicular steatosis, and (e) presentation of epitheloid cell granuloma. There are no significant differences in liver histopathology between acute and chronic DILI groups, except that the fibrosis and the ductular proliferation are different.

DAMAGE MECHANISMS

Drug metabolites can be free radicals or electrophilic chemicals that undergo or promote a variety of chemical reactions, such as the depletion of reduced glutathione; covalent binding to proteins, lipids, or nucleic acids; or induction of lipid peroxidation. All of these have consequent direct effects on cellular organelles such as mitochondria, the endoplasmic reticulum, the cytoskeleton, microtubules, or on the nucleus. They may also indirectly influence cellular structures through the activation and inhibition of signaling kinases, transcription factors, and gene-expression profiles. The subsequent intracellular stress leads to cell death caused by either cell shrinkage and nuclear disassembly (apoptosis) or swelling and lysis (necrosis). Liver injury is characterized by hepatocyte death; that is the main event, although bile duct epithelium or sinusoidal endothelial cells may also be involved.

CYP-mediated Biotransformation: the common substrate

While several CYPs are involved in the synthesis of bile acids and steroid hormones and the metabolism of fatty acids, retinoic acid, prostaglandins and eicosanoids, a limited number of CYPs (15 in humans) are primarily involved in xenobiotic metabolism. The xenobiotic-metabolizing CYPs are found in families 1-4. Since a single CYP can metabolize a large number of structurally diverse compounds these enzymes can collectively metabolize chemicals found in the diet, environment and administered as drugs. While metabolism of xenobiotics such as drugs is required to efficiently eliminate them from the body, as noted earlier, certain chemicals are metabolically activated to reactive derivatives that cause cell toxicity and cancer. Among these, the CYPs that metabolically activate toxicants and carcinogens are limited to some forms including CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2E1, and to a limited extent the CYP3A subfamily.

ATP depletion

Acetaminophen overdose causes liver injury by mechanisms involving glutathione depletion, oxidative stress and mitochondrial dysfunction. Acetaminophen-induced decreases in mitochondrial reduced glutathione and ATP content, and cytosolic leakage of cytochrome c are attenuated by cyclosporin A, suggesting that mitochondrial oxidative stress and ATP depletion resulting from mitochondrial permeability transition (MPT) are the principal mechanisms involved in acetaminophen-induced liver injury[18].

The role of ATP-depletion-dependent necrosis has further been ascertained. Recently, using TUNEL labeling and caspase 3 activation, it was observed that acetaminophen induced the MPT and ATP-depletion-dependent necrosis or caspase-dependent apoptosis as determined, in part, by ATP availability from glycolysis[19].

Binding to nuclear or cytoplasmatic constituents

Inducers can increase the bioactivation of drugs that contribute to hepatotoxicity *via* reactive intermediates. Nuclear receptors are key mediators of drug-induced changes in the expression of drug clearance pathways. However, species differences in nuclear receptor activation make the

prediction of CYP induction in humans from data derived from animal models problematic. Drugs have the capacity to alter nuclear receptor expression (modulators) and/or serve as ligands for the receptors (agonists or antagonists), and thus can have synergistic or antagonistic effects on the expression of drug-metabolizing enzymes and transporters. Co-administration of drugs that are nuclear receptor agonists or antagonists can lead to severe toxicity, a loss of therapeutic efficacy or an imbalance in physiological substrates, providing a novel molecular mechanism for drug-drug interactions[20].

RNA interference

Authors suggested that the lack of p53 response may confer a growth advantage on preneoplastic hepatocytes and may be an important factor in hepatic tumor promotion by 2-acetylaminofluorene and other genotoxic compounds. Inhibition of RNA polymerase Ⅱ driven transcription by DNA lesions may constitute one of the mechanisms leading to accumulation of the tumor suppressor p53[21].

Oxidative stress and lipid peroxidation

Redox signals are important in the modulation of cell function. Reactive oxygen species (ROS) generation influences many signaling proteins, interfering with molecular and biochemical processes responsible for cell differentiation, proliferation and death. Protein kinase (PKC) is a crucial signaling protein which is subject to redox regulation and controls these responses. ROS are products of normal cellular metabolism and are recognized to be harmful or beneficial to living systems. The harmful effect of ROS is termed oxidative stress and it occurs when there is an overproduction of these species or a deficiency of antioxidants. Enzymatic systems that contribute to ROS formation in the liver include CYP monooxygenases and NADPH oxidase. In the healthy liver, hepatocytes produce low amounts of ROS and Kupffer cells are well equipped to release ROS in response to infection. Antioxidants such as superoxide dismutase and catalase efficiently remove ROS surplus to maintain the normal cell homeostasis. Different stimuli are able to modify the redox state of liver cells modulating signal transduction pathways able to trigger many aspects of liver pathologies. In a recent study, evidence of the central role of PKC as a redox-sensitive molecule implicated in the pathogenesis and progression of toxic liver diseases was provided[22].

The free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in membranes, setting off a free radical chain reaction sequence. Lipid peroxidation is known to cause membrane disruption, resulting in the loss of membrane integrity and leakage of microsomal enzymes. By-products of lipid peroxidation include reactive aldehydes that can form protein and DNA adducts and may contribute to hepatotoxicity and carcinogenicity, respectively. Natural antioxidants, including glutathione, are capable of quenching the lipid peroxidation reaction[23].

Immune-mediated hypersensitivity

Because liver undergoes continuous exposure to xenobiotics, it possesses a variety of local immune mechanisms to face these challenges. In the liver there are both innate and adaptive immune cells, including tissue macrophages (KC), natural Killer (NK) cells, and non-NK (NKT) cells, which account for nearly 50% of intrahepatic leukocytes[24]. KC produce various cytokines and other mediators, including prostanoids, nitric oxide and ROS that play roles in promoting and regulating hepatic inflammation. Furthermore, KC represent a major population of antigen-presenting cells (APCs) having an important role in the balance between the induction of immunity and tolerance within the liver. It has been demonstrated that freshly isolated liver NK cells spontaneously induce the cytotoxicity of various cell lines, whereas NKT cells are cytotoxic in the presence of interleukin (IL)-2[25]. This cytotoxicity is further enhanced by IL-12 and IL-18, which are produced by activated KC. Another function ascribed to NK and NKT cells is their ability to produce high levels of T helper (Th) 1 and Th2 cytokines upon stimulation[26]. NK cells have been shown to represent a major source of interferon (IFN)-γ in many types of liver disease[27]. NKT cells produce either IFN-γ or IL-4, or in some cases both cytokines, depending on the differentiation state of the cells and the stimuli[25]. It has also been demonstrated that IL-4 produced by NKT cells may be associated with the initiation and regulation of Th2 responses. Various mechanisms have been suggested to explain this tolerance[28], such as immune deviation, active suppression and apoptosis of activated T cells. Regarding immune deviation, it has been shown that Th2 cytokine production is preferentially maintained when adoptively transferred Th1 and Th2 cells are recovered from the liver. It has also been reported that liver sinusoidal endothelial cells (LSEC) are capable of selectively suppressing IFN-γ-producing Th1 cells while concurrently promoting the outgrowth of IL-4-expressing Th2 cells[29]. Good evidence suggests that hepatic dendritic cells are also important in the induction of tolerance, rather than the activation of T-cell responses. It has been further demonstrated that although LSEC are capable of presenting antigens to T cells, LSEC-activated $CD4^+$ or $CD8^+$ T cells fail to differentiate into Th1 cells or cytotoxic effector cells, respectively[30]. Most drugs are not chemically reactive but can be activated metabolically to reactive species which, after binding to cellular macromolecules, become immunogenic and can elicit an effective immune response. Immune-mediated mechanisms have been proposed for idiosyncratic reactions observed with sulfonamides, halothane and phenytoins. Presentation of drug-protein adducts by professional cells to Th lymphocytes, and/or a direct association between the drug and major histocompatibility complex (MHC) proteins of hepatocytes could be involved in the activation of the immune system. As a consequence of this, drug-directed antibodies and/or T-lymphocytes able to recognize drug-derived haptens arise which are responsible for the clinical manifestations of hepatitis. Drug-directed antibodies can be detected in sera of allergic patients by solid-phase immunoassays. Sensitized T-lymphocytes can be shown by hapten-induced cell proliferation experiments and by the early expression of CD69 antigen[31]. Despite the

possible detection of drug-specific antibodies, it is difficult to directly prove the pathogenic role of the adaptive immune system in DILI, partly because of the lack of animal models. A difficulty in developing animal models is the fact that the default response of the liver to antigens is immunological tolerance. This could also explain the relatively low occurrence of this type of DILI in human beings.

Inflammation

A recent paper emphasizes the imbalance between Th1 cells producing cytokines associated with a cell-mediated response and Th2 cells associated with an antibody response, leading to a shift in immune response to one that may participate in DILI during administration of certain drugs, especially in subjects with genetic polymorphisms in drug-metabolizing enzymes. In fact, several cases of DILI related to administration of drugs appear to be initiated or intensified by respiratory inflammation states, which stimulate sometimes dysregulated production of IFN-γ and/or other proinflammatory cytokines/growth factors. This ends up in down-regulation of various induced and constitutive isoforms of CYPs and other enzymes involved in the metabolism of several drugs, thus having an important impact on the alterations in bioactivation and detoxication processes. DILI may eventually be prevented by screening methods that can identify genetic polymorphisms of drug-metabolizing enzymes and gene polymorphisms or RNA-expression profiles of some proinflammatory cytokines before patients take any drug[32].

Apoptosis

Although CYP-generated reactive metabolites can cause hepatocyte apoptosis, the mechanism of this effect has only recently been elucidated. Male rat hepatocytes were incubated with skullcap diterpenoids. This treatment decreased cellular glutathione and protein thiols and increased cellular Ca^{2+}. This activated Ca^{2+}-dependent tissue transglutaminase formed a cross-linked protein scaffold, and also opened the mitochondrial permeability transition pore, causing outer mitochondrial membrane rupture, increased cytosolic cytochrome c, activation of procaspase 3, internucleosomal DNA fragmentation, and ultrastructural features of apoptosis. Cell death was increased by a CYP3A inducer (dexamethasone) increasing glutathione depletion. In contrast, cell death was prevented by decreasing CYP3A activity (with troleandomycin), preventing glutathione depletion (with cysteine), blocking Ca^{2+}-modulated events (with calmidazolium), preventing mitochondrial permeability transition (with cyclosporin A), or inhibiting caspase 3 (with acetyl-Asp-G u-Va-Asp-a dehyde). Both calmidazolium and cyclosporin A also prevented the increase in cytosolic cytochrome c and procaspase 3 activation[33].

Calcium homeostasis imbalance

When glutathione and other antioxidants are depleted, however, opportunities for lipid peroxidation are enhanced. Weakened cellular membranes allow sufficient leakage of Ca^{2+} into the cytosol to disrupt intracellular Ca^{2+} homeostasis. High Ca^{2+} levels in the cytosol activate Ca^{2+}-dependent proteases and phospholipases that further increase the breakdown of the membranes. Similarly, the increase in intracellular Ca^{2+} can activate endonucleases that can cause chromosomal damage and also contribute to cell death. Sustained cell regeneration and proliferation following cell death may increase the likelihood of unrepaired spontaneous, lipid peroxidation- or endonuclease-derived mutations that can lead to cancer.

FACTORS INFLUENCING PATIENT SUSCEPTIBILITY TO HEPATOXICITY

Determination of DILI also includes an individual susceptibility. This susceptibility is governed by genetic, pre-existing and environmental factors. Predisposing factors are generally thought to be important to somehow explain the unpredictability of the phenomena through which substances turn into hepatotoxins, and consist of ethnic and racial factors, CYP polymorphisms, concomitant liver diseases, age, nutritional status and diet, gender and pregnancy.

Ethnic and racial factors

These factors have important implications for susceptibility to acetaminophen hepatotoxicity following overdosage especially in a small subgroup showing extensive metabolic activation. An exemplary study indicates markedly reduced metabolic activation of acetaminophen in Africans. These ethnic differences in acetaminophen metabolism may be related to genetics even though environmental factors, including differences in diet and protein intake, should not be excluded. There were no ethnic differences in the sulphate conjugation of acetaminophen, but the mean fractional recovery of the glucuronide conjugate in Caucasians was less than in Africans[34].

Concomitant chronic liver diseases

In liver diseases, pharmacokinetics are generally impaired. Pathogenetic factors include alterations in intestinal absorption, plasma protein binding, hepatic extraction ratio, liver blood flow and portal-systemic shunting, biliary excretion, enterohepatic circulation, and renal clearance. The key point is, however, the reduction of functional hepatic mass that may have complex effects on drug clearance, particularly biotransformation. Net results for an individual drug are unpredictable and do not correlate well with the type of liver damage, its severity, or liver laboratory test results. Thus, no general rules are available for modifying drug dosage in patients with liver disease. Recently, NonAlcoholic Fatty Liver Disease (NAFLD) has been found to be a fertile soil for the development of hepatotoxicity. With NAFLD now linked to obesity and metabolic syndrome, the impact of this observation should not be overlooked[35].

Oxidative stress has been detected in patients affected by alcohol abuse, hepatitis C virus (HCV) infections, iron overload and chronic cholestasis[36]. Alcohol-induced liver

disease (ALD) has been associated with the synergistic induction of oxidative stress by alcohol metabolites, iron accumulation and antioxidant depletion[37].

HCV infection may generate oxidative stress by chronic inflammation and by disruption of glutathione efflux. Therefore, oxidative stress is not only a consequence of chronic liver injury but it also contributes to fibrogenesis and it appears as a key player in the pathogenesis of hepatic diseases. Vitamin E could prevent the decrease in O_2 uptake[38].

But what is the role of stress in determining hepatotoxicity? Its regulation of several enzymatic systems which are involved in the biotransformation of xenobiotics in the liver was recently investigated in a study using restraint stress as a stress model in animals. The results demonstrated that stress suppressed total basal CYP content of one third of animals and basal ethoxyresorufin 7-dealkylase activity. On the other hand, restraint stress increased total CYP content in 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene-treated mice, while slightly suppressing PROD activity. In addition, CYP2E1 dependent p-nitrophenol hydroxylation was suppressed by stress in the same animals and cytosolic aldehyde dehydrogenases were not affected. Although stress had no effect on basal CYP2A5 activity, the inducibility of this hepatic activity increased 2-fold after stress exposure. In addition, a slight suppression in liver glutathione content was found. Northern blot analysis revealed that restraint stress had a relatively suppressive effect on control CYP1A2 expression in the liver. In conclusion, stress was found to significantly interfere with the expression processes of some CYP450s[39].

Age

Increased risk of DILI may result from age-related changes in pharmacokinetics or pharmacodynamics. Overall hepatic metabolism of many drugs through the CYP enzyme system decreases with aging. For drugs with decreased hepatic metabolism, clearance typically decreases by 30%-40%. Theoretically, maintenance drug doses should be decreased by this percentage; however, the rate of drug metabolism varies greatly from person to person, and individual titration is required. Risk of any adverse effect and obviously of DILI increases exponentially with the number of drugs used, partly because multiple drug therapy reflects the presence of many diseases and increases risk of drug-disease and drug-drug interactions.

Nutritional status and diet

Increasing evidence implicates dietary factors in the progression of diseases, including certain cancers, diabetes and obesity. Diet also regulates the expression and function of CYP genes which impacts on drug elimination and may also significantly affect disease pathogenesis. Upregulation of CYPs 2E1 and 3A4 occurs after feeding of experimental diets that are high in fats or carbohydrates; these diets also promote hepatic lipid infiltration, which is a component of the metabolic syndrome that characterizes obesity. Increased availability of lipid substrates for CYPs can enhance free radical production and exacerbate tissue injury. Similar processes may also occur in other models of experimental disease states that exhibit a component of altered nutrient utilization. Food-derived chemicals, including constituents of cruciferous vegetables and fruits, modulate CYP expression and the expression of genes that encode cytoprotective phase Ⅱ enzymes. Certain dietary indoles and flavonoids activate CYP1A expression either by direct ligand interaction with the aryl hydrocarbon receptor (AhR) or by augmenting the interaction of the AhR with xenobiotic response elements in CYP1A1 and other target genes. Other dietary chemicals, including methylenedioxyphenyl (MDP) compounds and isothiocyanates also modulate CYP gene expression. Apart from altered CYP regulation, a number of dietary agents also inhibit CYP enzyme activity, leading to pharmacokinetic interactions with coadministered drugs. A well described example is that of grapefruit juice, which contains psoralens and possibly other chemicals that inactivate intestinal CYP3A4. Decreased presystemic oxidation by this CYP increases the systemic bioavailability of drug substrates and the likelihood of drug toxicity. Dietary interactions may complicate drug therapy but inhibition of certain CYP reactions may also protect the individual against toxic metabolites and free radicals generated by CYPs. Chemicals in teas and cruciferous vegetables may also inhibit human CYP enzymes that have been implicated in the bioactivation of chemical carcinogens. Thus, food constituents modulate CYP expression and function by a range of mechanisms, with the potential for both deleterious and beneficial outcomes[40].

As previously emphasised, obesity is considered an important risk factor for DILI. Paradoxically, earlier studies have shown highly exaggerated mechanism-based liver injury by thioacetamide (TA) in rats following moderate diet restriction (DR). The objective of this significant study was to investigate the mechanism of higher liver injury of TA in DR rats. When male rats were maintained on DR (35% of ad libitum diet for 21 d), the total hepatic CYP was increased 2-fold along and there was a 4.6-fold increase in CYP2E1 protein, which corresponded with a 3-fold increase in CYP2E1 activity as measured by chlorzoxazone hydroxylation. To further test the involvement of CYP2E1, 24 and 18 h after pretreatment with pyridine (PYR) and isoniazid (INZ), specific inducers of CYP2E1, rats received a single administration of 50 mg of TA/kg. TA liver injury was > 2.5- and > 3-fold higher at 24 h in PYR + TA and INZ + TA groups, respectively, compared with the rats receiving TA alone. Pyridine pretreatment resulted in significantly increased total CYP content accompanied by a 2.2-fold increase in CYP2E1 protein and 2-fold increase in enzyme activity concordant with increased liver injury of TA, suggesting mechanism-based bioactivation of TA by CYP2E1. Hepatic injury of TA in DR rats pretreated with diallyl sulfide (DAS), a well known irreversible *in vivo* inhibitor of CYP2E1, was significantly decreased (60%) at 24 h. Carbon tetrachloride (CCl_4), a known substrate of CYP2E1, caused less liver injury and greater animal survival, confirming inhibition of CYP2E1 by DAS pretreatment[41].

Gender

Some authors clarified this issue, studying 50 patients diagnosed with active tuberculosis infection with normal pretreatment liver laboratory tests; they were monitored clinically as well as biochemically in a prospective cohort analysis. Antitubercular drug-induced hepatotoxicity was found more often in females (OR 4.2). Younger patients were also at a higher risk (OR 2.75). Nutritional status, assessed by body mass index and serum albumin level, was the next most common predisposing factor[42].

PHENOTYPING AND GENOTYPING STUDIES

The genetic polymorphisms of human drug metabolizing enzymes have been firmly established. Based on the metabolic handling of certain probe drugs, the population can be divided into two phenotypes: the rapid acetylator/extensive metabolizer (EM) and slow acetylator/poor metabolizer (PM). For some authors, a quadrimodal behaviour is possible, that is, poor, intermediate, extensive and ultrarapid.

These polymorphisms have provided useful tools to study the relationship between genetically determined differences in the activity of drug metabolizing enzymes and the risk for adverse drug reactions and certain types of chemically-induced diseases.

With regard to the susceptibility of the two phenotypes, DILI can be anticipated for the following scenarios: (1) the drug toxicity is caused by the parent compound and the elimination of the drug proceeds exclusively *via* the polymorphic enzyme, there being no alternate pathways of biotransformation available. Thus the poor metabolizer phenotype will be more prone to such a type of toxicity since, at the same level of exposure, this phenotype will accumulate the drug as a result of impaired metabolism; (2) the polymorphic pathway is a major route of detoxification, so impairment of this pathway shifts the metabolism to an alternate pathway *via* which a reactive intermediate is formed. In such a situation the PM phenotype constitutes a major risk factor for toxicity (i.e. INZ hepatotoxicity); the toxicity is mediated by a reactive intermediate generated by a polymorphic enzyme. Hence EMs are at a much higher risk than PMs of developing toxicity or cancer (for example, smokers)[43].

A further problem with PMs that the same dose of drug could yield a sustained plasma concentration. Poor metabolism is especially problematic with drugs that have a narrow therapeutic index like debrisoquine, phenformin or captopril. It has been estimated that psychiatric patients with CYP2D6 deficiency encounter more adverse drug incidents than those who are EMs[44].

With estimates of the percentage of pharmaceuticals that are subject to metabolism by the CYP in excess of 80%, the relative activities of these enzymes in various subpopulations and even in individual patients can have important ramifications in matters ranging from dose selection to prediction of toxicity to suitability of a new chemical entity for continued drug development.

As previously emphasized, CYP1A2, 2C9, 2C19, 2D6 and 3A are the major isoforms responsible for the metabolism of more than 90% of marketed drugs. Polymorphism of drug metabolism represents an important source of interindividual and interethnic variation in drug response.

CYP2D6, CYP2C9 and CYP2C19 are three polymorphic CYP enzymes. The EM phenotype occurs when there is at least one wild type allele at the relevant gene locus. The PM phenotype occurs when both alleles of either CYP2D6 or CYP2C19 carry inactivating mutations and give rise to synthesis of enzyme with impaired activity or no synthesis of enzyme at all.

Phenotyping is often based on high-performance liquid chromatography (HPLC) such as the determination of the dextromethorphan/total dextrorphan molar ratios as metabolic ratios (MRs) in plasma samples collected at 3 h after oral administration of 30 mg dextromethorphan hydrobromide. In this situation PMs and extensive EMs can be identified distinctly. To determine the real-time activity of the CYP isozymes, specific probe drugs can be employed. Recently, the use of multiple probe drugs, that is, a 'cocktail' approach, has become popular in pharmacogenetic studies as this provides a high-throughput approach in evaluating CYP isozyme activities. A number of cocktails (from five to six drugs) have been described in the literature.

These include the Pittsburgh cocktail, GW cocktail, Cooperstown cocktail and Karolinska cocktail. So far most of the analytical methods for these cocktails usually require a separate HPLC, gas chromatography (GC) or liquid chromatography/mass spectrometry (LC/MS) technique for each probe drug and its metabolite.

Recently, a fast gradient LC/MS method for the simultaneous determination of CYP substrates and metabolites in the GW cocktail was reported[45]. However, this cocktail has several practical limitations. First of all, the use of diclofenac as a CYP2C9 marker is undesirable due to its variable absorption in humans. Secondly, the use of mephenytoin is inconvenient as this drug is no longer commercially available in many parts of the world; besides, its sedative side effect is prominent, especially in PMs. Thirdly, chlorzoxazone, a probe drug in the cocktail, can significantly inhibit the CYP3A-mediated first-pass metabolism of midazolam in the gut and its use for the present purpose is not recommended.

A rapid LC/tandem MS method has been developed for the determination of six CYP probe substrate metabolites including acetaminophen for CYP1A2, 4-hydroxytolbutamide for CYP2C9, 5-hydroxyomeprazole for CYP2C19, dextrorphan for CYP2D6, 6-hydroxychlorzoxazone for CYP2E1 and dehydronifedipine for CYP3A4[46].

What is the clinical importance of studying the CYP polymorphisms? It has been shown that the cholesterol-lowering effect as well as the efficacy and tolerability of simvastatin is influenced by CYP2D6 genetic polymorphism[47]. Because the different HMG-CoA reductase in-

hibitors differ with respect to the degree of metabolism by the different CYP enzymes, genotyping may help to select the appropriate HMG-CoA reductase inhibitor and the optimal dosage during the start of the treatment and will allow for more efficient individual therapy, also taking in account the eventual DILI. To clarify this point, CYP polymorphisms of fluvastatin were studied.

More than the hepatotoxicity, the pharmacokinetics of both enantiomers of fluvastatin depended on the CYP2C9 genotype with a 3-fold mean difference in the active enantiomer and even greater differences in the inactive enantiomer. Differences in plasma concentrations were not reflected in cholesterol lowering after 14 d of fluvastatin intake in healthy volunteers[48]. In fact, the authors did not find any evidence to support CYP2C9 and CYP2C19 genetic polymorphisms as predictable potential risk factors for DILI[49].

PREDICTING VARIABILITY IN PHARMACOKINETICS

Obviously, it is impossible to genotype all individuals due to the cost, even though for some drugs it should be taken into serious consideration because a single serious incident (hepatic failure) could lead to an excessive health care involvement (liver transplantation). Several examples have been reported.

CYP2C19 is highly polymorphic, with variations in both the expression of mRNA and enzyme, plus actual differences in the protein coding region that give rise to differing rates of catalysis. As with most polymorphisms, there appear to be differences in expression in different ethnic groups. For example, the frequency of PMs among Asians is nearly 20% of Caucasians[50]. The proton pump inhibitor omeprazole and related ulcer drugs are oxidized by CYP2C19, and PMs show a better response to these drugs[51].

Not all drug interactions are genetically determined. In some cases, an inhibitor can block metabolism of a drug and produce the same effect as would poor metabolism. In retrospect, every marketed drug is a relatively successful drug in terms of the limited problems associated with widespread use, the deaths of some individuals notwithstanding-as in the case of "ultra" rapid metabolizers-or, more commonly, arising from enzyme induction. A classic example involves CYP3A4 and 17α-ethynylestradiol, the estrogenic component of oral contraceptives. Similarly, hyperforin, a potent CYP inducer found in the herbal medicine St. John's wort, greatly increases the expression of CYPs that metabolize drugs used for AIDS treatment and organ transplantation. Cases for enhanced drug toxicity due to elevated levels of CYPs are probably less clear; however, CYP3A4 converts the antidiabetic drug troglitazone into toxic products, although the mechanism of toxicity is still unclear. Troglitazone has since been removed from the market. In any event, the drug development process now incorporates a variety of *in vitro* studies designed to predict bioavailability, inhibition of CYP reaction, and the effects of any induction prior to consideration of clinical trials. Conclusively, genotyping can provide useful information about the expected behavior of a drug.

HAS CYP A ROLE IN THE "DIRECT" HEPATOTOXICITY?

CCl_4 is a well-known model compound for producing chemical hepatic injury. CYP is an important monooxygenase in biology. Recent research investigated CYP protein expression in the *in vivo* hepatotoxicity of rats induced by CCl_4. In this experiment, CCl_4 was administered to male rats, and their livers at 24 h post-dosing were studied using proteomic analysis. Blood biochemistry and histopathology were examined to identify specific changes. At the same time, a novel acetylation stable isotopic labeling method coupled with LTQ-FTICR MS was applied to disclose the changes in CYP expression amounts. The quantitative proteomics method demonstrated its correlation coefficient was 0.9998 in a 100-fold dynamic range and the average ratio of the labeled peptides was 1.04, which was very close to the theoretical ratio of 1.00 and the standard deviation (SD) of 0.21. With this approach, 17 CYP proteins were identified and quantified with high confidence. Among them, the expression amounts of 2C11, 3A2, and 2 E1 were down-regulated, while those of 2C6, 2B2, and 2B1 were up-regulated[52].

AN EXAMPLE OF DAMAGE MECHANISMS

CCl_4 continues to provide an important service today as a model substance to elucidate the mechanisms of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. CCl_4 is activated by CYP2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCl_3*. This radical can bind to cellular molecules (protein, lipid, nucleic acids), impairing crucial cellular processes such as lipid metabolism, leading to fatty degeneration (hepatic steatosis). Adduct formation between CCl_3* and DNA is thought to possibly induce hepatic cancer. This radical can also react with O_2 to form the trichloromethylperoxy radical CCl_3OO*, a highly reactive species, also called ROS. CCl_3OO* initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular Ca^{2+} sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Among the degradation products of fatty acids are reactive aldehydes, especially 4-hydroxynonenal, which bind easily to functional groups of proteins and inhibit important enzyme activities. CCl_4 intoxication also leads to hypomethylation of cellular components; in the case of RNA the outcome is thought to be inhibition of protein synthesis, and in the

case of phospholipids it plays a role in the inhibition of lipoprotein secretion. None of these processes per se is considered the ultimate cause of CCl_4-induced cell death; it is by cooperation that they achieve a fatal outcome, provided the toxicant acts in a high single dose, or over longer periods of time at low doses. At the molecular level CCl_4 activates tumor necrosis factor (TNF)-α, nitric oxide, and transforming growth factor (TGF)-α and -β in the cell, processes that appear to direct the cell primarily toward (self-)destruction or fibrosis. TNF-α pushes toward apoptosis, whereas TGF-β appears to direct toward fibrosis. IL-6, although induced by TNF-α, has a clearly antiapoptotic effect, and IL-10 also counteracts TNF-α action. Thus, both interleukins have the potential to initiate recovery of the CCl_4-damaged hepatocyte. Several of the above-mentioned toxicological processes can be specifically interrupted with the use of antioxidants and mitogens, respectively, by restoring cellular methylation, or by preserving calcium sequestration. Chemicals that induce CYPs that metabolize CCl_4, or delay tissue regeneration when co-administered with CCl_4 will potentiate its toxicity thoroughly, while appropriate CYP inhibitors will alleviate much of the toxicity. O_2 partial pressure can also direct the course of CCl_4 hepatotoxicity. Pressures between 5 and 35 mmHg favor lipid peroxidation, whereas the absence of O_2, as well as a partial pressure above 100 mmHg, both prevent lipid peroxidation entirely. Consequently, the location of CCl_4-induced damage mirrors the O_2 gradient across the liver lobule. Mixed halogenated methanes and ethanes, found as so-called disinfectant by-products at low concentrations in drinking water, elicit symptoms of toxicity very similar to CCl_4, including carcinogenicity[23].

TOTAL DOSE OF DRUG

Among drugs that are feared of inducing hepatoxicity, mainly when taken for a very long period, statins are largely under-dosed, but they do in rare cases cause significant liver injury whereas antiretroviral therapy is associated with hepatotoxicity in 10% of treated patients[16].

In a previous study, liver morphology was examined in 41 patients with vitamin A hepatoxicity. Cirrhosis was found in 17, mild chronic hepatitis in 10, noncirrhotic portal hypertension in 5, and "increased storage" alone in nine cases. During a mean follow-up period of 4.6 years, six patients died of causes related to the liver disease. A precise appraisal of drug consumption was obtained in 29 cases. Among them the total cumulative intake was highest in patients with cirrhosis ($423 \pm 103 \times 10^6$ IU) and significantly lower in those with noncirrhotic liver disease (88.5 ± 41; $P < 0.02$). The smallest continuous daily consumption leading to cirrhosis was 25 000 IU during 6 years, whereas higher daily doses (⩾ 100 000 IU) taken over 2.5 years resulted in similar histological lesions. It was concluded that prolonged and continuous consumption of doses in the low "therapeutic" range can result in life-threatening liver damage[53].

Recently, an interesting paper, reporting data from both USA and Sweden, showed a clear relationship between daily doses of oral prescription medications and idiosyncratic DILI, particularly as regards daily doses > 50 mg/d[54].

INDUCTION AND INHIBITION OF CYP ACTIVITY

The possible pharmacokinetic consequences of enzyme induction depend on the localization of the enzyme. They include decreased or absent bioavailability for orally administered drugs, increased hepatic clearance or accelerated formation of reactive metabolites, which is usually related to local toxicity. The toxicological consequences of enzyme induction in humans are fortunately rare, and appear to be mainly limited to hepatoxicity in ethanol-type induction[55].

Diclofenac sodium (DF-Na) is an NSAID used in various aspects of inflammatory disease. The effects of phenobarbital (PB) on metabolism and toxicity of DF-Na *in vitro* and the potential mechanism of DF-Na induced hepatotoxicity have been examined. The decline of CYP 3A was partially reversed by CYP inducer PB, and the maximum induction of CYP 3A was 2.2-fold over control after continuous exposure of hepatocytes to 2 mmol/L PB for 48 h. These findings suggest that the hepatotoxicity and metabolism of DF-Na in rat hepatocytes are increased when hepatic CYP 3A activity is increased[56]. Itraconazole and fluconazole, two antifungal drugs with potent inhibitory effect on CYP, induce hepatotoxicity clinically, but the mechanism underlying the hepatotoxicity is unknown. Pretreatment with SKF 525A, an inhibitor of CYPs, induced more severe hepatotoxicity with both itraconazole and fluconazole *in vivo*[57].

THE IMPACT OF DILI ON DRUG DEVELOPMENT

The inability to predict if a metabolically bioactivated compound will cause toxicity in later stages of drug development or post-marketing is of great importance. One approach for improving the predictive success of compound toxicity could be to compare the gene expression profile in preclinical models dosed with novel compounds to a gene expression database generated from compounds with known toxicity.

A current study[58] in mice utilized a known hepatotoxic compound N-methylformamide and its two analogs labeled with deuterium at different positions to block metabolic oxidation at the formyl [d (1)] and methyl [d (3)] moieties. The data set generated from the different groups of animals enabled authors to determine which gene expression changes were attributed to the bioactivating pathway. The metabolic pathway leading to the production of reactive methyl isocyanate resulted in distinct expression patterns that correlated with histopathologic findings. There was a clear correlation between the expression of certain genes involved in the cell cycle/apoptosis and inflammatory pathways and

the presence of reactive metabolite. These genes may serve as potential genomic biomarkers of hepatotoxicity induced by soft-electrophile-producing compounds. However, the robustness of these potential genomic biomarkers will need to be validated before being adopted into the drug discovery screening process.

AN OPINION ABOUT WIDELY USED DRUGS

Although liver injury has been associated with the "statins", the frequency of such toxicity is lower than that of the control population and the value of biochemical monitoring remains unproved[59]. Clinicians may be concerned about prescribing statins to patients with chronic liver disease, but there is little evidence to suggest that DILI from statins is increased in these patients. Thus, we should prescribe statins for the same indications in patients with chronic liver disease as in patients without, but with closer monitoring[60].

HERBAL REMEDIES AND OTHER DIETARY SUPPLEMENTS

A dietary supplement is defined as any product in pill or liquid form containing a herb, vitamin, amino acid or mineral that complements the normal diet. Indeed, every agency regulates dietary supplements differently from drugs, i.e. only ensuring quality control and good manufacturing processes but not standardization of the active ingredients. Dietary supplements are commonly used primarily because they are widely available and can be bought without consulting a physisician. However, a few supplements are now proven to be safe and useful complements to standard drugs. The supplement may have unlisted ingredients, which may be inert or harmful, or it may contain variable amounts of active ingredients, especially when whole herbs are ground or made into extracts. Most herbal products are mixtures of several substances, and which ingredients are active is not always known. Additional areas of concern include stability of supplements (especially herbal products) once manufactured, use of dietary supplements instead of conventional drugs, toxicity in children and the elderly, and interactions between supplements and drugs. Patients may not think to disclose or may wish to conceal their use of dietary supplements. For this reason, the patient's history should periodically include explicit questions about past and recent consumption of dietary supplements. Recently the use of herbal preparations as remedies for various medical conditions has been increasing rapidly. In one study, 38.9% of patients with chronic liver disease were found to use some sort of herbal preparation[61].

Efficacy and safety of medicinal plants naturally represent the object of interest for pharmacologists, and it is surely this aspect which gives the most important information on herbal medicine use[62].

Many plants have been studied and results published showing variable degrees of efficacy. Toxicity aspects of some of the most frequently used plants are now well known[63].

Among others, a recent report emphasizes the potentially severe hepatotoxicity of Kava which has recently led to the retraction of Kava-containing drugs by the pharmacovigilance authorities in Germany[64]. Authors reported two cases of acute liver injury along with the intake of greater celandine (*Chelidonium majus*), a well-known herbal remedy frequently used for irritable bowel syndrome. All other possible causes of acute liver damage were excluded in both patients. In one patient, cholestatic hepatitis recurred rapidly after involuntary re-exposure. Both patients fully recovered after the withdrawal of greater celandine. The two cases add to the existing database about the potential hepatotoxicity of drugs containing greater celandine and raise the question whether the approval of this drug should be re-evaluated in the light of a lack of evidence for a therapeutic benefit[65].

SOME CONSIDERATIONS ON CAUSALITY ASSESSMENT

Many methods have been proposed to assess the individual causality between a drug treatment and the occurrence of adverse drug events (ADRs), including hepatoxicity. Briefly, these methods may be classified into the following approaches, i.e. expert judgement, probabilistic methods and algorithms.

In expert judgement or global introspection (GI), an expert expresses a judgement about possible drug causation after having taken into account all the available and relevant information on the considered case.

Theoretically, it is possible to apply pre-existing Causality Assessment Methods (CAMs) to the assessment of causality in cases with diagnostic difficulties.

We have historical scales such as Naranjo probability scale[66], Danan's international consensus criteria[67], Maria's and Victorino's scales[68] or Beers criteria for ADRs[69] to make such events predictable and often preventable. Still, on the basis of a global score, four categories of preventability of ADRs ("preventable", "potentially preventable", "unclassable", "not preventable") were proposed by other researchers[70].

Standards are lacking for validation of drug CAMs. An original model has been proposed using a positive rechallenge as an external standard[71]. The GI approach suffers from marked subjectivity leading to poor reproducibility and intra- and inter-rater disagreements. A study confirms that experts express marked disagreements when assessing drug causality independently. The agreement rate was lower for intermediate levels of causality, especially when strong evidence was lacking for confirming or ruling out drug causality[72]. Probabilistic methods are usually regarded as the most rigorous[73]. The probabilistic approach is based on the Bayes theorem and makes it possible to directly assess the odds of drug causation. However, these methods are rather troublesome to routinely use because information for

assessing the probability of drug causation is rarely available. Unlike the Bayesian approach, algorithms have appealing simplicity and are much more widely used for the operational assessment of ADRs. The main reason for their use is to increase inter- and intra-rater agreement. The overall observed agreement between algorithm and GI was moderate although poorly different from chance, confounding variables being a shortcoming of algorithms ability in assessing causality[74].

Drawbacks of animal models

The doubtful assumption that animal models are reasonably predictive of human outcomes has provided the basis for their widespread use in toxicity testing and in biomedical research aimed at developing cures for human diseases. To investigate the validity of this assumption, comprehensive bibliographic databases were searched for published systematic reviews of the human clinical or toxicological utility of animal experiments. In 20 reviews in which clinical utility was examined, the authors concluded that animal models were either significantly useful in contributing to the development of clinical interventions, or were substantially consistent with clinical outcomes, in only two cases, one of which was contentious. These reviews failed to clearly demonstrate utility in predicting human toxicological outcomes; consequently, animal data may not generally be assumed to be substantially useful for these purposes. Possible causes include interspecies differences, the distortion of outcomes arising from experimental environments and protocols, and the poor methodological quality of many animal experiments. What is more, very few reviews existed in which the majority of animal experiments were of good methodological quality. The poor human clinical and toxicological utility of most animal models for which data exists, in conjunction with economic costs, justify a perplexity on animal models[75].

In numerous cases, researchers are simply not aware of the limitations of the animal experiment as such. For example, many animal experiments are dramatically "under-powered", that is, carried out with groups that are too small to allow conclusions to be drawn from the outcome. This stands in marked contrast to *in vitro* experiments where replicate experiments usually represent no major problem. Since *in vitro* models are generally more prone to artefacts due to the numerous variables, for example, of cell culture, the key requirement for their application is their validation and quality control. Sadly, many methods, even if published in the scientific literature, are little standardised and reproducible. Due to limitations in space, many scientific journals cannot publish detailed methodological descriptions. However, nowadays a supplementary central deposit of methods could easily be linked to the respective article[76].

ETHICAL AND LEGAL PROBLEMS ABOUT DRUG-INDUCED LIVER INJURY

Patients should be especially cautious about combining multiple drugs, and tell their doctor about any drugs or other substances they are taking, including prescription and over-the-counter medications, recreational drugs, herbal remedies, and nutritional supplements. Health care professionals are encouraged to report all ADRs, mainly hepatoxicity, and to pay much more attention in prescribing and administering drugs.

Pharmacovigilance is a key step in discovering DILI. But, it is also concerned with pharmacological, pathological, epidemiological and legal respects, other ADRs and interactions as well as problems relating to ineffectiveness, inappropriate use, dependence or poisoning. Physicians should always think of this ADR in the absence of other clear hepatic disease.

LIVER DISEASES POTENTIALLY CAUSED BY DRUGS

Acute hepatitis

Dose-dependent: Acetaminophen[77,78], salicylates[79], (high doses i.e. > 2 g/d).

Dose-independent: Acebutolol[80], allopurinol[81], carbamazepine[82], cimetidine[83], dantrolene[84], diclofenac[79], ethambutol[85], ethionamide[86], enflurane[87], phenelzine[88], phenindione[89], phenobarbital[90], phenytoin[91], phenylbutazone[79], halothane[92], ibuprofen[79], indomethacin[79], isoniazid[85], ketoconazole[93], labetalol[94], maprotiline[95], metoprolol[96], mianserin[97], naproxen[79], para-aminosalicylic acid[98], piroxicam[79], pyrazinamide[85], quinidine[99], penicillins[100], ranitidine[101], sulfonamides[102], sulindac[79], tricyclic antidepressants[103], trimethoprim-sulfamethoxazole[104], valproic acid[105], verapamil[106].

Acute fatty liver

Adrenocortical steroids[107], phenytoin[108], salicylates[79].

Fatty liver

Amiodarone[109], asparaginase[110], ibuprofen[79], indometacin[79], ketoconazole[111], methyldopa[112], naproxen[79], nifedipine[113], acetaminophen[77], perhexeline[114], rifampicin[85], sulindac[79], tetracyclin[115], valproic acid[116], zidovulin[117].

Cholestatic syndrome

Amoxicillin/clavulanate[118], azathioprine[119], captopril[120], carbamazepine[121], carbimazole[122], cephalosporins[123], chlordiazepoxide[124], chlorpropamide[125], cloxacillin[126], cyclosporine[127], danazol[128], disopyramide[129], enalapril[130], erythromycin[131], flecainide, flucoxacillin[132], flurazepam[133], flutamide[134], gold[135], griseofulvin[136], glyburide[137], imipramine[138], haloperidol[139], ketoconazole[140], megestrol[141], mercaptopurine[119], methimazole[142], methyltestosterone[143], nifedipine[144], nitrofurantoin[145], norethandrolone[146], nonsteroidal anti-inflammatory drugs[79], oral contraceptives[147], phenothiazines[148], phenytoin[149], penicillamine[150], propoxyphene[151], sulfonamides[152], tamoxifen[153], thiabendazole[154], tolbutamide[155], tricyclic antidepressants[156], troleandomycin[157], verapamil[158].

Liver granulomas

Allopurinol[159], aspirin[79], carbamazepine[160], chlorpromazine[161], diltiazem[162], gold[163], hydralazine[164], nitrofurantoin[165], penicillin[166], phenylbutazone[79], phenytoin[167], pyrazinamide[168], quinidine[169], sulfasalazine[170].

Chronic liver disease

Acetaminophen (in chronic use or at high doses)[79], dantrolene[171], isoniazid[172], methyldopa[173], phenytoin[174].

Liver cirrhosis/fibrosis

Methotrexate[175], nitrofurantoin[176], terbinafine[177].

Liver tumors

Anabolic steroids[178], danazol[179], oral contraceptives[178], testosterone[178], thorotrast[180].

Vascular reactions

Anabolic steroids[181], azathioprine[182], cyclophosphamide/cyclophosphamide combination[183], dacarbazine[184], oral contraceptives[185], thioquanine[186], vincristine[187].

Fulminant hepatitis and hepatic failure

Lamotrigine[188], nimesulide[189], carbamzepine and levetiracetam[190], isoniazid[191], clarithromycin[192], ecstasy[193].

LIVER DISEASES EVENTUALLY CAUSED BY DIETARY SUPPLEMENT (MAINLY IN OBESE PATIENTS)

Germander (Teucrium chamaedrys) extracts, widely used in Europe in the last decades as a weight loss agent, cause DILI probably mediated by furano neoclerodane diterpenoids[194]. Chaparral (creosote bush, greasewood, or Larrea tridentata) is a desert shrub traditionally used by Native Americans for treatment of several ailments. More recently, preparations of chaparral leaves have been marketed for use as weight loss agents. Reports of chaparral hepatotoxicity were first seen in 1992. The mechanism of chaparral toxicity is unclear but may involve its active ingredient, nordihydroguaiaretic acid[195]. Kava (kava kava, awa, or kew), derived from the dried root and rhizome of Piper methysticum, has recently been marketed as an anxiolytic and mood enhancer. Recent series from Europe have described more than 30 cases of kava-associated hepatic injury, including five cases leading to OLT. The mechanism of hepatic injury appears to be immune-mediated, with CYP2D6 deficiency perhaps being a risk factor[196]. *Ma huang* (from Ephedra sinica and other Ephedra species) is a traditional Chinese extract used for treatment of asthma, nasal congestion, and fever. Recent Western marketing has focused on the stimulatory effects of *Ma huang*, which contains 0.15%-2% of ephedrine-like alkaloids by weight. Although most adverse effects of *Ma huang* are cardiovascular or neurological (e.g. hypertension, stroke, myocardial infarction, seizures, and psychosis), 4% of reports mentioned acute hepatitis. *Ma huang* contains phytochemicals which are thought to modify its toxic activity[197]. In addition to the above supplements, liver injury has been attributed to other botanical agents. The pyrrolizidine alkaloids found in comfrey leaves and Heliotropium, Senecio, and Crotalaria species are known to cause veno-occlusive disease of the liver *via* a toxic effect[198]. Mixtures of valerian and skullcap (Valeriana officinalis and Scutellaria lateriflora) have induced hepatitis *via* alkylating agents. LipoKinetix, was marketed as a dietary supplement for weight loss. Following reports of seven cases of severe hepatotoxicity associated with its use, the FDA moved to remove it from the market in November 2001. Hepatic injury appears to be due to an idiosyncratic reaction, perhaps related to phenylpropanolamine[199]. Among other weight loss agents, usnic acid should be suspected in cases of severe hepatotoxicity[200].

For further details about the topics of this report, we advise the readers to consider the review by Bleibel *et al*[201] and/or to connect to: http://www.fda.gov/cder/guidance/7507dft.htm.

REFERENCES

1 **Gunawan B**, Kaplowitz N. Clinical perspectives on xenobiotic-induced hepatotoxicity. *Drug Metab Rev* 2004; **36**: 301-312

2 **Liu YT**, Hao HP, Liu CX, Wang GJ, Xie HG. Drugs as CYP3A probes, inducers, and inhibitors. *Drug Metab Rev* 2007; **39**: 699-721

3 **Floby E**, Briem S, Terelius Y, Sohlenius-Sternbeck AK. Use of a cocktail of probe substrates for drug-metabolizing enzymes for the assessment of the metabolic capacity of hepatocyte preparations. *Xenobiotica* 2004; **34**: 949-959

4 **Dixit V**, Hariparsad N, Desai P, Unadkat JD. In vitro LC-MS cocktail assays to simultaneously determine human cytochrome P450 activities. *Biopharm Drug Dispos* 2007; **28**: 257-262

5 **Jones JO**, Diamond MI. Design and implementation of cell-based assays to model human disease. *ACS Chem Biol* 2007; **2**: 718-724

6 **Li J**, Liu Y, Zhang JW, Wei H, Yang L. Characterization of hepatic drug-metabolizing activities of Bama miniature pigs (Sus scrofa domestica): comparison with human enzyme analogs. *Comp Med* 2006; **56**: 286-290

7 **Fujita K**, Kamataki T. Genetically engineered bacterial cells co-expressing human cytochrome P450 with NADPH-cytochrome P450 reductase: prediction of metabolism and toxicity of drugs in humans. *Drug Metab Pharmacokinet* 2002; **17**: 1-22

8 **Meier Y**, Cavallaro M, Roos M, Pauli-Magnus C, Folkers G, Meier PJ, Fattinger K. Incidence of drug-induced liver injury in medical inpatients. *Eur J Clin Pharmacol* 2005; **61**: 135-143

9 **Andrade RJ**, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, Garcia-Munoz B, Gonzalez-Grande R, Pizarro A, Duran JA, Jimenez M, Rodrigo L, Romero-Gomez M, Navarro JM, Planas R, Costa J, Borras A, Soler A, Salmeron J, Martin-Vivaldi R. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* 2005; **129**: 512-521

10 **De Valle MB**, Av Klinteberg V, Alem N, Olsson R, Bjornsson E. Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic. *Aliment Pharmacol Ther* 2006; **24**: 1187-1195

11 **Marti L**, Del Olmo JA, Tosca J, Ornia E, Garcia-Torres ML, Serra MA, Rodriguez F, Lluch P, Escudero A, Rodrigo JM. Clinical evaluation of drug-induced hepatitis. *Rev Esp En-*

ferm Dig 2005; **97**: 258-265
12 **Chan KA**, Truman A, Gurwitz JH, Hurley JS, Martinson B, Platt R, Everhart JE, Moseley RH, Terrault N, Ackerson L, Selby JV. A cohort study of the incidence of serious acute liver injury in diabetic patients treated with hypoglycemic agents. *Arch Intern Med* 2003; **163**: 728-734
13 **Aithal PG**, Day CP. The natural history of histologically proved drug induced liver disease. *Gut* 1999; **44**: 731-735
14 **Garcia Rodriguez LA**, Ruigomez A, Jick H. A review of epidemiologic research on drug-induced acute liver injury using the general practice research data base in the United Kingdom. *Pharmacotherapy* 1997; **17**: 721-728
15 **Li B**, Wang Z, Fang JJ, Xu CY, Chen WX. Evaluation of prognostic markers in severe drug-induced liver disease. *World J Gastroenterol* 2007; **13**: 628-632
16 **Hussaini SH**, Farrington EA. Idiosyncratic drug-induced liver injury: an overview. *Expert Opin Drug Saf* 2007; **6**: 673-684
17 **Kaplowitz N**. Biochemical and cellular mechanisms of toxic liver injury. *Semin Liver Dis* 2002; **22**: 137-144
18 **Masubuchi Y**, Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. *J Hepatol* 2005; **42**: 110-116
19 **Kon K**, Ikejima K, Okumura K, Aoyama T, Arai K, Takei Y, Lemasters JJ, Sato N. Role of apoptosis in acetaminophen hepatotoxicity. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S49-S52
20 **Wang H**, LeCluyse EL. Role of orphan nuclear receptors in the regulation of drug-metabolising enzymes. *Clin Pharmacokinet* 2003; **42**: 1331-1357
21 **van Gijssel HE**, Mullenders LH, van Oosterwijk MF, Meerman JH. Blockage of transcription as a trigger for p53 accumulation by 2-acetylaminofluorene DNA-adducts. *Life Sci* 2003; **73**: 1759-1771
22 **Jeong DH**, Lee SJ, Lee JH, Bae IH, Jeong KS, Jang JJ, Lim IK, Kim MR, Lee MJ, Lee YS. Subcellular redistribution of protein kinase C isozymes is associated with rat liver cirrhotic changes induced by carbon tetrachloride or thioacetamide. *J Gastroenterol Hepatol* 2001; **16**: 34-40
23 **Weber LW**, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2003; **33**: 105-136
24 **Mehal WZ**, Azzaroli F, Crispe IN. Immunology of the healthy liver: old questions and new insights. *Gastroenterology* 2001; **120**: 250-260
25 **Doherty DG**, Norris S, Madrigal-Estebas L, McEntee G, Traynor O, Hegarty JE, O'Farrelly C. The human liver contains multiple populations of NK cells, T cells, and CD3+CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J Immunol* 1999; **163**: 2314-2321
26 **Chen H**, Paul WE. Cultured NK1.1+ CD4+ T cells produce large amounts of IL-4 and IFN-gamma upon activation by anti-CD3 or CD1. *J Immunol* 1997; **159**: 2240-2249
27 **Li Z**, Diehl AM. Innate immunity in the liver. *Curr Opin Gastroenterol* 2003; **19**: 565-571
28 **Calne RY**, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; **223**: 472-476
29 **Klugewitz K**, Blumenthal-Barby F, Schrage A, Knolle PA, Hamann A, Crispe IN. Immunomodulatory effects of the liver: deletion of activated CD4+ effector cells and suppression of IFN-gamma-producing cells after intravenous protein immunization. *J Immunol* 2002; **169**: 2407-2413
30 **Limmer A**, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000; **6**: 1348-1354
31 **Castell JV**. Allergic hepatitis: a drug-mediated organ-specific immune reaction. *Clin Exp Allergy* 1998; **28** Suppl 4: 13-19
32 **Prandota J**. Important role of proinflammatory cytokines/other endogenous substances in drug-induced hepatotoxicity: depression of drug metabolism during infections/inflammation states, and genetic polymorphisms of drug-metabolizing enzymes/cytokines may markedly contribute to this pathology. *Am J Ther* 2005; **12**: 254-261
33 **Haouzi D**, Lekehal M, Moreau A, Moulis C, Feldmann G, Robin MA, Letteron P, Fau D, Pessayre D. Cytochrome P450-generated reactive metabolites cause mitochondrial permeability transition, caspase activation, and apoptosis in rat hepatocytes. *Hepatology* 2000; **32**: 303-311
34 **Critchley JA**, Nimmo GR, Gregson CA, Woolhouse NM, Prescott LF. Inter-subject and ethnic differences in paracetamol metabolism. *Br J Clin Pharmacol* 1986; **22**: 649-657
35 **Tarantino G**, Conca P, Basile V, Gentile A, Capone D, Polichetti G, Leo E. A prospective study of acute drug-induced liver injury in patients suffering from non-alcoholic fatty liver disease. *Hepatol Res* 2007; **37**: 410-415
36 **Parola M**, Robino G. Oxidative stress-related molecules and liver fibrosis. *J Hepatol* 2001; **35**: 297-306
37 **Arteel GE**. Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* 2003; **124**: 778-790
38 **Chen Q**, Cederbaum AI. Cytotoxicity and apoptosis produced by cytochrome P450 2E1 in Hep G2 cells. *Mol Pharmacol* 1998; **53**: 638-648
39 **Konstandi M**, Marselos M, Radon-Camus AM, Johnson E, Lang MA. The role of stress in the regulation of drug metabolizing enzymes in mice. *Eur J Drug Metab Pharmacokinet* 1998; **23**: 483-490
40 **Murray M**. Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis. *Curr Drug Metab* 2006; **7**: 67-81
41 **Ramaiah SK**, Apte U, Mehendale HM. Cytochrome P4502E1 induction increases thioacetamide liver injury in diet-restricted rats. *Drug Metab Dispos* 2001; **29**: 1088-1095
42 **Shakya R**, Rao BS, Shrestha B. Incidence of hepatotoxicity due to antitubercular medicines and assessment of risk factors. *Ann Pharmacother* 2004; **38**: 1074-1079
43 **Eichelbaum M**, Kroemer HK, Mikus G. Genetically determined differences in drug metabolism as a risk factor in drug toxicity. *Toxicol Lett* 1992; **64-65** Spec No: 115-122
44 **Chou WH**, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, Pho M, Xiao V, Ryder TB, Liu WW, Teiling C, Wedlund PJ. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. *J Clin Psychopharmacol* 2000; **20**: 246-251
45 **Scott RJ**, Palmer J, Lewis IA, Pleasance S. Determination of a 'GW cocktail' of cytochrome P450 probe substrates and their metabolites in plasma and urine using automated solid phase extraction and fast gradient liquid chromatography tandem mass spectrometry. *Rapid Commun Mass Spectrom* 1999; **13**: 2305-2319
46 **Yin OQ**, Lam SS, Lo CM, Chow MS. Rapid determination of five probe drugs and their metabolites in human plasma and urine by liquid chromatography/tandem mass spectrometry: application to cytochrome P450 phenotyping studies. *Rapid Commun Mass Spectrom* 2004; **18**: 2921-2933
47 **Mulder AB**, van Lijf HJ, Bon MA, van den Bergh FA, Touw DJ, Neef C, Vermes I. Association of polymorphism in the cytochrome CYP2D6 and the efficacy and tolerability of simvastatin. *Clin Pharmacol Ther* 2001; **70**: 546-551
48 **Kirchheiner J**, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I, Brockmoller J. Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther* 2003; **74**: 186-194
49 **Pachkoria K**, Lucena MI, Ruiz-Cabello F, Crespo E, Cabello MR, Andrade RJ. Genetic polymorphisms of CYP2C9 and CYP2C19 are not related to drug-induced idiosyncratic liver injury (DILI). *Br J Pharmacol* 2007; **150**: 808-815

50 **Eichelbaum M**, Evert B. Influence of pharmacogenetics on drug disposition and response. *Clin Exp Pharmacol Physiol* 1996; **23**: 983-985

51 **Schwab M**, Schaeffeler E, Klotz U, Treiber G. CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based quadruple therapy for eradication of Helicobacter pylori. *Clin Pharmacol Ther* 2004; **76**: 201-209

52 **Jia N**, Liu X, Wen J, Qian L, Qian X, Wu Y, Fan G. A proteomic method for analysis of CYP450s protein expression changes in carbon tetrachloride induced male rat liver microsomes. *Toxicology* 2007; **237**: 1-11

53 **Geubel AP**, De Galocsy C, Alves N, Rahier J, Dive C. Liver damage caused by therapeutic vitamin A administration: estimate of dose-related toxicity in 41 cases. *Gastroenterology* 1991; **100**: 1701-1709

54 **Lammert C**, Einarsson S, Saha C, Niklasson A, Bjornsson E, Chalasani N. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. *Hepatology* 2008; **47**: 2003-2009

55 **Fuhr U**. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet* 2000; **38**: 493-504

56 **Wang AG**, Xia T, Yuan J, Yu RA, Yang KD, Chen XM, Qu W, Waalkes MP. Effects of phenobarbital on metabolism and toxicity of diclofenac sodium in rat hepatocytes in vitro. *Food Chem Toxicol* 2004; **42**: 1647-1653

57 **Somchit N**, Wong CW, Zuraini A, Ahmad Bustamam A, Hasiah AH, Khairi HM, Sulaiman MR, Israf DA. Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. *Drug Chem Toxicol* 2006; **29**: 237-253

58 **Mutlib A**, Jiang P, Atherton J, Obert L, Kostrubsky S, Madore S, Nelson S. Identification of potential genomic biomarkers of hepatotoxicity caused by reactive metabolites of N-methylformamide: Application of stable isotope labeled compounds in toxicogenomic studies. *Chem Res Toxicol* 2006; **19**: 1270-1283

59 **Chitturi S**, George J. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetic agents, anticonvulsants, lipid-lowering agents, psychotropic drugs. *Semin Liver Dis* 2002; **22**: 169-183

60 **Russo MW**, Jacobson IM. How to use statins in patients with chronic liver disease. *Cleve Clin J Med* 2004; **71**: 58-62

61 **Ahn BM**. [Herbal preparation-induced liver injury] *Korean J Gastroenterol* 2004; **44**: 113-125

62 **Calapai G**, Caputi AP. Herbal medicines: can we do without pharmacologist? *Evid Based Complement Alternat Med* 2007; **4**: 41-43

63 **Furbee RB**, Barlotta KS, Allen MK, Holstege CP. Hepatotoxicity associated with herbal products. *Clin Lab Med* 2006; **26**: 227-241, x

64 **Stickel F**, Baumuller HM, Seitz K, Vasilakis D, Seitz G, Seitz HK, Schuppan D. Hepatitis induced by Kava (Piper methysticum rhizoma). *J Hepatol* 2003; **39**: 62-67

65 **Stickel F**, Poschl G, Seitz HK, Waldherr R, Hahn EG, Schuppan D. Acute hepatitis induced by Greater Celandine (Chelidonium majus). *Scand J Gastroenterol* 2003; **38**: 565-568

66 **Naranjo CA**, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981; **30**: 239-245

67 **Danan G**, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993; **46**: 1323-1330

68 **Maria VA**, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997; **26**: 664-669

69 **Fick DM**, Cooper JW, Wade WE, Waller JL, Maclean JR, Beers MH. Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. *Arch Intern Med* 2003; **163**: 2716-2724

70 **Olivier P**, Caron J, Haramburu F, Imbs JL, Jonville-Bera AP, Lagier G, Sgro C, Vial T, Montastruc JL, Lapeyr-Mestre M. [Validation of a measurement scale: example of a French Adverse Drug Reactions Preventability Scale] *Therapie* 2005; **60**: 39-45

71 **Benichou C**, Danan G, Flahault A. Causality assessment of adverse reactions to drugs--II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. *J Clin Epidemiol* 1993; **46**: 1331-1336

72 **Arimone Y**, Begaud B, Miremont-Salame G, Fourrier-Reglat A, Moore N, Molimard M, Haramburu F. Agreement of expert judgment in causality assessment of adverse drug reactions. *Eur J Clin Pharmacol* 2005; **61**: 169-173

73 **Lanctot KL**, Naranjo CA. Comparison of the Bayesian approach and a simple algorithm for assessment of adverse drug events. *Clin Pharmacol Ther* 1995; **58**: 692-698

74 **Macedo AF**, Marques FB, Ribeiro CF, Teixeira F. Causality assessment of adverse drug reactions: comparison of the results obtained from published decisional algorithms and from the evaluations of an expert panel. *Pharmacoepidemiol Drug Saf* 2005; **14**: 885-890

75 **Knight A**. Systematic reviews of animal experiments demonstrate poor human clinical and toxicological utility. *Altern Lab Anim* 2007; **35**: 641-659

76 **Gruber FP**, Dewhurst DG. Alternatives to animal experimentation in biomedical education. *ALTEX* 2004; **21** Suppl 1: 33-48

77 **Larson AM**. Acetaminophen hepatotoxicity. *Clin Liver Dis* 2007; **11**: 525-548, vi

78 **Rumack BH**. Acetaminophen hepatotoxicity: the first 35 years. *J Toxicol Clin Toxicol* 2002; **40**: 3-20

79 **Brass EP**. Hepatic toxicity of antirheumatic drugs. *Cleve Clin J Med* 1993; **60**: 466-472

80 **Tanner LA**, Bosco LA, Zimmerman HJ. Hepatic toxicity after acebutolol therapy. *Ann Intern Med* 1989; **111**: 533-534

81 **Al-Kawas FH**, Seeff LB, Berendson RA, Zimmerman HJ, Ishak KG. Allopurinol hepatotoxicity. Report of two cases and review of the literature. *Ann Intern Med* 1981; **95**: 588-590

82 **Gawlikowski T**, Hydzik P. [Carbamazepine hepatoxicity--a case report] *Przegl Lek* 2007; **64**: 318-319

83 **Hashimoto F**, Davis RL, Egli D. Hepatitis following treatments with famotidine and then cimetidine. *Ann Pharmacother* 1994; **28**: 37-39

84 **Wilkinson SP**, Portmann B, Williams R. Hepatitis from dantrolene sodium. *Gut* 1979; **20**: 33-36

85 **Tostmann A**, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol* 2008; **23**: 192-202

86 **See A**, Hervio P, Bouvry M. [The hepatotoxicity of ethionamide remains a topical subject. Apropos of a case of acute hepatitis] *Ann Gastroenterol Hepatol* (Paris) 1986; **22**: 129-130

87 **Sinha A**, Clatch RJ, Stuck G, Blumenthal SA, Patel SA. Isoflurane hepatotoxicity: a case report and review of the literature. *Am J Gastroenterol* 1996; **91**: 2406-2409

88 **Robinson DS**, Kurtz NM. What is the degree of risk of hepatotoxicity for depressed patients receiving phenelzine therapy? Is the risk sufficient to require that we modify the written advice (as to diet and risks) that we regularly give our patients before we institute this therapy? *J Clin Psychopharmacol* 1987; **7**: 61-62

89 **Portal RW**, Emanuel RW. Phenindione hepatitis complicating anticoagulant therapy. *Br Med J* 1961; **2**: 1318-1319

90 **Roberts EA**, Spielberg SP, Goldbach M, Phillips MJ. Phenobarbital hepatotoxicity in an 8-month-old infant. *J Hepatol* 1990; **10**: 235-239

91 **Brackett CC**, Bloch JD. Phenytoin as a possible cause of acetaminophen hepatotoxicity: case report and review of the

literature. *Pharmacotherapy* 2000; **20**: 229-233
92 **Ray DC**, Drummond GB. Halothane hepatitis. *Br J Anaesth* 1991; **67**: 84-99
93 **Lake-Bakaar G**, Scheuer PJ, Sherlock S. Hepatic reactions associated with ketoconazole in the United Kingdom. *Br Med J* (Clin Res Ed) 1987; **294**: 419-422
94 **Clark JA**, Zimmerman HJ, Tanner LA. Labetalol hepatotoxicity. *Ann Intern Med* 1990; **113**: 210-213
95 **Weinstein RP**, Gosselin JY. Case report of hepatotoxicity associated with maprotiline. *Can J Psychiatry* 1988; **33**: 233-234
96 **Lennard MS**. Metoprolol-induced hepatitis: is the rate of oxidation related to drug-induced hepatotoxicity? *Hepatology* 1989; **9**: 163-164
97 **Barbare JC**, Biour M, Cadot T, Latrive JP. [Hepatotoxicity of mianserin: a case with positive reintroduction] *Gastroenterol Clin Biol* 1992; **16**: 486-488
98 **Poli M**, Cordie L. [Liver disease caused by PAS: toxic manifestations of PAS.] *Arch Sci Med* (Torino) 1952; **93**: 391-424
99 **Handler SD**, Hirsch NR, Haas K, Davidson FZ. Quinidine hepatitis. *Arch Intern Med* 1975; **135**: 871-872
100 **Larrey D**, Vial T, Micaleff A, Babany G, Morichau-Beauchant M, Michel H, Benhamou JP. Hepatitis associated with amoxycillin-clavulanic acid combination report of 15 cases. *Gut* 1992; **33**: 368-371
101 **Ribeiro JM**, Lucas M, Baptista A, Victorino RM. Fatal hepatitis associated with ranitidine. *Am J Gastroenterol* 2000; **95**: 559-560
102 **Mainra RR**, Card SE. Trimethoprim-sulfamethoxazole-associated hepatotoxicity - part of a hypersensitivity syndrome. *Can J Clin Pharmacol* 2003; **10**: 175-178
103 **Lucena MI**, Carvajal A, Andrade RJ, Velasco A. Antidepressant-induced hepatotoxicity. *Expert Opin Drug Saf* 2003; **2**: 249-262
104 **Zaman F**, Ye G, Abreo KD, Latif S, Zibari GB. Successful orthotopic liver transplantation after trimethoprim-sulfamethoxazole associated fulminant liver failure. *Clin Transplant* 2003; **17**: 461-464
105 **Tennison MB**, Miles MV, Pollack GM, Thorn MD, Dupuis RE. Valproate metabolites and hepatotoxicity in an epileptic population. *Epilepsia* 1988; **29**: 543-547
106 **Odeh M**, Oliven A. [Verapamil-associated liver injury] *Harefuah* 1998; **134**: 36-37
107 **Dourakis SP**, Sevastianos VA, Kaliopi P. Acute severe steatohepatitis related to prednisolone therapy. *Am J Gastroenterol* 2002; **97**: 1074-1075
108 **de Leval L**, Lambermont B, D'Orio V, Boniver J. Fatal massive liver steatosis--a clinicopathological case report. *Acta Gastroenterol Belg* 1997; **60**: 180-183
109 **Berson A**, De Beco V, Letteron P, Robin MA, Moreau C, El Kahwaji J, Verthier N, Feldmann G, Fromenty B, Pessayre D. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. *Gastroenterology* 1998; **114**: 764-774
110 **Pratt CB**, Johnson WW. Duration and severity of fatty metamorphosis of the liver following L-asparaginase therapy. *Cancer* 1971; **28**: 361-364
111 **Gradon JD**, Sepkowitz DV. Massive hepatic enlargement with fatty change associated with ketoconazole. *DICP* 1990; **24**: 1175-1176
112 **Arranto AJ**, Sotaniemi EA. Morphologic alterations in patients with alpha-methyldopa-induced liver damage after short- and long-term exposure. *Scand J Gastroenterol* 1981; **16**: 853-863
113 **Babany G**, Uzzan F, Larrey D, Degott C, Bourgeois P, Rene E, Vissuzaine C, Erlinger S, Benhamou JP. Alcoholic-like liver lesions induced by nifedipine. *J Hepatol* 1989; **9**: 252-255
114 **Deschamps D**, DeBeco V, Fisch C, Fromenty B, Guillouzo A, Pessayre D. Inhibition by perhexiline of oxidative phosphorylation and the beta-oxidation of fatty acids: possible role in pseudoalcoholic liver lesions. *Hepatology* 1994; **19**: 948-961
115 **Wenk RE**, Gebhardt FC, Bhagavan BS, Lustgarten JA, McCarthy EF. Tetracycline-associated fatty liver of pregnancy, including possible pregnancy risk after chronic dermatologic use of tetracycline. *J Reprod Med* 1981; **26**: 135-141
116 **Walia KS**, Khan EA, Ko DH, Raza SS, Khan YN. Side effects of antiepileptics--a review. *Pain Pract* 2004; **4**: 194-203
117 **Bienvenu L**, Burel F, Hofman V, Itchai C, Amaro J, Hofman P. [A rare etiology of hepatic steatosis associated with lactic acidosis: the toxicity of antiviral nucleoside analogues] *Ann Pathol* 2001; **21**: 160-163
118 **Alexander P**, Roskams T, Van Steenbergen W, Peetermans W, Desmet V, Yap SH. Intrahepatic cholestasis induced by amoxicillin/clavulanic acid (Augmentin): a report on two cases. *Acta Clin Belg* 1991; **46**: 327-332
119 **Eisenbach C**, Goeggelmann C, Flechtenmacher C, Stremmel W, Encke J. Severe cholestatic hepatitis caused by azathioprine. *Immunopharmacol Immunotoxicol* 2005; **27**: 77-83
120 **Schattner A**, Kozak N, Friedman J. Captopril-induced jaundice: report of 2 cases and a review of 13 additional reports in the literature. *Am J Med Sci* 2001; **322**: 236-240
121 **Larrey D**, Hadengue A, Pessayre D, Choudat L, DeGott C, Benhamou JP. Carbamazepine-induced acute cholangitis. *Dig Dis Sci* 1987; **32**: 554-557
122 **Chan AO**, Ng IO, Lam CM, Shek TW, Lai CL. Cholestatic jaundice caused by sequential carbimazole and propylthiouracil treatment for thyrotoxicosis. *Hong Kong Med J* 2003; **9**: 377-380
123 **Skoog SM**, Smyrk TC, Talwalkar JA. Cephalexin-induced cholestatic hepatitis. *J Clin Gastroenterol* 2004; **38**: 833
124 **Lo KJ**, Eastwood IR, Eidelman S. Cholestatic jaundice associated with chlordiazepoxide hydrochloride (Librium) therapy. Report of a case and review of the literature. *Am J Dig Dis* 1967; **12**: 845-849
125 **Gupta R**, Sachar DB. Chlorpropamide-induced cholestatic jaundice and pseudomembranous colitis. *Am J Gastroenterol* 1985; **80**: 381-383
126 **Lotric S**, Lejko-Zupanc T, Jereb M. Cloxacillin-induced cholestasis. *Clin Infect Dis* 1994; **19**: 981-982
127 **Day C**, Hewins P, Sheikh L, Kilby M, McPake D, Lipkin G. Cholestasis in pregnancy associated with ciclosporin therapy in renal transplant recipients. *Transpl Int* 2006; **19**: 1026-1029
128 **Silva MO**, Reddy KR, McDonald T, Jeffers LJ, Schiff ER. Danazol-induced cholestasis. *Am J Gastroenterol* 1989; **84**: 426-428
129 **Bakris GL**, Cross PD, Hammarsten JE. Disopyramide-associated liver dysfunction. *Mayo Clin Proc* 1983; **58**: 265-267
130 **Todd P**, Levison D, Farthing MJ. Enalapril-related cholestatic jaundice. *J R Soc Med* 1990; **83**: 271-272
131 **Derby LE**, Jick H, Henry DA, Dean AD. Erythromycin-associated cholestatic hepatitis. *Med J Aust* 1993; **158**: 600-602
132 **Devereaux BM**, Crawford DH, Purcell P, Powell LW, Roeser HP. Flucloxacillin associated cholestatic hepatitis. An Australian and Swedish epidemic? *Eur J Clin Pharmacol* 1995; **49**: 81-85
133 **Reynolds R**, Lloyd DA, Slinger RP. Cholestatic jaundice induced by flurazepam hydrochloride. *Can Med Assoc J* 1981; **124**: 893-894
134 **Lee HW**, Chung JP, Lee KS, Kim KC, Lee KS, Chon CY, Park IS, Kim HG. A case of flutamide-induced acute cholestatic hepatitis--a case report. *Yonsei Med J* 1996; **37**: 225-229
135 **Basset C**, Vadrot J, Denis J, Poupon J, Zafrani ES. Prolonged cholestasis and ductopenia following gold salt therapy. *Liver Int* 2003; **23**: 89-93
136 **Chiprut RO**, Viteri A, Jamroz C, Dyck WP. Intrahepatic cholestasis after griseofulvin administration. *Gastroenterology* 1976; **70**: 1141-1143
137 **van Basten JP**, van Hoek B, Zeijen R, Stockbrugger R. Glyburide-induced cholestatic hepatitis and liver failure. Case-report and review of the literature. *Neth J Med* 1992; **40**: 305-307
138 **Horst DA**, Grace ND, LeCompte PM. Prolonged cholestasis and progressive hepatic fibrosis following imipramine

therapy. *Gastroenterology* 1980; **79**: 550-554
139 **Dincsoy HP**, Saelinger DA. Haloperidol-induced chronic cholestatic liver disease. *Gastroenterology* 1982; **83**: 694-700
140 **Benson GD**, Anderson PK, Combes B, Ishak KG. Prolonged jaundice following ketoconazole-induced hepatic injury. *Dig Dis Sci* 1988; **33**: 240-246
141 **Foitl DR**, Hyman G, Lefkowitch JH. Jaundice and intrahepatic cholestasis following high-dose megestrol acetate for breast cancer. *Cancer* 1989; **63**: 438-439
142 **Schmidt G**, Borsch G, Muller KM, Wegener M. Methimazole-associated cholestatic liver injury: case report and brief literature review. *Hepatogastroenterology* 1986; **33**: 244-246
143 **Lucey MR**, Moseley RH. Severe cholestasis associated with methyltestosterone: a case report. *Am J Gastroenterol* 1987; **82**: 461-462
144 **Kiire CF**, Rutherford D. Nifedipine-associated jaundice: a second case. *East Afr Med J* 1986; **63**: 560-561
145 **Mulberg AE**, Bell LM. Fatal cholestatic hepatitis and multisystem failure associated with nitrofurantoin. *J Pediatr Gastroenterol Nutr* 1993; **17**: 307-309
146 **Gilbert EF**, Dasilva AQ, Queen DM. Intrahepatic cholestasis with fatal termination following norethandrolone therapy. *JAMA* 1963; **185**: 538-539
147 **Lieberman DA**, Keeffe EB, Stenzel P. Severe and prolonged oral contraceptive jaundice. *J Clin Gastroenterol* 1984; **6**: 145-148
148 **Moradpour D**, Altorfer J, Flury R, Greminger P, Meyenberger C, Jost R, Schmid M. Chlorpromazine-induced vanishing bile duct syndrome leading to biliary cirrhosis. *Hepatology* 1994; **20**: 1437-1441
149 **Altuntas Y**, Ozturk B, Erdem L, Gunes G, Karul S, Ucak S, Sengul A. Phenytoin-induced toxic cholestatic hepatitis in a patient with skin lesions: case report. *South Med J* 2003; **96**: 201-203
150 **Gefel D**, Harats N, Lijovetsky G, Eliakim M. Cholestatic jaundice associated with D-penicillamine therapy. *Scand J Rheumatol* 1985; **14**: 303-306
151 **Rosenberg WM**, Ryley NG, Trowell JM, McGee JO, Chapman RW. Dextropropoxyphene induced hepatotoxicity: a report of nine cases. *J Hepatol* 1993; **19**: 470-474
152 **Kouklakis G**, Mpoumponaris A, Zezos P, Moschos J, Koulaouzidis A, Nakos A, Pehlivanidis A, Iosiphidis M, Molyvas E, Nikolaidis N. Cholestatic hepatitis with severe systemic reactions induced by trimethoprim-sulfamethoxazole. *Ann Hepatol* 2007; **6**: 63-65
153 **Lasso De La Vega MC**, Zapater P, Such J, Sola-Vera J, Paya A, Horga JF, Perez-Mateo M. [Toxic hepatitis associated with tamoxifen use. A case report and literature review] *Gastroenterol Hepatol* 2002; **25**: 247-250
154 **Eland IA**, Kerkhof SC, Overbosch D, Wismans PJ, Stricker BH. [Cholestatic hepatitis ascribed to the use of thiabendazole] *Ned Tijdschr Geneeskd* 1998; **142**: 1331-1334
155 **Nakao NL**, Gelb AM, Stenger RJ, Siegel JH. A case of chronic liver disease due to tolazamide. *Gastroenterology* 1985; **89**: 192-195
156 **Randeva HS**, Bangar V, Sailesh S, Hillhouse EW. Fatal cholestatic jaundice associated with amitriptyline. *Int J Clin Pract* 2000; **54**: 405-406
157 **Larrey D**, Amouyal G, Danan G, Degott C, Pessayre D, Benhamou JP. Prolonged cholestasis after troleandomycin-induced acute hepatitis. *J Hepatol* 1987; **4**: 327-329
158 **Burgunder JM**, Abernethy DR, Lauterburg BH. Liver injury due to verapamil. *Hepatogastroenterology* 1988; **35**: 169-170
159 **Vanderstigel M**, Zafrani ES, Lejonc JL, Schaeffer A, Portos JL. Allopurinol hypersensitivity syndrome as a cause of hepatic fibrin-ring granulomas. *Gastroenterology* 1986; **90**: 188-190
160 **Levy M**, Goodman MW, Van Dyne BJ, Sumner HW. Granulomatous hepatitis secondary to carbamazepine. *Ann Intern Med* 1981; **95**: 64-65
161 **Ben-Yehuda A**, Bloom A, Lijovetzky G, Flusser D, Tur-Kaspa R. Chlorpromazine-induced liver and bone marrow granulomas associated with agranulocytosis. *Isr J Med Sci* 1990; **26**: 449-451
162 **Sarachek NS**, London RL, Matulewicz TJ. Diltiazem and granulomatous hepatitis. *Gastroenterology* 1985; **88**: 1260-1262
163 **Harats N**, Ehrenfeld M, Shalit M, Lijovetzky G. Gold-induced granulomatous hepatitis. *Isr J Med Sci* 1985; **21**: 753-756
164 **Jori GP**, Peschile C. Hydralazine disease associated with transient granulomas in the liver. A case report. *Gastroenterology* 1973; **64**: 1163-1167
165 **Sippel PJ**, Agger WA. Nitrofurantoin-induced granulomatous hepatitis. *Urology* 1981; **18**: 177-178
166 **Silvain C**, Fort E, Levillain P, Labat-Labourdette J, Beauchant M. Granulomatous hepatitis due to combination of amoxicillin and clavulanic acid. *Dig Dis Sci* 1992; **37**: 150-152
167 **Cook IF**, Shilkin KB, Reed WD. Phenytoin induced granulomatous hepatitis. *Aust N Z J Med* 1981; **11**: 539-541
168 **Knobel B**, Buyanowsky G, Dan M, Zaidel L. Pyrazinamide-induced granulomatous hepatitis. *J Clin Gastroenterol* 1997; **24**: 264-266
169 **Bramlet DA**, Posalaky Z, Olson R. Granulomatous hepatitis as a manifestation of quinidine hypersensitivity. *Arch Intern Med* 1980; **140**: 395-397
170 **Namias A**, Bhalotra R, Donowitz M. Reversible sulfasalazine-induced granulomatous hepatitis. *J Clin Gastroenterol* 1981; **3**: 193-198
171 **Seeff LB**. Drug-induced chronic liver disease, with emphasis on chronic active hepatitis. *Semin Liver Dis* 1981; **1**: 104-115
172 **Black M**, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology* 1975; **69**: 289-302
173 **Balazs M**, Kovach G. Chronic aggressive hepatitis after methyldopa treatment. Case report with electron-microscopic study. *Hepatogastroenterology* 1981; **28**: 199-202
174 **Roy AK**, Mahoney HC, Levine RA. Phenytoin-induced chronic hepatitis. *Dig Dis Sci* 1993; **38**: 740-743
175 **Aponte J**, Petrelli M. Histopathologic findings in the liver of rheumatoid arthritis patients treated with long-term bolus methotrexate. *Arthritis Rheum* 1988; **31**: 1457-1464
176 **Volbeda F**, Jonker AM, Vecht J, Groeneveld PH. [Liver cirrhosis due to chronic use of nitrofurantoin] *Ned Tijdschr Geneeskd* 2004; **148**: 235-238
177 **Anania FA**, Rabin L. Terbinafine hepatotoxicity resulting in chronic biliary ductopenia and portal fibrosis. *Am J Med* 2002; **112**: 741-742
178 **Giannitrapani L**, Soresi M, La Spada E, Cervello M, D'Alessandro N, Montalto G. Sex hormones and risk of liver tumor. *Ann N Y Acad Sci* 2006; **1089**: 228-236
179 **Bartley J**, Loddenkemper C, Lange J, Mechsner S, Radke C, Neuhaus P, Ebert AD. Hepatocellular adenoma and focal nodular hyperplasia after long-term use of danazol for endometriosis: a case report. *Arch Gynecol Obstet* 2004; **269**: 290-293
180 **Zhu AX**, Lauwers GY, Tanabe KK. Cholangiocarcinoma in association with Thorotrast exposure. *J Hepatobiliary Pancreat Surg* 2004; **11**: 430-433
181 **Chopra S**, Edelstein A, Koff RS, Zimelman AP, Lacson A, Neiman RS. Peliosis hepatis in hematologic disease. Report of two cases. *JAMA* 1978; **240**: 1153-1155
182 **Sebagh M**, Debette M, Samuel D, Emile JF, Falissard B, Cailliez V, Shouval D, Bismuth H, Reynes M. "Silent" presentation of veno-occlusive disease after liver transplantation as part of the process of cellular rejection with endothelial predilection. *Hepatology* 1999; **30**: 1144-1150
183 **Essell JH**, Thompson JM, Harman GS, Halvorson RD, Snyder MJ, Johnson RA, Rubinsak JR. Marked increase in veno-occlusive disease of the liver associated with methotrexate use for graft-versus-host disease prophylaxis in patients receiving busulfan/cyclophosphamide. *Blood* 1992; **79**: 2784-2788

184 **Voigt H**, Caselitz J, Janner M. [Veno-occlusive syndrome with acute liver dystrophy following decarbazine therapy of malignant melanoma (author's transl)] *Klin Wochenschr* 1981; **59**: 229-236
185 **Spormann H**, Willgeroth C, Tautenhahn P. [Peliosis hepatis with liver rupture] *Zentralbl Allg Pathol* 1985; **130**: 545-550
186 **Lennard L**, Richards S, Cartwright CS, Mitchell C, Lilleyman JS, Vora A. The thiopurine methyltransferase genetic polymorphism is associated with thioguanine-related veno-occlusive disease of the liver in children with acute lymphoblastic leukemia. *Clin Pharmacol Ther* 2006; **80**: 375-383
187 **Sulis ML**, Bessmertny O, Granowetter L, Weiner M, Kelly KM. Veno-occlusive disease in pediatric patients receiving actinomycin D and vincristine only for the treatment of rhabdomyosarcoma. *J Pediatr Hematol Oncol* 2004; **26**: 843-846
188 **Ouellet G**, Tremblay L, Marleau D. Fulminant hepatitis induced by lamotrigine. *South Med J* 2009; **102**: 82-84
189 **Tan HH**, Ong WM, Lai SH, Chow WC. Nimesulide-induced hepatotoxicity and fatal hepatic failure. *Singapore Med J* 2007; **48**: 582-585
190 **Skopp G**, Schmitt HP, Pedal I. [Fulminant liver failure in a patient on carbamazepine and levetiracetam treatment associated with status epilepticus] *Arch Kriminol* 2006; **217**: 161-175
191 **Barcena R**, Oton E, Angeles Moreno M, Fortun J, Garcia-Gonzalez M, Moreno A, de Vicente E. Is liver transplantation advisable for isoniazid fulminant hepatitis in active extrapulmonary tuberculosis? *Am J Transplant* 2005; **5**: 2796-2798
192 **Tietz A**, Heim MH, Eriksson U, Marsch S, Terracciano L, Krahenbuhl S. Fulminant liver failure associated with clarithromycin. *Ann Pharmacother* 2003; **37**: 57-60
193 **Garbino J**, Henry JA, Mentha G, Romand JA. Ecstasy ingestion and fulminant hepatic failure: liver transplantation to be considered as a last therapeutic option. *Vet Hum Toxicol* 2001; **43**: 99-102
194 **Stickel F**, Egerer G, Seitz HK. Hepatotoxicity of botanicals. *Public Health Nutr* 2000; **3**: 113-124
195 **Gordon DW**, Rosenthal G, Hart J, Sirota R, Baker AL. Chaparral ingestion. The broadening spectrum of liver injury caused by herbal medications. *JAMA* 1995; **273**: 489-490
196 **Russmann S**, Lauterburg BH, Helbling A. Kava hepatotoxicity. *Ann Intern Med* 2001; **135**: 68-69
197 **Lee MK**, Cheng BW, Che CT, Hsieh DP. Cytotoxicity assessment of Ma-huang (Ephedra) under different conditions of preparation. *Toxicol Sci* 2000; **56**: 424-430
198 **Whiting PW**, Clouston A, Kerlin P. Black cohosh and other herbal remedies associated with acute hepatitis. *Med J Aust* 2002; **177**: 440-443
199 **Lake CR**, Gallant S, Masson E, Miller P. Adverse drug effects attributed to phenylpropanolamine: a review of 142 case reports. *Am J Med* 1990; **89**: 195-208
200 **Sanchez W**, Maple JT, Burgart LJ, Kamath PS. Severe hepatotoxicity associated with use of a dietary supplement containing usnic acid. *Mayo Clin Proc* 2006; **81**: 541-544
201 **Bleibel W**, Kim S, D'Silva K, Lemmer ER. Drug-induced liver injury: review article. *Dig Dis Sci* 2007; **52**: 2463-2471

S- Editor Li LF **L- Editor** O'Neill M **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2834

World J Gastroenterol 2009 June 21; 15(23): 2834-2838
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Consequences of dysthyroidism on the digestive tract and viscera

Ronald Daher, Thierry Yazbeck, Joe Bou Jaoude, Bassam Abboud

Ronald Daher, Thierry Yazbeck, Bassam Abboud, Department of General Surgery, Hotel Dieu de France Hospital, Faculty of Medicine, Saint-Joseph University, Beirut 16-6830, Lebanon
Joe Bou Jaoude, Department of Gastroenterology, Hotel Dieu de France Hospital, Faculty of Medicine, Saint-Joseph University, Beirut 16-6830, Lebanon
Author contributions: Abboud B designed the research; Daher R, Abboud B, Bou Jaoude J and Yazbeck T performed the research; Daher R, Abboud B and Yazbeck T wrote the paper.
Correspondence to: Bassam Abboud, MD, Department of General Surgery, Hotel Dieu de France Hospital, Alfred Naccache Street, PO Box 16-6830, Beirut, Lebanon. dbabboud@yahoo.fr
Telephone: +961-1-615300 Fax: +961-1-615295
Received: March 13, 2009 Revised: April 7, 2009
Accepted: April 14, 2009
Published online: June 21, 2009

Abstract

Thyroid hormones define basal metabolism throughout the body, particularly in the intestine and viscera. Gastrointestinal manifestations of dysthyroidism are numerous and involve all portions of the tract. Thyroid hormone action on motility has been widely studied, but more complex pathophysiologic mechanisms have been indicated by some studies although these are not fully understood. Both thyroid hormone excess and deficiency can have similar digestive manifestations, such as diarrhea, although the mechanism is different in each situation. The liver is the most affected organ in both hypo- and hyperthyroidism. Specific digestive diseases may be associated with autoimmune thyroid processes, such as Hashimoto's thyroiditis and Grave's disease. Among them, celiac sprue and primary biliary cirrhosis are the most frequent although a clear common mechanism has never been proven. Overall, thyroid-related digestive manifestations were described decades ago but studies are still needed in order to confirm old concepts or elucidate undiscovered mechanisms. All practitioners must be aware of digestive symptoms due to dysthyroidism in order to avoid misdiagnosis of rare but potentially lethal situations.

Key words: Hypothyroidism; Hyperthyroidism; Gastrointestinal motility; Intestine; Liver; Viscera

Peer reviewer: Serhan Karvar, MD, Assistant Professor of Medicine, University of Southern California, Keck School of Medicine, Division of Gastrointestinal & Liver Diseases, 2011 Zonal Avenue, HMR 101, Los Angeles, CA 90089, United States

Daher R, Yazbeck T, Bou Jaoude J, Abboud B. Consequences of dysthyroidism on the digestive tract and viscera. *World J Gastroenterol* 2009; 15(23): 2834-2838 Available from: URL: http://www.wjgnet.com/1007-9327/15/2834.asp DOI: http://dx.doi.org/10.3748/wjg.15.2834

INTRODUCTION

Thyroid hormones act on almost all organs throughout the body and regulate the basal metabolism of the organism[1]. The gut and viscera are not spared, and disturbances in thyroid function have numerous gastrointestinal manifestations, the true incidence of which is unknown[2]. Digestive symptoms or signs may also reveal clues to thyroid disease and, when ignored or underestimated, diagnosis may be delayed and serious consequences may occur[3-5]. Additionally, patients with dysthyroidism are at an increased risk of developing specific pathologies in the digestive system, whether due to thyroid hormone disturbances or associated with a particular thyroid disease[6-17].

Thyroid interactions with the gastrointestinal system have been widely reported but the literature lacks an exhaustive report on different consequences of dysthyroidism. Gastrointestinal motor dysfunction has been widely accepted as the main cause of symptoms but many complex phenomena have not yet been completely elucidated[4,11,18-21]. This review aims to gather up-to-date knowledge about the effects of dysthyroidism on the gut and viscera.

HYPERTHYROIDISM

As thyroid hormones act on almost all organs within the gastrointestinal tract (gut and viscera), hyperthyroidism induces several symptoms and signs, and causes different biologic and metabolic derangements. Digestive symptoms may represent the only manifestations of hyperthyroidism. A lack of cardinal features of the disease and the presence of persistent abdominal pain, intractable vomiting, weight loss and altered bowel habits are designated as apathetic hyperthyroidism[22].

Esophagus and stomach

Dysphagia is a rare manifestation of hyperthyroidism and can have an acute or chronic pattern[3]. It may be related to direct compression from goiter or to altered neurohormonal regulation[23,24]. Excess thyroid hormone may cause myopathy which involves striated muscles of the pharynx and the upper third of the esophagus[23]. Subsequently, the oropharyngeal phase of deglutition is predominantly impaired and patients are predisposed to nasal regurgitation and aspiration pneumonia. Correction of the endocrine disorder is believed to reverse dysphagia[3,23,24].

In the esophagus, thyroid hormone excess increases the propagation velocity of contractions[25]. Thyrotoxic patients may frequently complain of chronic dyspeptic symptoms such as epigastric pain, fullness and eructation[2]. Tachygastria has been incriminated in upper gastrointestinal symptoms but the true mechanism is not yet fully understood[19,20]. Vomiting is rarely intractable and may involve neurohormonal mediators along with direct action[26]. Studies have yielded variable, even contradictory results concerning gastric emptying in thyrotoxicosis[18,19,27,28]. A significant increase in the dominant electrical frequency and dysrhythmia was shown through a myoelectrical activity study[19,20] but lack of correlation between electrogastrography (EGG) findings and gastric emptying by scintigraphy may be the result of intervening factors such as a smooth muscle disorder, electro-mechanical dissociation, pylorospasm or incoordination of the antrum and duodenum[20,29]. Hypergastrinemia found in hyperthyroidism may also influence gastric and intestinal motility[30].

Intestine and colon

Appetite increase is common but may not be adequate to maintain weight in severe disease[31]. Up to 25% of patients with hyperthyroidism have mild-to-moderate diarrhea with frequent bowel movements[22,32]. Some degree of fat malabsorption is usually present and may reach 35 g/d[33]. Intestinal hypermotility in thyrotoxicosis reduces small bowel transit time, especially when diarrhea is present[18]. Increased appetite and excessive fat-rich food intake may contribute to excessive fecal fat[34]. Moreover, diarrhea may be related to a hypersecretory state within the intestinal mucosa[22,35]. The adrenergic system may contribute to diarrhea as suggested by correction of transit in hyperthyroid patients treated with the β-adrenergic antagonist propranolol[36]. A reduction in mixing of food with digestive secretions may also contribute to decreased fat absorption. Alterations in intestinal absorptive function are still a matter of debate, as absorption may be increased for glucose[34,37] but decreased for calcium[38]. Anorectal physiology is impaired in hyperthyroidism; when compared to controls, mean anal resting and squeeze pressures are lower as is the rectal threshold of sensation[39].

Liver

Increases in aspartate aminotransferase and alanine aminotransferase in 27% and 37%, respectively, of hyperthyroid patients have been reported[40]. These disturbances are attributed to a hypoxic state with disproportionately increased liver activity compared to blood flow[41]. Mild elevation of alkaline phosphatase is encountered in up to 64% of patients with hyperthyroidism[42-44]. This elevation is not specific to the liver since a high turnover in bones may contribute. Elevations of γ-glutamyl transferase and bilirubin do not exceed 20% of normal values[44]. Increases in liver enzymes and hepatic injury related to anti-thyroid therapy is well documented[45]. Mild histological changes are common[46], but cases of fulminant hepatic failure with centrizonal necrosis have been described[46,47]. Long term untreated hyperthyroidism can ultimately lead to cirrhosis[48]. Quantitative 99mTc-HIDA cholescintigraphy in hyperthyroid rats without a gallbladder showed accelerated bile flow to the duodenum[21].

Hyperthyroidism and associated gastrointestinal diseases

Ch'ng *et al*[6] found that patients with Grave's disease were at a 5-fold added risk of developing celiac disease when compared to sex- and age-matched controls. In such cases, celiac disease may contribute to diarrhea and malabsorption. Thyrotoxicosis has been reported in 3.8% of patients with ulcerative colitis while the incidence of ulcerative colitis in hyperthyroid patients varies around 1%[17]. Thyroid disease may exacerbate ulcerative colitis symptoms or alter the response to therapy. Moreover, a positive correlation between Grave's disease and ulcerative colitis has been reported[12], but a common autoimmune origin could not be proven[11]. Isolated instances of an association between Grave's disease and Crohn's disease have been reported, but a common pathogenesis is still to be identified[16]. Primary biliary cirrhosis in association with hyperthyroidism is extremely rare and has only described as isolated case reports[8]. One study showed a prevalence of pernicious anemia of 5% in thyrotoxic patients, mainly resulting from Grave's disease[49], but parietal cell antibodies have been found in up to 30% of patients[50].

HYPOTHYROIDISM

Hypothyroidism occurs mostly secondary to an autoimmune disease or as a consequence of therapy for hyperthyroidism. It manifests throughout the body with decreased metabolic functions. It is biochemically characterized by the accumulation of glycosaminoglycans, mainly hyaluronic acid, in soft tissues[51]. Interstitial edema predominating in the skin and muscles (including the heart and intestinal muscular layer) will follow. Clinical presentation of the disease is related to the severity of the disease (biochemical derangement) but harbors significant individual variation[52]. Gastrointestinal manifestations are not rare and involve different digestive organs.

Esophagus and stomach

Severe hypothyroidism may lead to disturbances in esophageal peristalsis. When the proximal portion is involved, myxedema causes oropharyngeal dysphagia[53] while esophagitis and hiatal hernia occur when the

distal esophagus is altered[22,54]. Esophageal motility disorders, reduced velocity and amplitude of esophageal peristalsis and a decrease in lower sphincter pressure all contribute to dysphagia[55]. Although it represents an extremely rare cause of dyspepsia, hypothyroidism should be investigated when all exploratory methods are negative[56]. A gastric myoelectrical study led by Gunsar *et al*[19] showed a positive correlation between dyspepsia and hypothyroid scores. Additionally, gastric dysmotility is significantly more frequent in hypothyroid patients and is a result of muscle edema and altered myoelectrical activity[57]. Despite a few contradictory results[58], the hypothyroid state seems to delay gastric emptying[19,59]. Phytobezoar due to hypothyroidism has also been reported[60]. Achlorhydria in hypothyroidism may be related to subnormal serum gastrin[61]. Finally, hypothyroidism is associated with a decrease in duodenal basal electrical rhythm[62].

Intestine and colon

Appetite is usually reduced, but weight gain may reach 10% because of fluid retention[31]. Vague abdominal discomfort and bloating may be erroneously attributed to functional bowel disease[2]. The effect of hypothyroidism on the gastrointestinal tract seems to be multifactorial with possible alterations in hormone receptors, neuromuscular disorders and myopathy caused by infiltration of the intestinal wall. Reduction of peristalsis in hypothyroidism is the main pathophysiologic process[62], and constipation remains the most frequent gastrointestinal complaint[22]. Up to 15% of patients have fewer than 3 bowel movements weekly[2]. Moreover, thyroid hormone deficiency may influence transepithelial flux transport by inhibiting Cl^-/HCO^{3-} anion exchange with a subsequent effect on intestinal motility[35]. Although rare, severe cases of hypothyroidism lead to ileus and colonic pseudo-obstruction with fecal impaction and megacolon[63,64]. Inadvertent surgery in these situations is harmful and may be lethal[5]. Absorption of specific substances may be decreased but the total quantity absorbed is usually normal or increased due to an extended time in bowel transit[31,65]. Diarrhea in the hypothyroid state is mainly the result of increased bacterial growth secondary to bowel hypomotility[66,67]. Exceptionally, hypothyroidism may be the cause of gastrointestinal bleeding refractory to usual treatments[68], most probably by means of acquired coagulopathy[69]. Deen *et al*[39] found that the anorectal physiology is altered in hypothyroid states. While maximal anal resting and squeeze pressures are normal, the threshold for rectal sensation is higher and the maximal tolerable volume is diminished when compared to controls.

Liver

Liver function tests are mildly disturbed in almost 50% of patients with hypothyroidism despite normal histological findings[22]. Decreased hepatic metabolism in hypothyroidism is reflected by reduced oxygen consumption[70] and causes a significant decrease in gluconeogenesis[71] and urea nitrogen production[72]. Myxedema ascites in hypothyroidism is rare and may be a long-standing overlooked and/or isolated sign of the disease[73]. The serum-to-ascites albumin gradient is usually > 1.1 g/dL with a high protein content[4,73]. Although considered to be the result of hypothyroidism-related chronic right-heart failure[74,75], it is mainly attributed to increased permeability of vascular endothelium[4,76]. Patients with a common bile duct stone and gallbladder stone have, respectively, 7-fold and 3-fold increases in the frequency of hypothyroidism[77]. This may be related to the triad: hypercholesterolemia, hypotonia of the gallbladder and reduced bilirubin excretion. Experiments in rats confirmed a thyroxine effect on bile composition[78,79], decreased hepatocytic bile salt excretion in hypothyroid state[80] and relaxation of the sphincter of Oddi[81]. Moreover, Laukkarinen *et al*[21] confirmed that bile flow to the duodenum was reduced in hypothyroid rats.

Hypothyroidism and associated gastrointestinal diseases

Compared to the general population, patients with autoimmune thyroiditis have an almost 5-fold increased risk of developing celiac disease[14,15,82,83]. Valentino *et al*[7] showed that as many as 43% of patients with Hashimoto's thyroiditis carry cellular markers for celiac disease. The prevalence of thyroid antibodies is extremely high in patients with pernicious anemia (57%)[13], and the prevalence of overt pernicious anemia among patients with primary hypothyroidism is 12%[31]. An association between hypothyroidism and primary biliary cirrhosis is well documented and ranges from 5% to 20%[9,10,84]. Among patients with primary biliary cirrhosis, antithyroid antibodies were present in 20%[10]. The coexistence of Hashimoto's thyroiditis and Crohn's disease is rare and the etiological background remains to be elucidated[16,85].

CONCLUSION

Dysthyroidism, whether in excess or deficiency, has clinical manifestations within different portions of the digestive tract and viscera. Whether these are related to hormone level disturbances alone or are associated with a specific thyroid disease, the underlying pathophysiology is often complex and not yet fully elucidated in current studies. Although most frequent manifestations are well known, some situations are often underdiagnosed, leading to serious illness and death.

Digestive diseases related to thyroid hormone abnormalities or associated with particular thyroid diseases must be recognized by most, if not all practitioners. Much research requires to be performed in order to add to our understanding of the scientific background of the older empirical works.

REFERENCES

1 **Guyton A**. The thyroid metabolic hormones. In: Textbook

of Medical Physiology. 8th edition. Philadelphia: Saunders, 1991: 831-841
2 **Maser C**, Toset A, Roman S. Gastrointestinal manifestations of endocrine disease. *World J Gastroenterol* 2006; **12**: 3174-3179
3 **Noto H**, Mitsuhashi T, Ishibashi S, Kimura S. Hyperthyroidism presenting as dysphagia. *Intern Med* 2000; **39**: 472-473
4 **Desramé J**, Mathurin P, Rozov R, Sabaté JM, Poynard T, Opolon P, Denis J. [Isolated ascites revealing a hypothyroidism. Study of 2 cases] *Gastroenterol Clin Biol* 1998; **22**: 732-735
5 **Abboud B**, Sayegh R, Medlej R, Halaby G, Saade C, Farah P. [A rare manifestation of hypothyroidism: intestinal obstruction. Report of 2 cases and review of the literature] *J Med Liban* 1999; **47**: 364-366
6 **Ch'ng CL**, Biswas M, Benton A, Jones MK, Kingham JG. Prospective screening for coeliac disease in patients with Graves' hyperthyroidism using anti-gliadin and tissue transglutaminase antibodies. *Clin Endocrinol* (Oxf) 2005; **62**: 303-306
7 **Valentino R**, Savastano S, Maglio M, Paparo F, Ferrara F, Dorato M, Lombardi G, Troncone R. Markers of potential coeliac disease in patients with Hashimoto's thyroiditis. *Eur J Endocrinol* 2002; **146**: 479-483
8 **Yaşar DG**, Ozenirler S, Doğan M. A patient with primary biliary cirrhosis accompanied by Graves disease and Hurthle cell adenoma. *Turk J Gastroenterol* 2007; **18**: 198-200
9 **Valera M JM**, Smok S G, Poniachik T J, Oksenberg R D, Silva P G, Ferrario B M, Buckel G E, Brahm B J. [Primary biliary cirrhosis: a thirteen years experience] *Rev Med Chil* 2006; **134**: 469-474
10 **Elta GH**, Sepersky RA, Goldberg MJ, Connors CM, Miller KB, Kaplan MM. Increased incidence of hypothyroidism in primary biliary cirrhosis. *Dig Dis Sci* 1983; **28**: 971-975
11 **Bonapace ES**, Srinivasan R. Simultaneous occurrence of inflammatory bowel disease and thyroid disease. *Am J Gastroenterol* 2001; **96**: 1925-1926
12 **Triantafillidis JK**, Cherakakis P, Zervakakis A, Theodorou M. Coexistence of hyperthyroidism and ulcerative colitis: report of 4 cases and a review of the literature. *Ital J Gastroenterol* 1992; **24**: 494-497
13 **Krassas G**, McHardy-Young S, Ramsay I, Florin-Christensen A. Thyroid function and antibody studies in pernicious anaemia. *Clin Endocrinol* (Oxf) 1977; **6**: 145-151
14 **Valentino R**, Savastano S, Tommaselli AP, Dorato M, Scarpitta MT, Gigante M, Micillo M, Paparo F, Petrone E, Lombardi G, Troncone R. Prevalence of coeliac disease in patients with thyroid autoimmunity. *Horm Res* 1999; **51**: 124-127
15 **Volta U**, Ravaglia G, Granito A, Forti P, Maioli F, Petrolini N, Zoli M, Bianchi FB. Coeliac disease in patients with autoimmune thyroiditis. *Digestion* 2001; **64**: 61-65
16 **Inokuchi T**, Moriwaki Y, Takahashi S, Tsutsumi Z, KA T, Yamamoto T. Autoimmune thyroid disease (Graves' disease and hashimoto's thyroiditis) in two patients with Crohn's disease: case reports and literature review. *Intern Med* 2005; **44**: 303-306
17 **Nishimura M**, Yamamoto T, Iijima H, Moriwaki Y, Takahashi S, Hada T. Basedow's disease and chronic ulcerative colitis: a case report and review of the Japanese literature. *Intern Med* 2001; **40**: 44-47
18 **Wegener M**, Wedmann B, Langhoff T, Schaffstein J, Adamek R. Effect of hyperthyroidism on the transit of a caloric solid-liquid meal through the stomach, the small intestine, and the colon in man. *J Clin Endocrinol Metab* 1992; **75**: 745-749
19 **Gunsar F**, Yilmaz S, Bor S, Kumanlioğlu K, Cetinkalp S, Kabalak T, Ozutemiz OA. Effect of hypo- and hyperthyroidism on gastric myoelectrical activity. *Dig Dis Sci* 2003; **48**: 706-712
20 **Pfaffenbach B**, Adamek RJ, Hagelmann D, Schaffstein J, Wegener M. Effect of hyperthyroidism on antral myoelectrical activity, gastric emptying and dyspepsia in man. *Hepatogastroenterology* 1997; **44**: 1500-1508
21 **Laukkarinen J**, Koobi P, Kalliovalkama J, Sand J, Mattila J, Turjanmaa V, Porsti I, Nordback I. Bile flow to the duodenum is reduced in hypothyreosis and enhanced in hyperthyreosis. *Neurogastroenterol Motil* 2002; **14**: 183-188
22 **Kim D**, Ryan J. Gastrointestinal manifestations of systemic diseases. In: Feldman M, Friedman L, Sleisenger M, eds. Gastrointestinal and Liver Disease: Pathophysiology/ Diagnosis/Management. 7th edition. Philadelphia: Saunders, 2002
23 **Chiu WY**, Yang CC, Huang IC, Huang TS. Dysphagia as a manifestation of thyrotoxicosis: report of three cases and literature review. *Dysphagia* 2004; **19**: 120-124
24 **Branski D**, Levy J, Globus M, Aviad I, Keren A, Chowers I. Dysphagia as a primary manifestation of hyperthyroidism. *J Clin Gastroenterol* 1984; **6**: 437-440
25 **Meshkinpour H**, Afrasiabi MA, Valenta LJ. Esophageal motor function in Graves' disease. *Dig Dis Sci* 1979; **24**: 159-161
26 **Hoogendoorn EH**, Cools BM. Hyperthyroidism as a cause of persistent vomiting. *Neth J Med* 2004; **62**: 293-296
27 **Miller LJ**, Owyang C, Malagelada JR, Gorman CA, Go VL. Gastric, pancreatic, and biliary responses to meals in hyperthyroidism. *Gut* 1980; **21**: 695-700
28 **Jonderko K**, Jonderko G, Marcisz C, Gołab T. Gastric emptying in hyperthyroidism. *Am J Gastroenterol* 1997; **92**: 835-838
29 **Rothstein RD**, Alavi A, Reynolds JC. Electrogastrography in patients with gastroparesis and effect of long-term cisapride. *Dig Dis Sci* 1993; **38**: 1518-1524
30 **Kaise M**, Sumitomo H, Hashimoto K, Takahashi Y, Matsui J, Tanaka S, Kobayashi Y, Nishimura M. [Hypergastrinemia and type A gastritis in Basedow's disease] *Nippon Shokakibyo Gakkai Zasshi* 1992; **89**: 1990-1995
31 **Larsen PR**, Davies TF, Hay ID. The thyroid gland. In: Wilson J, Foster D, Kronenberg H, Larsen PR, eds. Williams textbook of endocrinology. 9th edition. Philadelphia: WB Saunders, 1998: 389-515
32 **Karaus M**, Wienbeck M, Grussendorf M, Erckenbrecht JF, Strohmeyer G. Intestinal motor activity in experimental hyperthyroidism in conscious dogs. *Gastroenterology* 1989; **97**: 911-999
33 **Hellesen C**, Friis T, Larsen E, Pock-Steen C. Small intestinal histology, radiology and absorption in hyperthyroidism. *Scand J Gastroenterol* 1969; **4**: 169-175
34 **Thomas FB**, Caldwell JH, Greenberger NJ. Steatorrhea in thyrotoxicosis. Relation to hypermotility and excessive dietary fat. *Ann Intern Med* 1973; **78**: 669-675
35 **Tenore A**, Fasano A, Gasparini N, Sandomenico ML, Ferrara A, Di Carlo A, Guandalini S. Thyroxine effect on intestinal Cl-/HCO3- exchange in hypo- and hyperthyroid rats. *J Endocrinol* 1996; **151**: 431-437
36 **Thomas FB**, Caldwell JH, Greenberger NJ. Steatorrhea in thyrotoxicosis. Relation to hypermotility and excessive dietary fat. *Ann Intern Med* 1973; **78**: 669-675
37 **Debiec H**, Cross HS, Peterlik M. D-glucose uptake is increased in jejunal brush-border membrane vesicles from hyperthyroid chicks. *Acta Endocrinol* (Copenh) 1989; **120**: 435-441
38 **Noble HM**, Matty AJ. The effect of thyroxine on the movement of calcium and inorganic phosphate through the small intestine of the rat. *J Endocrinol* 1967; **37**: 111-117
39 **Deen KI**, Seneviratne SL, de Silva HJ. Anorectal physiology and transit in patients with disorders of thyroid metabolism. *J Gastroenterol Hepatol* 1999; **14**: 384-387
40 **Thompson P Jr**, Strum D, Boehm T, Wartofsky L. Abnormalities of liver function tests in tyrotoxicosis. *Mil Med* 1978; **143**: 548-551
41 **Myers JD**, Brannon ES, Holland BC. A correlative study of the cardiac output and the hepatic circulation in

hyperthyroidism. *J Clin Invest* 1950; **29**: 1069-1077
42 **Biscoveanu M**, Hasinski S. Abnormal results of liver function tests in patients with Graves' disease. *Endocr Pract* 2000; **6**: 367-369
43 **Gürlek A**, Cobankara V, Bayraktar M. Liver tests in hyperthyroidism: effect of antithyroid therapy. *J Clin Gastroenterol* 1997; **24**: 180-183
44 **Doran GR**. Serum enzyme disturbances in thyrotoxicosis and myxoedema. *J R Soc Med* 1978; **71**: 189-194
45 **Malik R**, Hodgson H. The relationship between the thyroid gland and the liver. *QJM* 2002; **95**: 559-569
46 **Huang MJ**, Liaw YF. Clinical associations between thyroid and liver diseases. *J Gastroenterol Hepatol* 1995; **10**: 344-350
47 **Choudhary AM**, Roberts I. Thyroid storm presenting with liver failure. *J Clin Gastroenterol* 1999; **29**: 318-321
48 **Sola J**, Pardo-Mindán FJ, Zozaya J, Quiroga J, Sangro B, Prieto J. Liver changes in patients with hyperthyroidism. *Liver* 1991; **11**: 193-197
49 **Furszyfer J**, McConahey WM, Kurland LT, Maldonado JE. On the increased association of Graves' disease with pernicious anemia. *Mayo Clin Proc* 1971; **46**: 37-39
50 **Burman P**, Kämpe O, Kraaz W, Lööf L, Smolka A, Karlsson A, Karlsson-Parra A. A study of autoimmune gastritis in the postpartum period and at a 5-year follow-up. *Gastroenterology* 1992; **103**: 934-942
51 **Smith TJ**, Bahn RS, Gorman CA. Connective tissue, glycosaminoglycans, and diseases of the thyroid. *Endocr Rev* 1989; **10**: 366-391
52 **Devdhar M**, Ousman YH, Burman KD. Hypothyroidism. *Endocrinol Metab Clin North Am* 2007; **36**: 595-615, v
53 **Wright RA**, Penner DB. Myxedema and upper esophageal dysmotility. *Dig Dis Sci* 1981; **26**: 376-377
54 **Savina LV**, Semenikhina TM, Korochanskaia NV, Klitinskaia IS, Iakovenko MS. [Hiatus hernia and gastroesophageal reflux disease as a manifestation of a newly revealed hypothyroidism] *Klin Med* (Mosk) 2006; **84**: 71-74
55 **Eastwood GL**, Braverman LE, White EM, Vander Salm TJ. Reversal of lower esophageal sphincter hypotension and esophageal aperistalsis after treatment for hypothyroidism. *J Clin Gastroenterol* 1982; **4**: 307-310
56 **Heikkinen M**, Pikkarainen P, Takala J, Räsänen H, Julkunen R. Etiology of dyspepsia: four hundred unselected consecutive patients in general practice. *Scand J Gastroenterol* 1995; **30**: 519-523
57 **Greenspan FS**, Rapaport B. Thyroid gland. In: Greenspan FS, Baxter JD, eds. Basic and Clinical Endocrinology. New York: Saunders, 1992: 188-246
58 **Dubois A**, Goldman JM. Gastric secretion and emptying in hypothyroidism. *Dig Dis Sci* 1984; **29**: 407-410
59 **Kahraman H**, Kaya N, Demirçali A, Bernay I, Tanyeri F. Gastric emptying time in patients with primary hypothyroidism. *Eur J Gastroenterol Hepatol* 1997; **9**: 901-904
60 **Kaplan LR**. Hypothyroidism presenting as a gastric phytobezoar. *Am J Gastroenterol* 1980; **74**: 168-169
61 **Seino Y**, Matsukura S, Inoue Y, Kadowaki S, Mori K, Imura H. Hypogastrinemia in hypothyroidism. *Am J Dig Dis* 1978; **23**: 189-191
62 **Shafer RB**, Prentiss RA, Bond JH. Gastrointestinal transit in thyroid disease. *Gastroenterology* 1984; **86**: 852-855
63 **Bassotti G**, Pagliacci MC, Nicoletti I, Pelli MA, Morelli A. Intestinal pseudoobstruction secondary to hypothyroidism. Importance of small bowel manometry. *J Clin Gastroenterol* 1992; **14**: 56-58
64 **Batke M**, Cappell MS. Adynamic ileus and acute colonic pseudo-obstruction. *Med Clin North Am* 2008; **92**: 649-670, ix
65 **Misra GC**, Bose SL, Samal AK. Malabsorption in thyroid dysfunctions. *J Indian Med Assoc* 1991; **89**: 195-197
66 **Goldin E**, Wengrower D. Diarrhea in hypothyroidism: bacterial overgrowth as a possible etiology. *J Clin Gastroenterol* 1990; **12**: 98-99
67 **Lauritano EC**, Bilotta AL, Gabrielli M, Scarpellini E, Lupascu A, Laginestra A, Novi M, Sottili S, Serricchio M, Cammarota G, Gasbarrini G, Pontecorvi A, Gasbarrini A. Association between hypothyroidism and small intestinal bacterial overgrowth. *J Clin Endocrinol Metab* 2007; **92**: 4180-4184
68 **Fukunaga K**. Refractory gastrointestinal bleeding treated with thyroid hormone replacement. *J Clin Gastroenterol* 2001; **33**: 145-147
69 **Dalton RG**, Dewar MS, Savidge GF, Kernoff PB, Matthews KB, Greaves M, Preston FE. Hypothyroidism as a cause of acquired von Willebrand's disease. *Lancet* 1987; **1**: 1007-1009
70 **Liverini G**, Iossa S, Barletta A. Relationship between resting metabolism and hepatic metabolism: effect of hypothyroidism and 24 hours fasting. *Horm Res* 1992; **38**: 154-159
71 **Comte B**, Vidal H, Laville M, Riou JP. Influence of thyroid hormones on gluconeogenesis from glycerol in rat hepatocytes: a dose-response study. *Metabolism* 1990; **39**: 259-263
72 **Marchesini G**, Fabbri A, Bianchi GP, Motta E, Bugianesi E, Urbini D, Pascoli A, Lodi A. Hepatic conversion of amino nitrogen to urea nitrogen in hypothyroid patients and upon L-thyroxine therapy. *Metabolism* 1993; **42**: 1263-1269
73 **Ji JS**, Chae HS, Cho YS, Kim HK, Kim SS, Kim CW, Lee CD, Lee BI, Choi H, Lee KM, Lee HK, Choi KY. Myxedema ascites: case report and literature review. *J Korean Med Sci* 2006; **21**: 761-764
74 **Kinney EL**, Wright RJ, Caldwell JW. Value of clinical features for distinguishing myxedema ascites from other forms of ascites. *Comput Biol Med* 1989; **19**: 55-59
75 **Klein I**, Levey GS. Unusual manifestations of hypothyroidism. *Arch Intern Med* 1984; **144**: 123-128
76 **Baker A**, Kaplan M, Wolfe H. Central congestive fibrosis of the liver in myxedema ascites. *Ann Intern Med* 1972; **77**: 927-929
77 **Inkinen J**, Sand J, Nordback I. Association between common bile duct stones and treated hypothyroidism. *Hepatogastroenterology* 2000; **47**: 919-921
78 **Andreini JP**, Prigge WF, Ma C, Gebbard RL. Vesicles and mixed micelles in hypothyroid rat bile before and after thyroid hormone treatment: evidence for a vesicle transport system for biliary cholesterol secretion. *J Lipid Res* 1994; **35**: 1405-1412
79 **Vlahcevic ZR**, Eggertsen G, Björkhem I, Hylemon PB, Redford K, Pandak WM. Regulation of sterol 12alpha-hydroxylase and cholic acid biosynthesis in the rat. *Gastroenterology* 2000; **118**: 599-607
80 **Van Steenbergen W**, Fevery J, De Vos R, Leyten R, Heirwegh KP, De Groote J. Thyroid hormones and the hepatic handling of bilirubin. I. Effects of hypothyroidism and hyperthyroidism on the hepatic transport of bilirubin mono- and diconjugates in the Wistar rat. *Hepatology* 1989; **9**: 314-321
81 **Inkinen J**, Sand J, Arvola P, Pörsti I, Nordback I. Direct effect of thyroxine on pig sphincter of Oddi contractility. *Dig Dis Sci* 2001; **46**: 182-186
82 **Guliter S**, Yakaryilmaz F, Ozkurt Z, Ersoy R, Ucardag D, Caglayan O, Atasoy P. Prevalence of coeliac disease in patients with autoimmune thyroiditis in a Turkish population. *World J Gastroenterol* 2007; **13**: 1599-1601
83 **Berti I**, Trevisiol C, Tommasini A, Città A, Neri E, Geatti O, Giammarini A, Ventura A, Not T. Usefulness of screening program for celiac disease in autoimmune thyroiditis. *Dig Dis Sci* 2000; **45**: 403-406
84 **Zeniya M**. [Thyroid disease in autoimmune liver diseases] *Nippon Rinsho* 1999; **57**: 1882-1887
85 **Shah SA**, Peppercorn MA, Pallotta JA. Autoimmune (Hashimoto's) thyroiditis associated with Crohn's disease. *J Clin Gastroenterol* 1998; **26**: 117-120

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2839

World J Gastroenterol 2009 June 21; 15(23): 2839-2854
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Dr. Shahid A Khan, *Series Editor*

Gastroenterology in developing countries: Issues and advances

Kate L Mandeville, Justus Krabshuis, Nimzing Gwamzhi Ladep, Chris JJ Mulder, Eamonn MM Quigley, Shahid A Khan

Kate L Mandeville, Centre for Infectious Diseases Epidemiology, Department of Primary Care and Population Sciences, University College London, Hampstead Campus, Royal Free Hospital, London NW3 2PF, United Kingdom
Justus Krabshuis, Highland Data, Les Charleix, 24390 Tourtoirac, Dordogne, France
Nimzing Gwamzhi Ladep, Department of Medicine, University of Jos and Jos University Teaching Hospital, Jos, Plateau State, P.M.B. 2076, Nigeria
Chris JJ Mulder, Department of Gastroenterology, VU University Medical Center, Amsterdam 1081 HV, Holland
Eamonn MM Quigley, World Gastroenterology Organisation and Department of Medicine, National University of Ireland, Cork University Hospital Clinical Sciences Building Wilton, Cork, Ireland
Shahid A Khan, Department of Hepatology and Gastroenterology, Faculty of Medicine, Imperial College London, St Mary's Campus, London W2 1NY, United Kingdom
Author contributions: Mandeville KL and Khan SA developed the structure of the paper; Mandeville KL wrote the manuscript; Quigley EMM, Mulder CJJ, Krabshuis J, Ladep NG and Khan SA contributed sections to the paper and reviewed the manuscript.
Supported by The NIHR Biomedical Research Centre funding scheme, the Higher Education Funding Council for England (HEFCE), the British Liver Trust and the Alan Morement Memorial Fund AMMF, Essex, UK
Correspondence to: Kate L Mandeville, MBBS, Centre for Infectious Diseases Epidemiology, Department of Primary Care and Population Sciences, University College London, Hampstead Campus, Royal Free Hospital, Rowland Hill Street, London NW3 2PF, United Kingdom. kate.mandeville@doctors.org.uk
Telephone: +44-20-78302239 Fax: +44-20-77941224
Received: February 3, 2009 Revised: April 21, 2009
Accepted: April 28, 2009
Published online: June 21, 2009

Abstract

Developing countries shoulder a considerable burden of gastroenterological disease. Infectious diseases in particular cause enormous morbidity and mortality. Diseases which afflict both western and developing countries are often seen in more florid forms in poorer countries. Innovative techniques continuously improve and update gastroenterological practice. However, advances in diagnosis and treatment which are commonplace in the West, have yet to reach many developing countries. Clinical guidelines, based on these advances and collated in resource-rich environments, lose their relevance outside these settings. In this two-part review, we first highlight the global burden of gastroenterological disease in three major areas: diarrhoeal diseases, hepatitis B, and *Helicobacter pylori*. Recent progress in their management is explored, with consideration of future solutions. The second part of the review focuses on the delivery of clinical services in developing countries. Inadequate numbers of healthcare workers hamper efforts to combat gastroenterological disease. Reasons for this shortage are examined, along with possibilities for increased specialist training. Endoscopy services, the mainstay of gastroenterology in the West, are in their infancy in many developing countries. The challenges faced by those setting up a service are illustrated by the example of a Nigerian endoscopy unit. Finally, we highlight the limited scope of many clinical guidelines produced in western countries. Guidelines which take account of resource limitations in the form of "cascades" are advocated in order to make these guidelines truly global. Recognition of the different working conditions facing practitioners worldwide is an important step towards narrowing the gap between gastroenterology in rich and poor countries.

Key words: *Helicobacter pylori*; Developing countries; Gastrointestinal diseases; Health care delivery; Practice guidelines

Peer reviewer: Roger Jones, Professor, Department of General Practice and Primary Care, King's College London, 5 Lambeth Walk, London SE11 6SP, United Kingdom

Mandeville KL, Krabshuis J, Ladep NG, Mulder CJJ, Quigley EMM, Khan SA. Gastroenterology in developing countries: Issues and advances. *World J Gastroenterol* 2009; 15(23): 2839-2854 Available from: URL: http://www.wjgnet.com/1007-9327/15/2839.asp DOI: http://dx.doi.org/10.3748/wjg.15.2839

INTRODUCTION

Despite political rhetoric, foreign aid, and increased global wealth, the disparity between the developing and developed world is more evident than ever before.

Recently, the economic successes of China and India have lessened poverty for millions of people. Nevertheless, these countries aside, international inequality in income has continued to rise over the past two decades[1].

Nowhere is this inequality clearer than in the arena of health. A child born in Angola in 2006 has a 26% chance of dying before its fifth birthday. In the UK, that risk is 0.6%. In 2006, the life expectancy for a woman from the United States of America was 80 years. If she was instead living in Zambia, her life expectancy declines to just 43 years[2].

In this review, we examine some of the main challenges in developing countries, and discuss potential and existing solutions. Our definition of developing countries is that set out by the International Monetary Fund; "countries with low levels of output, living standards, and technology; per capita GDPs are generally below $5000 and often less than $1500"[3]. The converse, developed countries, will be referred to as "western countries" for clarity. In common practice, these include most of Europe, North America, Japan and Australasia[3] (Figure 1).

The focus of the review will be on gastroenterological problems which head the global disease burden. Although challenges such as war, inadequate water and sanitation, and economic failure all undoubtedly impact on global health, it is beyond the scope of this article to discuss these factors in detail. Moreover, whilst problems such as unstable governments, sectarian violence, and environmental catastrophe undeniably compound health issues, they are by no means confined to developing countries.

In the first part of this review, we will focus on three significant areas of gastroenterological disease which highlight particular problems in developing countries: diarrhoea, hepatitis B and *Helicobacter pylori* (*H pylori*).

The second part of the review will consider the implementation of clinical services in developing nations, encompassing the health workforce, endoscopy services, and the relevance of resource-blind guidelines.

GASTROENTEROLOGICAL DISEASE BURDEN OF DEVELOPING COUNTRIES

Diarrhoeal diseases

The global burden of diarrhoeal diseases outweighs any of the more complex diseases seen in gastroenterology clinics. Every year, there are an estimated 1.5 billion episodes of diarrhoea worldwide[4]. These episodes result in the deaths of approximately 2.2 million people, mostly children in developing countries[4]. This mortality rate has improved from the early 1980s, when diarrhoea is estimated to have caused 4.5 million deaths in children alone[5]. However, it is still the third leading cause of death in under-5 years old, after neonatal causes and pneumonias[6].

Developing countries bear the brunt of this burden. Diarrhoea causes 17.9% of deaths in low-income countries compared to 1.6% in high income countries[6]. Most of these cases are due to the lack of safe water, sanitation and hygiene. Only 34% of people in low-income countries have access to adequate sanitation[6]. As mortality rates from diarrhoea are now so low in western countries, the scale of disease is often expressed in terms of financial costs instead: hospitalisation rates and doctors' consultation time[7]. However, these can be overused resources in the West, and are thus poor comparison measures between countries.

Figure 1 Distribution of countries as per International Monetary Fund (IMF) definitions of economic development (IMF statistical database[3]: reproduced with kind permission of IMF).

Diarrhoeal diseases are caused by a wide variety of pathogens. In 1991, the World Health Organization (WHO) performed a case-control study of the aetiology of diarrhoea in children under 36 mo of age, in five countries: China, India, Mexico, Myanmar and Pakistan. The pathogens most strongly associated with disease were rotavirus, Shigella species and enterotoxigenic *Escherichia coli*[8]. These enteric pathogens, with cholera and typhoid fever, have been identified as the highest priorities for vaccination development by WHO[9].

Diarrhoeal episodes are usually acute and self-limiting. However, they can cause fluid and electrolyte loss from the small intestine so severe that it results in death from dehydration. In some cases, diarrhoea can become persistent: usually defined as lasting at least 14 d[10]. There is evidence that persistent diarrhoea in children can lead to malnutrition[11,12], growth stunting[13,14], and effects on cognitive function[15,16]. A Brazilian study found that children with persistent diarrhoea in the first 2 years of life scored significantly lower on intelligence tests at age 6-10 years, even when controlling for maternal education and helminthic infection[16].

In the late 1980s, oral rehydration therapy (ORT) transformed the management of acute diarrhoea. Physiological studies conducted during the 1950s and 1960s identified the co-transport of sodium and glucose in the small intestine[17-19], which were then harnessed into the oral rehydration solution (ORS) developed at the International Centre for Diarrhoeal Diseases Research in Bangladesh in 1968[20]. WHO adopted and started distribution of a standard ORS in 1975, and set up the WHO Programme for Diarrhoeal Control in 1979[21].

ORT has been heralded as one of the most important therapeutic advances of the past century and has undoubtedly contributed towards the reduction in global child mortality rates described above[22,23] (Figure 2). However it has not reduced the morbidity associated with diarrhoea. Neither stool volume nor

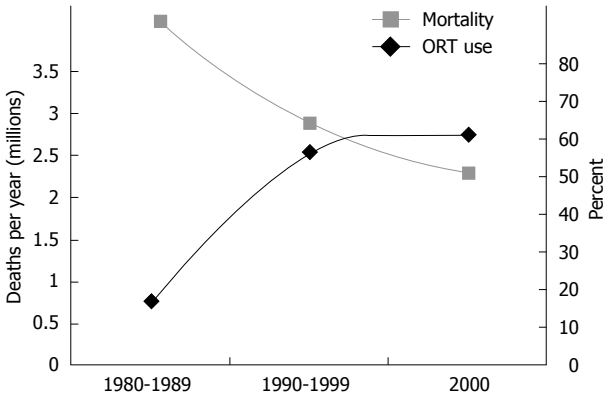

Figure 2 Association between coverage of oral rehydration therapy (ORT) use and mortality rates from diarrhoea in selected countries (from Podewils *et al*[23], 2004. Reproduced with kind permission of Elsevier).

duration of illness are significantly reduced with ORT use, and there may even be a paradoxical increase in stool volume. Therefore, further research has been performed on modifying ORT formulations, including the development of amino acid-containing, starch-based, and reduced sodium solutions. Glutamine, an amino acid, stimulates sodium absorption in experimental models of cholera and rotavirus diarrhoea[24-27]. However, there is conflicting evidence on the efficacy of glutamine containing ORT[20,28], and it is not recommended over WHO ORS at present. The rationale for starch-based solutions (either rice or cereals) is that the polysaccharides would provide more glucose at the intestinal mucosa without the large osmotic load of glucose-based formulations. In addition, they provide nutrition early in the illness. Cereal-based formulations have been shown to reduce total fluid requirements and duration of illness, and are recommended over standard ORS in patients with cholera (but not for non-cholera diarrhoea)[28]. Although cereal-based ORT should be more accessible in rural locations, one study showed that mothers found it more time-consuming to prepare and used standard ORT in preference[29]. In 2003, WHO modified its ORS formulation to contain a reduced amount of sodium and glucose. This hypotonic solution has been associated with less vomiting, decreased stool volume, and reduced need for intravenous fluids[30], and has been recommended for patients with non-cholera diarrhoea. However, concerns have been raised that exclusion of cholera can be difficult in under-resourced areas and use of this formula will lead to hyponatremia in these patients[31]. Clearly, there is still research to be done into the definitive formulation of ORT.

Despite the efficacy of ORT, uptake in developing countries can be variable[32,33]. Difficulties include remembering the correct quantities of ingredients involved in preparing an ORT and high levels of illiteracy[34-36]. Continued effort is required to provide ongoing education at a community level in order to bring about long-term changes[37-40].

More recently, it has been shown that zinc deficiency complicates a significant proportion of diarrhoeal cases[41]. Zinc is not stored in the body, and may be lost from the intestine during diarrhoea[42]. It has a role in immune function[43], however the physiological mechanism linking zinc deficiency with diarrhoea has not yet been elucidated. Several meta-analyses have shown that zinc replacement in acute and persistent diarrhoea reduces both the duration and severity of diarrhoea[43,44], and short (14 d) courses prevent further diarrhoea for 2-3 mo[45]. The WHO has recently recommended that zinc supplementation should be given to all children with acute diarrhoea persisting for at least 14 d[29]. Zinc supplementation has also been shown to significantly reduce the duration of lower respiratory infections[46], the second largest cause of child mortality worldwide.

Universal clean water, hygiene, and sanitation would be the ultimate solution to the global burden of diarrhoea, however in their continued absence, considerable interest has been shown in a more immediate intervention to prevent diarrhoea: vaccination. Of all the pathogens mentioned above, rotavirus is the leading cause of severe diarrhoea in children worldwide. By age 5 years, virtually all children will have been infected by rotavirus, and one in 293 children will have died from it. More than 80% of deaths from rotavirus infection occur in developing countries[47,48]. It also causes a significant financial burden in western countries. Each year in the United States, there are more than 400 000 consultations and up to 70 000 hospital admissions due to rotavirus[49]. Therefore, there has been substantial investment in the development of vaccines against rotavirus infections, both for western and developing countries.

In 2000 and 2001, China introduced a monovalent lamb-derived live attenuated oral vaccine[50]. However, the efficacy of this vaccine is not known, as it was not tested against placebo in the final stages. The focus of research in other countries has been on developing a vaccine against multiple rotavirus serotypes, in order to provide heterotypic protection[48]. The first multivalent live oral reassortant vaccine developed was RotaShield, which was highly effective in field trials in the United States, Finland and Venezuela[51-54]. It was included in the USA immunisation programme in 1998[55], however several cases of intussusception were reported, and the vaccine was subsequently withdrawn[56]. This risk was estimated to be only one per 10 000 vaccinated infants[57], however trials in Ghana, Bangladesh, and India were also stopped at that time, and it was thus not possible to do a risk-benefit analysis for developing countries[48]. Two further rotavirus vaccines have since come onto the market; Rotateq, a human-bovine live-attenuated oral vaccine, and Rotarix, a human live-attenuated oral vaccine. Trials in medium and high-income countries have produced good efficacy results for both these vaccines[58,59], however more trials are needed in developing countries. A large commitment to funding from donor countries will also be required to further reduce global child mortality from diarrhoea.

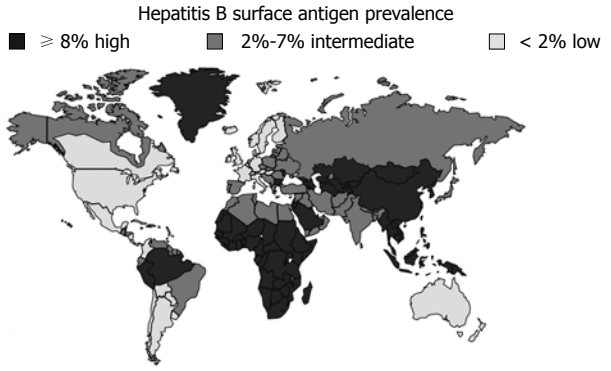

Figure 3 Geographical distribution of the prevalence of chronic hepatitis B virus infection, 2002 (from Mast *et al*[67], 2004. Reproduced with kind permission of Elsevier).

Hepatitis B virus (HBV)

Hepatitis B is the foremost hepatological health problem in the developing world. Up to two billion people worldwide have serological evidence of past or present HBV infection, and 360 million have chronic infection[60,61]. Through its long-term sequelae of liver cirrhosis and hepatocellular carcinoma (HCC), HBV causes 500 000-700 000 deaths each year[60] and is an accessible target for cancer prevention on a massive scale. Although hepatitis C virus (HCV) is increasing in importance, particularly in the western world, HBV is still estimated to account for 50%-55% of HCC worldwide compared to 25%-30% for HCV[62-64].

HBV varies in its prevalence worldwide. Countries can be divided by their level of endemicity, which is based on the percentage of the general population that is seropositive for HBsAg (chronic carriers). Countries with high endemicity have more than 7% seropositivity levels, intermediate 2%-7%, low 0.5%-2% and very low endemicity countries have < 0.5% seropositivity[65,66] (Figure 3[67]). Developing nations make up the bulk of high endemicity countries, including much of sub-Saharan Africa and South East Asia. Notable pre-vaccination examples include the Gambia, where 36% of children were chronic carriers[68], and Taiwan, with 15%-20% chronic carriage in the general population[69-71].

Of particular relevance to developing countries, the likelihood of acquiring chronic HBV infection depends on the age of acquisition of the virus[60,72,73]. For children under 1 year, the risk of chronic infection is 90%. For 1-5 years old, the risk is 30% and for children older than 5 years and adults, the risk decreases to only 6%. This feature accounts for most of the disparity in prevalence outlined above.

The main modes of transmission also vary between countries of high and low endemicity. In high endemicity countries, and in stark contrast to the West, perinatal and horizontal transmission (exposure from close household contacts or play with other children) are the dominant modes[66,74]. In these countries, 70%-90% of the population show serological evidence of previous or current HBV infection. In lower endemicity countries, HBV transmission is mainly limited to high risk groups, such as intravenous drug users and healthcare workers, or is acquired sexually. Although not the main transmission mode, healthcare-acquired infections can assume greater importance in developing countries due to lack of resources for disposable equipment and sterilisation, or lack of awareness of infection control practices[65,75]. However, blood products in most parts of the world are now screened for HBsAg[76].

Chronic infection is responsible for the main burden of disease associated with HBV. Approximately 20% of chronic carriers will die prematurely from cirrhosis leading to liver failure or HCC[77]. Although therapies are available which can suppress HBV replication or modulate the immune reaction, these are expensive and not widely available in much of the developing world. There is currently no therapy which results in virus eradication.

Therefore, the emergence of a plasma-derived vaccine against HBV in the early 1980s was a significant event. This was the world's first cancer prevention vaccine and the first vaccine to prevent a sexually transmitted disease, both functions now echoed by the recently licensed human papillomavirus vaccines. Most current vaccines are produced by recombinant technology[65], and the vaccine prevents HBV infection in 90%-100% of people who produce sufficient antibody responses[78]. It is also highly effective as post-exposure prophylaxis in cases of possible perinatal transmission, even where HBV immunoglobulin co-administration is not possible[79]. Current consensus is that booster doses are not necessary to maintain immunity[60]. Finally, although susceptible to freezing, present vaccines are heat stable, a great advantage in developing countries where access to cold storage facilities is often difficult[60].

In 1992, WHO's Global Advisory Group of the Expanded Programme on Immunization recommended that all highly endemic countries included hepatitis B vaccination into their national childhood immunisation programs by 1995, and all other countries by 1997[80,81]. As of 2006, more than 160 countries had implemented universal hepatitis B vaccination[82]. Several western countries with very low endemicity, such as the United Kingdom, have chosen to pursue a policy of targeted vaccination of high-risk groups rather than universal vaccination[83].

In countries which implemented universal childhood vaccination early on, such as Taiwan, the Gambia, and Malaysia, HBV vaccination was found to be very effective, both in terms of disease prevention and health costs[66,84]. The ultimate goal of these programmes is to prevent the long-term consequences of cirrhosis and HCC, therefore it will be some years before a complete evaluation can be carried out on the first vaccinated cohorts. However, indicators such as HBV seroprevalence and hospital records of acute HBV infections, provide early evidence of their successful impact.

In Malaysia, where universal vaccination was introduced in 1990, HBsAg seroprevalence among children aged 7-12 years decreased from 1.6% in 1997 to

0.3% in 2003[85]. In the Gambia, where HCC is the most common tumour in men[86], vaccination was introduced progressively between 1986 and 1990. Childhood HBsAg seroprevalence has since decreased from 10% in 1986 to 0.6% in 1991[87-89]. Similar declines have been shown in Senegal, China, Indonesia, and Thailand[90].

The best example of the effectiveness of a HBV vaccination programme is probably Taiwan, which had very high levels of chronic carriage in the pre-vaccination era[91]. Over 90% of the population under the age of 40 years had been infected by HBV[92]. Universal infant vaccination was introduced in Taiwan in 1984, one of the first regions to do so[91,93]. 15 years after implementation, HBsAg seroprevalence amongst children 1-15 years decreased from 9.8% in 1984 to 0.7%[94]. In addition, the incidence of fulminant hepatitis amongst infants also decreased. The average mortality from fulminant hepatitis in infants between 1975 and 1984 (pre-vaccination) and from 1985-1998 (post-vaccination) was 5.36 and 1.71 per 100 000 infants, respectively[95].

These evaluations show that HBV can be effectively prevented through a universal vaccination programme. As humans are the only known natural host of the virus, it is feasible that vaccination could eradicate HBV from the world. The major obstacle to global coverage of HBV vaccination is funding. Although the cost of monovalent HBV vaccines had decreased from approximately US $3.00 per dose in 1990 to US $0.30 per dose in 2001[96]. the cost is still higher than the other vaccines included in the extended programme on immunization (e.g. measles, oral polio) which cost between US $0.06 to 0.10 per dose. Several manufacturers have produced combination vaccines containing hepatitis B antigen which allow the addition of hepatitis B vaccine into existing childhood immunisation programmes, however again these are expensive and beyond the capacity of many developing countries.

The Global Alliance for Vaccines and Immunization (GAVI) was founded in 1999 to address this funding gap. GAVI is a consortium between WHO, the World Bank, UNICEF, the Bill and Melinda Gates Foundation, governments of both developing and developed nations, and the vaccine industry[97]. By 2007, it had provided funding for 67 countries out of 69 eligible for support towards the introduction of HBV vaccination programmes[98]. As a result, global three dose vaccine coverage has nearly doubled since 1999 (Figure 4[82]). However, the millions currently infected with HBV in the developing world carry an impending disease burden that will be substantial in the near future.

H pylori infection

It is estimated that 50% of the world's population is infected by *H pylori*[99]. Although most infections are not associated with clinical disease, a significant proportion will go on to develop some of the commonest problems in gastroenterology: gastritis, peptic ulcer disease, and gastric cancer[100-103]. Although less than one percent of infected persons will develop gastric cancer, this is the fourth most common malignancy in the world[104]. It is

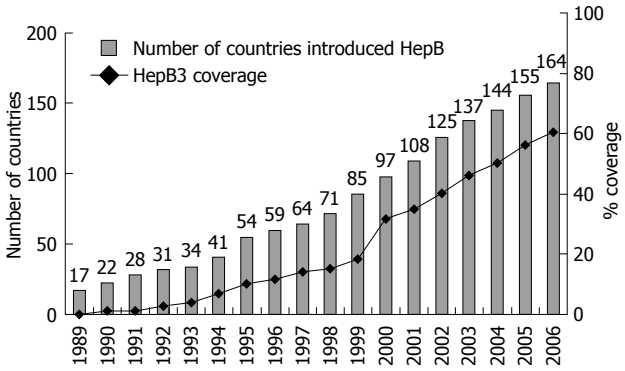

Figure 4 Graph showing number of countries who have introduced hepatitis B vaccination programme and global infant three dose vaccine coverage ("HepB3") (reproduced with kind permission of World Health Organisation).

Table 1 Estimated *H pylori* infection prevalence globally (reproduced with kind permission of World Gastroenterology Organisation)

Country/region	Estimated prevalence of *H pylori* infection (%)
Mexico, Central/South America	70-90
Africa	70-90
Asia	50-80
Eastern Europe	70
Western Europe	50-30
United States and Canada	30
Australia	20

these strong disease associations which establish *H pylori* infection as a leading gastroenterological public health problem.

There are distinct differences in the pattern of *H pylori* infection between developing and western countries. The prevalence of infection in the West has been declining for some years, however it is still very high in developing countries[99], with the majority of the global burden of infection found here (Table 1[105]). This is not surprising, given that risk factors for the infection include low socio-economic status, crowded living conditions, several children sleeping in one bed, large number of siblings and unclean water-all conditions common in the developing world[106-110]. Low education levels have also been positively associated with *H pylori* infection in several studies[111-113]. Prevalence levels in developing countries are therefore associated with the circumstances induced by poverty-and are unlikely to follow the decreasing trend of the western world without alleviation of this factor.

Another marked difference between *H pylori* infection in developing and western countries is the age at acquisition of the infection. Individuals tend to be infected much younger in developing countries than in western countries. In many developing countries, the prevalence of infection exceeds 50% by 5 years of age[114]. In a study of Bangladeshi children, the prevalence of *H pylori* infection was 42% by 2 years of age. Another study of Gambian children using the

diagnostic ^{13}C-urea breath test (UBT) found prevalence levels of over 75% in the second year of life[115] (although very young ages may produce false positive results in the UBT[116]). Comparative studies in western countries show prevalences ranging from 6% in Finland[117], 11% in Scotland[118], 13% in Germany[119], and 23% in Italy[120,121].

Epidemiological studies support person-to-person transmission, which is likely to be *via* faecal-oral and oral-oral routes[122]. The oral-oral route is supported by the finding that pre-mastication of food (the chewing of food by mothers before feeding their babies) is associated with increased prevalence of *H pylori* in infants[106,123,124]. Pre-mastication of food is a common practice in both South-East Asia and Africa. Water sources have also been implicated as a potential mode of transmission, possibly through faecal contamination. An early Peruvian study showed that children living in households with a municipal water supply had a markedly higher risk of *H pylori* infection compared to those who had access to well water[125]. This was supported by findings from a study in Bolivia which found that children living in families using containers which prevented direct contact with this drinking water were significantly less likely to have *H pylori* infection compared to families without this container[126]. Iatrogenic transmission through contaminated endoscopes has been documented both in western and developing countries[127-129], and may be a particular problem in those countries where lack of resources hinders full disinfection procedures[130].

Worldwide, 90% of duodenal ulcers and up to 70% of gastric ulcers are associated with *H pylori* infection[100]. However, peptic ulcer disease is more likely to be reported in western countries, whereas gastric carcinoma is the more common disease association in developing countries[131] In 1994, the International Agency for Research into Cancer designated *H pylori* as a Class I Carcinogen[132].

It is hypothesized that the global variance in disease presentation is related to the age of acquisition of *H pylori* infection[101,122,133]. Infections acquired early in childhood, as in most developing countries, may cause persistent chronic low-grade inflammation which is linked with gastric cancer. Conversely, infections acquired later in life or in adulthood are associated with a more acute inflammatory response and thus ulcer disease.

However, differing incidence rates of gastric cancer globally has led to the description of the "African enigma": that despite a high prevalence of *H pylori* infection, this region has a relatively low incidence of gastric cancer[114,134]. However, this description has been disputed as the average life expectancy on the continent is low (51 years in 2006)[6]. Therefore, individuals may die of other causes before an age at which gastric cancer would become apparent. Indeed, a recent review by Agha *et al*[135] of endoscopy studies carried out on the continent found that *H pylori*-associated peptic ulcers and gastric cancer occurred at similar rates to Western levels.

H pylori infection can have significant sequelae in children in developing countries in addition to the long-term effects of chronic inflammation. Acute *H pylori* infection induces hypochlorhydria, which can be persistent[136-138]. Hypochlorhydria is associated with an increased risk of diarrhoeal diseases, as the gastric acid barrier is effective against many enteric pathogens[139,140]. Therefore, children infected with *H pylori* may be more likely to suffer from diarrhoeal diseases, both acute and persistent[140-143]. In fact, similar findings of malnutrition and decreased growth to those described above for diarrhoeal diseases have been shown in children infected with *H pylori*[142,144-146].

Spontaneous remission of *H pylori* is rare. For symptomatic infections, eradication is usually achieved by a course of antibiotics (typically clarithromycin, amoxicillin or metronidazole) combined with a proton-pump inhibitor[147]. There are other regional guidelines which recommend specific combinations, some of which are directed towards cost issues[148,149]. However, low-cost options may not be as effective as more expensive regimes and may necessitate repeat treatment, leading to higher costs overall[105]. In addition, although eradication may be achievable in western countries with a 7 d regime, treatments of 14 d may be required in developing countries. A study from Brazil showed eradication rates of only 50% if therapy was less than 10 d[150]. Subsequently, a meta-analysis showed a 12% higher eradication rate for 14 d *versus* 7 d regimes[151]. This must be balanced against the likelihood of patient compliance to a complex regime of drugs for 2 wk.

Unfortunately, eradication has been increasingly affected by antibiotic resistance levels worldwide. Many antibiotics are available "over the counter" in developing countries, i.e. not subject to prescription from a doctor. Metronidazole is also used to treat common enteric infections such as amoebiasis and giardiasis: often empirically. Metronidazole resistance is an increasing problem worldwide, although may not affect eradication as much as clarithromycin resistance[114,147]. Antibiotic resistance is the main reason for treatment failure. If sensitivity testing is available, this should guide choices for local first-choice and rescue therapy[105,147].

Re-infection rates after eradication can be as low as 1% in western countries[114]. Given the much greater prevalence of *H pylori* infection in developing countries, it is not surprising that re-infection rates there are also markedly higher. Studies from Chile and Bangladesh have found re-infection rates of around 13%[152,153]. It was difficult, however, to distinguish re-infection from recrudescence in these studies[114].

These issues highlight the need for a vaccine against *H pylori*. As for HBV, there is a need for both preventative and therapeutic vaccines, with the preventative vaccine used primarily on young children in high prevalence areas. Rupnow *et al*[154] modelled the population impact of a prophylactic vaccine. For a typical developing country, they found that with continuous vaccination, *H pylori*-attributable gastric cancer would decrease from 31.8 per 100 000 to 5.8 per 100 000 by 2100. Unfortunately, the current status of *H pylori* vaccines is disappointing. A number of trials have been conducted examining the safety and immunogenicity of various

formulations including recombinant urease, killed whole cells, and live vectors expressing *H pylori* antigens[155]. However, the vast majority of these showed low immunogenicity. Further research is needed to elucidate the mechanism of immune protection and the role of adjuvants.

In conclusion, *H pylori* infection is recognised as a significant public health problem in developing countries. Both antibiotic resistance and re-infection rates threaten the efficacy of existing eradication therapy. Definitive treatment in the form of both prophylactic and therapeutic vaccines is urgently needed in order to alleviate the burden of disease associated with this bacterium.

DELIVERY OF CLINICAL SERVICES IN DEVELOPING COUNTRIES

The first part of this review described three major gastroenterological diseases in the developing world: diarrhoeal disease, hepatitis B, and *H pylori*. Given this burden of gastroenterological disease, specialists in gastroenterology and hepatology are particularly important in developing countries. However, as in most specialities, there is a shortage of trainees.

Mass education and vaccination programmes against diarrhoeal infections, HBV, and potentially *H pylori*, all require immense resources for delivery. Dissemination of the ORT message requires health workers trained in education and prepared to work in remote areas. The consequences of HBV and *H pylori* infection are optimally treated by specialist referral and endoscopy services. However, one of the most limiting factors in the delivery of clinical services in developing countries is the severe lack of trained healthcare personnel.

Health workers

WHO estimates that there is a global shortage of 4.3 million healthcare workers. Africa alone needs an estimated 1.5 million more health workers in order to provide just basic health services[156]. For many years, the strengthening of national health systems and training of personnel have not been included as part of international aid programmes[157]. Staff have been mainly trained intensively for a particular programme's focus, with little integration into a comprehensive national system of health workers.

The result is an uneven distribution of health workers, inverse to the world's health needs. The Americas, including Canada and the United States, have 10% of the global burden of disease, yet almost 37% of the world's health workers. Sub-Saharan Africa, conversely, has more than 24% of the disease burden, yet has only 3% of health workers and less than 1% of the world's financial resources[156]. Ethiopia, for example, has two doctors per 100 000 population. The UK has more than 230 per 100 000[2] (Figure 5[156]). Fifty-seven countries are estimated to have critical shortages of health workers: 36 in sub-Saharan Africa[156].

Figure 5 Geographical distribution of countries with a critical shortage of health service providers (doctors, nurses and midwives) (reproduced with kind permission of World Health Organisation).

There is also a great imbalance within countries. Many hospitals and medical centres are centred in urban areas, whilst populations of many developing countries are still predominantly rural. In both developing and western countries, highly skilled workers can resist rural postings, as there is a higher quality of life and opportunity for money in the urban areas[157,158]. In Malawi, 85% of the population live in rural areas[2]. However, out of Malawi's 156 public sector doctors, 81 are working in central hospitals[159,160]. This leaves many districts without any doctors at all.

There is a general perception that economic migration by skilled health professionals is the main cause of the global shortage. There has been debate in western countries over the ethics of wealthy, more developed countries with a relative density of health workers accepting highly skilled medical migrants from countries with a severe need and valuable investment in their initial training[161]. It is estimated that the UK has saved some £65 million in training costs between 1998 and 2002 by recruiting Ghanaian doctors[162], whilst Ghana has lost £35 million of its training investment to the UK. However, although migration certainly plays a part, it is not the major factor. If Ethiopia had all the doctors it had trained over the last 30 years, there would still only be approximately ten doctors per 100 000 population[157]. The total number of African-born doctors and nurses working in Organisation for Economic Co-operative and Development countries account for less than 12% of the estimated shortages in Africa[163]. (While migration may not account for overall shortages in medical manpower its effects appear to be especially felt among highly trained specialists. In some developing countries almost 100% of specialists leave the country; most never to return).

There are not enough healthcare professionals being trained in developing countries to sustain the needs of the population. Ethiopia produces about 200 doctors per year for 75 million population[164]. The UK produces 6000 per year for 60 million people. Two thirds of Sub-Saharan African countries have only one medical school, whilst some still have none[165]. This inadequate production of health professionals, combined with the accumulative effects of migration and losses due

to the HIV/AIDS epidemic (44 Malawian nurses died in 1999-44% of the annual number trained)[166] have resulted in the severe shortages seen today.

There are several reasons for this low production rate of health workers. Foremost of these, inevitably, is funding. WHO estimates that it would cost $26.4 billion dollars to train the extra 1.5 million health workers needed for the African region, not including provision for future salary costs[156]. Any extra training or production of healthcare workers would require great motivation by donor agencies and governments to provide sustainable funding. Firstly, there is a high rate of student and teacher attrition. Students often find it difficult to find sufficient funding for tuition fees, even with government subsidies. Students who have received poorer quality secondary education may struggle with a medical course, and there is often little support available. Health worker shortages inevitably affect teacher numbers, with remaining lecturers shouldering an increased workload[157].

Once qualified, new graduates are faced with difficult working conditions. Lack of resources can lead to poor job satisfaction and high levels of HIV seropositivity make practical procedures hazardous[156,158]. There has also been a tendency to focus on pre-service training to produce health workers, compared to postgraduate education. Opportunities for specialisation and career progression are few[167]. For example, in Malawi, only 5% of medical specialists and 8% of paediatric posts are filled[159,160]. Salaries which are not adequate to cover living costs make posts in western countries very appealing. In 2004, a junior doctor in Malawi earned approximately £1900 per annum[160]. Basic annual pay for a pre-registration house officer in the UK is £21 000[168]. Indeed, there may be strong family pressures to take a job overseas and provide valuable remittances for those who remain in the country. Some countries have actively embraced health worker migration as a source of revenue. The Philippines operates a managed migration policy and is now the largest provider of registered nurses working overseas[158]. However, 30 000 nurse posts are currently unfilled in the Philippines[158].

The consequences of all these factors on a medical specialty are amply illustrated by the situation in gastroenterology. The world-wide burden of digestive illness is tremendous; for example, digestive cancers, collectively, are the most common malignant diseases. Furthermore, while infectious diseases, as illustrated in the first section, represent a major and persisting challenge for developing nations, urbanization and westernization now threaten to inflict the gastroenterological problems of the West, such as those related to obesity[169] on these already underserved populations. In addition, in certain developing countries prevalence rates for gastroenterological disorders which are very rare in the West, such as gall bladder and biliary cancer, are high and a public health issue. Yet many African countries possess not a single gastroenterologist. Though some would argue that more basic medical care should be the priority in these countries, the World Gastroenterology Organisation (WGO) would counter that to deprive these countries of the expertise that specialists and sub-specialists can provide is condescending, to say the least. If nothing else, such expertise is needed to assist in health care provision planning. It should come as no surprise, therefore, that the many advances in the field of gastroenterology which have so dramatically advanced patient care and improved mortality and morbidity in the West, have not been evenly bestowed on the world's population; some areas of our planet have barely felt the impact of advances in diagnostics and therapeutics. A very striking example is provided by the failure of the laparoscopic era which has so revolutionized digestive surgery elsewhere to even dawn in many African countries. Lack of resources is certainly a factor but lack of skilled personnel is also contributory. Similarly, in many African countries none save for the most privileged have access to diagnostic services, such as endoscopy and ultrasound, that would be deemed routine elsewhere.

More global attention has been paid to the issue of health worker shortages over the last few years. The World Health Report in 2006[156] was dedicated to the health worker crisis. The Global Workforce Alliance, which is hosted by WHO, was set up later in 2006 in order to collate and implement effective strategies to tackle the shortages. In 2008, the first Global Forum on Human Resources for Health was held in Uganda.

Given the urgency of the problem, there is a consensus that innovative solutions are needed rather than simply increasing the number of medical training places. One approach, which has been successful in several developing countries, has been to shift away from a western-style distribution of health workers. Low- and medium-level workers, such as community workers and nursing auxiliaries, can be more appropriate for the needs of the population rather than dependence on high-level workers such as doctors and nurses[157]. Not only is there a greatly reduced training time for these lower-level workers, but more workers can be produced for the same training investment and salary costs are lower. In addition, these workers do not have internationally recognised qualifications, and therefore are less likely to emigrate[157,158]. Although high-level workers are still needed for supervision, some countries have had great success in achieving basic health coverage with community workers.

In 1994, Pakistan created the Lady Health Worker (LHW) cadre, aiming to train 100 000 female community health workers by 2005[157]. These workers are recommended by their community, usually in rural and urban slum areas, and are trained for 15 mo in the prevention and treatment of common illnesses. An evaluation of the scheme in 2002 found that populations served by a LHW are more likely to adopt antenatal care, receive medical assistance at birth, and use family planning services[170]. In Malawi, there is a high rate of trauma associated with farming and road traffic accidents, but only nine orthopaedic surgeons[171]. Orthopaedic clinical officers (OCO) are specifically

trained over 18 mo to be able to fulfil most of the orthopaedic roles required in rural district hospitals, including the conservative management of fractures and dislocations, and some external fixation methods. Since the programme began in 1985, it has trained 117 OCOs, who now manage an estimated 80% to 90% of the orthopaedic workload in Malawi[171]. Indeed, a reliance on western-style models of health workforces has meant that in sub-Saharan Africa low and mid-level workers make up only 7% of the workforce, compared to around 20% in Brazil and Iran[172].

It has also been commented that western models of medical curricula may not be appropriate for countries with an urgent need for health workers. A 5-year course with a strong focus on basic sciences may be a luxury in developing countries with high levels of communicable diseases and limited resources. The St Paul's Millennium Medical School in Ethiopia was set up by the government as part of its aim to increase the national production of doctors to 1000 per year[157]. Here, the curriculum has been cut down from 5.5 to 3.5 years, with an emphasis on practical skills, in order to better prepare graduates for their 5 years service in rural hospitals (Professor Gordon Williams, Dean of St Paul's, personal communication).

Internationally, there have been several bilateral agreements which aim to promote ethical recruitment in response to criticism of western countries' active recruitment of foreign health workers. The UK-South Africa Memorandum of Understanding was signed in 2003 and aims to decrease the efflux of South African health workers through efforts to offer time-limited placements as alternatives and to promote UK self-sufficiency[173]. Norway is also developing a policy which will invest in health worker development projects, whilst increasing the number of national training places to encourage self-sufficiency[173]. Changes under the new UK career progression scheme Modernising Medical Careers is also likely to have an effect on international recruitment. For all training posts, UK graduates and those from the European Economic Area are prioritised over international medical graduates[174]. Although prompted by efforts to improve NHS stability and secure training places for all UK graduates rather than ethical concerns, this policy is likely to decrease the attractiveness of the UK medical job market abroad.

If health workers increasingly remain in their home country, further training must be made available in order to provide adequate numbers of specialists and promote more advanced skills. For gastroenterology specialist training, the main advocate is the WGO. The current objectives of WGO are enshrined in its mission statement: "to promote, to the general public and health care professionals alike, an awareness of the world wide prevalence and optimal care of digestive disorders through the provision of high quality, accessible and independent education and training", which signals the commitment of WGO to address two challenges: firstly, providing the gastroenterologist of the future with an optimal training and, secondly, and most pertinent to this review, bringing the benefits of digestive health care to those who currently struggle or, indeed, fail to achieve access to it. The primary emphasis of WGO, therefore, is on education and training; these objectives are achieved through three distinctive, though closely inter-related, programmes: Training Centres, Train-the-Trainers, and Global Guidelines (described later in review).

Figure 6 Map showing location of World Gastroenterology Training Centres worldwide (reproduced with kind permission of World Gastroenterology Organisation).

Training Centres most directly address the issue of training specialists in gastroenterology or individuals with additional expertise in gastroenterology to serve previously underserved areas. Each centre represents a direct collaboration between local experts, international faculty and several national and regional societies from Europe and North America to deliver regionally relevant training to those who have limited, or in some cases, no access to such opportunities. Our centres in South Africa, Morocco, Egypt, Bolivia, Pakistan, Thailand, Mexico City and Fiji (Figure 6) provide training of variable duration to several hundred young and aspiring gastroenterologists and digestive surgeons from underserved nations in their region. In some of these instances, such as in the centres in Soweto and Suva, the focus is on providing training opportunities to young doctors from areas where little or no gastroenterological expertise exists (in these cases Sub-Saharan Africa and Oceania, respectively). More recently, WGO has established partnerships with centres in Italy, Chile, Argentina, and Brazil to provide more advanced training opportunities to the young doctor who has already completed basic training.

Activities at these training centres are supported by other linked WGO programmes. Train-the-Trainers courses are uniquely devoted to bringing the very latest in educational techniques to those who will train the gastroenterologists of the future, including those who teach and train at our Training Centres. These networks should be accessible to all who seek to train in our specialty, thereby, ensuring the highest standards of care for those who suffer from digestive disorders through the world.

Clinical services

The limiting effect of the shortage of trained health workers and specialists can be seen in the central clinical service of gastroenterology: endoscopy.

In western countries, endoscopy services have

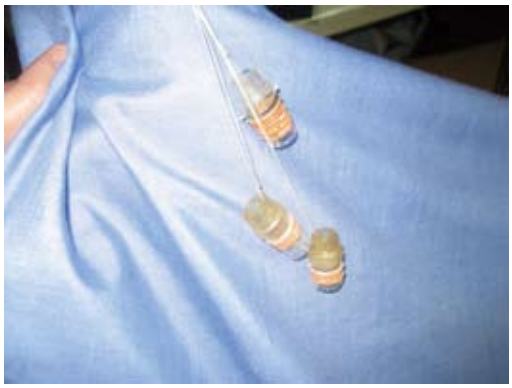

Figure 7 Cut Foley urethral catheter reloaded unto the Opti-vu cap for variceal band ligation at the Endoscopy Unit of Jos University Teaching Hospital, Nigeria.

become such a routine procedure that facilities are readily available, even in some primary care centers. However, in developing countries, services are only available in so-called "centers of excellence" and are rudimentary in most circumstances. They often comprise of direct viewing fibre-optic endoscopes only and are mostly restricted to diagnostic gastroscopies.

This difference is not surprising due to the numerous challenges posed by establishing endoscopy units, including training needs, adequate disinfection facilities and equipment. There is also a lack of awareness amongst most healthcare professionals of the usefulness of therapeutic endoscopy.

While the overall picture looks bleak, there have been some attempts by developing country gastroenterologists to establish endoscopy services. Variceal haemorrhage from portal hypertension is associated with high mortality in most West African countries, due to the lack of endoscopic banding facilities[175]. The Endoscopy Unit at Jos University Teaching Hospital in Nigeria has recently started to perform oesophageal variceal banding and injection sclerotherapy. It costs approximately $300 USD for a single use variceal band ligator, which is vastly prohibitive for most developing country workers. Therefore, the gastroenterologists working at the Endoscopy Unit have modified the normal variceal banding technique by cutting size 14 Foley urethral catheters to size and reloading these on previously used Opti-vu caps (Figure 7). Although not optimal practice, this has reduced the cost to only $30 per session. The modified technique has allowed much greater uptake of the procedure, with improved clinical outcomes. If such interventions become widespread nationally, it has the potential to markedly improve the prognosis in complications of end-stage liver disease, which currently carries a significantly burden in Nigeria.

The training programme for Nigerian gastroenterologists in endoscopic therapies has been bolstered by Royal College of Physicians educational bursaries, which have allowed visits to the UK and reciprocal visits to Nigeria. The Tropical Health and Education Trust also provides training for frontline health workers in the poorest settings, and develops the institutional capacity of local health institutions. This is achieved by focusing on the goals of local health care specialists in individual hospitals, clinics and primary health care projects and offering specialist support and training from UK-based health professionals on a one-to-one basis. Finally, the World Organization of Digestive Endoscopy has set up training centres in Cairo, Egypt and Soweto, South Africa with the aim of improving the management of gastrointestinal disorders in sub Saharan Africa.

It is evident that effective clinical services can exist in developing countries despite being tailored to available resources. Difficulty in adhering to western-defined standards should not necessarily inhibit medical action with benefit to the population.

Guidelines and cascades

Numerous guidelines are produced annually by prestigious medical bodies. The vast majority of these outline "gold standard" practice and are aimed at physicians in resource-rich environments. As such, they are inaccessible and irrelevant for many clinicians in developing countries. As in the case of endoscopy above, many clinicians in developing countries have to "make do" with available resources: in full knowledge that this falls below the gold standard. However, by failing to acknowledge this situation and providing the "next-best option", western guidelines may be preventing the dissemination of knowledge and evidence to its full global audience[176-179].

In order to make guidelines more applicable to differing resource environments, the concept of "cascades" have been developed[177,180]. A cascade is a collection of related diagnostic and treatment options arranged hierarchically in terms of conditions and available resources. Whilst guidelines should continue to summarise best known practice, they could also include alternatives for clinicians with limited funding. These alternatives are usually on the basis of cost, but could also take account of local availability, technology, and infrastructure. Cascades can range from a simple list of options (Table 2) to more complex parallel diagnostic and treatment pathways (Figure 8). In this way, they transform guidelines from being "resource-blind" to "resource-sensitive". Inevitably, cascades are more heavily based on empirical evidence than gold standard options. Research funding is usually spent on trying to improve on best practice-rather than the practicalities of delivery in developing countries. However, with strong involvement from experienced clinicians in developing countries, a consensus is usually reached. More widespread use of cascades in guidelines may also motivate research into the best options for resource-limited services.

Several organizations are now using cascades regularly in their work. The WGO is aiming to include cascades in all of its new guidelines, and has so far published on *H pylori*, acute diarrhoea, treatment of oesophageal varices, colorectal cancer, and hepatitis B[105,181-184]. To aid dissemination, most WGO guidelines are available in at

Table 2 Cascade for treatment of oesophageal varices (reproduced with kind permission of WGO)

Endoscopic band ligation plus octreotide or terlipressin (gold standard)
Endoscopic band ligation alone
Endoscopic sclerotherapy
Balloon tamponade

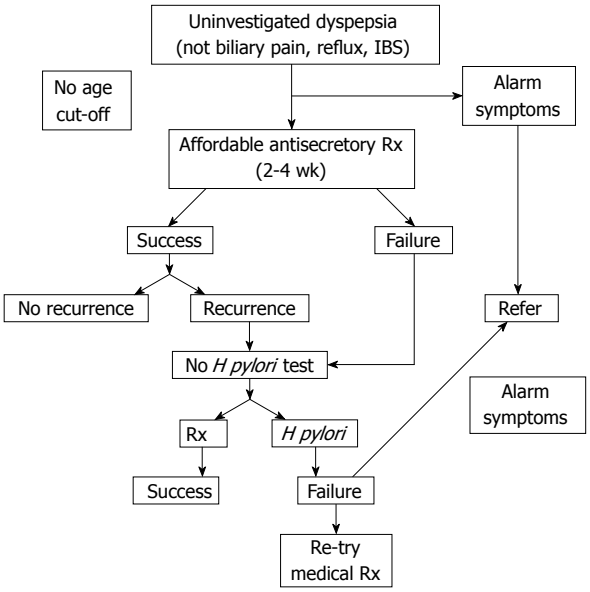

Figure 8 A more complicated cascade for management of dyspepsia in a region with a high prevalence of *H pylori* infection but limited access to endoscopy (reproduced with kind permission of WGO). IBS: Irritable bowel syndrome; Rx: Retreatment; Success: Symptoms resolve; Failure: Symptoms persist.

least four languages; downloads of non-English versions now account for more than 50% of website traffic. The Asian Pacific Consensus on the msanagement of *H pylori* was an early piece of work produced in response to the need for regional guidelines which took account of resource limitations[149]. A programme which uses cascades in response to the urgent need for improved early detection and basic treatment of breast cancer in developing countries is the Breast Health Global Initiative[185,186]. This is an international alliance of health organizations, government agencies, and leading clinicians, which recognises the inflexibility of western-developed screening programmes and aims to produce evidence-based and economically feasible guidelines for medium and low resource regions.

CONCLUSION

The first part of this review has described the disparity between developing and developed countries for three prominent public health problems: diarrhoeal diseases, hepatitis B, and *H pylori*. In this second section, we have considered some of the major obstacles developing countries face in the delivery of gastroenterological services. Increased investment into health workers worldwide is long overdue. Using endoscopy as an example, some of the current difficulties in clinical services implementation in developing countries were highlighted. Finally, guidelines which acknowledge and adapt to the reality of resource limitations would greatly improve information delivery worldwide.

Ultimately, however, perhaps the most important factor needed to improve healthcare in developing countries is the alleviation of poverty. A report in 2001 from the Commission on Macroeconomics and Health[187] showed a negative correlation between the infant mortality rate of a country and the rate of growth of their gross domestic product-the lower the mortality rate, the faster the growth of the economy. An improved economy would allow more investment in sanitation, housing, water quality: all factors which would effectively reduce the prevalence of the diseases discussed above. Until then, however, we must increase recognition of the varying situations facing gastroenterological colleagues worldwide.

REFERENCES

1 **Ferreira FH**, Ravallion M. Global Poverty and Inequality: A review of the evidence. Policy research working paper for the World Bank. Washington: World Bank, 2008
2 **World Health Organization**. World Health Organization Statistical Information System (WHOSIS). Accessed August 4, 2008. Available from: URL: http://www.who.int/whosis/en/
3 International Monetary Fund Statistical Database. Accessed August 5, 2008. Available from: URL: http://www.imfstatistics.org/imf/
4 **World Health Organization (WHO)**. World Health Report 2000 - Health systems: improving performance. Geneva: WHO, 2000. Available from: URL: http://www.who.int/whr/2000/en/
5 **Snyder JD**, Merson MH. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull World Health Organ* 1982; **60**: 605-613
6 **World Health Organization (WHO)**. World Health Statistics 2008. Geneva: WHO, 2008. Available from: URL: http://www.who.int/whosis/whostat/2008/en/index.html
7 **Farthing MJ**. Diarrhoea: a significant worldwide problem. *Int J Antimicrob Agents* 2000; **14**: 65-69
8 **Huilan S**, Zhen LG, Mathan MM, Mathew MM, Olarte J, Espejo R, Khin Maung U, Ghafoor MA, Khan MA, Sami Z. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. *Bull World Health Organ* 1991; **69**: 549-555
9 **Levine MM**. Enteric infections and the vaccines to counter them: future directions. *Vaccine* 2006; **24**: 3865-3873
10 **World Health Organization**. Persistent diarrhoea in children: CCD/DDM/85.1. Diarrhoeal disease control. Geneva: World Health Organization, 1985
11 **Bairagi R**, Chowdhury MK, Kim YJ, Curlin GT, Gray RH. The association between malnutrition and diarrhoea in rural Bangladesh. *Int J Epidemiol* 1987; **16**: 477-481
12 **Mittal SK**. Chronic diarrhea in tropics. *Indian J Pediatr* 1999; **66**: S4-S15
13 **Moore SR**, Lima AA, Conaway MR, Schorling JB, Soares AM, Guerrant RL. Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering. *Int J Epidemiol* 2001; **30**: 1457-1464
14 **Checkley W**, Buckley G, Gilman RH, Assis AM, Guerrant RL, Morris SS, Mølbak K, Valentiner-Branth P, Lanata CF, Black RE. Multi-country analysis of the effects of diarrhoea

on childhood stunting. *Int J Epidemiol* 2008; **37**: 816-830
15 **Guerrant DI**, Moore SR, Lima AA, Patrick PD, Schorling JB, Guerrant RL. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *Am J Trop Med Hyg* 1999; **61**: 707-713
16 **Niehaus MD**, Moore SR, Patrick PD, Derr LL, Lorntz B, Lima AA, Guerrant RL. Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. *Am J Trop Med Hyg* 2002; **66**: 590-593
17 **Fisher RB**, Parsons DS. Glucose movements across the wall of the rat small intestine. *J Physiol* 1953; **119**: 210-223
18 **Riklis E**, Quastel JH. Effects of cations on sugar absorption by isolated surviving guinea pig intestine. *Can J Biochem Physiol* 1958; **36**: 347-362
19 **Crane RK**. Hypothesis for mechanism of intestinal active transport of sugars. *Fed Proc* 1962; **21**: 891-895
20 **Guerrant RL**, Carneiro-Filho BA, Dillingham RA. Cholera, diarrhea, and oral rehydration therapy: triumph and indictment. *Clin Infect Dis* 2003; **37**: 398-405
21 ICDDR, B and ORS: the history of a miracle discovery. *Glimpse* 1994; **16**: 3-4
22 **Victora CG**, Bryce J, Fontaine O, Monasch R. Reducing deaths from diarrhoea through oral rehydration therapy. *Bull World Health Organ* 2000; **78**: 1246-1255
23 **Podewils LJ**, Mintz ED, Nataro JP, Parashar UD. Acute, infectious diarrhea among children in developing countries. *Semin Pediatr Infect Dis* 2004; **15**: 155-168
24 **Lima AA**, Soares AM, Freire Júnior JE, Guerrant RL. Cotransport of sodium with glutamine, alanine and glucose in the isolated rabbit ileal mucosa. *Braz J Med Biol Res* 1992; **25**: 637-640
25 **Silva AC**, Santos-Neto MS, Soares AM, Fonteles MC, Guerrant RL, Lima AA. Efficacy of a glutamine-based oral rehydration solution on the electrolyte and water absorption in a rabbit model of secretory diarrhea induced by cholera toxin. *J Pediatr Gastroenterol Nutr* 1998; **26**: 513-519
26 **Abely M**, Dallet P, Boisset M, Desjeux JF. Effect of cholera toxin on glutamine metabolism and transport in rabbit ileum. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G789-G796
27 **Rhoads JM**, Keku EO, Quinn J, Woosely J, Lecce JG. L-glutamine stimulates jejunal sodium and chloride absorption in pig rotavirus enteritis. *Gastroenterology* 1991; **100**: 683-691
28 **Bhan MK**, Mahalanabis D, Fontaine O, Pierce NF. Clinical trials of improved oral rehydration salt formulations: a review. *Bull World Health Organ* 1994; **72**: 945-955
29 **Chowdhury AM**, Karim F, Rohde JE, Ahmed J, Abed FH. Oral rehydration therapy: a community trial comparing the acceptability of homemade sucrose and cereal-based solutions. *Bull World Health Organ* 1991; **69**: 229-234
30 **World Health Organization**. The treatment of diarrhoea: A manual for physicians and other senior health workers. Geneva: World Health Organization, 2005. Available from: URL: http://whqlibdoc.who.int/publications/2005/9241593180.pdf
31 **Murphy C**, Hahn S, Volmink J. Reduced osmolarity oral rehydration solution for treating cholera. *Cochrane Database Syst Rev* 2004; CD003754
32 **Banwell JG**. Worldwide impact of oral rehydration therapy. *Clin Ther* 1990; **12** Suppl A: 29-36; discussion 36-37
33 **Sarkar K**, Sircar BK, Roy S, Deb BC, Biswas AB, Biswas R. Global review on ORT (oral rehydration therapy) programme with special reference to Indian scene. *Indian J Public Health* 1990; **34**: 48-53
34 **Nyatoti V**, Nyati Z, Mtero S. Knowledge, attitudes and practices of mothers and health workers in relation to the use of sugar and salt solution in Masvingo Province, Zimbabwe. *Cent Afr J Med* 1993; **39**: 95-102
35 **Widarsa KT**, Muninjaya AA. Factors associated with the use of oral rehydration solution among mothers in west Lombok, Indonesia. *J Diarrhoeal Dis Res* 1994; **12**: 261-264
36 **Rao KV**, Mishra VK, Retherford RD. Mass media can help improve treatment of childhood diarrhoea. *Natl Fam Health Surv Bull* 1998; 1-4
37 **Gupta DN**, SenGupta PG, Sircar BK, Mondal S, Sarkar S, Deb BC. Implementation of ORT: some problems encountered in training of health workers during an operational research programme. *Indian J Public Health* 1994; **38**: 69-72
38 **Koul PB**, Murali MV, Gupta P, Sharma PP. Evaluation of social marketing of oral rehydration therapy. *Indian Pediatr* 1991; **28**: 1013-1016
39 **Chowdhury AM**, Karim F, Sarkar SK, Cash RA, Bhuiya A. The status of ORT (oral rehydration therapy) in Bangladesh: how widely is it used? *Health Policy Plan* 1997; **12**: 58-66
40 **Nations MK**, de Sousa MA, Correia LL, da Silva DM. Brazilian popular healers as effective promoters of oral rehydration therapy (ORT) and related child survival strategies. *Bull Pan Am Health Organ* 1988; **22**: 335-354
41 **Bahl R**, Bhandari N, Hambidge KM, Bhan MK. Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. *Am J Clin Nutr* 1998; **68**: 414S-417S
42 **Naveh Y**, Lightman A, Zinder O. Effect of diarrhea on serum zinc concentrations in infants and children. *J Pediatr* 1982; **101**: 730-732
43 **Lukacik M**, Thomas RL, Aranda JV. A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. *Pediatrics* 2008; **121**: 326-336
44 **Bhutta ZA**, Bird SM, Black RE, Brown KH, Gardner JM, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. *Am J Clin Nutr* 2000; **72**: 1516-1522
45 **Bhutta ZA**, Black RE, Brown KH, Gardner JM, Gore S, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. *J Pediatr* 1999; **135**: 689-697
46 **Brooks WA**, Yunus M, Santosham M, Wahed MA, Nahar K, Yeasmin S, Black RE. Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial. *Lancet* 2004; **363**: 1683-1688
47 **Parashar UD**, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003; **9**: 565-572
48 **Dennehy PH**. Rotavirus vaccines: an overview. *Clin Microbiol Rev* 2008; **21**: 198-208
49 **Fischer TK**, Viboud C, Parashar U, Malek M, Steiner C, Glass R, Simonsen L. Hospitalizations and deaths from diarrhea and rotavirus among children <5 years of age in the United States, 1993-2003. *J Infect Dis* 2007; **195**: 1117-1125
50 **Ling-Qiao Z**. A rotavirus vaccine licensed in China. *Health News* 2001; **31**: 1
51 **Rennels MB**, Glass RI, Dennehy PH, Bernstein DI, Pichichero ME, Zito ET, Mack ME, Davidson BL, Kapikian AZ. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines--report of the National Multicenter Trial. United States Rotavirus Vaccine Efficacy Group. *Pediatrics* 1996; **97**: 7-13
52 **Santosham M**, Moulton LH, Reid R, Croll J, Weatherholt R, Ward R, Forro J, Zito E, Mack M, Brenneman G, Davidson BL. Efficacy and safety of high-dose rhesus-human reassortant rotavirus vaccine in Native American populations. *J Pediatr* 1997; **131**: 632-638
53 **Joensuu J**, Koskenniemi E, Pang XL, Vesikari T. Randomised

placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. *Lancet* 1997; **350**: 1205-1209
54 **Pérez-Schael I**, Guntiñas MJ, Pérez M, Pagone V, Rojas AM, González R, Cunto W, Hoshino Y, Kapikian AZ. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. *N Engl J Med* 1997; **337**: 1181-1187
55 Rotavirus vaccine for the prevention of rotavirus gastroenteritis among children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999; **48**: 1-20
56 **Centers for Disease Control and Prevention (CDC)**. Intussusception among recipients of rotavirus vaccine--United States, 1998-1999. *MMWR Morb Mortal Wkly Rep* 1999; **48**: 577-581
57 **Peter G**, Myers MG. Intussusception, rotavirus, and oral vaccines: summary of a workshop. *Pediatrics* 2002; **110**: e67
58 **Vesikari T**, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB, Shinefield HR, Christie CD, Ylitalo S, Itzler RF, Coia ML, Onorato MT, Adeyi BA, Marshall GS, Gothefors L, Campens D, Karvonen A, Watt JP, O'Brien KL, DiNubile MJ, Clark HF, Boslego JW, Offit PA, Heaton PM. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006; **354**: 23-33
59 **Ruiz-Palacios GM**, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, López P, Macías-Parra M, Ortega-Barría E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavía-Ruz N, Salmerón J, Rüttimann R, Tinoco JC, Rubio P, Nuñez E, Guerrero ML, Yarzábal JP, Damaso S, Tornieporth N, Sáez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006; **354**: 11-22
60 **World Health Organization**. Hepatitis B vaccines. *Wkly Epidemiol Rec* 2004; **79**: 255-263
61 **World Health Organization**. Hepatitis B. Fact Sheet 204. Accessed August 4, 2008. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/index.html
62 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16
63 **Poynard T**, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; **362**: 2095-2100
64 **World Health Organization**. World Health Report 1996: Fighting disease, fostering development. Geneva: World Health Organization, 1996. Available from: URL: http://www.who.int/whr/1996/en/index.html
65 **Shepard CW**, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006; **28**: 112-125
66 **Beutels P**. Economic evaluations of hepatitis B immunization: a global review of recent studies (1994-2000). *Health Econ* 2001; **10**: 751-774
67 **Mast E**, Mahoney F, Kane M, Margolis H. Hepatitis B vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. 4th ed. Philadelphia: Saunders, 2004: 299-337
68 **Whittle HC**, Bradley AK, McLauchlan K, Ajdukiewicz AB, Howard CR, Zuckerman AJ, McGregor IA. Hepatitis B virus infection in two Gambian villages. *Lancet* 1983; **1**: 1203-1206
69 **Chen DS**, Sung JL, Lai MY. A seroepidemiologic study of hepatitis B virus infection in Taiwan. *Taiwan Yixuehui Zazhi* 1978; **77**: 908-918
70 **Sung JL**. Hepatitis B virus infection and its sequelae in Taiwan. *Gastroenterol Jpn* 1984; **19**: 363-366
71 **Beasley RP**, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. *Am J Epidemiol* 1977; **105**: 94-98
72 **Hyams KC**. Risks of chronicity following acute hepatitis B virus infection: a review. *Clin Infect Dis* 1995; **20**: 992-1000
73 **McMahon BJ**, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; **151**: 599-603
74 **Francis DP**, Favero MS, Maynard JE. Transmission of hepatitis B virus. *Semin Liver Dis* 1981; **1**: 27-32
75 **Hauri AM**, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. *Int J STD AIDS* 2004; **15**: 7-16
76 **Busch MP**, Kleinman SH, Nemo GJ. Current and emerging infectious risks of blood transfusions. *JAMA* 2003; **289**: 959-962
77 **Kane M**. Global programme for control of hepatitis B infection. *Vaccine* 1995; **13** Suppl 1: S47-S49
78 **André FE**. Overview of a 5-year clinical experience with a yeast-derived hepatitis B vaccine. *Vaccine* 1990; **8** Suppl: S74-S78; discussion S79-S80
79 **Marion SA**, Tomm Pastore M, Pi DW, Mathias RG. Long-term follow-up of hepatitis B vaccine in infants of carrier mothers. *Am J Epidemiol* 1994; **140**: 734-746
80 Expanded programme on immunization. Global Advisory Group--Part I. *Wkly Epidemiol Rec* 1992; **67**: 11-15
81 **World Health Organization**. 2004 global immunization data. Geneva: World Health Organization, 2005. Available from: URL: http://www.who.int/immunization_monitoring/data/GlobalImmunizationData.pdf
82 **World Health Organization**. Immunization surveillance, assessment and monitoring. Accessed July 28, 2008. Available from: URL: http://www.who.int/immunization_monitoring/data/en/
83 **English P**. Should universal hepatitis B immunisation be introduced in the UK? *Arch Dis Child* 2006; **91**: 286-289
84 **Aggarwal R**, Ghoshal UC, Naik SR. Assessment of cost-effectiveness of universal hepatitis B immunization in a low-income country with intermediate endemicity using a Markov model. *J Hepatol* 2003; **38**: 215-222
85 **Ng KP**, Saw TL, Baki A, Rozainah K, Pang KW, Ramanathan M. Impact of the Expanded Program of Immunization against hepatitis B infection in school children in Malaysia. *Med Microbiol Immunol* 2005; **194**: 163-168
86 **Bah E**, Parkin DM, Hall AJ, Jack AD, Whittle H. Cancer in the Gambia: 1988-97. *Br J Cancer* 2001; **84**: 1207-1214
87 **Viviani S**, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, Whittle HC. Hepatitis B vaccination in infancy in The Gambia: protection against carriage at 9 years of age. *Vaccine* 1999; **17**: 2946-2950
88 **Chotard J**, Inskip HM, Hall AJ, Loik F, Mendy M, Whittle H, George MO, Lowe Y. The Gambia Hepatitis Intervention Study: follow-up of a cohort of children vaccinated against hepatitis B. *J Infect Dis* 1992; **166**: 764-768
89 **Fortuin M**, Chotard J, Jack AD, Maine NP, Mendy M, Hall AJ, Inskip HM, George MO, Whittle HC. Efficacy of hepatitis B vaccine in the Gambian expanded programme on immunisation. *Lancet* 1993; **341**: 1129-1131
90 **Kane MA**. Status of hepatitis B immunization programmes in 1998. *Vaccine* 1998; **16** Suppl: S104-S108
91 **Chien YC**, Jan CF, Kuo HS, Chen CJ. Nationwide hepatitis B vaccination program in Taiwan: effectiveness in the 20 years after it was launched. *Epidemiol Rev* 2006; **28**: 126-135
92 **Beasley RP**, Hwang LY, Lin CC, Leu ML, Stevens CE, Szmuness W, Chen KP. Incidence of hepatitis B virus infections in preschool children in Taiwan. *J Infect Dis* 1982; **146**: 198-204
93 **Chan CY**, Lee SD, Lo KJ. Legend of hepatitis B vaccination: the Taiwan experience. *J Gastroenterol Hepatol* 2004; **19**: 121-126
94 **Ni YH**, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, Tsai KS, Chen DS. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med* 2001; **135**: 796-800

95 **Kao JH**, Hsu HM, Shau WY, Chang MH, Chen DS. Universal hepatitis B vaccination and the decreased mortality from fulminant hepatitis in infants in Taiwan. *J Pediatr* 2001; **139**: 349-352

96 **Centers for Disease Control and Prevention (CDC)**. Global progress toward universal childhood hepatitis B vaccination, 2003. *MMWR Morb Mortal Wkly Rep* 2003; **52**: 868-870

97 **Martin JF**, Marshall J. New tendencies and strategies in international immunisation: GAVI and The Vaccine Fund. *Vaccine* 2003; **21**: 587-592

98 **Global Alliance for Vaccines and Immunization**. Performance. Accessed August 5, 2008. Available from: URL: http://www.gavialliance.org/performance

99 **Torres J**, Pérez-Pérez G, Goodman KJ, Atherton JC, Gold BD, Harris PR, la Garza AM, Guarner J, Muñoz O. A comprehensive review of the natural history of Helicobacter pylori infection in children. *Arch Med Res* 2000; **31**: 431-469

100 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186

101 **Blaser MJ**, Chyou PH, Nomura A. Age at establishment of Helicobacter pylori infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. *Cancer Res* 1995; **55**: 562-565

102 **Blaser MJ**. The role of Helicobacter pylori in gastritis and its progression to peptic ulcer disease. *Aliment Pharmacol Ther* 1995; **9** Suppl 1: 27-30

103 **Graham DY**. Benefits from elimination of Helicobacter pylori infection include major reduction in the incidence of peptic ulcer disease, gastric cancer, and primary gastric lymphoma. *Prev Med* 1994; **23**: 712-716

104 **Parkin DM**. Global cancer statistics in the year 2000. *Lancet Oncol* 2001; **2**: 533-543

105 **World Gastroenterology Organisation**. Practice guidelines: Helicobacter pylori in developing countries. 2006. Available from: URL: http://www.worldgastroenterology.org/helicobacter-pylori-in-developing-countries.html

106 **Lindkvist P**, Enquselassie F, Asrat D, Muhe L, Nilsson I, Giesecke J. Risk factors for infection with Helicobacter pylori--a study of children in rural Ethiopia. *Scand J Infect Dis* 1998; **30**: 371-376

107 **Dominici P**, Bellentani S, Di Biase AR, Saccoccio G, Le Rose A, Masutti F, Viola L, Balli F, Tiribelli C, Grilli R, Fusillo M, Grossi E. Familial clustering of Helicobacter pylori infection: population based study. *BMJ* 1999; **319**: 537-540

108 **Nabwera HM**, Nguyen-Van-Tam JS, Logan RF, Logan RP. Prevalence of Helicobacter pylori infection in Kenyan schoolchildren aged 3-15 years and risk factors for infection. *Eur J Gastroenterol Hepatol* 2000; **12**: 483-487

109 **Webb PM**, Knight T, Greaves S, Wilson A, Newell DG, Elder J, Forman D. Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life. *BMJ* 1994; **308**: 750-753

110 **Malaty HM**, Paykov V, Bykova O, Ross A, Graham DP, Anneger JF, Graham DY. Helicobacter pylori and socioeconomic factors in Russia. *Helicobacter* 1996; **1**: 82-87

111 **Torres J**, Leal-Herrera Y, Perez-Perez G, Gomez A, Camorlinga-Ponce M, Cedillo-Rivera R, Tapia-Conyer R, Muñoz O. A community-based seroepidemiologic study of Helicobacter pylori infection in Mexico. *J Infect Dis* 1998; **178**: 1089-1094

112 Epidemiology of, and risk factors for, Helicobacter pylori infection among 3194 asymptomatic subjects in 17 populations. The EUROGAST Study Group. *Gut* 1993; **34**: 1672-1676

113 **Malaty HM**, Evans DG, Evans DJ Jr, Graham DY. Helicobacter pylori in Hispanics: comparison with blacks and whites of similar age and socioeconomic class. *Gastroenterology* 1992; **103**: 813-816

114 **Frenck RW Jr**, Clemens J. Helicobacter in the developing world. *Microbes Infect* 2003; **5**: 705-713

115 **Thomas JE**, Dale A, Harding M, Coward WA, Cole TJ, Weaver LT. Helicobacter pylori colonization in early life. *Pediatr Res* 1999; **45**: 218-223

116 **Kindermann A**, Demmelmair H, Koletzko B, Krauss-Etschmann S, Wiebecke B, Koletzko S. Influence of age on 13C-urea breath test results in children. *J Pediatr Gastroenterol Nutr* 2000; **30**: 85-91

117 **Rehnberg-Laiho L**, Rautelin H, Valle M, Kosunen TU. Persisting Helicobacter antibodies in Finnish children and adolescents between two and twenty years of age. *Pediatr Infect Dis J* 1998; **17**: 796-799

118 **Patel P**, Mendall MA, Khulusi S, Northfield TC, Strachan DP. Helicobacter pylori infection in childhood: risk factors and effect on growth. *BMJ* 1994; **309**: 1119-1123

119 **Rothenbacher D**, Bode G, Berg G, Gommel R, Gonser T, Adler G, Brenner H. Prevalence and determinants of Helicobacter pylori infection in preschool children: a population-based study from Germany. *Int J Epidemiol* 1998; **27**: 135-141

120 **Rothenbacher D**, Brenner H. Burden of Helicobacter pylori and H. pylori-related diseases in developed countries: recent developments and future implications. *Microbes Infect* 2003; **5**: 693-703

121 **Perri F**, Pastore M, Leandro G, Clemente R, Ghoos Y, Peeters M, Annese V, Quitadamo M, Latiano A, Rutgeerts P, Andriulli A. Helicobacter pylori infection and growth delay in older children. *Arch Dis Child* 1997; **77**: 46-49

122 **Brown LM**. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev* 2000; **22**: 283-297

123 **Clemens J**, Albert MJ, Rao M, Huda S, Qadri F, Van Loon FP, Pradhan B, Naficy A, Banik A. Sociodemographic, hygienic and nutritional correlates of Helicobacter pylori infection of young Bangladeshi children. *Pediatr Infect Dis J* 1996; **15**: 1113-1118

124 **Mégraud F**. Transmission of Helicobacter pylori: faecal-oral versus oral-oral route. *Aliment Pharmacol Ther* 1995; **9** Suppl 2: 85-91

125 **Klein PD**, Graham DY, Gaillour A, Opekun AR, Smith EO. Water source as risk factor for Helicobacter pylori infection in Peruvian children. Gastrointestinal Physiology Working Group. *Lancet* 1991; **337**: 1503-1506

126 **Glynn MK**, Friedman CR, Gold BD, Khanna B, Hutwagner L, Iihoshi N, Revollo C, Quick R. Seroincidence of Helicobacter pylori infection in a cohort of rural Bolivian children: acquisition and analysis of possible risk factors. *Clin Infect Dis* 2002; **35**: 1059-1065

127 **Rohr MR**, Castro R, Morais M, Brant CQ, Castelo Filho A, Ferrari Júnior AP. Risk of Helicobacter pylori transmission by upper gastrointestinal endoscopy. *Am J Infect Control* 1998; **26**: 12-15

128 **Katoh M**, Saito D, Noda T, Yoshida S, Oguro Y, Yazaki Y, Sugimura T, Terada M. Helicobacter pylori may be transmitted through gastrofiberscope even after manual Hyamine washing. *Jpn J Cancer Res* 1993; **84**: 117-119

129 **Langenberg W**, Rauws EA, Oudbier JH, Tytgat GN. Patient-to-patient transmission of Campylobacter pylori infection by fiberoptic gastroduodenoscopy and biopsy. *J Infect Dis* 1990; **161**: 507-511

130 **Tytgat GN**. Endoscopic transmission of Helicobacter pylori. *Aliment Pharmacol Ther* 1995; **9** Suppl 2: 105-110

131 **Burstein M**, Monge E, León-Barúa R, Lozano R, Berendson R, Gilman RH, Legua H, Rodriguez C. Low peptic ulcer and high gastric cancer prevalence in a developing country with a high prevalence of infection by Helicobacter pylori. *J Clin Gastroenterol* 1991; **13**: 154-156

132 **International Agency for Research on Cancer**. Schistosomes, liver flukes and Helicobacter pylori. In: IARC monographs on the evaluation of carcinogenic risks to humans. Vol 61. Lyon: International Agency for Research on Cancer, 1994: 177-241

133 **McColl KE**. What remaining questions regarding Helicobacter pylori and associated diseases should be addressed by future research? View from Europe. *Gastroenterology* 1997; **113**: S158-S162

134 **Kuipers EJ**, Meijer GA. Helicobacter pylori gastritis in Africa. *Eur J Gastroenterol Hepatol* 2000; **12**: 601-603
135 **Agha A**, Graham DY. Evidence-based examination of the African enigma in relation to Helicobacter pylori infection. *Scand J Gastroenterol* 2005; **40**: 523-529
136 **McColl KE**, el-Omar E, Gillen D. Interactions between H. pylori infection, gastric acid secretion and anti-secretory therapy. *Br Med Bull* 1998; **54**: 121-138
137 **El-Omar EM**, Oien K, El-Nujumi A, Gillen D, Wirz A, Dahill S, Williams C, Ardill JE, McColl KE. Helicobacter pylori infection and chronic gastric acid hyposecretion. *Gastroenterology* 1997; **113**: 15-24
138 **Iijima K**, Ohara S, Sekine H, Koike T, Kato K, Asaki S, Shimosegawa T, Toyota T. Changes in gastric acid secretion assayed by endoscopic gastrin test before and after Helicobacter pylori eradication. *Gut* 2000; **46**: 20-26
139 **Gilman RH**, Partanen R, Brown KH, Spira WM, Khanam S, Greenberg B, Bloom SR, Ali A. Decreased gastric acid secretion and bacterial colonization of the stomach in severely malnourished Bangladeshi children. *Gastroenterology* 1988; **94**: 1308-1314
140 **Howden CW**, Hunt RH. Relationship between gastric secretion and infection. *Gut* 1987; **28**: 96-107
141 **Windle HJ**, Kelleher D, Crabtree JE. Childhood Helicobacter pylori infection and growth impairment in developing countries: a vicious cycle? *Pediatrics* 2007; **119**: e754-e759
142 **Sullivan PB**, Thomas JE, Wight DG, Neale G, Eastham EJ, Corrah T, Lloyd-Evans N, Greenwood BM. Helicobacter pylori in Gambian children with chronic diarrhoea and malnutrition. *Arch Dis Child* 1990; **65**: 189-191
143 **Passaro DJ**, Taylor DN, Meza R, Cabrera L, Gilman RH, Parsonnet J. Acute Helicobacter pylori infection is followed by an increase in diarrheal disease among Peruvian children. *Pediatrics* 2001; **108**: E87
144 **Bravo LE**, Mera R, Reina JC, Pradilla A, Alzate A, Fontham E, Correa P. Impact of Helicobacter pylori infection on growth of children: a prospective cohort study. *J Pediatr Gastroenterol Nutr* 2003; **37**: 614-619
145 **Demir H**, Saltik IN, Kocak N, Yuce A, Ozen H, Gurakan F. Subnormal growth in children with Helicobacter pylori infection. *Arch Dis Child* 2001; **84**: 89-90
146 **Taşar A**, Kibrisli E, Dallar Y. Seroprevalence of Helicobacter pylori in children with constitutional height retardation. *Turk J Gastroenterol* 2006; **17**: 7-12
147 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
148 **Chinese Society of Gastroenterology, Chinese Medical Association**. Consensus on the management of Helicobacter pylori infection: Tongcheng, Anhui Province, 2003. *Chin J Dig Dis* 2004; **5**: 186-188
149 **Lam SK**, Talley NJ. Report of the 1997 Asia Pacific Consensus Conference on the management of Helicobacter pylori infection. *J Gastroenterol Hepatol* 1998; **13**: 1-12
150 **Kawakami E**, Ogata SK, Portorreal AC, Magni AM, Pardo ML, Patrício FR. Triple therapy with clarithromycin, amoxicillin and omeprazole for Helicobacter pylori eradication in children and adolescents. *Arq Gastroenterol* 2001; **38**: 203-206
151 **Ford A**, Moayyedi P. How can the current strategies for Helicobacter pylori eradication therapy be improved? *Can J Gastroenterol* 2003; **17** Suppl B: 36B-40B
152 **Rollan A**, Giancaspero R, Fuster F, Acevedo C, Figueroa C, Hola K, Schulz M, Duarte I. The long-term reinfection rate and the course of duodenal ulcer disease after eradication of Helicobacter pylori in a developing country. *Am J Gastroenterol* 2000; **95**: 50-56
153 **Hildebrand P**, Bardhan P, Rossi L, Parvin S, Rahman A, Arefin MS, Hasan M, Ahmad MM, Glatz-Krieger K, Terracciano L, Bauerfeind P, Beglinger C, Gyr N, Khan AK. Recrudescence and reinfection with Helicobacter pylori after eradication therapy in Bangladeshi adults. *Gastroenterology* 2001; **121**: 792-798
154 **Rupnow MF**, Shachter RD, Owens DK, Parsonnet J. Quantifying the population impact of a prophylactic Helicobacter pylori vaccine. *Vaccine* 2001; **20**: 879-885
155 **Kabir S**. The current status of Helicobacter pylori vaccines: a review. *Helicobacter* 2007; **12**: 89-102
156 **World Health Organization (WHO)**. World Health Report 2006: working together for health. Geneva: WHO, 2006. Available from: URL: http://www.who.int/whr/2006/whr06_en.pdf
157 **Task Force for Scaling Up Education and Training for Health Workers, Global Health Workforce Alliance**. Scaling up, saving lives. Geneva: Global Health Workforce Alliance, 2008. Available from: URL: http://www.who.int/workforcealliance/documents/Global_Health%20SUMMARY.pdf
158 **Hongoro C**, McPake B. How to bridge the gap in human resources for health. *Lancet* 2004; **364**: 1451-1456
159 **Ministry of Health - Republic of Malawi**. Human Resources in the Health Sector: Towards a Solution. Lilongwe: Ministry of Health, 2004
160 **Record R**, Mohiddin A. An economic perspective on Malawi's medical "brain drain". *Global Health* 2006; **2**: 12
161 **Mills EJ**, Schabas WA, Volmink J, Walker R, Ford N, Katabira E, Anema A, Joffres M, Cahn P, Montaner J. Should active recruitment of health workers from sub-Saharan Africa be viewed as a crime? *Lancet* 2008; **371**: 685-688
162 **Martineau T**, Decker K, Bundred P. "Brain drain" of health professionals: from rhetoric to responsible action. *Health Policy* 2004; **70**: 1-10
163 **Organization for Economic Cooperation and Development (OECD)**. Immigrant health workers in OECD countries in the broader context of highly skilled migration, Part III of International Migration Outlook 2007. Paris: OECD, 2007
164 **Crisp N**, Gawanas B, Sharp I. Training the health workforce: scaling up, saving lives. *Lancet* 2008; **371**: 689-691
165 **Narasimhan V**, Brown H, Pablos-Mendez A, Adams O, Dussault G, Elzinga G, Nordstrom A, Habte D, Jacobs M, Solimano G, Sewankambo N, Wibulpolprasert S, Evans T, Chen L. Responding to the global human resources crisis. *Lancet* 2004; **363**: 1469-1472
166 **World Health Organization (WHO)**. World Health Report 2004: changing history. Geneva: WHO, cited 2008-07-22; 170. Available from: URL: http://www.who.int/whr/2004/en/report04_en.pdf
167 **Awases M**, Gbary A, Nyoni J, Chatora R. Migration of health professionals in six countries: A synthesis report. Brazzaville: World Health Organization Regional Office for Africa, 2004
168 **Department of Health (England)**. Review body on doctors and dentists remuneration: Review for 2008. London: Department of Health, 2007
169 **Hussain Z**, Quigley EMM. Gastrointestinal issues in the assessment and management of the obese patient. *Gastroenterol Hepatol* 2007; **3**: 559-569
170 **Oxford Policy Management**. External evaluation of the National Programme for Family Planning and Primary Health Care. Islamabad: Lady Health Worker Programme, 2002
171 **Mkandawire N**, Ngulube C, Lavy C. Orthopaedic clinical officer program in Malawi: a model for providing orthopaedic care. *Clin Orthop Relat Res* 2008; **466**: 2385-2391
172 **Conway M**, Gupta S, Khajavi K. Addressing Africa's health workforce crisis. The McKinsey Quarterly. 2007. Available from: URL: http://www.mckinseyquarterly.com
173 **Robinson M**, Clark P. Forging solutions to health worker migration. *Lancet* 2008; **371**: 691-693
174 **Modernising Medical Careers (MMC) England**. Recruitment to foundation and speciality training - Proposals for managing applications from medical graduates from outside the European Economic Area. London: Department

of Health, 2008
175 **Ladep NG**, Sule J, Umar SM, Obienu O, Anyanechi C, Okeke EN. Oesophageal variceal band ligation using a saeed six-shooter multiband ligator; experience at Jos University Teaching Hospital, Nigeria: case report. *Niger J Med* 2008; **17**: 110-111
176 **Fried M**, Farthing M, Krabshuis J, Quigley E. Global guidelines: is gastroenterology leading the way? *Lancet* 2006; **368**: 2041-2042
177 **Fried M**, Quigley EM, Hunt RH, Guyatt G, Anderson BO, Bjorkman DJ, Farthing MJ, Fedail SS, Green-Thompson R, Hampton J, Krabshuis J, Laine L, Horton R. Are global guidelines desirable, feasible and necessary? *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 2-3
178 **Fried M**, Quigley EM, Hunt RH, Guyatt G, Anderson BO, Bjorkman DJ, Farthing MJ, Fedail SS, Green-Thompson R, Hampton J, Krabshuis J, Laine L, Horton R. Can global guidelines change health policy? *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 120-121
179 **Fried M**, Quigley EM, Hunt RH, Guyatt G, Anderson BO, Bjorkman DJ, Farthing MJ, Fedail SS, Green-Thompson R, Hampton J, Krabshuis J, Laine L, Horton R. Is an evidence-based approach to creating guidelines always the right one? *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 60-61
180 **Fried M**, Krabshuis J. Can 'Cascades' make guidelines global? *J Eval Clin Pract* 2008; **14**: 874-879
181 **World Gastroenterology Organisation (WGO)**. Practice guidelines: Acute diarrhoea. Munich: WGO, 2008: 28. Available from: URL: http://www.worldgastroenterology.org/acute-diarrhoea-in-adults.html
182 **World Gastroenterology Organisation (WGO)**. Practice guidelines: Colorectal cancer screening. Paris: WGO, 2007: 18. Available from: URL: http://www.worldgastroenterology.org/colorectal-cancer-screening.html
183 **World Gastroenterology Organisation (WGO)**. Practice guideline: Esophageal varices. Munich: WGO, 2008: 17. Available from: URL: http://www.worldgastroenterology.org/treatment-of-esophageal-varices.html
184 **World Gastroenterology Organisation (WGO)**. Practice guideline: Hepatitis B. Munich: WGO, 2008. Available from: URL: http://www.worldgastroenterology.org/hepatitis-b.html
185 **Anderson BO**, Carlson RW. Guidelines for improving breast health care in limited resource countries: the Breast Health Global Initiative. *J Natl Compr Canc Netw* 2007; **5**: 349-356
186 **Yip CH**, Anderson BO. The Breast Health Global Initiative: clinical practice guidelines for management of breast cancer in low- and middle-income countries. *Expert Rev Anticancer Ther* 2007; **7**: 1095-1104
187 **Sachs JD**. Macroeconomics and health: investing in health for economic development. Report of the Commission on Macroeconomics and Health. Geneva: World Health Organization, 2001

S- Editor Li LF **L- Editor** Webster JR **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2855

World J Gastroenterol 2009 June 21; 15(23): 2855-2861
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

CD74 in antigen presentation, inflammation, and cancers of the gastrointestinal tract

Ellen J Beswick, Victor E Reyes

Ellen J Beswick, Department of Pediatrics, University of Texas Medical Branch, Galveston, TX 77555, United States
Victor E Reyes, Department of Pediatrics and Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, United States
Author contributions: Beswick EJ and Reyes VE wrote the paper.
Supported by The National Institutes of Health Grant K22AI068712, the Texas Board of Higher Education, and the John Sealy Memorial Endowment Fund for Biomedical Research
Correspondence to: Dr. Ellen J Beswick, Department of Pediatrics, Children's Hospital, Room 2.300, University of Texas Medical Branch, 301 University Blvd. Galveston, TX 77555. United States. ejbeswic@utmb.edu
Telephone: +1-409-7720423 Fax: +1-409-7721761
Received: February 28, 2009 Revised: April 24, 2009
Accepted: May 3, 2009
Published online: June 21, 2009

Abstract

CD74 is a protein whose initial role in antigen presentation was recognized two decades ago. Recent studies have revealed that it has additional functions as a receptor for macrophage migration inhibitory factor and as a receptor for an important human pathogen, *Helicobacter pylori* (*H pylori*). The role of CD74 as a receptor is important because after binding of migration inhibitory factor or *H pylori*, NF-κB and Erk1/2 activation occurs, along with the induction of proinflammatory cytokine secretion. This review provides an up-to-date account of the functions of CD74 and how it might be involved in inflammation and cancer within the gastrointestinal tract.

Key words: CD74; Invariant chain; Cancer; Inflammation; *Helicobacter pylori*; Gastrointestinal tract

Peer reviewer: Dr. Francesco Costa, Dipartimento di Medicina Interna, U.O. di Gastroenterologia, Università di Pisa, Via Roma, 67-56122 Pisa, Italy

Beswick EJ, Reyes VE. CD74 in antigen presentation, inflammation, and cancers of the gastrointestinal tract. *World J Gastroenterol* 2009; 15(23): 2855-2861 Available from: URL: http://www.wjgnet.com/1007-9327/15/2855.asp DOI: http://dx.doi.org/10.3748/wjg.15.2855

INTRODUCTION

CD74, also known as the invariant chain or Ii, is a non-polymorphic glycoprotein that has diverse immunological functions. The most well-known function of CD74 is regulating the trafficking of class Ⅱ major histocompatibility complex (MHC) proteins in antigen presenting cells. More recently, CD74 expression has been examined in cell types other than antigen presenting cells (APCs), such as epithelial cells[1]. Some studies also suggest that CD74 might be expressed independently of class Ⅱ MHC, indicating additional functions[2]. Various studies have indicated that CD74 is highly expressed in inflammatory disorders and cancers. It also acts as a receptor for macrophage migration inhibitory factor (MIF) and facilitates adhesion of *Helicobacter pylori* (*H pylori*) to gastric epithelial cells (GECs)[3,4]. CD74 expression is increased during *H pylori* infection, chronic inflammatory conditions of the gastrointestinal (GI) tract, and gastric and colon cancers. One critical function it has in carcinogenesis is to act as an accessory signaling molecule for cell proliferation. This molecule is particularly important in the complex immunological mechanisms of the gastrointestinal tract and in the link between chronic inflammation and carcinogenesis in the GI tract.

THE ROLE OF CD74 IN ANTIGEN PRESENTATION

CD74 was initially characterized for its role in regulating class Ⅱ MHC folding and intracellular sorting and has been studied in most detail in APCs. Newly synthesized CD74 self-assembles as a trimer and this trimer serves as a scaffold onto which class Ⅱ MHC molecules assemble. CD74 blocks the peptide binding cleft of class Ⅱ MHC and prevents premature binding of antigenic peptides. Upon endocytosis of antigens, CD74 directs the class Ⅱ MHC complex to the endosomal pathway using two di-leucine-based signals[5]. Within an endosomal compartment CD74 is digested, leaving just one residual peptide, CLIP (amino acids 91-99), blocking the peptide binding cleft of class Ⅱ MHC. A class Ⅱ MHC-like molecule, HLA-DM, then binds to the class Ⅱ MHC and CLIP is released leaving the peptide binding cleft open for antigenic peptide binding. Class Ⅱ MHC molecules with bound peptides are then exported to the surface of the

antigen presenting cell (APC) for presentation of foreign peptides to T cells. CD74 plays a crucial role in antigen presentation, as class Ⅱ MHC processing and regulation cannot properly occur in the absence of CD74.

In addition to APCs, other cells of the gastrointestinal tract, such as epithelial cells, express class Ⅱ MHC proteins and CD74 and act as APCs, which is an unusual trait of the GI tract. We have previously shown that gastric epithelial cells express class Ⅱ MHC proteins and are capable of processing antigens for presentation to T cells[6,7]. Expression of class Ⅱ MHC and the potential for antigen presentation have also been shown in intestinal epithelial cells. In one elaborate study by Hershberg *et al*, the trafficking of these molecules in epithelial cells was followed in a polarized manner outlining a functional system for antigen presentation[8]. Another important group of cells that express class Ⅱ MHC proteins are the subepithelial intestinal myofibroblasts (IMF)[9,10]. These cells are α-smooth actin positive stromal cells that exist in the lamina propria of the gut[11]. They also act as antigen presenting cells and play an important role in inflammatory diseases and carcinogenesis by releasing cytokines and growth factors and interacting with the immune cells of the lamina propria.

In conventional APCs, CD74 surface expression is low as CD74 is proteolytically removed in endosomes, as we and others[12,13] have shown. However, gastric epithelial cells express readily detectable levels of CD74 on the surface. When we examined human gastric biopsy sections by immunohistochemistry for epithelial expression of CD74, gastric biopsy samples from 44 random patients stained with anti-CD74 monoclonal antibodies (mAb) showed the expression of CD74 in the epithelium. In the samples that were positive for *H pylori* or had gastritis, CD74 staining was heavily increased[14]. This was corroborated by confocal microscopy studies of gastric epithelial cells grown as a polarized monolayer where expression was higher on the apical side of the cells[1].

CD74 ISOFORMS

CD74 is post-translationally glycosylated and exists in different isoforms. As evidence for this, our previous studies showed proteins with different mobilities when immunoprecipitated and subjected to gel electrophoresis[1]. After chemical deglycosylation, only the isoforms that result from alternative translation initiation or splicing were observed. The most common isoform is 33 kDa (p33), but p35, p41 and p43 isoforms also exist[15]. The p35 isoform contains a longer cytoplasmic tail due to the use of an alternative translation initiation site, while the p41 isoform results from alternative splicing, and p43 has both. Both the p33 and p35 isoforms appear to function in regulating class Ⅱ MHC antigen presentation. However, the p41 isoform might also play an important role in T cell selection in the thymus[16]. An important posttranslational modification of CD74 commonly seen is the addition of a glycosaminoglycan side chain, chondroitin sulfate. This isoform has been reported to act as an accessory protein during T cell activation through interaction with CD44 on T cells[17].

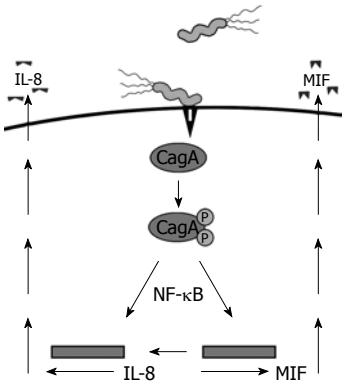

Figure 1 ***H pylori*** **induces MIF and IL-8 production by injecting CagA into GECs** ***via*** **a type Ⅳ secretion system.**

CD74 AS A RECEPTOR

CD74 has begun to emerge as a more versatile molecule beyond its well-known function of regulating class Ⅱ MHC trafficking. Multiple studies have revealed that cell surface expression of CD74 is not always dependent on class Ⅱ MHC[2,18]. This was found to be true in studies of colorectal mucosa and different types of lymphocytes by immunohistochemistry, immunoprecipitation, and a mutant cell line that did not express class Ⅱ MHC products. The expression of cell surface CD74 in the absence of class Ⅱ MHC suggests alternative functions for CD74 apart from antigen presentation.

CD74 as a cytokine receptor

Recently, CD74 has emerged as an integral component of a receptor complex for macrophage MIF[3]. MIF is a versatile cytokine-like molecule that mediates both innate and adaptive immunity, plays a role in chronic inflammation, and has also been linked to carcinogenesis. MIF is expressed by the GI tract during inflammatory conditions, *H pylori* infection, and cancers, suggesting the importance of this interaction[19-21]. *H pylori* utilizes a type Ⅳ secretion system to inject the CagA protein into GECs, which we have shown induces MIF production[19] (Figure 1). CagA has also been shown to induce NF-κB activation and IL-8 production[22]. This protein is not only important in inflammation by increasing proinflammatory cytokine production, such as IL-8, but is also associated with gastric cancer[23]. The current model of this receptor complex suggests that CD44 is required in order for MIF to induce signaling events[24]. This model might require the chondroitin sulfate modified isoform of CD74, since CD44 has been shown to bind only to this isoform thus far.

Another study has suggested that CD74 complexes with CXCR2, an interleukin-8 (IL-8) receptor, which is commonly expressed on macrophages and functions to recruit leukocytes to sites of infection[25]. CXCR2 is also expressed by the gastric epithelium[26]. Since gastric epithelial cells are central players in the inflammatory response, IL-8 may act via the gastric epithelium in various processes associated with gastric inflammation linked

to *H pylori* infection. The role of CXCR2 on the CD74 receptor complex has only recently been suggested and should be further investigated.

CD74 as a bacterial receptor

CD74 is an interesting example of a host cell receptor usurped by a pathogen because *H pylori* uses it to adhere to gastric epithelial cells (GECs). *H pylori* is a gram-negative spiral bacterium that colonizes the human gastroduodenal mucosa. Infection with *H pylori* usually begins in childhood and persists for decades if untreated. *H pylori* is recognized as a major contributor to chronic gastritis and peptic ulcer formation and is strongly associated with gastric carcinoma and lymphoma[27,28]. Due to the strength of the evidence supporting an association between adenocarcinomas of the gastric mucosa and *H pylori* infection, *H pylori* was classified as a class I carcinogen by the International Agency for Research on Cancer in affiliation with the World Health Organization[29]. Gastric cancer remains among the most common forms of cancer and is the second deadliest cancer worldwide. Gastric cancer accounts for approximately 700 000 deaths annually worldwide and in the US there are 24 000 new cases and 14 000 deaths annually[30]. The prevalence rates of *H pylori* seropositivity and the incidence of gastric cancer are highly associated within several populations from various countries. For instance, seropositivity can be as high as 80%-100% in some age groups in some countries or in minorities with lower incomes in the United States[31]. These groups have the highest risk of developing gastric cancer and/or gastric ulcers. Thus, *H pylori*-associated diseases represent a significant global and domestic problem and result in considerable morbidity, mortality, and societal costs.

H pylori adhesion to the gastric epithelium is important in successful colonization of the gastric mucosa. Adherent strains survive in the gastric mucosa, reach high bacterial densities, and can re-colonize, whereas non-adherent strains are cleared[32]. These observations support the notion that adhesion is essential in *H pylori* persistence and disease induction. An assortment of molecules on epithelial cells have been proposed as receptors for *H pylori* adherence including carbohydrate moieties [such as Lewis^b (Le^b) blood group antigen and sialyl-dimeric-Lewis^x (Le^x)], lysophospholipid, and other structures[33-35]. Our studies have indicated that *H pylori* also utilizes CD74 to attach to gastric epithelial cells (GEC)[4]. The binding of *H pylori* to CD74 on gastric epithelial cells was confirmed by a series of independent approaches. For instance, blocking of CD74 with antibodies significantly reduced the binding of *H pylori* to gastric epithelial cells. Immunoprecipitation revealed that *H pylori* predominantly binds to the 33 kDa isoform of CD74, but further investigation is needed to test for attachment to the CS modified isoform. As *H pylori* has been reported to bind to various glycoconjugates, including glycosaminoglycans[36], this isoform of CD74 might contribute to the overall interaction of *H pylori* with the host gastric epithelium. We also revealed that urease is the protein on *H pylori* that binds to CD74[37]. This interaction is particularly interesting because many bacteria express urease, so the possibility exists that there might be wider applications of this type of interaction with CD74 depending on urease sequence variation between bacteria.

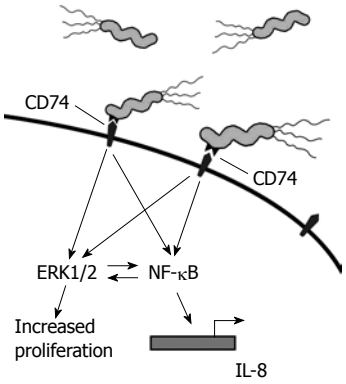

Figure 2 *H pylori* binds to CD74 on gastric epithelial cells and induces Nf-κB and Erk1/2 activation and IL-8 production.

After adhesion of *H pylori* to GEC, the expression of cell surface CD74 is increased[14]. We further showed that CD74 expression increases in gastric epithelial cells of infected humans and a recent study confirmed that this increase in CD74 expression also occurred in a mouse model of *H pylori* infection[38]. Upon *H pylori* binding to CD74, NF-κB activation occurs resulting in the production of proinflammatory cytokines, including IL-8. IL-8 plays a major role in the proinflammatory immune response to *H pylori* infection, therefore, the interaction of *H pylori* with the gastric epithelial cells might be of critical importance in the immune response to infection.

THE ROLE OF CD74 IN SIGNALING EVENTS

The role of CD74 in signal transduction was initially hypothesized when it was found to be phosphorylated and associated with proteins that coordinate various signal transduction pathways[39-41]. Interestingly, the observation that CD74 deficient mice have a defect in B cell development that results in decreased levels of follicular B cells provided insights into the important role of signals delivered through CD74 in B cell development. The cytosolic domain of CD74 alone was noted to induce B cell maturation by activation of NF-κB[42]. CD74 appears to promote B cell survival; therefore, it has been implicated in B cell neoplasms such as gastric mucosa-associated lymphoid tissue (MALT) lymphomas associated with *H pylori* infection.

Our studies have shown that the interaction of *H pylori* with CD74 on GECs induces NF-κB activation and IL-8 production, as shown in Figure 2. MIF has also been shown to bind to CD74[3]. One study outlines a role for CD44 in CD74 signaling. CD44 was required to initiate ERK1 and ERK2 signaling after MIF binding to CD74. Cells deficient in CD44 or transfected with truncated CD44 were not able to induce ERK signaling[24]. In B cells, MIF binding to CD74 led to AKT, Syk, and NF-κB activation, and proliferation in a CD44-dependant manner[43]. CXCR2 has also been shown to complex with

CD74 on monocytes[25]. This study also illustrated that MIF bound directly to CXCR2 and induced monocyte arrest. This study further showed that MIF might interact with CXCR4 on T cells and induce effector T cell arrest. However, it is not yet clear how CD44 is involved in the complexing of CD74 with CXCR2 and what signaling may be induced through CXCR4 since CXCR2 and CXCR4 are G protein-coupled receptors.

Other studies suggest that MIF signaling may also occur by non-receptor mediated endocytosis in addition to the above described receptors[44]. In this proposed mechanism, endocytosed or endogenous MIF interacts with the Jun activation domain-binding protein (Jab1), which is a transcriptional activator for AP-1[45]. Activation of AP-1 might affect cell cycle events by inducing degradation of the cyclin dependent kinase inhibitor, which is a tumor suppresser gene.

THE ROLE OF CD74 IN GI INFLAMMATION

CD74 is highly expressed in inflammatory disorders. We have shown it to be expressed on the gastric epithelium and up-regulated during *H pylori* infection[14]. Others have shown expression to be increased in ulcerative colitis, where overexpression was shown in DNA microarray profiles[46]. Additionally, CD74 is increased during inflammation associated cancers, such as gastric and colon[47,48]. Concurrently, MIF is highly expressed during many inflammatory conditions of the GI tract. We and others have shown that production is increased during *H pylori* infection[19,49]. MIF is also highly expressed during inflammatory bowel disorders (IBD), such as ulcerative colitis and Crohn's disease, where it is induced by intestinal bacteria[50]. In one study, elevated MIF levels were found in Crohn's disease patients at approximately six times higher levels than in healthy controls. Crohn's disease is an inflammatory bowel disorder where the immune system attacks part of the GI tract, and is accompanied by chronic inflammation. Also, this group went on to study MIF in murine colitis where they found colitis to be dependent on continuous MIF production, as evidenced by the protection from colitis by MIF-deficient mice or blocking MIF with monoclonal antibodies in mice with established colitis leading to reduced inflammation.

MIF or *H pylori* binding to CD74 induces NF-κB and subsequent cellular responses, such as the secretion of proinflammatory cytokines. MIF also increases inflammatory responses by overriding glucocortocoid suppression of inflammatory immune responses, including cytokines such as IL-1, IL-6, IL-8, and TNF-α[51]. One of the major proinflammatory cytokines produced after engagement of CD74 and receptor complexing is IL-8. IL-8 is a chemoattractant for neutrophils to a site of inflammation or infection. Upon arrival, they endocytose the antigen and form a phagosome in which reactive oxygen species and hydrolytic enzymes are released. While this process is crucial in fighting infections, it might also exacerbate inflammation in *H pylori* infection and inflammatory bowel disease[52-54]. In another study of glucocortocoid resistant ulcerative colitis, MIF was found to increase IL-8 production through the p38 MAPK pathway with isolated lamina propria mononuclear cells from patient biopsies[55]. When MIF was blocked with monoclonal antibodies after prednisolone treatment, activation of the p38 pathway and IL-8 decreased.

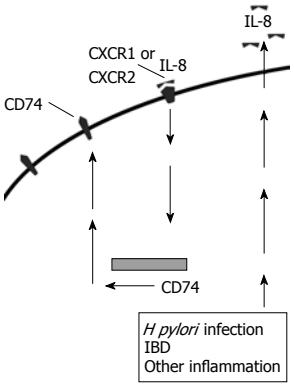

Figure 3 IL-8 produced by epithelial cells binds to CXCR1 and CXCR2 and up-regulates CD74 expression in an autocrine manner.

MIF might also contribute to inflammation by regulating Toll Like Receptor 4 (TLR4) expression on immune cells. TLR4 engagement by ligands such as bacterial LPS leads to proinflammatory cytokine production. This mechanism might be especially important in IBD, in which intestinal bacteria are a major contributor to the induction of the strong inflammatory response. In an *in vivo* study in mice, TLR4 expression in colonic tissue was not seen in MIF knockout mice, although it was in wild-type mice[56]. When the MIF knockout mice were administered rMIF, TLR4 expression was restored and further increased in colonic mice. In a human study of macrophages, neutralizing MIF or deleting the MIF gene resulted in decreased expression of TLR4 and a decreased response to LPS and gram negative bacteria; in broader experiments a decreased response to staphylococcal toxic shock and septic shock was demonstrated[57]. In addition, these cells did not respond well to LPS or gram negative bacteria and had a decreased expression of TLR4. The role of this receptor in the inflammation seen during *H pylori* infection is not clear because although *H pylori* LPS has been suggested to induce only weak responses, there are some studies suggesting it might contribute to the overall immune response to *H pylori*[58,59].

In addition to the role IL-8 plays in inflammation, we have previously shown that IL-8 increases expression of CD74 by gastric epithelial cells, both at the cell surface and mRNA levels[14] (Figure 3). Similarly, we found increased CD74 expression *in vivo*. Most of the *H pylori* infected samples and the samples with gastritis for reasons other than *H pylori* infection had much higher expression of CD74 than uninfected samples not exhibiting signs of gastritis. Other studies have further shown the expression of CD74 increased in ulcerative colitis and Crohn's Disease[46]. Increased CD74 expression could then go on to intensify inflammation by providing more free receptors for MIF or *H pylori* attachment.

THE ROLE OF CD74 IN GI CANCERS

CD74 has a strong link to carcinogenesis, as does MIF.

This ligand/receptor combination might be an important link between chronic inflammation and carcinogenesis. CD74 expression and MIF production have been shown to be increased during *H pylori* infection and gastric cancer[14,47]. High expression has also been noted in colon cancer along with highly increased serum concentrations of MIF in patients with colorectal cancers[60,61]. The contribution of CD74 to carcinogenesis is multifaceted. High levels of CD74 expression associated with class Ⅱ MHC expression might prevent tumor antigen presentation by blocking the peptide binding cleft and preventing antigenic peptide binding for presentation to T cells, rendering tumors less immunogenic. One study suggested this to be the case with colon neoplasms where expression was even increased from low to high grade neoplasms[48]. Chronic inflammation and IL-8 production leads to a prolonged increase in CD74 expression, which might not only decrease antigen presentation, but also exacerbate IL-8 production upon engagement by MIF or *H pylori*. MIF binding to the CD74 receptor complex also promotes proliferation of epithelial cells[19,62]. Long term increased proliferation overtakes natural cell cycle events and sets the stage for carcinogenesis.

MIF binding to CD74 might contribute to carcinogenesis in chronic conditions through the up-regulation of proinflammatory cytokines, including IL-8, which up-regulates CD74 and has its own mechanisms leading to increased proliferation, tumor growth, and angiogenesis. MIF or IL-8 binding to their receptors on epithelial cell surfaces induces the shedding of EGFR ligands in a mellatoprotease-dependent manner, and activation of EGFR through engagement of these ligands[63]. We and others have shown that this pathway is activated during *H pylori* infection[62,64,65]. Additionally, we found that EGFR expression is up-regulated in gastric epithelial cells during *H pylori* infection by MIF and IL-8" after the word infection. The EGFR is highly expressed in various cancers and is involved in pro-inflammatory responses and pro-carcinogenic events, including cell proliferation, migration, and invasion. Expression and activation of this receptor is well-documented in gastric and intestinal cancers[66,67]. One study suggests a correlation between EGFR expression on tumor cells, proliferation, and prognosis in gastric cancer[68]. Another study showed that treatment with antisense RNA for EGFR inhibited gastric tumor growth in a mouse model[69]. Blocking EGFR or its ligands is being studied in order to develop more effective treatments for GI cancers.

MIF also increases epithelial cell proliferation after binding to CD74. One way MIF might affect proliferation and cell cycle events is by regulating p53 tumor suppressor. Numerous cell cycle and apoptosis genes are controlled by p53. We have shown that phosphorylation of the p53 is decreased after MIF binding to CD74[19]. Others have shown that MIF can interact directly with p53 and prevent translocation to the nucleus where it becomes activated and acts as a transcription factor for apoptotic genes[70]. When p53 is blocked from transport to the nucleus, apoptotic pathways are decreased and proliferation increases. Suppression of p53 in macrophages results in a more robust inflammatory response, implying a further link between p53 and inflammation[71].

CONCLUSION

CD74 is a much more versatile molecule than originally thought, playing many important roles in the immune system. Of crucial importance is the role it plays in class Ⅱ MHC processing and the regulation of antigen presentation. This is important in the GI tract because epithelial cells and subepithelial myofibroblasts express CD74 and act as antigen presenting cells. Furthermore, the expression of CD74 on the cell surface might increase chronic inflammatory responses important in both *H pylori* infection and IBD. As a receptor for MIF, CD74 also plays and crucial role in chronic inflammation and might represent a major link between chronic inflammation and carcinogenesis in gastric and intestinal cancers. Development of therapeutics for cancer involving blocking CD74 might provide effective treatments for GI cancers.

REFERENCES

1 **Barrera CA**, Beswick EJ, Sierra JC, Bland D, Espejo R, Mifflin R, Adegboyega P, Crowe SE, Ernst PB, Reyes VE. Polarized expression of CD74 by gastric epithelial cells. *J Histochem Cytochem* 2005; **53**: 1481-1489

2 **Henne C**, Schwenk F, Koch N, Moller P. Surface expression of the invariant chain (CD74) is independent of concomitant expression of major histocompatibility complex class II antigens. *Immunology* 1995; **84**: 177-182

3 **Leng L**, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, Delohery T, Chen Y, Mitchell RA, Bucala R. MIF signal transduction initiated by binding to CD74. *J Exp Med* 2003; **197**: 1467-1476

4 **Beswick EJ**, Bland DA, Suarez G, Barrera CA, Fan X, Reyes VE. Helicobacter pylori binds to CD74 on gastric epithelial cells and stimulates interleukin-8 production. *Infect Immun* 2005; **73**: 2736-2743

5 **Barrera C**, Ye G, Espejo R, Gunasena S, Almanza R, Leary J, Crowe S, Ernst P, Reyes VE. Expression of cathepsins B, L, S, and D by gastric epithelial cells implicates them as antigen presenting cells in local immune responses. *Hum Immunol* 2001; **62**: 1081-1091

6 **Pieters J**, Bakke O, Dobberstein B. The MHC class II-associated invariant chain contains two endosomal targeting signals within its cytoplasmic tail. *J Cell Sci* 1993; **106** (Pt 3): 831-846

7 **Barrera C**, Espejo R, Reyes VE. Differential glycosylation of MHC class II molecules on gastric epithelial cells: implications in local immune responses. *Hum Immunol* 2002; **63**: 384-393

8 **Hershberg RM**, Cho DH, Youakim A, Bradley MB, Lee JS, Framson PE, Nepom GT. Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. *J Clin Invest* 1998; **102**: 792-803

9 **Barrera CA**, Pinchuk IV, Saada JI, Suarez G, Bland DA, Beswick E, Adegboyega PA, Mifflin RC, Powell DW, Reyes VE. Class II MHC-expressing myofibroblasts play a role in the immunopathogenesis associated with staphylococcal enterotoxins. *Ann N Y Acad Sci* 2004; **1029**: 313-318

10 **Saada JI**, Pinchuk IV, Barrera CA, Adegboyega PA, Suarez G, Mifflin RC, Di Mari JF, Reyes VE, Powell DW. Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. *J Immunol* 2006; **177**: 5968-5979

11 **Powell DW**, Adegboyega PA, Di Mari JF, Mifflin RC.

Epithelial cells and their neighbors I. Role of intestinal myofibroblasts in development, repair, and cancer. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G2-G7
12 **Blum JS**, Cresswell P. Role for intracellular proteases in the processing and transport of class II HLA antigens. *Proc Natl Acad Sci USA* 1988; **85**: 3975-3979
13 **Reyes VE**, Lu S, Humphreys RE. Cathepsin B cleavage of Ii from class II MHC alpha- and beta-chains. *J Immunol* 1991; **146**: 3877-3880
14 **Beswick EJ**, Das S, Pinchuk IV, Adegboyega P, Suarez G, Yamaoka Y, Reyes VE. Helicobacter pylori-induced IL-8 production by gastric epithelial cells up-regulates CD74 expression. *J Immunol* 2005; **175**: 171-176
15 **Warmerdam PA**, Long EO, Roche PA. Isoforms of the invariant chain regulate transport of MHC class II molecules to antigen processing compartments. *J Cell Biol* 1996; **133**: 281-291
16 **Wright RJ**, Bikoff EK, Stockinger B. The Ii41 isoform of invariant chain mediates both positive and negative selection events in T-cell receptor transgenic mice. *Immunology* 1998; **95**: 309-313
17 **Naujokas MF**, Morin M, Anderson MS, Peterson M, Miller J. The chondroitin sulfate form of invariant chain can enhance stimulation of T cell responses through interaction with CD44. *Cell* 1993; **74**: 257-268
18 **Momburg F**, Koretz K, Von Herbay A, Moller P. Nonimmune human cells can express MHC class II antigens in the absence of invariant chain--an immunohistological study on normal and chronically inflamed small intestine. *Clin Exp Immunol* 1988; **72**: 367-372
19 **Beswick EJ**, Pinchuk IV, Suarez G, Sierra JC, Reyes VE. Helicobacter pylori CagA-dependent macrophage migration inhibitory factor produced by gastric epithelial cells binds to CD74 and stimulates procarcinogenic events. *J Immunol* 2006; **176**: 6794-6801
20 **Ishiguro Y**, Yamagata K, Sakuraba H, Munakata A, Nakane A, Morita T, Nishihira J. Macrophage migration inhibitory factor and activator protein-1 in ulcerative colitis. *Ann N Y Acad Sci* 2004; **1029**: 348-349
21 **Wilson JM**, Coletta PL, Cuthbert RJ, Scott N, MacLennan K, Hawcroft G, Leng L, Lubetsky JB, Jin KK, Lolis E, Medina F, Brieva JA, Poulsom R, Markham AF, Bucala R, Hull MA. Macrophage migration inhibitory factor promotes intestinal tumorigenesis. *Gastroenterology* 2005; **129**: 1485-1503
22 **Kim SY**, Lee YC, Kim HK, Blaser MJ. Helicobacter pylori CagA transfection of gastric epithelial cells induces interleukin-8. *Cell Microbiol* 2006; **8**: 97-106
23 **Shmuely H**, Passaro D, Figer A, Niv Y, Pitlik S, Samra Z, Koren R, Yahav J. Relationship between Helicobacter pylori CagA status and colorectal cancer. *Am J Gastroenterol* 2001; **96**: 3406-3410
24 **Shi X**, Leng L, Wang T, Wang W, Du X, Li J, McDonald C, Chen Z, Murphy JW, Lolis E, Noble P, Knudson W, Bucala R. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity* 2006; **25**: 595-606
25 **Bernhagen J**, Krohn R, Lue H, Gregory JL, Zernecke A, Koe nen RR, Dewor M, Georgiev I, Schober A, Leng L, Kooistra T, Fingerle-Rowson G, Ghezzi P, Kleemann R, McColl SR, B ucala R, Hickey MJ, Weber C. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med* 2007; **13**: 587-596
26 **Backhed F**, Torstensson E, Seguin D, Richter-Dahlfors A, Rokbi B. Helicobacter pylori infection induces interleukin-8 receptor expression in the human gastric epithelium. *Infect Immun* 2003; **71**: 3357-3360
27 **Solnick JV**, Tompkins LS. Helicobacter pylori and gastroduodenal disease: pathogenesis and host-parasite interaction. *Infect Agents Dis* 1992; **1**: 294-309
28 **Talley NJ**, Zinsmeister AR, Weaver A, DiMagno EP, Carpenter HA, Perez-Perez GI, Blaser MJ. Gastric adenocarcinoma and Helicobacter pylori infection. *J Natl Cancer Inst* 1991; **83**: 1734-1739
29 Infection with Helicobacter pylori. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 177-240
30 **Cancer Database.** Available from: URL: http://www-dep.iarc.fr/ 2005
31 **Blaser MJ**. Helicobacter pylori phenotypes associated with peptic ulceration. *Scand J Gastroenterol Suppl* 1994; **205**: 1-5
32 **Hayashi S**, Sugiyama T, Asaka M, Yokota K, Oguma K, Hirai Y. Modification of Helicobacter pylori adhesion to human gastric epithelial cells by antiadhesion agents. *Dig Dis Sci* 1998; **43**: 56S-60S
33 **Boren T**, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895
34 **Ilver D**, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998; **279**: 373-377
35 **Van den Brink GR**, Tytgat KM, Van der Hulst RW, Van der Loos CM, Einerhand AW, Buller HA, Dekker J. H pylori colocalises with MUC5AC in the human stomach. *Gut* 2000; **46**: 601-607
36 **Ascencio F**, Fransson LA, Wadstrom T. Affinity of the gastric pathogen Helicobacter pylori for the N-sulphated glycosaminoglycan heparan sulphate. *J Med Microbiol* 1993; **38**: 240-244
37 **Beswick EJ**, Pinchuk IV, Minch K, Suarez G, Sierra JC, Yamaoka Y, Reyes VE. The Helicobacter pylori urease B subunit binds to CD74 on gastric epithelial cells and induces NF-kappaB activation and interleukin-8 production. *Infect Immun* 2006; **74**: 1148-1155
38 **Kobayashi M**, Lee H, Schaffer L, Gilmartin TJ, Head SR, Takaishi S, Wang TC, Nakayama J, Fukuda M. A distinctive set of genes is upregulated during the inflammation-carcinoma sequence in mouse stomach infected by Helicobacter felis. *J Histochem Cytochem* 2007; **55**: 263-274
39 **Anderson HA**, Roche PA. Phosphorylation regulates the delivery of MHC class II invariant chain complexes to antigen processing compartments. *J Immunol* 1998; **160**: 4850-4858
40 **Kuwana T**, Peterson PA, Karlsson L. Exit of major histocompatibility complex class II-invariant chain p35 complexes from the endoplasmic reticulum is modulated by phosphorylation. *Proc Natl Acad Sci USA* 1998; **95**: 1056-1061
41 **Spiro RC**, Quaranta V. The invariant chain is a phosphorylated subunit of class II molecules. *J Immunol* 1989; **143**: 2589-2594
42 **Matza D**, Wolstein O, Dikstein R, Shachar I. Invariant chain induces B cell maturation by activating a TAF(II)105-NF-kappaB-dependent transcription program. *J Biol Chem* 2001; **276**: 27203-27206
43 **Gore Y**, Starlets D, Maharshak N, Becker-Herman S, Kaneyuki U, Leng L, Bucala R, Shachar I. Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. *J Biol Chem* 2008; **283**: 2784-2792
44 **Kleemann R**, Grell M, Mischke R, Zimmermann G, Bernhagen J. Receptor binding and cellular uptake studies of macrophage migration inhibitory factor (MIF): use of biologically active labeled MIF derivatives. *J Interferon Cytokine Res* 2002; **22**: 351-363
45 **Kleemann R**, Hausser A, Geiger G, Mischke R, Burger-Kentischer A, Flieger O, Johannes FJ, Roger T, Calandra T, Kapurniotu A, Grell M, Finkelmeier D, Brunner H, Bernhagen J. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature* 2000; **408**: 211-216
46 **Lawrance IC**, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. *Hum Mol Genet* 2001; **10**: 445-456
47 **Ishigami S**, Natsugoe S, Tokuda K, Nakajo A, Iwashige H,

Aridome K, Hokita S, Aikou T. Invariant chain expression in gastric cancer. *Cancer Lett* 2001; **168**: 87-91
48 **Jiang Z**, Xu M, Savas L, LeClair P, Banner BF. Invariant chain expression in colon neoplasms. *Virchows Arch* 1999; **435**: 32-36
49 **Xia HH**, Lam SK, Chan AO, Lin MC, Kung HF, Ogura K, B erg DE, Wong BC. Macrophage migration inhibitory factor stimulated by Helicobacter pylori increases proliferation of gastric epithelial cells. *World J Gastroenterol* 2005; **11**: 1946-1950
50 **de Jong YP**, Abadia-Molina AC, Satoskar AR, Clarke K, Rietdijk ST, Faubion WA, Mizoguchi E, Metz CN, Alsahli M, ten Hove T, Keates AC, Lubetsky JB, Farrell RJ, Michetti P, van Deventer SJ, Lolis E, David JR, Bhan AK, Terhorst C. Development of chronic colitis is dependent on the cytokine MIF. *Nat Immunol* 2001; **2**: 1061-1066
51 **Calandra T**, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* 1995; **377**: 68-71
52 **Ina K**, Kusugami K, Yamaguchi T, Imada A, Hosokawa T, Ohsuga M, Shinoda M, Ando T, Ito K, Yokoyama Y. Mucosal interleukin-8 is involved in neutrophil migration and binding to extracellular matrix in inflammatory bowel disease. *Am J Gastroenterol* 1997; **92**: 1342-1346
53 **Leakey A**, La Brooy J, Hirst R. The ability of Helicobacter pylori to activate neutrophils is determined by factors other than H pylori neutrophil-activating protein. *J Infect Dis* 2000; **182**: 1749-1755
54 **Mazzucchelli L**, Hauser C, Zgraggen K, Wagner H, Hess M, Laissue JA, Mueller C. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. *Am J Pathol* 1994; **144**: 997-1007
55 **Ishiguro Y**, Ohkawara T, Sakuraba H, Yamagata K, Hiraga H, Yamaguchi S, Fukuda S, Munakata A, Nakane A, Nishihira J. Macrophage migration inhibitory factor has a proinflammatory activity via the p38 pathway in glucocorticoid-resistant ulcerative colitis. *Clin Immunol* 2006; **120**: 335-341
56 **Ohkawara T**, Takeda H, Miyashita K, Nishiwaki M, Nakayama T, Taniguchi M, Yoshiki T, Tanaka J, Imamura M, Sugiyama T, Asaka M, Nishihira J. Regulation of Toll-like receptor 4 expression in mouse colon by macrophage migration inhibitory factor. *Histochem Cell Biol* 2006; **125**: 575-582
57 **Roger T**, Froidevaux C, Martin C, Calandra T. Macrophage migration inhibitory factor (MIF) regulates host responses to endotoxin through modulation of Toll-like receptor 4 (TLR4). *J Endotoxin Res* 2003; **9**: 119-123
58 **Ishihara S**, Rumi MA, Kadowaki Y, Ortega-Cava CF, Yuki T, Yoshino N, Miyaoka Y, Kazumori H, Ishimura N, Amano Y, Kinoshita Y. Essential role of MD-2 in TLR4-dependent signaling during Helicobacter pylori-associated gastritis. *J Immunol* 2004; **173**: 1406-1416
59 **Smith MF Jr**, Mitchell A, Li G, Ding S, Fitzmaurice AM, Ryan K, Crowe S, Goldberg JB. Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. *J Biol Chem* 2003; **278**: 32552-32560
60 **Degener T**, Momburg F, Moller P. Differential expression of HLA-DR, HLA-DP, HLA-DQ and associated invariant chain (Ii) in normal colorectal mucosa, adenoma and carcinoma. *Virchows Arch A Pathol Anat Histopathol* 1988; **412**: 315-322
61 **Yasasever V**, Camlica H, Duranyildiz D, Oguz H, Tas F, Dalay N. Macrophage migration inhibitory factor in cancer. *Cancer Invest* 2007; **25**: 715-719
62 **Beswick EJ**, Reyes VE. Macrophage migration inhibitory factor and interleukin-8 produced by gastric epithelial cells during Helicobacter pylori exposure induce expression and activation of the epidermal growth factor receptor. *Infect Immun* 2008; **76**: 3233-3240
63 **Itoh Y**, Joh T, Tanida S, Sasaki M, Kataoka H, Itoh K, Oshima T, Ogasawara N, Togawa S, Wada T, Kubota H, Mori Y, Ohara H, Nomura T, Higashiyama S, Itoh M. IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells. *Cytokine* 2005; **29**: 275-282
64 **Joh T**, Kataoka H, Tanida S, Watanabe K, Ohshima T, Sasaki M, Nakao H, Ohhara H, Higashiyama S, Itoh M. Helicobacter pylori-stimulated interleukin-8 (IL-8) promotes cell proliferation through transactivation of epidermal growth factor receptor (EGFR) by disintegrin and metalloproteinase (ADAM) activation. *Dig Dis Sci* 2005; **50**: 2081-2089
65 **Keates S**, Keates AC, Nath S, Peek RM Jr, Kelly CP. Transactivation of the epidermal growth factor receptor by cag+ Helicobacter pylori induces upregulation of the early growth response gene Egr-1 in gastric epithelial cells. *Gut* 2005; **54**: 1363-1369
66 **Svrcek M**, Cosnes J, Tiret E, Bennis M, Parc Y, Flejou JF. Expression of epidermal growth factor receptor (EGFR) is frequent in inflammatory bowel disease (IBD)-associated intestinal cancer. *Virchows Arch* 2007; **450**: 243-244
67 **Tokunaga A**, Onda M, Okuda T, Teramoto T, Fujita I, Mizutani T, Kiyama T, Yoshiyuki T, Nishi K, Matsukura N. Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric cancer. *Cancer* 1995; **75**: 1418-1425
68 **Jonjic N**, Kovac K, Krasevic M, Valkovic T, Ernjak N, Sasso F, Melato M. Epidermal growth factor-receptor expression correlates with tumor cell proliferation and prognosis in gastric cancer. *Anticancer Res* 1997; **17**: 3883-3888
69 **Hirao T**, Sawada H, Koyama F, Watanabe A, Yamada Y, Sakaguchi T, Tatsumi M, Fujimoto H, Emoto K, Narikiyo M, Oridate N, Nakano H. Antisense epidermal growth factor receptor delivered by adenoviral vector blocks tumor growth in human gastric cancer. *Cancer Gene Ther* 1999; **6**: 423-427
70 **Jung H**, Seong HA, Ha H. Critical role of cysteine residue 81 of macrophage migration inhibitory factor (MIF) in MIF-induced inhibition of p53 activity. *J Biol Chem* 2008; **283**: 20383-20396
71 **Mitchell RA**, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, Bucala R. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc Natl Acad Sci USA* 2002; **99**: 345-350

S- Editor Li LF **L- Editor** Stewart GJ **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2862

World J Gastroenterol 2009 June 21; 15(23): 2862-2869
World Journal of Gastroenterology ISSN 1007-9327

ORIGINAL ARTICLES

Protective effect of *Radix Astragali* injection on immune organs of rats with obstructive jaundice and its mechanism

Rui-Ping Zhang, Xi-Ping Zhang, Yue-Fang Ruan, Shu-Yun Ye, Hong-Chan Zhao, Qi-Hui Cheng, Di-Jiong Wu

Rui-Ping Zhang, Department of Orthopaedics or Department of Radiology, First Clinical Medical College of Shanxi Medical University, Taiyuan 030001, Shanxi Province, China
Xi-Ping Zhang, Department of General Surgery, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Yue-Fang Ruan, Shu-Yun Ye, Di-Jiong Wu, Zhejiang University of Traditional Chinese Medicine, Hangzhou 310053, Zhejiang Province, China
Hong-Chan Zhao, Department of test, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Qi-Hui Cheng, Department of Gynaecology and Obstetrics, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Author contributions: Zhang RP, Zhang XP and Cheng QH designed the research; Zhang RP, Zhang XP, Ruan YF, Cheng QH and Ye SY wrote the paper; Zhao HC collected the analyzed data; Wu DJ picked the color figures in the paper; all authors contributed to the intellectual context and approved the final version.
Supported by Technological Foundation Project of Traditional Chinese Medicine of Zhejiang Province, No. 2003C130, No. 2004C142; Foundation Project for Medical Science and Technology of Zhejiang Province, No. 2003B134; Grave foundation project for Technology and Development of Hangzhou, No. 2003123B19; Intensive Foundation Project for Technology of Hangzhou, No. 2004Z006; Foundation Project for Medical Science and Technology of Hangzhou, No. 2003A004; and Foundation Project for Technology of Hangzhou, No. 2005224
Correspondence to: Dr. Rui-Ping Zhang, Department of Orthopaedics or Department of Radiology, First Clinical Medical College of Shanxi Medical University, Taiyuan 030001, Shanxi Province, China. zxp99688@vip.163.com
Telephone: +86-351-4639504 Fax: +86-351-8263016
Received: March 27, 2009 Revised: April 15, 2009
Accepted: April 22, 2009
Published online: June 21, 2009

Abstract

AIM: To observe the protective effect of *Radix Astragali* injection on immune organs (lymph nodes, spleen and thymus) of rats with obstructive jaundice (OJ) and its mechanism.

METHODS: SD rats were randomly divided into sham-operation group, model control group and *Radix Astragali* treatment group. On days 7, 14, 21 and 28 after operation, mortality rate of rats, pathological changes in immune organs, expression levels of Bax and nuclear factor (NF)-κB p65 proteins, apoptosis indexes and serum tumor necrosis factor (TNF)-α level in spleen and thymus were observed, respectively.

RESULTS: Compared to model control group, the number of dead OJ rats in *Radix Astragali* treatment group decreased ($P > 0.05$). The TNF-α level (27.62 ± 12.61 *vs* 29.55 ± 18.02, 24.61 ± 9.09 *vs* 31.52 ± 10.95) on days 7 and 21, the pathological severity score for spleen [0.0 (0.0) *vs* 0.0 (2.0) on days 7 and 14 and for lymph nodes [0.0 (1.0) *vs* 1.0 (2.0), 1.0 (0.0) *vs* 2.0 (1.0)] on days 21 and 28, the product staining intensity and positive rate of Bax protein in spleen [0.0 (0.0) *vs* 1.0 (2.0), 0.0 (1.0) *vs* 2.0 (1.5) and thymus [0.0 (0.0) *vs* 1.0 (2.0), 0.0 (1.0) *vs* 2.0 (1.5)] on days 14 and 28, the apoptotic indexes [0.0 (0.0) *vs* 0.0 (0.01)] in spleen and thymus [0.0 (0.0) *vs* 0.0 (0.01) on days 14 and 21 were significantly lower in *Radix Astragali* treatment group than in model control group ($P < 0.05$).

CONCLUSION: *Radix Astragali* has protective effects on immune organs of OJ rats by relieving the pathological changes in immune organs, reducing TNF-α level and inhibiting Bax expression and apoptosis in spleen and thymus.

Key words: *Radix Astragali*; Traditional Chinese medicine; Obstructive jaundice; Rat; Immune organ; Tumor necrosis factor-α; Bax; Nuclear factor-κB; Apoptosis; Tissue microarry

Peer reviewers: Sharon DeMorrow, Assistant Professor, Division of Research and Education, Scott and White Hospital and The Texas A&M University System, Health Science Center College of Medicine, Temple, Texas 76504, United States; Alain L Servin, PhD, Faculty of Pharmacy, French National Institute of Health and Medical Research, Unit 756, Rue J.-B. Clément, F-922296 Châtenay-Malabry, France

Zhang RP, Zhang XP, Ruan YF, Ye SY, Zhao HC, Cheng QH, Wu DJ. Protective effect of *Radix Astragali* injection on immune organs of rats with obstructive jaundice and its mechanism. *World J Gastroenterol* 2009; 15(23): 2862-2869 Available from: URL: http://www.wjgnet.com/1007-9327/15/2862.asp DOI: http://dx.doi.org/10.3748/wjg.15.2862

INTRODUCTION

Obstructive jaundice (OJ) is a kind of common clinical manifestation. The pathogenesis and treatment of OJ have been a hot topic in medical field for a long time[1-3]. Since

systemic inflammatory response syndrome and multiple organ dysfunction syndrome were studied in recent years, immune function impairment concomitant with OJ has gradually attracted wide attention and is considered as a cause of death in OJ patients[4-7]. Therefore, one of the important approaches to treatment of OJ is to restore the functions of immune organs[8-10].

Development and utilization of traditional Chinese medicine have good prospects in therapy for OJ since it has a lower cost, more extensive pharmacological effects and fewer side effects. *Radix Astragali,* dried root of *Astragalus membranaceus*, is sweet in taste with a warm property and mainly produced in Inner Mongolia Autonomous Region, Shanxi, Gansu and Heilongjiang Provinces of China. The raw *Radix Astragali* can be used to consolidate the exterior of body. *Radix Astragali* can regulate sweating, warm muscles, strengthen striae, invigorate Qi (vital energy), and alleviate heat in muscles due to Qi deficiency. *Radix Astragali* can also be used in treatment of chicken pox or other diseases with vesicular-papules due to inadequate "dispersing of the evils". *Radix Astragali* invigorates Qi, ascends the Yang-Qi, protects Qi and consolidates the exterior of body, promotes diuresis, relieves edema (generalized swelling of the body), supports Qi to promote skin wound/ulcer healing and tissue/muscle regeneration. *Astragalus* injection is made of extraction from *Radix Astragali*. Since *Astragalus* injection contains polysaccharide, saponin, flavone and trace elements, it has a variety of pharmacological effects and increases the immunity and protects the liver and kidneys[11-15]. It has been shown that cellular immune function decreases in OJ rats[16], which can be successfully treated with *Astragalus* injection.

At present, studies about the effects of *Astragalus* injection on immune organs during OJ are not available. This study was to investigate the protective effect of *Astragalus* injection on immune organs of OJ rats and its mechanism. The results may provide an experimental basis for its application in clinical practice.

MATERIALS AND METHODS

Materials

Healthy male SD rats of clean grade, weighing 270-330 g, were provided by Laboratory Animal Research Center, Zhejiang University of Traditional Chinese Medicine (China). Sodium pentobarbital was purchased from Sigma Corporation (USA). *Radix Astragali* injection (10 mL vial contains active components equivalent to 20 g of the original medicine) was purchased from Chiatai Qingchunbao Pharmaceutical Co, Ltd (China). Serum tumor necrosis factor (TNF)-α ELISA kits were purchased from Shanghai Senxiong Technological Company (China). Anti-nuclear factor (NF)-κB P65 and anti-Bax antibodies were purchased from Santa Cruz Biotechnology, Inc (USA). TUNEL assay kits were purchased from Takara Bio Inc (Jingdu, Japan).

Animal grouping and preparation of OJ models

One hundred and eighty OJ rats, enrolled in this study, were randomly divided into sham-operation group, model control group, and treatment group (n = 60), which were further subdivided into 7, 14, 21 and 28 d groups (n = 15) according to the time after operation. After the rats were anesthetized with intraperitoneal injection of 2.5% sodium pentobarbital (0.2 mL/100 g), their abdominal cavity was opened to identify and dissociate the common bile duct along the hepatoduodenal ligament. The proximal end of the common bile duct of rats in the model control and treatment groups was double-ligated with surgical threads, the common bile duct was cut off, and a layered suture of the abdominal wall was performed to close the abdominal cavity. The common bile duct of rats in the sham-operation group was dissociated but not ligated, and a layered suture of the abdominal wall was also performed to close the abdominal cavity. Rats in the treatment group received intraperitoneal *Radix Astragali* injection at a dose of 0.75 mL/100 g per day, while those in the sham-operation and model control groups received an equal volume of physiological saline solution until the end of 7-, 14-, 21- and 28-d observation periods in the corresponding groups.

Determination of experimental parameters

Mortality rates of rats in different groups were recorded. Rats were killed after anesthesia with sodium pentobarbital in batches, serum was collected to measure TNF-α level by ELISA, and pathological changes in immune organs (lymph nodes, spleen and thymus) were observed. Pathological severity of immune organs was scored according to related standards. Tissues of spleen, thymus and lymph nodes were cut into sections, but the sections of lymph node were not stained. Changes in expression levels of Bax and NF-κB P65 proteins, as well as apoptosis index of spleen and thymus were observed, respectively.

Immunohistochemical staining of Bax and NF-κB P65 proteins in intestinal mucosa

Envision two-step method was used to detect the expression levels of Bax and NF-κB P65 proteins in intestinal mucosa. The staining intensity was evaluated according to the extent of cell coloration: "-" represents negative staining; "+" represents mild staining with positively stained cells showing a yellow pigment; "++" represents moderate staining with positively stained cells showing a brown pigment; "+++" represents intense staining with positively stained cells showing a dark brown pigment; (-) indicates no positive cells; (+) indicates less than 25% of positive cells; (++) indicates 26%-50% of positive cells; and (+++) indicates over 50% of positive cells, each of which was scored as 0, 1, 2 and 3 points, respectively.

Detection of apoptotic index in intestinal mucosa with TUNEL staining

DNA nicking in tissue sections was observed with *in situ* end-labeling (TUNEL) staining. In brief, sections were

Table 1 Comparison of serum TNF-α levels in different groups (ng/L, mean ± SD)

Group	7 d	14 d	21 d	28 d
Sham-operation group	12.89 ± 1.63	12.25 ± 3.37	14.21 ± 3.24	15.61 ± 4.84
Model control group	29.55 ± 18.02[b]	34.10 ± 8.94[b]	31.52 ± 10.95[b]	57.66 ± 12.99[b]
Treatment group	27.62 ± 12.61[a,b]	27.20 ± 9.34[b]	24.61 ± 9.09[a,b]	39.01 ± 9.62[b]

[a]$P < 0.05$ *vs* model control group; [b]$P < 0.01$ *vs* sham-operation group.

baked at 60℃ for 30 min, deparaffinaged, and washed with Milli-Q for 5 min. Tissue was processed with protease K (10 μg/μL) at room temperature for 15 min and washed with phosphate-buffered saline (PBS) for 5 min. A 3% H_2O_2 solution was used to block endogenous peroxydase for 5 min, washed twice with PBS, 5 min each time. Thirty microliters of reaction solution at freezing condition (TdT enzyme : labeling safe buffer = 1:10) was added, incubated at 37℃ for 90 min, and washed twice with PBS, 5 min each time. Fifty microliters of anti-FITC HRP conjugate was added, incubated at 37℃ for 30 min, and washed twice with PBS, 5 min each time. DAB was colored, washed with Milli-Q, counterstained with hematoxylin, and washed with water after differentiation till it became blue. The DAB was routinely dehydrated and mounted onto neutral gum. Apoptotic index was calculated following the equation: Apoptotic index = apoptotic cell count/total cell count × 100%.

Statistical analysis

Data were input into the Excel sheet and read into SPSS 15.0 for further analysis. Normal data were expressed as mean ± SD while abnormal data were expressed as median (interquartile range). Analysis of variance and pairwise comparison were used for normal data, whereas abnormal data were subjected to non-parametric tests, of which Kruskal-Wallis H test was used for pairwise comparison and Mann-Whitney *U* test was used for multiple comparisons. Yates' χ^2 test was used for inter-group comparisons of mortality rates.

RESULTS

Comparison of mortality rates

All rats in the sham-operation group were alive after operation. Two rats in the model control group and one rat in the treatment group died on day 7 after operation. Four rats in the model control group and three rats in the treatment group died on day 14 after operation. Four rats in the model control group and four rats in the treatment group died on day 21 after operation. Seven rats in the model control group and six rats in the treatment group died on day 28 after operation. The total mortality rate of rats in the model control and treatment groups on day 28 was significantly higher than that of rats in the sham-operation group ($P < 0.001$). No significant difference was found in mortality rate between the model control and treatment groups.

Comparison of serum TNF-α levels

The serum TNF-α level was significantly lower in sham-operation group than in model control and treatment groups at different time points after operation ($P < 0.01$), and was significantly lower in treatment group than in model control group on days 7 and 21 after operation ($P < 0.05$, Table 1).

Pathological changes in spleen

In the sham-operation group, the morphology of spleen was normal with no gross pathological changes under light microscope.

In the model control group, the size of spleen increased by 1.2-1.5 folds and the texture of spleen became fragile with no change in color on day 7 after operation. The spleen became enlarged with a thickness of above 0.6 cm and a deeper color and its texture became fragile on day 14 after operation. The spleen was 4 cm × 1 cm × 1 cm in size and its texture became fragile with a purple black color on days 21 and 28 after operation. Under light microscope, the spleen of all rats was grossly normal on day 7 after operation. Fusion, enlargement or spotty necrosis of follicular germinal center in the white pulp of spleen, hyperplasia of fibrous tissue in sinus, and arteriolar sclerosis in spleen of most rats were observed on day 14 after operation. The spleen of few rats was grossly normal. Fusion, enlargement or spotty necrosis of follicular germinal centers in the white pulp of spleen, hyperplasia of fibrous tissue in sinus, and arteriolar sclerosis in spleen of few rats were seen on days 21 and 28 after operation. The spleen of some rats was grossly normal (Figure 1A).

In the treatment group, no significant difference was found in pathological changes at all time points after operation compared to the model control group. Under light microscope**,** no significant difference in pathological changes was noted at each time point after operation. The spleen of most rats was grossly normal. Arteriolar sclerosis in spleen of few rats was seen (Figure 1B).

The pathological scoring standards for spleen are listed in Table 2. The pathological scores were significantly lower for sham-operation group than for model control group on day 14 after operation ($P < 0.05$), and were significantly lower for treatment group than for model control group on days 7 and 14 after operation ($P < 0.05$, Table 3).

No significant difference was found in product staining intensity and in positive rate of NF-κB protein in spleens of all groups (Table 3).

The product staining intensity and positive rate of Bax protein were significantly lower in sham-operation and treatment groups than in model control group on day 28 after operation ($P < 0.05$, Table 3).

The apoptosis index was significantly lower in sham-

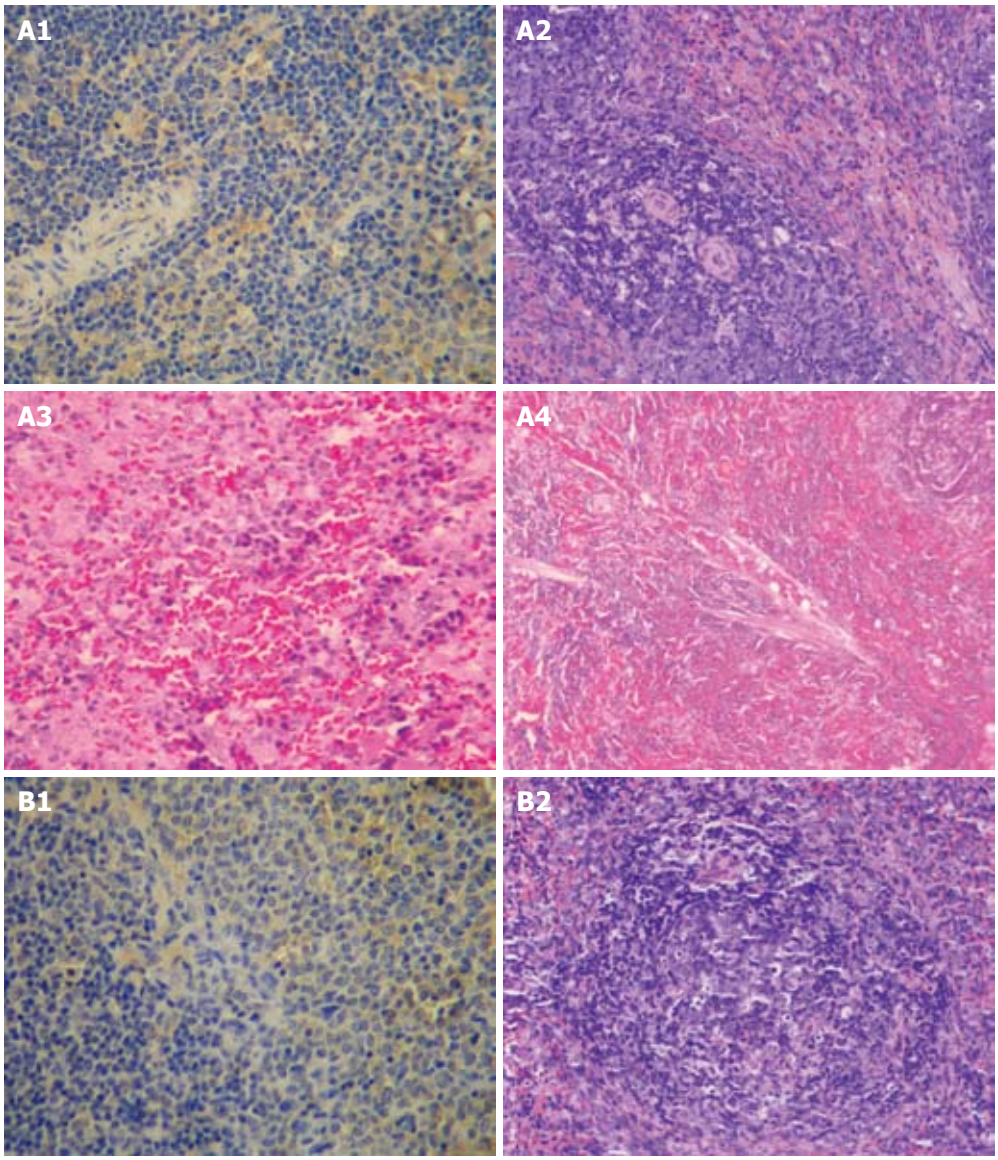

Figure 1 Pathological changes in spleen of model control group (A) and treatment group (B). A1: 21 d. Spleen (++) (Bax, × 200); A2: 28 d. Thickening of the wall of small spleen arteries as well as expansion and congestion of the red pulp (HE, × 200); A3: 28 d. Enlargement of spleen sinusoid, hyperplasia of cells in the spleen sinus as well as inflammatory cell infiltration and hemorrhage (HE, × 200); A4: 28 d. Enlargement of spleen sinusoid and hyperplasia of fibrous tissue (HE, × 100); B1: 21 d. Spleen (+) (Bax, × 200); B2: 28 d. Focal necrosis in spleen lymphoid follicles (HE, × 100).

Table 2 Pathological severity scoring standards for spleen

Score standards	Observation indexes
0	Normal
1	Necrosis of follicle center
2	Blood sinus dilation or arteriolar sclerosis
3	Necrosis of follicle center, blood sinus dilation and arteriolar sclerosis

operation group than in model control group on days 7, 14 and 28 after operation ($P < 0.05$), and was significantly lower in treatment group than in model control group on day 14 after operation ($P < 0.05$, Table 3).

Pathological changes in lymph nodes

In sham-operation group, the gross morphology of lymph nodes was normal. Under light microscope, no significant difference was observed in pathological changes at different time points after operation. The morphology and structure of lymph nodes were grossly normal. Enlargement of follicular germinal centers and hyperplasia of sinus cells were seen in few rats (Figure 2A).

Table 3 Comparison of different pathological indexes for spleen, M (Q_R)

Index	Time (d)	Sham-operation group	Model control group	Treatment group
Pathological severity score	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[c]
	14	0.0 (1.0)[c]	0.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)	1.0 (2.0)	0.0 (0.0)
	28	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)
Product staining intensity and positive rate of Bax	7	0.0 (0.0)[c]	1.0 (2.0)	0.5 (1.0)[a]
	14	0.0 (1.0)	1.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	1.0 (2.0)	0.0 (2.0)
	28	0.0 (0.0)[c]	2.0 (1.5)	0.0 (1.0)[c]
Apoptosis index	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	14	0.0 (0.0)	0.0 (0.01)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	0.0 (0.01)	0.0 (0.0)
	28	0.0 (0.0)[c]	0.01 (0.02)	0.0 (0.0)
Product staining intensity and positive rate of NF-κB	7	0.0 (0.0)	0.0 (1.0)	0.0 (0.0)
	14	0.0 (1.0)	0.0 (2.0)	0.0 (0.0)
	21	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	28	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a]$P < 0.05$ *vs* sham-operation group; [c]$P < 0.05$ *vs* model control group.

In model control group, lymph nodes became yellow in 50% of the rats on day 7 after operation, but no

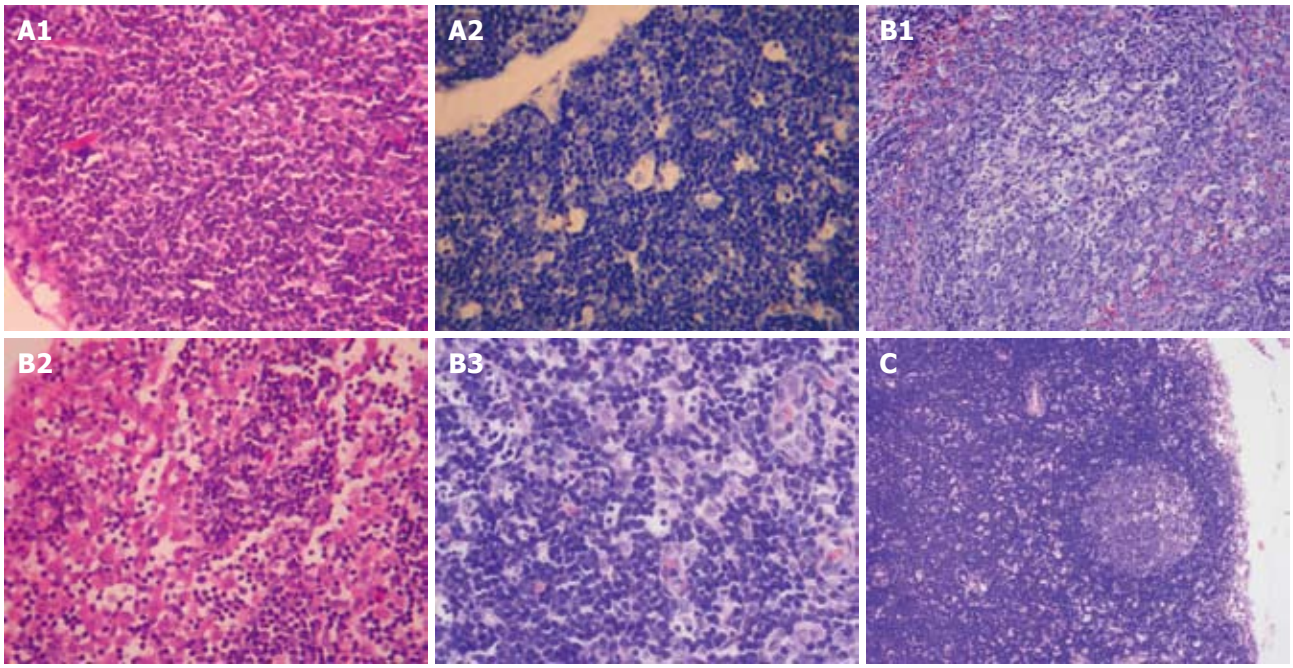

Figure 2 Pathological changes in lymph nodes of sham-operating group (A), model control group (B), and treatment group (C). A1: 28 d. Mainly normal lymph nodes (HE, × 200); A2: 28 d. No apoptotic cells in lymph node (TUNEL, × 200); B1: 21 d. Focal necrosis in lymphoid follicles and formation of germinal centers (HE, × 200); B2: 21 d. Expansion of lymph sinus, sinus cell hyperplasia and inflammatory cell infiltration (HE, × 200); B3: 28 d. Enlargement and spotty necrosis in germinal centers of lymph nodes, expansion of lymph sinus, hyperplasia of sinus cells and infiltration of neutrophils in lymph sinus (HE, × 200); C: 28 d. Clear follicular structure and fewer necrotic spots in lymph nodes (HE, × 100).

Table 4 Comparison of pathological severity scores for lymph nodes

Groups	7 d	14 d	21 d	28 d
Sham-operation group	0.0 (2.0)[c]	0.0 (1.0)[c]	0.0 (1.0)[c]	0.0 (1.0)
Model control group	1.0 (2.0)	1.0 (1.0)	1.0 (2.0)	2.0 (1.0)
Treatment group	0.0 (0.0)[a]	0.0 (1.0)	0.0 (1.0)[c]	1.0 (0.0)[c]

[a]$P < 0.05$ *vs* sham-operation group; [c]$P < 0.05$ *vs* model control group.

changes were found in the texture of lymph nodes at all time points after operation. Under light microscope, no significant difference in pathological changes was observed at all time points after operation. Enlargement of follicular germinal centers and hyperplasia of sinus cells were seen in most rats and few rats showed no obvious pathological changes in lymph nodes with spotty necrosis in the mantle zone and germinal centers on days 7, 14, 21 and 28 after operation (Figure 2B).

In the treatment group, no significant difference in pathological changes was observed at all time points after operation compared to the model control group. Under light microscope, no obvious difference was found in lymph node pathological changes at all time points after operation. In most rats, enlargement of lymph nodes in germinal centers and hyperplasia of cells in lymph sinus were observed with spotty necrosis of lymph nodes in the mantle zone and germinal centers of (Figure 2C).

The pathological scoring standards for lymph nodes have been described elsewhere[17]. The pathological score was significantly lower for sham-operation group than for model control group on days 7, 14 and 21 after operation ($P < 0.05$). The pathological score was significantly lower for sham-operation group than for treatment group on day 7 after operation ($P < 0.05$) and was significantly lower for treatment group than for model control group on days 21 and 28 after operation ($P < 0.05$, Table 4).

Pathological changes in thymus

In sham-operation group, no significant difference was found in thymus pathological changes at all time points after operation compared to model control group, and the thymus tissue of all rats was grossly normal (Figure 3A).

In model control group, the thymus of rats was mildly shrunken on day 7 after operation, moderately shrunken and jaundiced on day 14 after operation, and severely shrunken and jaundiced on days 21 and 28 after operation. Under light microscope, no significant difference was noted in thymus pathological changes at all time points after operation. The thymus tissue of most rats was grossly normal. An obscure boundary between thymus cortex and medulla was occasionally seen. The thymus tissue was grossly normal on day 14 after operation. The thymus pathological changes were similar on days 14, 21 and 28 after operation (Figure 3B).

In treatment group, no significant difference in thymus pathological changes was observed on days 7 and 14 after operation compared with model control group. The thymus became mildly jaundiced with no obvious shrinkage on days 21 and 28 after operation. Under light microscope, the thymus tissue of most rats was grossly

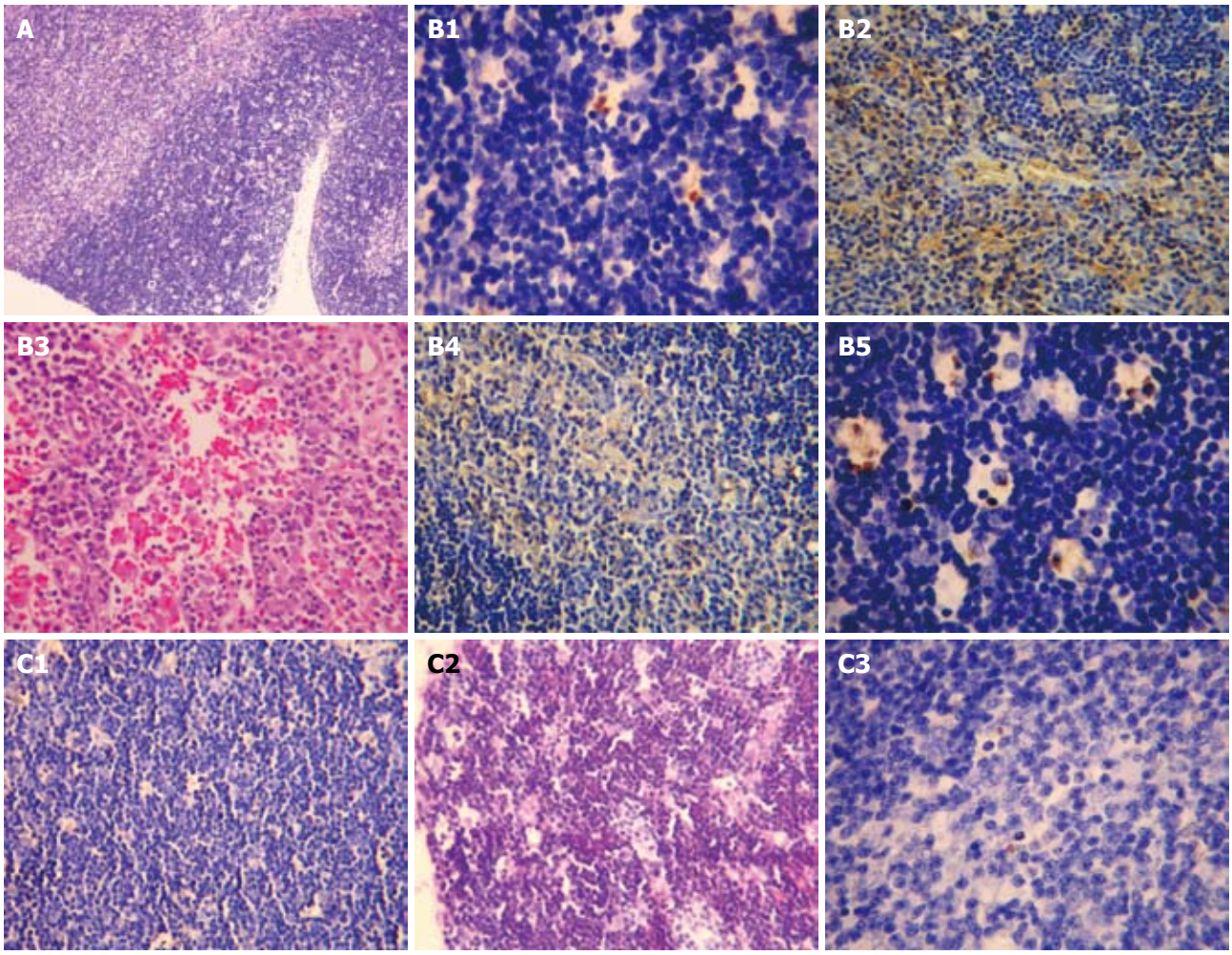

Figure 3 Pathological changes in thymus of sham-operated group (A), model control group (B), and treatment group (C). A1: 28 d. Clear structure of thymus lobules and a clear boundary between thymus cortex and medulla (HE, × 100); B1: 7 d. Sporadic apoptotic cells in thymus (TUNEL, × 400); B2: 21 d. Thymus (++) (NF-κB p65, × 200); B3: 28 d. An obscure boundary between the thymus cortex and medulla with hemorrhage in the medulla (HE, × 200); B4: 28 d. Thymus (++) (Bax, × 200); B5: 28 d. Sporadic apoptotic cells in thymus (TUNEL, × 400); C1: 21 d. Thymus (not shown) (NF-κB p65, × 200); C2: 28 d. Normal thymus (HE, × 200); C3: 28 d. Sporadic apoptotic cells in thymus (TUNEL, × 400).

Table 5 Comparison of different pathological indexes for thymus, *M* (Q_R)

Index	Time (d)	Sham-operation group	Model control group	Treatment group
Product staining intensity and positive rate of Bax	7	0.0 (0.0)[c]	1.0 (2.0)	0.5 (1.0)[a]
	14	0.0 (1.0)	1.0 (2.0)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	1.0 (2.0)	0.0 (2.0)
	28	0.0 (0.0)[c]	2.0 (1.5)	0.0 (1.0)[c]
Apoptosis index	7	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	14	0.0 (0.0)	0.0 (0.01)	0.0 (0.0)[c]
	21	0.0 (0.0)[c]	0.0 (0.01)	0.0 (0.0)[c]
	28	0.0 (0.0)[c]	0.01 (0.02)	0.0 (0.0)
Product of the staining intensity and positive rate of NF-κB	7	0.0 (0.0)	0.0 (2.0)	0.0 (0.0)
	14	0.0 (4.0)	2.0 (4.0)	0.0 (2.0)
	21	0.0 (2.0)	0.0 (2.0)	0.0 (0.0)
	28	0.0 (1.0)	0.0 (3.0)	0.0 (2.0)

[a]$P < 0.05$ *vs* sham-operating group; [c]$P < 0.05$ *vs* model control group.

normal. An obscure boundary between thymus cortex and medulla was seen in few rats (Figure 3C).

The pathological scoring standards for thymus have been described elsewhere [18]. No significant difference was observed in pathological scores for different groups (Table 5).

No significant difference was found in product staining intensity and positive rate of NF-κB protein in thymus of different groups (Table 5).

The product staining intensity and positive rate of Bax protein were significantly lower in sham-operation group than in model control group on days 7, 21 and 28 after operation ($P < 0.05$), in sham-operation group than in treatment group on day 7 after operation ($P < 0.05$), and in treatment group than in model control group on days 14 and 28 after operation ($P < 0.05$).

The apoptosis index for thymus was significantly lower in sham-operation group than in model control group on days 21 and 28 after operation ($P < 0.05$), and in treatment group than in model control group on days 14 and 21 after operation ($P < 0.05$).

DISCUSSION

It has been shown that the incidence rate of endotoxemia in OJ patients is as high as 39.3%[19], which is mainly

due to insufficient intestinal bile salt that leads to excessive multiplication of intestinal bacteria and decreased inactivation of endotoxins. Since the function of reticuloendothelial system is inhibited, gut-derived endotoxins are not effectively eliminated. As a result, large amounts of endotoxin enter into the blood resulting in endotoxemia. Endotoxin is a main factor for immune function impairment during OJ since it can directly stimulate Kupffer cells to release inflammatory mediators, including oxygen free radicals, TNF-α, IL-6 and IL-8, and thereby aggravates the inflammatory response of body[19-24]. TNF-α is the most important factor for mediating toxic effects of endotoxin. Excessive release of TNF-α can induce multiple organ injuries. We speculate that immune function impairment results from the damage to immune organs. The results of this study show that the serum TNF-α level and pathological scores for spleen and lymph nodes were significantly lower in sham-operation and treatment groups than in model control group, suggesting that TNF-α is involved in OJ-induced damage to immune organs, and *Astragalus* injection can significantly lessen the toxic effects of TNF-α on and improve the pathological changes in immune organs. We think that *Astragalus* injection exerts, to a certain degree, its protective effects on immune organs by suppressing the production of TNF-α. Although no statistically significant difference was observed in pathological scores for thymus, the pathological changes in thymus of *Astragalus* treatment group showed varying degrees of improvement compared with model control group. In model control group, the thymus showed varying degrees of jaundice and atrophy at all time points after operation, and an obscure boundary between the thymus cortex and medulla in parts of thymus tissue under light microscope. In contrast, the thymus in treatment group became jaundiced with no atrophy on days 21 and 28 after operation, and the boundary between the thymus cortex and medulla was obscure in few parts of thymus tissue under light microscope, suggesting that *Astragalus* has protective effects on immune organs.

NF-κB p65, a protein that is extensively distributed in cytoplasm of many cells, can regulate gene transcription in nuclei. It is a member of Rel family of transcriptional regulatory proteins and is involved in gene expression regulation of many inflammatory factors. When the body is under stress, NF-κB p65 is activated and binds to specific κB gene sequences, and thereby promotes gene transcription and protein synthesis of pro-inflammatory molecules, causes strong expression of inflammatory cytokines such as TNF-α and IL-6mRNA, accelerates toxic effect on cells in multiple organs, eventually leading to multiple organ dysfunction. Based on the expression levels of NF-κB p65 protein in spleen and thymus, we speculate that *Astragalus* has no inhibitory effects on the expression of NF-κB p65 protein in spleen and thymus of OJ rats.

Apoptosis is a self-protective strategy employed by the body for removal of the destroyed cells through initiating programmed gene expression under certain pathophysiological conditions[25]. In contrast to cell necrosis, apoptosis is an active and spontaneous process and does not induce dramatic inflammatory reaction. However, apoptosis as a mode of cell loss can also induce functional impairment of immune organs. Bax, a soluble protein encoded by a recently discovered apoptosis-promoting gene, shares the same protein family as Bcl-2 and is able to promote cell apoptosis[26,27]. In this study, the expression level of Bax protein was higher in spleen and thymus of model group than in those of sham-operation group. As a result, the apoptosis index was increased and pathological injury was aggravated, suggesting that Bax protein is involved in physiological or pathological cellular apoptosis of spleen and thymus. After treatment with *Astragalus* injection, the pathological changes in immune organs were improved and the expression level of Bax protein in spleen and thymus, apoptosis index and pathological scores for spleen and thymus were significantly lower in treatment group than in model control group, indicating that *Astragalus* injection can down-regulate the expression of Bax protein, suppress cell apoptosis and exert protective effects on immune organs.

In summary, *Astragalus* injection can improve pathological changes in immune organs, reduce serum TNF-α level, down-regulate expression of Bax protein in spleen and thymus, and suppress cell apoptosis, thereby exerting its protective effects on immune organs of OJ rats. Since *Astragalus* has diverse pharmacological actions, low cost and few side effects, it has a better application prospect and economic value.

COMMENTS

Background

Obstructive jaundice (OJ) is a kind of common clinical manifestation. The pathogenesis and treatment of OJ have been a hot topic in medical field for a long time. As systemic inflammatory response syndrome and multiple organ dysfunction syndrome were studied in recent years, immune function impairment concomitant with OJ has gradually attracted wide attention and is considered as a cause of death in OJ patients. Therefore, one of the important approaches to treatment of OJ is to restore the functions of immune organs.

Research frontiers

Development and utilization of traditional Chinese medicine have good prospects in therapy for OJ since it has lower cost, more extensive pharmacological effects and fewer side effects. Since *Astragalus* injection contains polysaccharide, saponin, flavone and trace elements, it has a variety of pharmacological effects and plays an important role in increasing the immunity of body and protecting the liver and kidney. This study demonstrated that *Radix Astragali* could exert its protective effects on immune organs of OJ rats by relieving the pathological changes in immune organs, reducing tumor necrosis factor (TNF)-α level, and inhibiting Bax expression and apoptosis in spleen and thymus.

Innovations and breakthroughs

At present, no studies about the effects of *Astragalus* on immune organs during OJ are available. In the present study, we investigated the protective effect of *Astragalus* injection on immune organs of OJ rats and its mechanism, which may provide an experimental basis for its application in clinical practice.

Applications

Astragalus has diverse pharmacological actions, low cost and few side effects, and thus can be applied in clinical practice.

Terminology

Nuclear factor (NF)-κB p65, a protein that is extensively distributed in cytoplasm of many cells, is able to regulate gene transcription in nuclei. TNF-α

is a most important factor for mediating the toxic effects of endotoxins. Bax, a soluble protein encoded by a recently discovered apoptosis-promoting gene, shares the same protein family as Bcl-2 and is able to promote cell apoptosis.

Peer review

The manuscript describes the protective effects of *Radix astragali* injection on immune organs of rats with OJ. *Radix astragali* injection could reverse elevated TNF-α level, and spleen, thymus and lymph node lesions. Bax immunoreactivity and apoptosis could be observed after obstructive jaundice. This manuscript is largely descriptive by providing novel insights into the mechanism underlying the beneficial effect *of Radix astragali* on obstructive jaundice.

REFERENCES

1 **Ljungdahl M**, Osterberg J, Ransjö U, Engstrand L, Haglund U. Inflammatory response in patients with malignant obstructive jaundice. *Scand J Gastroenterol* 2007; **42**: 94-102

2 **Tsuyuguchi T**, Takada T, Miyazaki M, Miyakawa S, Tsukada K, Nagino M, Kondo S, Furuse J, Saito H, Suyama M, Kimura F, Yoshitomi H, Nozawa S, Yoshida M, Wada K, Amano H, Miura F. Stenting and interventional radiology for obstructive jaundice in patients with unresectable biliary tract carcinomas. *J Hepatobiliary Pancreat Surg* 2008; **15**: 69-73

3 **Comert M**, Ustundag Y, Tekin IO, Gun BD, Barut F. Obstructive jaundice leads to accumulation of oxidized low density lipoprotein in human liver tissue. *World J Gastroenterol* 2006; **12**: 5094-5095

4 **Sano T**, Ajiki T, Takeyama Y, Kuroda Y. Internal biliary drainage improves decreased number of gut mucosal T lymphocytes and MAdCAM-1 expression in jaundiced rats. *Surgery* 2004; **136**: 693-699

5 **Sakrak O**, Akpinar M, Bedirli A, Akyurek N, Aritas Y. Short and long-term effects of bacterial translocation due to obstructive jaundice on liver damage. *Hepatogastroenterology* 2003; **50**: 1542-1546

6 **Veligostkiĭ NN**, Veligotsiĭ AN, Obuobi RB, Okleĭ DV, Maslov SP, Komarchuk VV. [The choice of surgical strategy for patients with obstructive jaundice and high risk of multi-organ insufficiency syndrome] *Klin Khir* 2001; 10-13

7 **Nehéz L**, Andersson R. Compromise of immune function in obstructive jaundice. *Eur J Surg* 2002; **168**: 315-328

8 **Padillo FJ**, Cruz A, Segura-Jiménez I, Ruiz-Rabelo J, Vázquez-Ezquerra MR, Perea-Alvarez MD, Peña J, Briceño J, Muntané J. Anti-TNF-alpha treatment and bile duct drainage restore cellular immunity and prevent tissue injury in experimental obstructive jaundice. *Int J Immunopathol Pharmacol* 2007; **20**: 855-860

9 **Zulfikaroglu B**, Zulfikaroglu E, Ozmen MM, Ozalp N, Berkem R, Erdogan S, Besler HT, Koc M, Korkmaz A. The effect of immunonutrition on bacterial translocation, and intestinal villus atrophy in experimental obstructive jaundice. *Clin Nutr* 2003; **22**: 277-281

10 **Jiang WG**, Puntis MC. Immune dysfunction in patients with obstructive jaundice, mediators and implications for treatments. *HPB Surg* 1997; **10**: 129-142

11 **Yuan W**, Zhang Y, Ge Y, Yan M, Kuang R, Zheng X. Astragaloside IV inhibits proliferation and promotes apoptosis in rat vascular smooth muscle cells under high glucose concentration in vitro. *Planta Med* 2008; **74**: 1259-1264

12 **Gao QT**, Cheung JK, Choi RC, Cheung AW, Li J, Jiang ZY, Duan R, Zhao KJ, Ding AW, Dong TT, Tsim KW. A Chinese herbal decoction prepared from Radix Astragali and Radix Angelicae Sinensis induces the expression of erythropoietin in cultured Hep3B cells. *Planta Med* 2008; **74**: 392-395

13 **Mou S**, Ni ZH, Zhang QY. [Expression of c-met in human kidney fibroblasts induced by high glucose in vitro and the regulation of Radix Astragali] *Zhongxiyi Jiehe Xuebao* 2008; **6**: 482-487

14 **Gui SY**, Wei W, Wang H, Wu L, Sun WY, Chen WB, Wu CY. Effects and mechanisms of crude astragalosides fraction on liver fibrosis in rats. *J Ethnopharmacol* 2006; **103**: 154-159

15 **Mou S**, Ni ZH, Zhang QY. [Expression of c-met in human kidney fibroblasts induced by high glucose in vitro and the regulation of Radix Astragali] *Zhongxiyi Jiehe Xuebao* 2008; **6**: 482-487

16 **Wang Y**, Hu ZQ, Cheng ZG, Zhang LZ, Wang YH. Effect of astragalus on cellular immune function of rats with obstructive jaundice. *Gandan Waike Zazhi* 2000, **8**: 64-66

17 **Zhang XP**, Xu HM, Jiang YY, Yu S, Cai Y, Lu B, Xie Q, Ju TF. Influence of dexamethasone on mesenteric lymph node of rats with severe acute pancreatitis. *World J Gastroenterol* 2008; **14**: 3511-3517

18 **Xiping Z**, Li C, Miao L, Hua T. Protecting effects of dexamethasone on thymus of rats with severe acute pancreatitis. *Mediators Inflamm* 2007; **2007**: 72361

19 **Zhang J**, Liu YH, Jiang XH, Xu KS. Relationship between endotoxemia and cellular immunity in obstructive jaundice. *Huaren Xiaohua Zazhi* 1998; **6**: 305-306

20 **Ding XZ**, Li H, Xiong ST, Zhang SX, Lu KZ, Shao JF, Shen GX, Yang J. Effects of cimetidine on IL-2 and T suppressor cell function in rats with obstructive jaundice. *J Tongji Med Univ* 1994; **14**: 94-97

21 **Zhan SL**. [Clinical study on the immune function of patients with obstructive jaundice] *Zhonghua Waike Zazhi* 1993; **31**: 480-483

22 **Greve JW**, Gouma DJ, Soeters PB, Buurman WA. Suppression of cellular immunity in obstructive jaundice is caused by endotoxins: a study with germ-free rats. *Gastroenterology* 1990; **98**: 478-485

23 **Li YG**, Li QL. Effect of obstructive jaundice on the blood and immune system. *Zhongguo Shiyong Waike Zazhi* 1996; **16**: 17-20

24 **Ji F**, Chen JX, Shi WJ. Changes in the body's immune function in obstructive jaundice complicated with endotoxemia. *Gandanyi Waike Zazhi* 1997; **9**: 100-102

25 **Thatte U**, Dahanukar S. Apoptosis: clinical relevance and pharmacological manipulation. *Drugs* 1997; **54**: 511-532

26 **Wolter KG**, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle RJ. Movement of Bax from the cytosol to mitochondria during apoptosis. *J Cell Biol* 1997; **139**: 1281-1292

27 **Maurer M**, Tsai M, Metz M, Fish S, Korsmeyer SJ, Galli SJ. A role for Bax in the regulation of apoptosis in mouse mast cells. *J Invest Dermatol* 2000; **114**: 1205-1206

S- Editor Tian L **L- Editor** Wang XL **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2870

World J Gastroenterol 2009 June 21; 15(23): 2870-2877
World Journal of Gastroenterology ISSN 1007-9327

ORIGINAL ARTICLES

Alisol B acetate induces apoptosis of SGC7901 cells *via* mitochondrial and phosphatidylinositol 3-kinases/Akt signaling pathways

Yong-Hong Xu, Li-Jie Zhao, Yan Li

Yong-Hong Xu, Li-Jie Zhao, Yan Li, Department of Digestive Diseases, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China
Author contributions: Xu YH and Li Y contributed equally to this work; Xu YH and Li Y designed the research; Xu YH performed the research; Xu YH and Zhao LJ analyzed data; Xu YH wrote the paper; Li Y helped organize, wrote and corrected the paper.
Correspondence to: Yan Li, Professor, Department of Digestive Diseases, Shengjing Hospital, China Medical University, Shenyang 110004, Liaoning Province, China. yanli0227@126.com
Telephone: +86-24-83956416 Fax: +86-24-23582697
Received: March 13, 2009 Revised: May 7, 2009
Accepted: May 14, 2009
Published online: June 21, 2009

Abstract

AIM: To examine the effect of alisol B acetate on the growth of human gastric cancer cell line SGC7901 and its possible mechanism of action.

METHODS: The cytotoxic effect of alisol B acetate on SGC7901 cells was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Phase-contrast and electron microscopy were used to observe the morphological changes. Cell cycle and mitochondrial transmembrane potential ($\Delta\Psi m$) were determined by flow cytometry. Western blotting was used to detect the expression of apoptosis-regulated gene Bcl-2, Bax, Apaf-1, caspase-3, caspase-9, Akt, P-Akt and phosphatidylinositol 3-kinases (PI3K).

RESULTS: Alisol B acetate inhibited the proliferation of SGC7901 cell line in a time- and dose-dependent manner. PI staining showed that alisol B acetate can change the cell cycle distribution of SGC7901, increase the proportion of cells in G0-G1 phase and decrease the proportion of S phase cells and G2-M phase cells. Alisol B acetate at a concentration of 30 μmol/L induced apoptosis after 24, 48 and 72 h incubation, with occurrence rates of apoptotic cells of 4.36%, 14.42% and 21.16%, respectively. Phase-contrast and electron microscopy revealed that the nuclear fragmentation and chromosomal condensed, cells shrank and attachment loss appeared in the SGC7901 treated with alisol B acetate. Apoptosis of SGC7901 cells was associated with cell cycle arrest, caspase-3 and caspase-9 activation, loss of mitochondrial membrane potential and up-regulation of the ratio of Bax/Bcl-2 and inhibition of the PI3K/Akt.

CONCLUSION: Alisol B acetate exhibits an anti-proliferative effect in SGC7901 cells by inducing apoptosis. Apoptosis of SGC7901 cells involves mitochondria-caspase and PI3K/Akt dependent pathways.

Key words: Alisol B acetate; Apoptosis; Mitochondria; Phosphatidylinositol 3-kinases/Akt; SGC7901 cells

Peer reviewer: Zsuzsa Szondy, Professor, Department of Biochemistry and Molecular Biol, University of Debrecen, Debrecen H-4012, Hungary

Xu YH, Zhao LJ, Li Y. Alisol B acetate induces apoptosis of SGC7901 cells *via* mitochondrial and phosphatidylinositol 3-kinases/Akt signaling pathways. *World J Gastroenterol* 2009; 15(23): 2870-2877 Available from: URL: http://www.wjgnet.com/1007-9327/15/2870.asp DOI: http://dx.doi.org/10.3748/wjg.15.2870

INTRODUCTION

Gastric cancer is one of the most common malignancies in mankind and its incidence and mortality rank first in China[1]. Recent data indicate that the mortality of gastric cancer in China is tending to increase and it severely threatens the health and life of people[2]. At present, the management of gastric cancer mainly includes surgery and chemotherapy, but the curative effect of the existing chemotherapeutic drugs is not good enough and they have numerous side effects. Therefore, it has become a focus to search the drugs capable of preventing and treating gastric cancer and other malignancies.

Herbal medicines are an important source of novel agents with pharmaceutical potential. Alisol B acetate is a major ingredient isolated from *Alismatis rhizoma* and has been used for urological diseases in traditional Chinese medicine. In recent years, the pharmacological characterization of alisol B acetate has been identified and several biological activities have been defined, such

as the inhibitory effects on lipopolysaccharide[3], the inhibition of complementary activity[4,5] and antibody-mediated allergic reaction[6]. Furthermore, it has been demonstrated that alisol B acetate induces cell death in hepatoma and leukemia cells[7,8]. Later studies have shown that alisol B acetate induces Bax nuclear translocation and apoptosis in human hormone-resistant prostate cancer PC-3 cells[9].

However, no detailed data are available about the role and mechanisms of alisol B acetate in gastric carcinoma. In order to understand the role and mechanisms of alisol B acetate in the treatment of gastric carcinoma, we investigated the effect of alisol B acetate on the growth of human SGC7901 cells and the underlying intracellular signal transduction pathways involved in regulating apoptosis. We found that alisol B acetate-induced apoptosis is accompanied by the modulation of the Bcl-2 family, mitochondrial dysfunction and activation of caspases; in SGC7901 cells, alisol B acetate induced apoptosis *via* the mitochondrial death pathway; and the apoptosis induced by alisol B acetate was sensitized through inhibition of the PI3K/Akt signaling pathway.

MATERIALS AND METHODS

Materials

Gastric adenocarcinoma cell line (SGC7901) was obtained from our laboratory. Alisol B acetate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Methyl thiazolyl tetrazolium (MTT), propidium iodide (PI), Tris-HCl, and Triton X-100 were obtained from Sigma Chemical Company (St. Louis, USA). Monoclonal antibodies to Bax, Bcl-2, Apaf-1 caspase-3 and caspase-9 were purchased from Santa Cruz Biotechnology Incorporation (Santa Cruz, CA, USA). PVDF membrane was obtained from Bio-Rad (CA, USA). Phospho-Akt (Thr308), Akt, PI3K antibodies were procured from Cell Signaling Technology (Beverly, MA, USA).

Cell culture and treatment with alisol B acetate

Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37℃ humidified atmosphere containing 5% CO_2. The chemical compounds (alisol B acetate) were dissolved in dimethyl sulfoxide (DMSO), and diluted to appropriate concentrations with culture medium. The final concentration of DMSO in the culture medium did not exceed 0.1%.

MTT assay

Cells were plated at a density of 2×10^3/well in 96-well plates. Twenty-four hours later, cells were treated with alisol B acetate at different final concentrations from 10 to 80 μmol/L for 24 h or at 30 μmol/L concentration for the indicated time courses. Control cell cultures were treated with DMSO. After addition of test compounds, 20 μL MTT was added to each well. Four hours later, 100 μL of DMSO was added to each well after the medium was removed. Finally, absorbance was detected with an enzyme calibrator at 570 nm and cell viability= (A of study group/A of control group) × 100%. Experiments were done in triplicate. There were six wells for each concentration.

Morphological changes examined by phase-contrast and electron microscopy

Phase-contrast microscopic studies: SGC7901 cells were grown in 35 mm sterile petri plates and treated with various doses of alisol B acetate for 24 h. Morphological changes were observed under phase-contrast microscope.

Electron microscopy: Cells were cultured with alisol B acetate at a concentration of 30 μmol/L for 24, 48 and 72 h, then fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4), followed by 1% osmium tetroxide. After dehydration, thin sections were stained with uranyl acetate and lead citrate for observation under a JEM 100 CX electron microscope (JEOL, Peabody, NY, USA).

Flow cytometric analysis of cell cycle

Cells were seeded in 6-well plates and treated with alisol B acetate at 30 μmol/L concentration for 24, 48 and 72 h. DMSO (0.1%)-treated cells served as control. After treatment, media were discarded. The adherent cells were washed with PBS, and 300 μL trypsin was added for 5 min at room temperature to detach the cells. Then, the cell suspension was centrifuged at 1500 r/min for 5 min at room temperature. Decanting of all the supernatant was followed by adding 1 mL of 70% methanol to the pellet. After incubation at 4℃ for at least 12 h, prior to the samples being analyzed by the flow cytometry (FCM) (Becton Dickinson), 1 mL of cold PI stain solution (20 g/mL PI, 20 g/mL RNase A, and 0.1% Triton X-100) was added to the mixture and it was incubated for 15 min in darkness at room temperature. The samples were analyzed by FCM (BD FACS CantoTM). The results were analyzed by Mod Fit LT 3.0 software.

Analysis of mitochondrial membrane potential

Changes of mitochondrial membrane potential were monitored by determination of the fluorescence of Rhodamine (Rh)-123. Cells were treated with or without alisol B acetate for the indicated time courses. At the end of treatment, the cells were finally harvested. Rh-123 was added to 1×10^6 cells in 5 mL complete growth medium to a final concentration of 5 g/L and cells were incubated at 37℃ in the dark for 30 min to allow Rh-123 uptake. Rh-123 loaded cells were washed with ice cold PBS and re-suspended in PBS. Changes in mitochondrial transmembrane potential as a result of mitochondrial perturbation were measured after staining with Rh-123[10]. Ten thousand events were examined by a FACSCAN flow cytometer and data were analyzed with the Macintosh Cell Quest software.

Western blotting analysis

After being treated with alisol B acetate for the indicated periods, the cells were washed with PBS and lysed in a buffer containing 20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, 1 mmol/L $NaVO_3$, 100 mmol/L NaF, 10% glycerol, 1mmol/L EGTA, 10 mmol/L sodium pyrophosphate, and 1 mmol/L phenylmethylsulfonyl fluoride, pH 7.5. Cell lysates were centrifuged at 12000 × *g* for 60 min at 4℃. The protein concentrations were determined using Bio-Rad protein assay (Bio-Rad Laboratories, USA). After SDS-PAGE, proteins were transferred to PVDF membranes for 2 h at 80 mA. The PVDF membrane was treated with TBST containing 50 g/L skimmed milk at room temperature for 2 h, followed by incubation with the first antibodies caspase-3, caspase-9, Apaf-1, Bcl-2, Bax, Akt, P-Akt, PI3K, respectively, at 4℃ overnight. After being washed with TBST for 30 min, the corresponding secondary antibody was added and incubated at room temperature for 1 h. The membrane was then washed three times for 15 min each with TBST and visualized with diaminobenzidine. Quantification of protein was detected with a Lumivision IMAGER (Aisin Seiki, Aichi). Each value represents the mean of triple experiments, and is presented as the relative density of protein bands normalized to β-actin.

Statistical analysis

Data were expressed as mean ± SD. Statistical correlation of data was checked for significance by ANOVA and Student's *t* test. Differences with $P < 0.05$ were considered significant. These analyses were performed using SPSS 11.0 software.

RESULTS

Alisol B acetate inhibited SGC7901 cell proliferation

To investigate the growth inhibition effects of alisol B acetate, the cells were treated with various concentrations of alisol B acetate for 24 h and 30 μmol/L for 8, 16, 24, 48 and 72 h. As shown in Figure 1A, cell viability was decreased remarkably after the cells were treated with 30, 50, 70 and 80 μmol/L alisol B acetate for 24 h. Only a minor inhibition of SGC7901 cell growth was observed in the presence of 20 μmol/L alisol B acetate. Growth was inhibited by more than 40% in cells exposed to 30 μmol/L alisol B acetate after 24, 48 and 72 h. Alisol B acetate had significant growth inhibitory effects on SGC7901 cells in a dose- and time-dependent manner. A concentration of 30 μmol/L alisol B acetate was used in all further experiments.

Alisol B acetate induces apoptosis

SGC7901 cells were treated with alisol B acetate (0, 30, 50 and 70 μmol/L) for 24 h, and phase-contrast microscopy revealed that some cells became round, blunt and smaller in size; light refraction was increased; and cells became detached and suspended in the medium, especially with 50 and 70 μmol/L alisol B acetate. In the control group, cells were regular in morphology and grew fully in patches and confluently, rarely sloughing off (Figure 2).

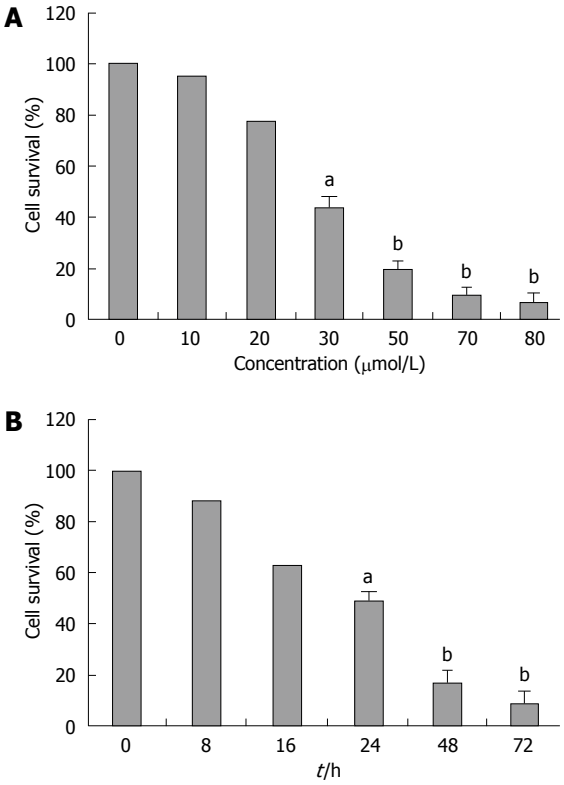

Figure 1 Effect of alisol B acetate on survival of SGC7901 cells. Cells were incubated in the absence or presence of various concentrations of alisol B acetate for 24 h (A), or at 30 μmol/L for different incubation times (B). $^aP < 0.05$, $^bP < 0.01$ *vs* control group (unpaired Student's *t* test).

The ultrastructural and morphological changes were also observed under electron microscope. As shown in Figure 3B and C, nuclear fragmentation, chromosome condensation and cell shrinkage were visible. Subsequent formation of apoptotic bodies were also observed (Figure 3D).

FCM with only PI staining showed (Figure 4) that treatment of SGC7901 cells with 30 μmol/L alisol B acetate for 72 h resulted in a higher number of cells in the G0/G1 phase (79.61%) compared with the control (40.46%). This increase was coupled with the decreased percentage of cells in S phase. After 72 h treatment, the percentage of S phase in alisol B acetate -treated cells was 16.55%, whereas 48.45% in the control cells. In addition, flow cytometric analysis also revealed the effect of alisol B acetate on the induction of apoptosis. As shown in Figure 4, the percentage of the sub/G1 fraction in alisol B acetate-treated cells was increased in a time-dependent manner, indicative of apoptotic cell death.

Alisol B-induced apoptosis is associated with mitochondrial pathways

Mitochondria played a major role in apoptosis triggered by many stimuli. The early loss of mitochondrial membrane potential is a hallmark of apoptosis[11]. We examined the effect of alisol B acetate on mitochondrial

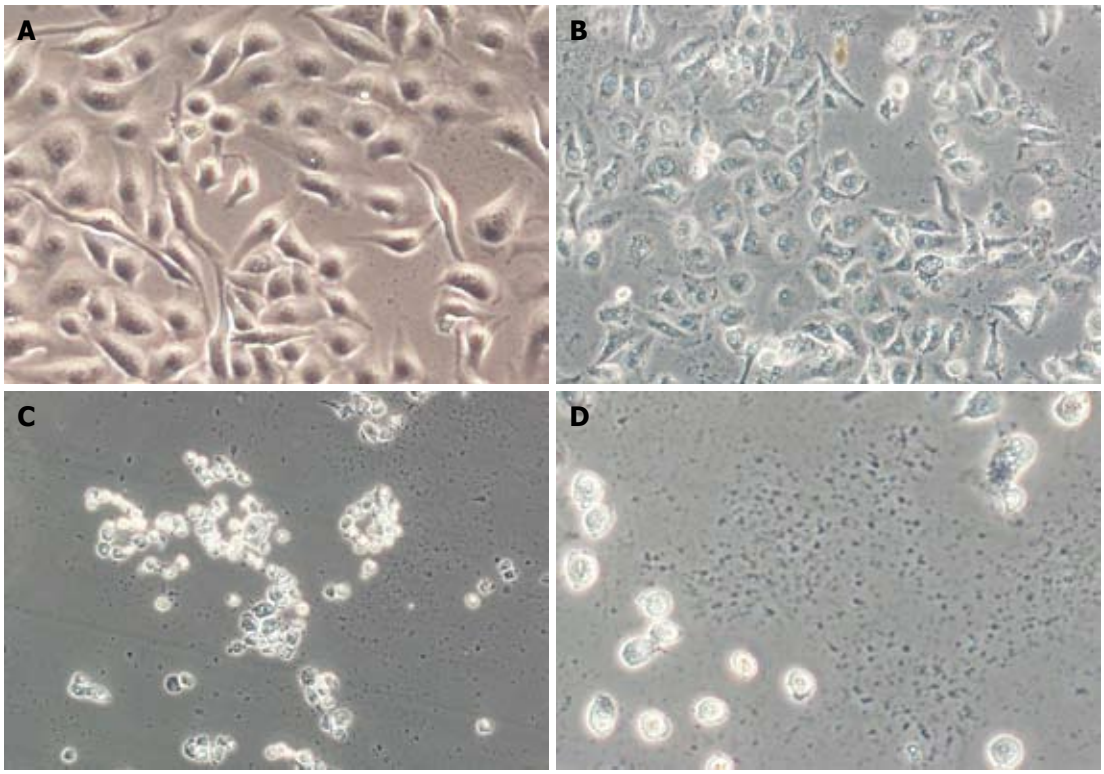

Figure 2 Morphology of SGC7901 cells exposed to alisol B acetate for different concentrations observed under phase-contrast microscope. A: Controls; B-D: SGC7901 cells were treated with 30, 50 and 70 μmol/L alisol B acetate for 24 h.

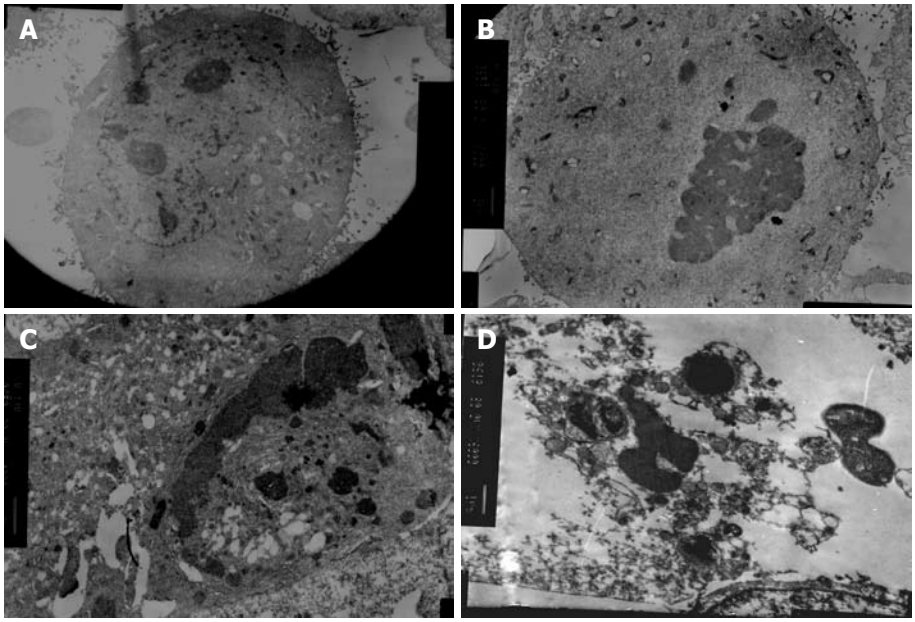

Figure 3 Morphological changes observed under electronic microscope. A: Control; B-D: Cells were incubated with 30 μmol/L alisol B acetate for 24, 48 and 72 h.

membrane potential by means of the potential sensitive Rh-123. After treatment of SGC7901 cells with 30 μmol/L alisol B acetate for 12, 24, 48 and 72 h, flow cytometric data revealed that disruption of mitochondrial membrane potential was 17.75%, 29.87%, 43.32% and 70.00%, respectively, while it was only 4.05% in the control group. The data demonstrated that alisol B acetate induced a time-dependent decrease in mitochondrial membrane potential, indicating the participation of mitochondria-related mechanism (Figure 5).

Up-regulation of Apaf-1 and Bax, activation of caspase-3 and caspase-9

To investigate the mechanism underlying apoptosis induced by alisol B acetate, we tested the effect of this compound on Bcl-2, Bax levels, two important regulators of apoptotic signaling pathways[12]. As shown in Figure 6, Western blotting analysis revealed that alisol B acetate -induced apoptosis did not alter Bcl-2 expression, but resulted in a time-dependent up-regulation of Bax expression, with a maximal up-

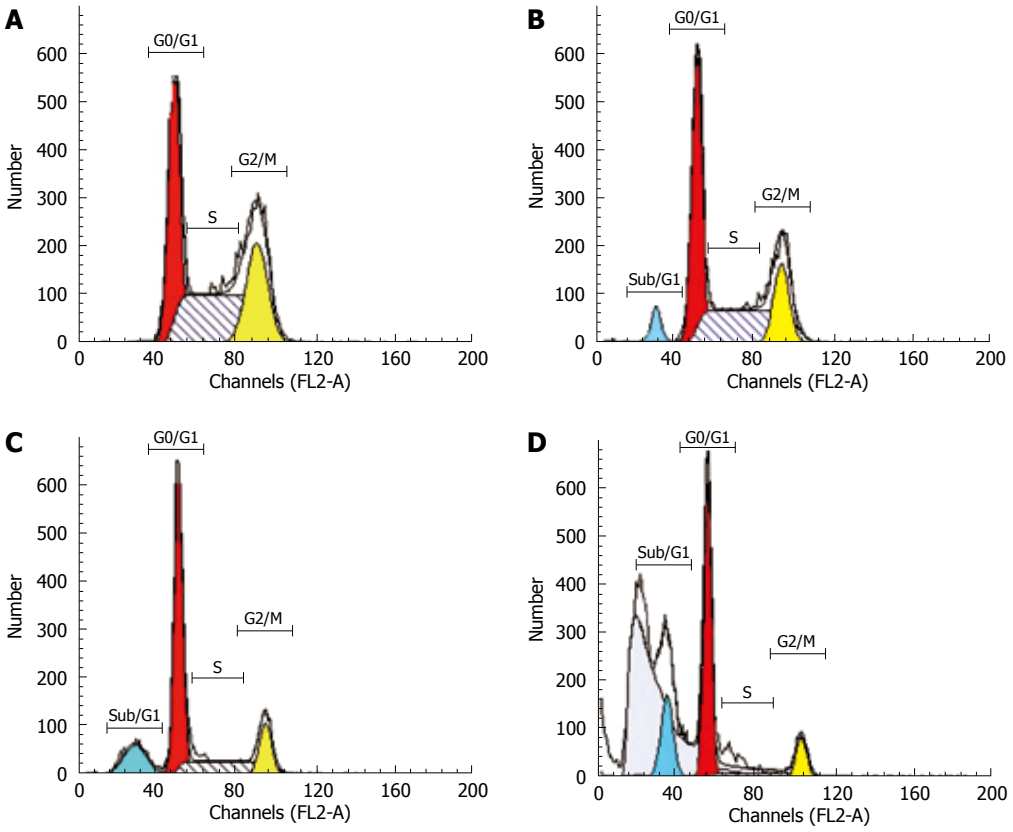

Figure 4 Effect of alisol B acetate on cell cycle distribution of SGC7901 cells. SGC7901 cells were treated with 0.1% DMSO (A) or with 30 μmol/L alisol B acetate for 24 h (B), 48 h (C), or 72 h (D). The cells were collected sequentially and stained with PI and analyzed by FCM.

regulation at 72 h after treatment that was associated with increased levels of Apaf-1, and activated caspases-3 and caspase-9. These results suggest that alisol B acetate induces SGC7901 apoptosis, at least partly, through up-regulation of pro-apoptotic Bax, resulting in up-regulation of the Bax/Bcl-2 ratio and activation of caspase-3 and caspase-9.

Effects of alisol B acetate on PI3K/Akt pathway

Akt, a major downstream target of PI3K, may be the best-characterized kinase known to promote cellular survival[13] and is dysregulated (mainly overexpressed) in a wide spectrum of human cancers, including gastric, hepatoma, and ovarian cancers[14-16]. To determine whether regulation of the Akt signal pathway is necessary for alisol B acetate-induced apoptosis, we investigated the expression of PI3K and Akt after treatment with 30 μmol/L Alisol B acetate. As shown in Figure 7, the levels of PI3K and phosphorylated Akt are time-dependently decreased in response to alisol B acetate. PI3K and phosphorylated Akt were rapidly decreased at 24 h, while the total Akt protein levels remained constant during alisol B acetate treatment. Therefore, these results suggested that Akt/PKB is associated with the survival of SGC7901 gastric cancer cells and inhibition of the PI3K/Akt signaling pathway increased the apoptosis induced by the alisol B acetate.

DISCUSSION

Apoptosis plays an important role in developmental processes, maintenance of homeostasis, and elimination of the damaged cells. Accumulated data indicate that many anticancer drugs can cause the death of tumor cells through the induction of apoptosis[15,17]. Alisol B acetate induces cell apoptosis in hepatoma and leukemia cells[7,8]. However, the effects of alisol B acetate on human gastric cells are still unclear. Therefore, the purpose of the present study was to find out the molecular mechanism of alisol B acetate underlying human gastric cancer cell line SGC7901.

In the present study, we first demonstrated that SGC7901 cells treated with alisol B acetate showed a dose- and time-dependent inhibition of the proliferation. Nuclear fragmentation, chromosome condensation, cell shrinkage and formation of apoptotic bodies were also observed. Flow cytometric analysis revealed that alisol B acetate treatment results in an increase of apoptotic cells. These results suggest that alisol B acetate-induced apoptosis contributes to the growth inhibition of SGC7901 cells.

Mitochondrion plays a critical role in apoptosis induced by some drugs[11]. The mitochondrial death pathway is associated with changes in the permeability of the outer mitochondrial membrane, the collapse of membrane potential[18,19]. Mitochondrial membrane permeability is mainly controlled by the Bcl-2 family of proteins, through regulation of the formation of apoptotic protein-conducting pores in the outer mitochondrial membrane[20-22]. Members of the Bcl-2 family proteins can be divided into two subfamilies; one is anti-apoptotic protein such as Bcl-2 and Bcl-X_L, the other is pro-apoptotic protein such as Bax, Bad, and Bid[23]. The expression of Bcl-2 and Bax is significantly involved in the balance of pro-apoptotic and anti-apoptotic signals at the mitochondrial level[24].

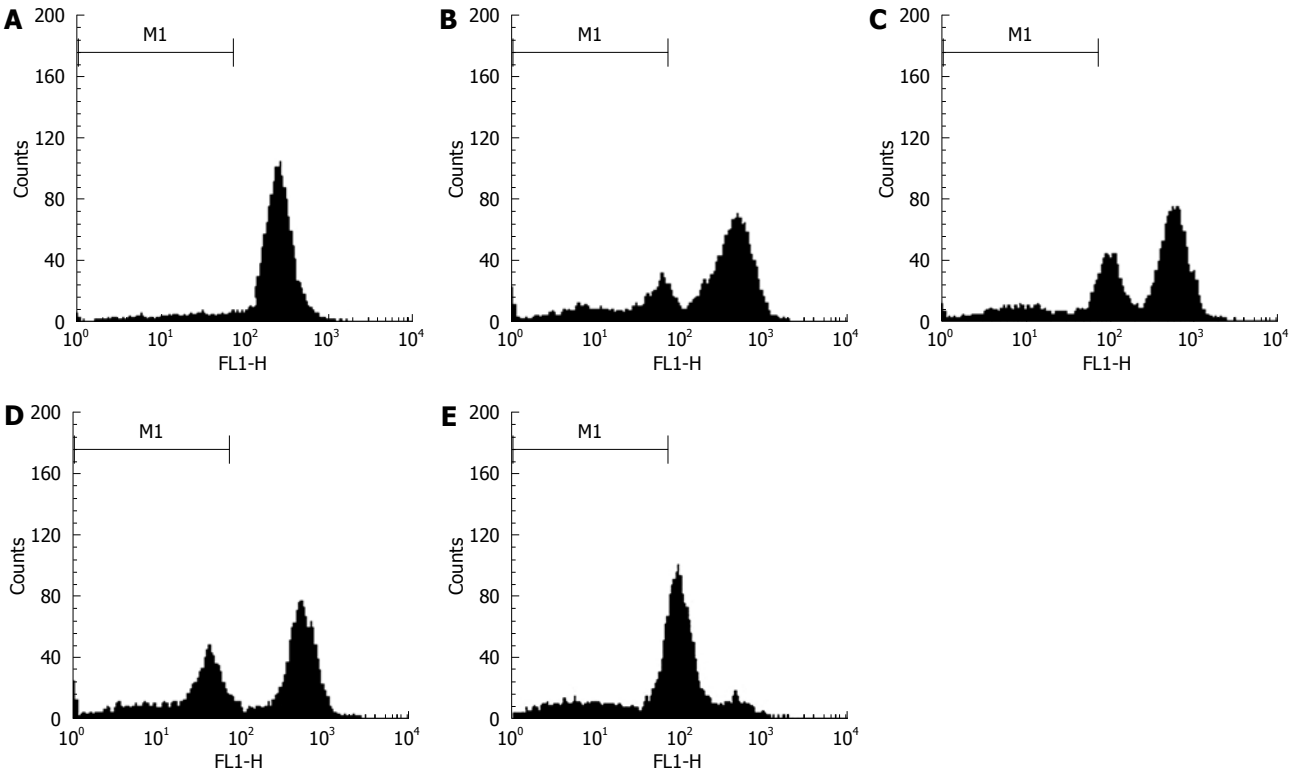

Figure 5 FCM analysis of mitochondrial membrane potential in human SGC7901 cells with 30 μmol/L alisol B acetate for various time periods. The zero concentration was defined as control. The percentage of cells stained with Rh-123 was determined by FCM as described in the Materials and Methods. A: Control; B-E: 12, 24, 48 and 72 h.

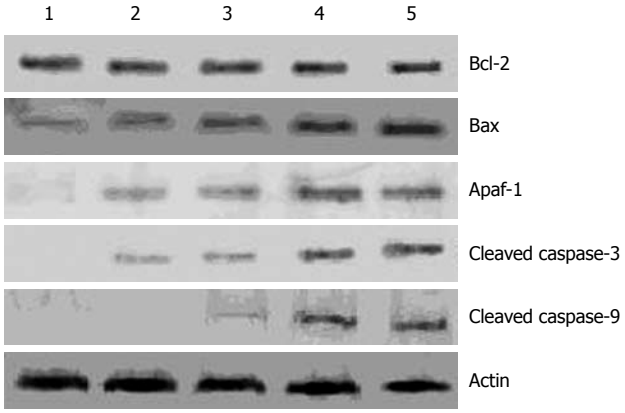

Figure 6 Effect of Alisol B acetate on the expression of Bcl-2, Bax, Apaf-1, cleaved caspase-3 and caspase-9. SGC7901 cells were treated with 30 μmol/L alisol B acetate for 12, 24, 48 and 72 h. The cells were collected and lysed. Western blotting analysis was conducted and probed with antibodies to Bcl-2, Bax, Apaf-1, caspase-3 and caspase-9. Lanes (from left to right): Control cells; 12 h; 24 h; 48 h; 72 h.

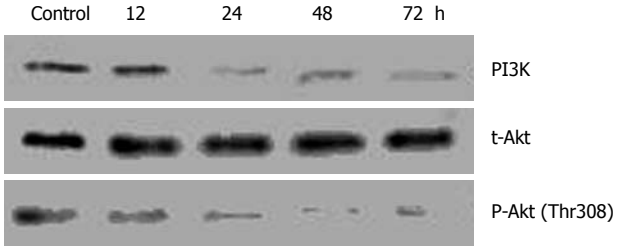

Figure 7 Effect of alisol B acetate on PI3K/Akt activation in SGC7901 cells. SGC7901 cells were treated with 0.1% DMSO (A) or 30 μmol/L alisol B acetate for 12, 24, 48 and 72 h. Western blotting analysis for PI3K, t-Akt and P-Akt was performed using specific antibodies with β-actin as a loading control.

The ratio of Bax/Bcl-2 is critical for the induction of apoptosis and this ratio determines whether cells will undergo apoptosis[25,26]. An increase in the ratio of Bax/Bcl-2 stimulates the release of cytochrome c from mitochondria into the cytosol. The cytosolic cytochrome c then binds to Apaf-1, leading to the activation of caspase-9, caspase-3 and poly (ADP-ribose) polymerase[27,28]. Thus, we examined the effect of alisol B acetate on Bax, Bcl-2 and mitochondrial membrane potential. In our experiments, alisol B acetate increased pro-apoptotic Bax expression without affecting Bcl-2 expression, leading to up-regulation of the ratio between pro-apoptotic (Bax) and anti-apoptotic (Bcl-2). This may be responsible for the concomitant execution phase of apoptosis that we observed, which included disruption of mitochondrial membrane potential.

Caspases, a family of cysteine proteases, are integral parts of the apoptotic pathway. Caspase-9 is the apical caspase in the intrinsic or mitochondria-initiated apoptosis pathway and requires the release of cytochrome C from the mitochondria as well as interactions with Apaf-1[18]. Activation of caspase-3 correlated with activation of caspase-9. Caspase-3 in particular, when activated, has many cellular targets[29]. During apoptosis, caspase-3 is one of the key executioners of apoptosis in response to various stimuli[30]. In many studies, it has been determined that a variety of chemotherapeutic agents induce apoptosis through the activation of caspases[15]. Previous studies have observed that alisol B

acetate induces apoptosis in human hepatoma Hep3B cells and human hormone-resistant prostate cancer PC-3 cells through an increase of caspase-3 and caspase-9 activity[7,9]. Consistent with an increase in the ratio of Bax/Bcl-2 and disruption of mitochondrial membrane potential, this study showed that alisol B acetate resulted in a time dependent up-regulation of Apaf-1, and activation of caspase-9 and caspase-3.

According to the present data, alisol B acetate may first increased the ratio of Bax/Bcl-2, which leads to disruption of mitochondrial membrane potential, and then activates mitochondria-mediated downstream molecular events including cytochrome c release and sequential activation of caspase-9 and caspase-3. These results demonstrated that a mitochondrial damage-mediated caspase pathway might be involved in alisol B acetate -induced apoptosis of SGC7901 cells.

The PI3K pathway regulates several cellular processes, including proliferation, growth[31], apoptosis[32] and cytoskeletal rearrangement. Akt, a major downstream target of PI3K, was originally reported as the cellular counterpart of the viral oncogene, which was amplified in gastric adenocarcinoma[14]. Overexpression of Akt isoforms has now been reported in ovarian, breast, prostate, and pancreatic cancers[16,33-35]. In addition, Akt activity is increased in cancers where the PTEN tumor suppressor is mutated[36]. In recent years, a number of cancer researchers have focused on the central importance of the PI3K/Akt pathway, and therapeutic strategies that target the PI3K/Akt pathway are now being developed[15,37]. PI3K consists of p85 regulatory subunit and a p110 catalytic subunit. Akt is activated by recruitment to the plasma membrane though direct contact of its Plecpstrinhomology domain with PIP3, and phosphorylation at Thr308 and Ser473. Thr308 is phosphorylated by the 3-phosphoinositide-dependent protein kinase PDKI[38]. Through its interaction with proteins in the Bcl-2 family such as BAD, Bax, Bcl-X_L and their downstream effectors, Akt provides a survival signal to the cell[39]. Additionally, Akt blocks cytochrome C release from the mitochondria through regulation of Bcl-2[40] and inhibits the catalytic activity of a pro-death protease, caspase 9 through phosphorylation[41].

In our study, we evaluated the effect of alisol B acetate on the PI3K/Akt pathways by measuring the PI3K and total Akt protein and sequential expression levels of phospho-Akt. We observed that alisol B acetate markedly inhibited PI3K and phospho-Akt after 24 h of treatment. No additional reduction in PI3K and phospho-Akt levels was seen when treatment was prolonged to 72 h and the total Akt protein levels remained constant throughout the course of the experiment. Meanwhile, our data showed that alisol B acetate treatment up-regulates Bax protein and down-regulates mitochondrial membrane potential. Furthermore, our data also showed activation of caspase-9 and caspase-3 in alisol B acetate-treated groups. Inhibition of PI3K/Akt signaling pathway is possibly preceded by modulations in Bax protein, leading to mitochondrial damage and activation of caspase-9, caspase-3 in favor of apoptosis.

In conclusion, we have demonstrated that alisol B acetate significantly induces apoptosis. This apoptotic response is associated with the up-regulation of the ratio of Bax/Bcl-2, loss of mitochondrial membrane and caspase activation. Moreover, the inhibition of PI3K/Akt pathway may play an important role in alisol B acetate-induced apoptosis. Therefore, we believe that alisol B acetate might be a promising molecule in cancer chemoprevention or chemotherapy; and further efforts to explore this therapeutic strategy are necessary.

COMMENTS

Background

Alisol B acetate is a major ingredient isolated from Alismatis rhizome. In recent years, alisol B acetate has shown various biological activities, including inhibitory effects on lipopolysaccharide, the inhibition of complementary activity, antibody-mediated allergic reaction and anti-tumor effects. However, its molecular mechanisms in the anti-tumor effects have not been well documented.

Research frontiers

Studies have confirmed that many Chinese herbs have anti-tumor properties and induce apoptosis. In the process of cell apoptosis induced by drugs, mitochondria plays a great role. phosphatidylinositol 3-kinases (PI3K)/Akt pathway is important in the development and proliferation of various human cancers. In recent years, a number of cancer researchers have focused on the importance of the PI3K/Akt pathway, and therapeutic strategies that target the PI3K/Akt pathway are being developed. In the present work, the authors investigated the effect of alisol B acetate on human SGC7901 cell proliferation, mitochondrial and PI3K/Akt signaling pathways.

Innovations and breakthroughs

The present study shows that alisol B acetate induces apoptosis and decreases proliferation in human gastric cancer cells. It was shown for the first time that alisol B acetate induced human gastric cancer apoptosis by modulation of the Bcl-2 family, loss of mitochondrial membrane potential, activation of caspases and inhibition of PI3K/Akt pathway.

Applications

The data of this article demonstrate the anti-cancer properties of alisol B acetate as well as its mechanism of action. By knowing the mechanism of action of alisol B acetate, it may provide a new therapeutic option, as a potential anticancer agent in the treatment of gastric cancer.

Terminology

PI3K is a major regulator of cell proliferation located in the Akt upstream, and together PI3K and Akt define a signal transduction pathway important in the pathogenesis of many different cancer types. Alisol B acetate is a natural herbal substance.

Peer review

This is a carefully performed study with novel findings that alisol B acetate has the potential for therapeutic application in gastric cancer. It examines the effect of alisol B acetate on the growth of human gastric cancer cell line and its possible mechanism of action. This paper is well-written. The experiments were well-designed and well-executed.

REFERENCES

1 **Sun XD**, Mu R, Zhou YS, Dai XD, Qiao YL, Zhang SW, Huangfu XM, Sun J, Li LD, Lu FZ. 1990-1992 mortality of stomach cancer in China. *Zhonghua Zhongliu Zazhi* 2002; **24**: 4-8

2 **Sun XD**, Mu R, Zhou YS, Dai XD, Zhang SW, Huangfu XM, Sun J, Li LD, Lu FZ, Qiao YL. Analysis of mortality rate of stomach cancer and its trend in twenty years in China. *Zhonghua Zhongliu Zazhi* 2004; **26**: 4-9

3 **Matsuda H**, Kageura T, Toguchida I, Murakami T, Kishi A, Yoshikawa M. Effects of sesquiterpenes and triterpenes from the rhizome of Alisma orientale on nitric oxide production in lipopolysaccharide-activated macrophages:

absolute stereostructures of alismaketones-B 23-acetate and -C 23-acetate. *Bioorg Med Chem Lett* 1999; **9**: 3081-3086
4 **Lee SM**, Kim JH, Zhang Y, An RB, Min BS, Joung H, Lee HK. Anti-complementary activity of protostane-type triterpenes from Alismatis rhizoma. *Arch Pharm Res* 2003; **26**: 463-465
5 **Matsuda H**, Tomohiro N, Yoshikawa M, Kubo M. Studies on Alismatis Rhizoma. II. Anti-complementary activities of methanol extract and terpene components from Alismatis Rhizoma (dried rhizome of Alisma orientale). *Biol Pharm Bull* 1998; **21**: 1317-1321
6 **Kubo M**, Matsuda H, Tomohiro N, Yoshikawa M. Studies on Alismatis rhizoma. I. Anti-allergic effects of methanol extract and six terpene components from Alismatis rhizoma (dried rhizome of Alisma orientale). *Biol Pharm Bull* 1997; **20**: 511-516
7 **Chou CC**, Pan SL, Teng CM, Guh JH. Pharmacological evaluation of several major ingredients of Chinese herbal medicines in human hepatoma Hep3B cells. *Eur J Pharm Sci* 2003; **19**: 403-412
8 **Chen HW**, Hsu MJ, Chien CT, Huang HC. Effect of alisol B acetate, a plant triterpene, on apoptosis in vascular smooth muscle cells and lymphocytes. *Eur J Pharmacol* 2001; **419**: 127-138
9 **Huang YT**, Huang DM, Chueh SC, Teng CM, Guh JH. Alisol B acetate, a triterpene from Alismatis rhizoma, induces Bax nuclear translocation and apoptosis in human hormone-resistant prostate cancer PC-3 cells. *Cancer Lett* 2006; **231**: 270-278
10 **Scaduto RC Jr**, Grotyohann LW. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J* 1999; **76**: 469-477
11 **Liu MJ**, Wang Z, Li HX, Wu RC, Liu YZ, Wu QY. Mitochondrial dysfunction as an early event in the process of apoptosis induced by woodfordin I in human leukemia K562 cells. *Toxicol Appl Pharmacol* 2004; **194**: 141-155
12 **Wong WW**, Puthalakath H. Bcl-2 family proteins: the sentinels of the mitochondrial apoptosis pathway. *IUBMB Life* 2008; **60**: 390-397
13 **Brazil DP**, Hemmings BA. Ten years of protein kinase B signalling: a hard Akt to follow. *Trends Biochem Sci* 2001; **26**: 657-664
14 **Staal SP**. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci USA* 1987; **84**: 5034-5037
15 **Lah JJ**, Cui W, Hu KQ. Effects and mechanisms of silibinin on human hepatoma cell lines. *World J Gastroenterol* 2007; **13**: 5299-5305
16 **Cheng JQ**, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci USA* 1992; **89**: 9267-9271
17 **Edderkaoui M**, Odinokova I, Ohno I, Gukovsky I, Go VL, Pandol SJ, Gukovskaya AS. Ellagic acid induces apoptosis through inhibition of nuclear factor kappa B in pancreatic cancer cells. *World J Gastroenterol* 2008; **14**: 3672-3680
18 **Martinou JC**, Green DR. Breaking the mitochondrial barrier. *Nat Rev Mol Cell Biol* 2001; **2**: 63-67
19 **Wang X**. The expanding role of mitochondria in apoptosis. *Genes Dev* 2001; **15**: 2922-2933
20 **Adams JM**, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998; **281**: 1322-1326
21 **Cory S**, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2002; **2**: 647-656
22 **Gross A**, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999; **13**: 1899-1911
23 **Robertson JD**, Orrenius S. Molecular mechanisms of apoptosis induced by cytotoxic chemicals. *Crit Rev Toxicol* 2000; **30**: 609-627
24 **Cory S**, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* 2003; **22**: 8590-8607
25 **Tang DG**, Porter AT. Target to apoptosis: a hopeful weapon for prostate cancer. *Prostate* 1997; **32**: 284-293
26 **Reed JC**. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. *Curr Opin Oncol* 1995; **7**: 541-546
27 **Yang J**, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 1997; **275**: 1129-1132
28 **Kluck RM**, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 1997; **275**: 1132-1136
29 **Cohen GM**. Caspases: the executioners of apoptosis. *Biochem J* 1997; **326** (Pt 1): 1-16
30 **Tewari M**, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS, Dixit VM. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 1995; **81**: 801-809
31 **Klippel A**, Reinhard C, Kavanaugh WM, Apell G, Escobedo MA, Williams LT. Membrane localization of phosphatidylinositol 3-kinase is sufficient to activate multiple signal-transducing kinase pathways. *Mol Cell Biol* 1996; **16**: 4117-4127
32 **Kauffmann-Zeh A**, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J, Evan G. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* 1997; **385**: 544-548
33 **Tacheau C**, Fontaine J, Loy J, Mauviel A, Verrecchia F. TGF-beta induces connexin43 gene expression in normal murine mammary gland epithelial cells via activation of p38 and PI3K/AKT signaling pathways. *J Cell Physiol* 2008; **217**: 759-768
34 **Wang S**, Yang Q, Fung KM, Lin HK. AKR1C2 and AKR1C3 mediated prostaglandin D2 metabolism augments the PI3K/Akt proliferative signaling pathway in human prostate cancer cells. *Mol Cell Endocrinol* 2008; **289**: 60-66
35 **Middleton G**, Ghaneh P, Costello E, Greenhalf W, Neoptolemos JP. New treatment options for advanced pancreatic cancer. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 673-696
36 **Yamada KM**, Araki M. Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis. *J Cell Sci* 2001; **114**: 2375-2382
37 **Morgan TM**, Koreckij TD, Corey E. Targeted therapy for advanced prostate cancer: inhibition of the PI3K/Akt/mTOR pathway. *Curr Cancer Drug Targets* 2009; **9**: 237-249
38 **Stokoe D**, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science* 1997; **277**: 567-570
39 **Datta SR**, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 1997; **91**: 231-241
40 **Davies MA**, Koul D, Dhesi H, Berman R, McDonnell TJ, McConkey D, Yung WK, Steck PA. Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC/PTEN. *Cancer Res* 1999; **59**: 2551-2556
41 **Cardone MH**, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC. Regulation of cell death protease caspase-9 by phosphorylation. *Science* 1998; **282**: 1318-1321

S- Editor Tian L **L- Editor** Ma JY **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2878

World J Gastroenterol 2009 June 21; 15(23): 2878-2881
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Comparison of reflux esophagitis and its complications between African Americans and non-Hispanic whites

Kenneth J Vega, Sian Chisholm, M Mazen Jamal

Kenneth J Vega, Sian Chisholm, Divisions of Gastroenterology and General Internal Medicine, University of Florida Health Sciences Center, Jacksonville, Florida 32207, United States
M Mazen Jamal, Gastroenterology Section, Veterans Affairs Medical Center, 5901 E. 7th St., Long Beach, CA 90822, United States
Author contributions: Vega KJ and Jamal MM designed the research and analyzed the data, Vega KJ and Chisholm S performed the research, Vega KJ, Chisholm S and Jamal MM wrote the manuscript.
Correspondence to: Kenneth J Vega, MD, Associate Professor, Divisions of Gastroenterology and General Internal Medicine, University of Florida Health Sciences Center, Jacksonville, Florida 32207, United States. kenneth.vega@jax.ufl.edu
Telephone: +1-904-6330087 Fax: +1-904-6330028
Received: March 9, 2009 Revised: May 7, 2009
Accepted: May 14, 2009
Published online: June 21, 2009

Abstract

AIM: To determine the effect of ethnicity on the severity of reflux esophagitis (RE) and its complications.

METHODS: A retrospective search of the endoscopy database at the University of Florida Health Science Center/Jacksonville for all cases of reflux esophagitis and its complications from January 1 to March 31, 2001 was performed. Inclusion criteria were endoscopic evidence of esophagitis using the LA classification, reflux related complications and self-reported ethnicity. The data obtained included esophagitis grade, presence of a hiatal hernia, esophageal ulcer, stricture and Barrett's esophagus, and endoscopy indication.

RESULTS: The search identified 259 patients with RE or its complications, of which 171 were non-Hispanic whites and 88 were African Americans. The mean ages and male/female ratios were similar in the two groups. RE grade, esophageal ulcer, stricture and hiatal hernia frequency were likewise similar in the groups. Barrett's esophagus was present more often in non-Hispanic whites than in African Americans (15.8% *vs* 4.5%; $P < 0.01$). Heartburn was a more frequent indication for endoscopy in non-Hispanic whites with erosive esophagitis than in African Americans (28.1% *vs* 7.9%; $P < 0.001$).

CONCLUSION: Distribution of RE grade and frequency of reflux-related esophageal ulcer, stricture and hiatal hernia are similar in non-Hispanic whites and African Americans. Heartburn was more frequently and nausea/vomiting less frequently reported as the primary endoscopic indication in non-Hispanic whites compared with African Americans with erosive esophagitis or its complications. African Americans have a decreased prevalence of Barrett's esophagus compared with non-Hispanic whites.

Key words: Reflux esophagitis; African American; Hiatal hernia; Barrett's esophagus

Peer reviewers: Siegfried Wagner, Professor, Medizinische Klinik II, Klinikum Deggendorf, Perlasberger Str. 41, Deggendorf 94469, Germany; Dr. Katerina Dvorak, Research Assistant Professor, Cell Biology and Anatomy, The University of Arizona, 1501 N. Campbell Ave, Tucson 85724, United States

Vega KJ, Chisholm S, Jamal MM. Comparison of reflux esophagitis and its complications between African Americans and non-Hispanic whites. *World J Gastroenterol* 2009; 15(23): 2878-2881 Available from: URL: http://www.wjgnet.com/1007-9327/15/2878.asp DOI: http://dx.doi.org/10.3748/wjg.15.2878

INTRODUCTION

Multiple studies suggest that the frequency of gastroesophageal reflux disease (GERD) complications such as erosive esophagitis, stricture and Barrett's esophagus (BE) is significantly lower in the US minority populations compared with non-Hispanic whites (nHw)[1-6]. Two studies from Veterans Affairs Medical Centers observed that severe GERD affected older nHw more commonly than non-whites[2,3]. Both of these investigations used the Department of Veterans Affairs patient treatment file as the data source.

Two groups of investigators have attempted to determine the frequency of GERD complications between ethnic groups seen at their institutions[7,8]. In one study[7], African Americans (AA), nHw and Asians significantly differed in heartburn prevalence, while the other[8] indicated that AA and nHw had equivalent heartburn prevalence rates. Both studies revealed that AA had decreased rates of erosive esophagitis compared with nHw. The prevalence of BE was only assessed in one investigation with the overwhelming majority seen in nHw.

Regarding BE, limited data exists about the prevalence of this entity in the United States minority populations. Initial studies compared non-Hispanic whites with African Americans. These revealed a predominant presence of BE in nHw when compared with AA[7-9]. In contrast, a report from the National Cancer Institute indicated that the incidence of esophageal adenocarcinoma is increasing in AA[10]. BE is considered the precursor lesion of esophageal adenocarcinoma, therefore one could speculate that the incidence of BE might also be increasing within the AA population.

There is minimal data evaluating the prevalence of GERD complications in any defined general population other than non-Hispanic whites[11,12]. The goal of this study is to compare the severity of reflux esophagitis and its complications in AA and nHw patients who underwent endoscopy at our institution.

MATERIALS AND METHODS

Patient population

A retrospective search of the endoscopy laboratory report database was performed to determine the total number of patients having upper gastrointestinal endoscopy (EGD) at the University of Florida Health Science Center/Jacksonville from 1 January to 31 March 2001. Inclusion criteria were endoscopic evidence of reflux esophagitis, esophageal stricture and/or ulcer, BE, and self reported ethnicity. Exclusion criteria were previous diagnostic EGD, history of non-GERD esophageal condition with the potential to cause esophageal injury (acquired immunodeficiency syndrome, esophageal infections, caustic ingestion, and thoracic radiation), and absence of demographic information within the patient record. The study was approved by the Institutional Review Board of the University of Florida Health Science Center/Jacksonville.

Symptom evaluation

Indication for EGD was recorded on the individual reports by all endoscopists. If multiple indications were listed, the first indication listed was used at the primary reason for performing the procedure. Patients with an indication such as follow-up of any previously noted esophageal lesion were not included in the analysis.

Classification of endoscopic findings

Esophagitis was graded using the Los Angeles system[13,14]. This scheme categorizes mucosal injury as follows: grade A defined as one or more mucosal breaks no longer than 5 mm which do not extend between the tops of two mucosal folds; grade B defined as one or more mucosal breaks more than 5 mm long that do not extend between the tops of two mucosal folds; grade C defined as one or more breaks that are continuous between the tops of two or more mucosal folds but involve < 75% of the esophageal circumference; and grade D defined as one or more mucosal breaks that involve at least 75% of the esophageal circumference. Four-quadrant biopsy specimens were taken at 2 cm intervals from any visible length of columnar lined esophagus to assess the presence of histologic BE. Biopsy sections were then stained with Alcian blue to detect the presence of specialized intestinal metaplasia, the characteristic feature of BE.

Esophageal stricture was defined as a narrowing of the esophageal lumen, which either did not allow passage of a 9 mm endoscope or allowed distal passage of the endoscope with difficulty. The length of the narrowing was measured using the 5 cm endoscope length markings as a reference. Hiatal hernia was defined as the presence of gastric mucosa above the level of the esophageal hiatus. The hernia length was measured using the 5 cm markings as a reference. Hiatal hernia length was considered small if < 2 cm, medium from 2 to 5 cm, and large if greater than 5 cm. The hernia was defined as sliding if the stomach re-entered the abdominal cavity during the endoscopy. Esophageal ulcer was defined as an excavation of the esophageal mucosa of at least 3 mm wide. The ulcer margin had to be raised from the base by at least 1 mm. Location of the ulcer was noted as distal, mid or proximal esophagus.

Table 1 Demographic data of the study population

	AA (n = 88)	nHw (n = 171)
Male/female (%)	38/62	48/52
Age range (yr)	26-101	18-90
Mean age (mean ± SD)	58.5 ± 16.9	55.8 ± 14.5

P = NS for all, NS represents not significant.

Statistical analysis

All values of esophagitis distribution frequency, presence of stricture, esophageal ulcer, hiatal hernia, BE and procedure indication were reported as percentage present for each group. Student's t-test was used for comparisons between groups. Differences between groups will be considered significant if $P < 0.05$. Data analysis was performed using JMP 5.0 for Windows (SAS Institute Inc., Cary, NC).

RESULTS

Demographics of the study population

During the study period, 716 patients had an EGD and 259 of the 716 patients met the criteria for study inclusion. Of the study group, 171 (66%) were nHw and 88 (34%) were AA. Males comprised 48% of the nHw and 38% of the AA groups, respectively. The groups were similar in age and gender distribution (Table 1).

Indication for endoscopy

Table 2 illustrates the indication for EGD between ethnic groups. Heartburn was noted as the primary indication significantly more frequently in nHw than in AA patients (nHw 28.1% and AA 8%; $P < 001$). Nausea and/or vomiting were noted significantly more frequently in AA than nHw patients (nHw 2.9% and AA 9%; $P < 0.04$). Other indications for the procedure were similar between ethnic groups.

Table 2 Indication for endoscopy by ethnicity *n* (%)

Indication	88 AA patients	171 nHw patients	*P* value
Heartburn	7 (8)	48 (28.1)	< 0.001
Dysphagia	14 (15.9)	37 (21.6)	NS
Upper GI bleeding	22 (25)	25 (14.6)	NS
Nausea/vomiting	8 (9.1)	5 (2.9)	< 0.040
Abdominal pain	15 (17)	31 (18.1)	NS
Abnormal X-ray	3 (3.4)	4 (2.3)	NS
Anemia	8 (9.1)	10 (5.9)	NS
Weight loss	4 (4.5)	2 (1.2)	NS
Other	7 (8)	9 (5.3)	NS

Reflux esophagitis grade distribution

Of the 259 patients, 204 had evidence of reflux esophagitis on EGD. Of those with esophagitis, 76 were AA and 128 were nHw. The distribution of esophagitis grade among those with esophagitis in both ethnic groups is illustrated in Table 3. There was no difference observed between the two groups regarding the severity of erosive esophagitis seen.

Table 3 Distribution of reflux esophagitis grade by ethnicity

Ethnicity	LA classification grade			
	A	B	C	D
AA (%) (*n* = 76)	63.2	18.4	7.9	10.5
nHw (%) (*n* = 128)	71.1	12.5	7.0	9.4

P = NS.

Table 4 Prevalence of other endoscopic findings by ethnicity *n* (%)

	AA patients	nHw patients	*P* value
Hiatal hernia	18 (20.4)	39 (22.8)	NS
Stricture	2 (2.2)	10 (5.8)	NS
Ulcer	7 (7.9)	12 (7)	NS
Endoscopic Barrett's esophagus	4 (4.5)	27 (15.8)	< 0.01
Histological Barrett's esophagus	0 (0)	9 (5.3)	0.03

Prevalence of other esophageal findings

Table 4 demonstrates the prevalence of hiatal hernia and endoscopic complications of GERD observed between ethnic groups. With regard to presence of hiatal hernia, esophageal stricture and ulcer, no difference was observed between the groups. However, endoscopic and histologic BE were present significantly more frequently in nHw than AA patients (endoscopic BE: nHw 15.8% and AA 4.5%, $P < 0.01$; histologic BE: nHw 5.8% and AA 0%, $P < 0.04$).

DISCUSSION

The current study comparing the spectrum of reflux esophagitis between nHw and AA patients was designed to test the hypothesis that reflux esophagitis severity and its complications vary between nHw and AA at EGD. The results indicate that the distribution of severity and reflux related complications, other than BE, are similar between AA and nHw patients. This similarity in reflux esophagitis severity, presence of hiatal hernia and complications of reflux esophagitis (except for BE) observed between ethnic groups has not been previously described[2,7,8]. Also, the primary indication for the diagnostic procedure (heartburn in nHw and upper GI bleeding in AA) revealed differences between AA and nHw patients not noted previously. Both of these factors provide insight into reflux disease within the Unites States as a whole and specifically in the African American community.

Similarities and differences in reported GERD symptoms between ethnicities have been reported previously[7,8]. Spechler and colleagues noted that AA complained, understood and met predefined criteria for heartburn more frequently than was observed in either nHw or Asian patients in the metropolitan Boston area. However, El Serag and associates observed that the occurrence of weekly heartburn and/or regurgitation was no different between AA, nHw and a multiethnic group who were all employees at the Houston VA Medical Center. Complicating this further was the use of a survey (GERQ) in the investigation of El Serag *et al* to assess GERD symptoms, which had only been previously validated in nHw or Spaniards[15,16]. It is well recognized that medical communication differs between AA and nHw[17]. Using a tool not validated in African Americans might have led to underestimation of the prevalence of reflux symptoms in that group. This is also suggested by the difference in clinical indication for EGD observed between AA and nHw in the present investigation.

The difference observed in the prevalence of endoscopic BE between AA and nHw in the present investigation corresponds with previous reports in the literature[7,18,19]. The prevalence of histologically confirmed BE among nHw patients is also consistent with the single publication that specifically addressed that issue[19]. The finding that endoscopic BE was only confirmed in 1/3 of cases strongly supports the need for histology in assessing BE.

There are limitations of this investigation that should be recognized. Only those who were referred and presented for EGD were eligible for inclusion in this study. As suggested by previous reports[7,8], AA patients may have not been referred for endoscopy as frequently. This could have led to an underestimation of reflux-related endoscopic disease in that group. The effect of obesity was not accounted for in our study. Multiple studies have indicated an association linking increasing body mass index and presence of reflux symptoms in women, in addition to existence of obesity as a likely risk factor in males[20,21]. A negative association between *H pylori* presence and GERD symptoms is well established[22]. Unfortunately, biopsies of the antrum were not routinely taken in our study, therefore the presence and impact of *H pylori* colonization could not be adequately assessed.

In summary, the results of this study indicate that AA have a similar distribution of esophagitis severity and complications from GERD when compared with nHw. However, the presence of Barrett's esophagus is more common in nHw than AA. Also, it appears that AA and nHw patients with reflux esophagitis and its complications on endoscopy have different indica-

tions for EGD. The reasons for the observed difference in procedure indication and the development of histologic Barrett's esophagus between racial groups are not currently known. However, our data suggests that ethnicity may influence both symptoms leading to endoscopy and the development of BE, a premalignant change in the esophagus due to GERD. Further ethnic-specific investigations are needed to completely understand the lower prevalence of Barrett's esophagus between African Americans and non-Hispanic whites.

COMMENTS

Background

There is minimal data evaluating the prevalence of gastroesophageal reflux disease (GERD) complications in any United States general population other than non-Hispanic whites (nHw). Presently, it is thought that such complications occur less frequently in African Americans (AA) than non-Hispanic whites.

Research frontiers

Barrett's esophagus is a well-recognized complication of GERD and is the principal risk factor for esophageal adenocarcinoma. It is well known that Barrett's esophagus is more frequently discovered in nHw than in other United States ethnic groups. However, differences in other GERD associated conditions between ethnic groups have not been well evaluated. In this study, the authors demonstrate reflux esophagitis and its complications, except for Barrett's esophagus, occur in an equal distribution between AA and nHw patients.

Innovations and breakthroughs

Recent reports have highlighted discrepancies in the prevalence of Barrett's esophagus. However, minimal data exists on the frequency of reflux esophagitis and its complications in communities with a significant component of African Americans. This is the first study to report that reflux esophagitis and its complications, other than Barrett's esophagus, occur at a similar frequency in nHw and AA. In addition, indication for the index endoscopy appears to be different in the above ethnic groups.

Applications

By understanding GERD and its complications among ethnic groups in the United States, this study might indicate future avenues for investigation to prevent the development of Barrett's esophagus and esophageal adenocarcinoma.

Terminology

Barrett's esophagus is the antecedent neoplastic lesion associated with the development of esophageal adenocarcinoma. Incidence of esophageal adenocarcinoma is 3-4 times more frequent in non-Hispanic whites than African Americans.

Peer review

In this paper the authors evaluated the effect of ethnicity on the severity of reflux esophagitis. It deals with an important subject. However, as an endoscopic study, it is limited by lack of information regarding body mass index, access to medication, type of medication used for GERD and socioeconomic status of the different ethnic groups seen.

REFERENCES

1 **Sonnenberg A**, Massey BT, Jacobsen SJ. Hospital discharges resulting from esophagitis among Medicare beneficiaries. *Dig Dis Sci* 1994; **39**: 183-188

2 **el-Serag HB**, Sonnenberg A. Associations between different forms of gastro-oesophageal reflux disease. *Gut* 1997; **41**: 594-599

3 **el-Serag HB**, Sonnenberg A. Opposing time trends of peptic ulcer and reflux disease. *Gut* 1998; **43**: 327-333

4 **Lind T**, Havelund T, Carlsson R, Anker-Hansen O, Glise H, Hernqvist H, Junghard O, Lauritsen K, Lundell L, Pedersen SA, Stubberod A. Heartburn without oesophagitis: efficacy of omeprazole therapy and features determining therapeutic response. *Scand J Gastroenterol* 1997; **32**: 974-979

5 **Venables TL**, Newland RD, Patel AC, Hole J, Wilcock C, Turbitt ML. Omeprazole 10 milligrams once daily, omeprazole 20 milligrams once daily, or ranitidine 150 milligrams twice daily, evaluated as initial therapy for the relief of symptoms of gastro-oesophageal reflux disease in general practice. *Scand J Gastroenterol* 1997; **32**: 965-973

6 **Galmiche JP**, Barthelemy P, Hamelin B. Treating the symptoms of gastro-oesophageal reflux disease: a double-blind comparison of omeprazole and cisapride. *Aliment Pharmacol Ther* 1997; **11**: 765-773

7 **Spechler SJ**, Jain SK, Tendler DA, Parker RA. Racial differences in the frequency of symptoms and complications of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2002; **16**: 1795-1800

8 **El-Serag HB**, Petersen NJ, Carter J, Graham DY, Richardson P, Genta RM, Rabeneck L. Gastroesophageal reflux among different racial groups in the United States. *Gastroenterology* 2004; **126**: 1692-1699

9 **Smith RR**, Hamilton SR, Boitnott JK, Rogers EL. The spectrum of carcinoma arising in Barrett's esophagus. A clinicopathologic study of 26 patients. *Am J Surg Pathol* 1984; **8**: 563-573

10 **Sarr MG**, Hamilton SR, Marrone GC, Cameron JL. Barrett's esophagus: its prevalence and association with adenocarcinoma in patients with symptoms of gastroesophageal reflux. *Am J Surg* 1985; **149**: 187-193

11 **Chalasani N**, Wo JM, Hunter JG, Waring JP. Significance of intestinal metaplasia in different areas of esophagus including esophagogastric junction. *Dig Dis Sci* 1997; **42**: 603-607

12 **Brown LM**. The role of race/ethnicity in the epidemiology of esophageal cancer. *J Assoc Acad Minor Phys* 2000; **11**: 32-37

13 **Armstrong D**, Bennett JR, Blum AL, Dent J, De Dombal FT, Galmiche JP, Lundell L, Margulies M, Richter JE, Spechler SJ, Tytgat GN, Wallin L. The endoscopic assessment of esophagitis: a progress report on observer agreement. *Gastroenterology* 1996; **111**: 85-92

14 **Lundell LR**, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, Johnson F, Hongo M, Richter JE, Spechler SJ, Tytgat GN, Wallin L. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut* 1999; **45**: 172-180

15 **Locke GR 3rd**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456

16 **Moreno Elola-Olaso C**, Rey E, Rodriguez-Artalejo F, Locke GR 3rd, Diaz-Rubio M. Adaptation and validation of a gastroesophageal reflux questionnaire for use on a Spanish population. *Rev Esp Enferm Dig* 2002; **94**: 745-758

17 **Johnson RL**, Roter D, Powe NR, Cooper LA. Patient race/ethnicity and quality of patient-physician communication during medical visits. *Am J Public Health* 2004; **94**: 2084-2090

18 **Lieberman D**, Fennerty MB, Morris CD, Holub J, Eisen G, Sonnenberg A. Endoscopic evaluation of patients with dyspepsia: results from the national endoscopic data repository. *Gastroenterology* 2004; **127**: 1067-1075

19 **Abrams JA**, Fields S, Lightdale CJ, Neugut AI. Racial and ethnic disparities in the prevalence of Barrett's esophagus among patients who undergo upper endoscopy. *Clin Gastroenterol Hepatol* 2008; **6**: 30-34

20 **Jacobson BC**, Somers SC, Fuchs CS, Kelly CP, Camargo CA Jr. Body-mass index and symptoms of gastroesophageal reflux in women. *N Engl J Med* 2006; **354**: 2340-2348

21 **Kulig M**, Nocon M, Vieth M, Leodolter A, Jaspersen D, Labenz J, Meyer-Sabellek W, Stolte M, Lind T, Malfertheiner P, Willich SN. Risk factors of gastroesophageal reflux disease: methodology and first epidemiological results of the ProGERD study. *J Clin Epidemiol* 2004; **57**: 580-589

22 **Raghunath A**, Hungin AP, Wooff D, Childs S. Prevalence of Helicobacter pylori in patients with gastro-oesophageal reflux disease: systematic review. *BMJ* 2003; **326**: 737

S- Editor Tian L **L- Editor** Stewart GJ **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2882

World J Gastroenterol 2009 June 21; 15(23): 2882-2886
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Factors associated with patient absenteeism for scheduled endoscopy

Victor K Wong, Hong-Bin Zhang, Robert Enns

Victor K Wong, Robert Enns, St. Paul's Hospital, University of British Columbia, Vancouver V6Z-2K5, BC, Canada
Hong-Bin Zhang, Centre for Health Evaluation and Outcomes Sciences, St. Paul's Hospital, University of British Columbia, Vancouver V6Z-2K5, BC, Canada
Author contributions: Wong VK and Enns R contributed to drafting of the article; Enns R contributed to the conception and design of the study and revision of the article, and Zhang HB contributed to statistical analysis of the data.
Correspondence to: Dr. Robert Enns, Clinical Associate Professor of Medicine, St. Paul's Hospital, University of British Columbia, #770-1190 Hornby St., Vancouver V6Z-2K5, BC, Canada. renns@interchange.ubc.ca
Telephone: +1-604-6886332-222 Fax: +1-604-6892004
Received: January 18, 2009 Revised: May 11, 2009
Accepted: May 18, 2009
Published online: June 21, 2009

Abstract

AIM: To identify risk factors to help predict which patients are likely to fail to appear for an endoscopic procedure.

METHODS: This was a retrospective, chart review, cohort study in a Canadian, tertiary care, academic, hospital-based endoscopy clinic. Patients included were: those undergoing esophagogastroduodenoscopy, colonoscopy or flexible sigmoidoscopy and patients who failed to appear were compared to a control group. The main outcome measure was a multivariate analysis of factors associated with truancy from scheduled endoscopic procedures. Factors analyzed included gender, age, waiting time, type of procedure, referring physician, distance to hospital, first or subsequent endoscopic procedure or encounter with gastroenterologist, and urgency of the procedure.

RESULTS: Two hundred and thirty-four patients did not show up for their scheduled appointment. Compared to a control group, factors statistically significantly associated with truancy in the multivariate analysis were: non-urgent *vs* urgent procedure (OR 1.62, 95% CI 1.06, 2.450), referred by a specialist *vs* a family doctor (OR 2.76, 95% CI 1.31, 5.52) and office-based consult prior to endoscopy *vs* consult and endoscopic procedure during the same appointment (OR 2.24, 95% CI 1.33, 3.78).

CONCLUSION: Identifying patients who are not scheduled for same-day consult and endoscopy, those referred by a specialist, and those with non-urgent referrals may help reduce patient truancy.

Key words: Absenteeism; Colonoscopy; Endoscopy; Esophagogastroduodenoscopy; Gastroenterologist

Peer reviewer: Atsushi Nakajima, Professor, Division of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan

Wong VK, Zhang HB, Enns R. Factors associated with patient absenteeism for scheduled endoscopy. *World J Gastroenterol* 2009; 15(23): 2882-2886 Available from: URL: http://www.wjgnet.com/1007-9327/15/2882.asp DOI: http://dx.doi.org/10.3748/wjg.15.2882

INTRODUCTION

Patient absenteeism from scheduled outpatient appointments is a major problem for all ambulatory clinics. Failure to attend an appointment results in inefficiency because the vacant appointment interval is often not used by another patient. This typically results in ongoing expenditures without concomitant reimbursement thereby decreasing appropriate resource utilization. This is particularly important for endoscopic procedures where a specific interval is scheduled and appropriate preparation is required for each patient. If a patient does not attend the prearranged endoscopy time, often the time is simply absorbed into the rest of the day without an appropriate substitute being found.

There are a number of maneuvers that various clinics have used in an attempt to decrease truancy from endoscopic appointments. Some sites notify all patients within a few days of their scheduled time, others will insist that the patient themselves confirm their appointment, other sites even "over-book" the endoscopy unit to account for a small percentage that will not appear on their scheduled day. Various methods such as telephone[1] or text message[2] reminders and mailed pre-procedural pamphlets[3] have been used successfully to decrease truancy from endoscopic appointments. Very little research has been devoted to enhancing our

understanding of why patients do not appear at their scheduled appointments, and that which has been done has demonstrated conflicting results[4-8].

Research based in adolescent outpatient clinics have found that telephone reminders the day before the scheduled appointment help to reduce "no-show" rates[9]. Other studies have shown that such reminder systems do not improve patient attendance rates[10,11]. One prospective study found that previous non-attendance for an outpatient appointment was the strongest predictor of future non-attendance behavior[8]. There has been limited research into the explanations or reasons for patient absenteeism for scheduled gastroenterology appointments[12]. If patients could be identified as "high-risk" for absenteeism, then specific targeted efforts could be developed to ensure their appropriate appearance at their procedure. The objective of this study is to identify risk factors that may help predict which patients are the most likely to be truant for a scheduled elective endoscopic procedure.

MATERIALS AND METHODS

This study involved all consecutive patients scheduled to undergo an elective esophagoduodenoscopy (EGD), flexible sigmoidoscopy or colonoscopy in a single Canadian tertiary care, gastroenterology clinic (hospital based) in the year 2003. A retrospective chart review was performed to identify all patients who did not appear for their scheduled outpatient endoscopic procedure and they were compared to a control group (randomly selected patients from the same time period who did show at their appointment) to generate predictors of patient absenteeism. It was felt that it was unnecessary, and in fact not practical, to assess all patients during the entire year that did appear for their examination. By using a random sample (selected from a similar time period as the truant group) comparison between the two groups was deemed statistically appropriate. Patients referred from other hospitals and those undergoing endoscopic retrograde cholangiopancreatography (ERCP) or endoscopic ultrasound (EUS) were excluded.

The factors analyzed included gender, age, duration of time on a waiting list, time of day of procedure (07:30-10:00, 10:00-12:30, 12:30-15:00), day of the week, type of procedure, referring physician (family physician *vs* other specialist), distance to hospital (divided into regional areas), whether the patient went direct to the endoscopy suite for a consult and endoscopy during the same appointment without consulting the gastroenterologist in his/her outpatient clinic prior to the procedure, urgency of the procedure (urgent procedures were defined as patients who were bleeding or who had radiological abnormalities warranting an endoscopic procedure) and whether the patient was undergoing a repeat procedure by the previous gastroenterologist or surgeon. Univariate analysis was then performed to determine independent associations of each factor to patient "no-shows". Odds ratios (OR), 95% confidence interval (95% CI) and their respective *P*-values were generated. Following this, multivariate analysis was performed using logistic regression analysis. Only factors that were statistically significant in multivariate analysis were reported in the model. The SPSS software package for Windows (Release 15.0.0-6 Sept 2006; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Ethics approval was obtained through St Paul's Hospital, University of British Columbia to conduct the study.

Table 1 Prevalence of patient characteristics among patient's who were and were not truant for scheduled endoscopic procedure

Factor		Number of patients (*n*)		
		Control group[1]	No-show	Total patients evaluated
Urgent procedure	No	219	198	417
	Yes	99	50	149
Direct to endoscopy	No	235	187	422
	Yes	81	75	156
Referring doctor	No referral	9	17	26
	GP	293	235	528
	Specialist	19	33	52
Sex	Female	176	152	328
	Male	150	133	283
Time of procedure	7:30-10:00	125	87	212
	10:00-12:30	115	106	221
	12:30-15:00	86	92	178
Type of procedure	Colonoscopy	177	129	306
	EGD	109	107	216
	Flex-sig	40	49	89
Weekday	Monday	53	61	114
	Tuesday	58	65	123
	Wednesday	72	56	128
	Thursday	73	61	134
	Friday	70	42	112
Distance living from hospital	Within 10 miles	201	194	395
	Within 60 miles	106	74	180
	Beyond 60 miles	18	13	31
Previous endoscopy	No	132	104	236
	Yes	184	144	328
New patient	No	153	135	288
	Yes	161	133	294

[1]Control group refers to a random sample of patients during the same interval who appeared at their appointment as scheduled; GP: General practitioner; Flex-sig: Flexible sigmoidoscopy.

RESULTS

Table 1 shows the prevalence of patient characteristics that were analyzed as potential predictive factors for truancy for scheduled endoscopic procedure. The absenteeism rate was 3.6% (n = 234) overall, 2.6% for colonoscopy, 2.9% for EGD and 4.3% for flexible sigmoidoscopy. Of the 234 patients, 50% were scheduled for colonoscopy, 35% for EGD and 15% for flexible sigmoidoscopy.

Univariate analysis was performed on each factor to determine the possibility of independent associations with patient "no shows" (Table 2, Figure 1) In the

Table 2 Univariate analysis of patient characteristics and their predictive value for patient absenteeism from scheduled endoscopic procedure

	Comparison	OR	95% CI	*P*-value
Urgency	Non-urgent *vs* urgent	1.790	1.211, 2.645	0.0035
Direct to endoscopy	Not direct *vs* direct	0.859	0.595, 1.242	0.4199
Referring physician	Specialist *vs* GP	2.165	1.200, 3.906	0.0063
	No referral *vs* GP	2.355	1.031, 5.379	
Age	Increments of 10 years	0.912	0.823, 1.010	0.0757
Sex	Female *vs* male	0.974	0.708, 1.340	0.8714
Time of procedure	7:30-10:00 *vs* 12:30-15:00	0.651	0.423, 1.001	0.0835
	10-12:30 *vs* 12:30-15:00	0.967	0.637, 1.468	
Type of procedure	Colonoscopy *vs* flex-sig	0.741	0.439, 1.250	0.2757
	EGD *vs* flex-sig	0.964	0.558, 1.667	
Weekday	Tues *vs* Mon	1.000	0.580, 1.723	0.9570
	Wed *vs* Mon	0.754	0.439, 1.293	
	Thurs *vs* Mon	0.809	0.476, 1.373	
	Fri *vs* Mon	0.493	0.278, 0.873	
Region	< 10 mile radius *vs* < 60 mile radius	1.537	0.685, 3.448	0.1110
	< 60 mile radius *vs* > 60 mile radius	1.054	0.454, 2.449	
Previous endoscopy	Yes *vs* no	1.039	0.738, 1.464	0.8252
Waiting time	3 mo increments	0.835	0.675, 1.034	0.0980
New patient	No *vs* yes	1.293	0.921, 1.815	0.1369

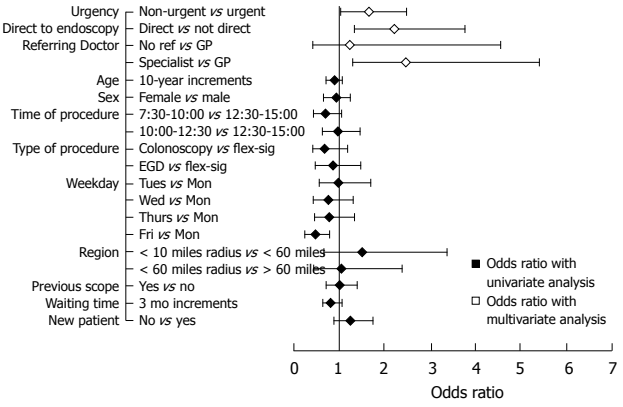

Figure 1 Predictive value for patient absenteeism of variables assessed in univariate (black lines) and multivariate (white lines) analyses. Three factors were statistically associated with patient absenteeism in the multivariate analysis: (1) patients referred for non-urgent *vs* urgent procedures (OR 1.62, 95% CI 1.06, 2.50); (2) patients referred by a specialist *vs* a family doctor (OR 2.76, 95% CI 1.38, 5.52); and (3) patients undergoing office-based consult prior to endoscopy *vs* consult and endoscopic procedure during the same appointment (OR 2.24, 95% CI 1.33, 3.78). In the univariate analysis, patients were more likely to show up for their scheduled appointment on Fridays, however, this was not significant in the multivariate analysis. GP: General practitioner; Flex-sig: Flexible sigmoidoscopy.

univariate analysis, a significant trend was determined towards truancy in those non-urgently referred (OR 1.79, 95% CI 1.2-2.6) and those referred from specialists (as opposed to family physicians) (OR 2.1, 95% CI 1.2-3.9). Interestingly, in the univariate analysis, having their procedure performed on Friday (as opposed to Monday to Thursday) was protective against truancy (OR 0.493, 95% CI 0.28-0.87).

Multivariate analysis was then performed to determine which factors were most associated with a positive outcome (Table 3, Figure 1). In the multivariate analysis three factors were statistically significant determinants in predicting "no shows": (1) patients referred to the clinic for a non-urgent compared to urgent procedures (OR 1.624, 95% CI 1.06, 2.45); (2) patients referred by a specialist compared to those referred by a family doctor (OR 2.76, 95% CI 1.31, 5.524); (3) patients who had an office-based consult prior to the endoscopy as compared to those who went direct to the endoscopy suite for a consult and procedure during the same appointment (OR 2.244, 95% CI 1.33, 3.78). In the multivariate analysis, day of the week the procedure was performed was no longer significant. Figure 1 summarizes the findings of the univariate analysis and the significant factors on the multivariate analysis.

Table 3 Multivariate analysis of patient characteristics and their predictive value for patient absenteeism from scheduled endoscopic procedure

	Comparison	OR	95% CI	*P*-value
Urgency	Non-urgent *vs* urgent	1.624	1.056, 2.497	0.027
Direct to endoscopy	Not direct *vs* direct	2.244	1.331, 3.783	0.002
Referring physician	Specialist *vs* GP	2.763	1.383, 5.519	0.058
	No referral *vs* GP	1.228	0.321, 4.706	0.666

DISCUSSION

There are many factors that are critical in maintaining the efficiency of an endoscopy unit. Many of these factors, such as emergency procedures, equipment failures and sedation difficulties are virtually impossible to predict. The multitasking, late physician is another major cause of an inefficient endoscopy unit and likewise, he/she is admittedly difficult to modify. On the other hand, truancy among patients who fail to attend their scheduled appointment is something that, in theory at least, has the capacity to be controlled.

We have determined the absenteeism rate in our endoscopy unit to be 3.6%, which encompassed 4.3% of flexible sigmoidoscopies, 2.6% of colonoscopies and 2.9% of upper endoscopies. This study was not

designed to compare the absenteeism rates between the different procedures; however, when compared, these rates are not statistically different. We determined three factors that resulted in a patient being considered "high risk" for truancy. It has become common for patients to have a consultation and endoscopy at the same time, just prior to the endoscopy (particularly for screening colonoscopies), and we have found that these patients are more likely to attend their appointment than those who have been previously seen in an office setting by the physician. At present, in our setting, we do not use physician assistants, however, all patients scheduled for a consult and endoscopic procedure at the same time are called by a secretary with subsequent explanation of the preparation and procedure. Those patients who continue to have additional questions that cannot be answered by the secretary are scheduled for an office visit prior to the procedure. The practice of "direct to endoscopy" has become commonplace and we note that this practice has indeed apparently minimized our truancy rates. It should also be recognized that in our Canadian system, many patients are booked for their endoscopic procedures weeks to months in advance and yet, despite this, we still have improved attendance rates for those patients that are booked directly to endoscopy, possibly demonstrating a more motivated patient group. It does confirm that this practice at least results in appropriate attendance to endoscopy and that these patients are compliant with their appointment.

The second factor found to be associated with absenteeism from endoscopy was referral from a specialist rather than a family physician. This is logical in that family physicians are very accustomed to referring patients to a specialist and have an organized system to arrange it. On the other hand, many specialists' offices are very adept at accepting referrals but not nearly as organized when it comes to referring a patient to another specialist. In addition, patients referred from other specialists often tend to have multiple health issues and more likely to be at a more acute state of illness. Just the fact that they have multiple health problems may put them at risk for absenteeism from their scheduled endoscopic appointment. This is another group of patients that can relatively easily be targeted as "high risk" for absenteeism and steps taken to ensure confirmation of their appointment.

The last group of patients who are more likely not to attend their appointments are those with non-urgent reasons for endoscopy. We defined urgent as those patients with bleeding or radiological abnormalities requiring endoscopic assessment. These patients are more likely to attend their procedure as opposed to the truly elective patient. This is logical in that typically, these patients have been told that there is a high likelihood that an abnormality is present and tissue confirmation is critical. These patients are therefore concerned enough to ensure their attendance at their endoscopic examination.

An Australian study demonstrated that patients with previous history of non-attendance were more likely not to attend[8], we have not found that in this study. This may be because if a patient doesn't attend the endoscopy clinic at a scheduled time, typically, the physician will not arrange another endoscopy until another office visit has been completed and an explanation for truancy extracted. A pediatric study demonstrated that social factors (social class, unmarried parents, poorer housing) played a larger role in increasing truancy than other factors such as severity of disease[13]. Due to the nature of this study, assessment of social factors was not performed.

There are several limitations of our study. It is retrospective and contains the usual limitations inherent within this study design. On the other hand, there is presently very limited data available from the literature to determine who is at high risk for truancy from endoscopy units. Many endoscopic sites have instituted measures to limit truancy such as calling all patients by phone or mailing reminders prior to the endoscopic examination to ensure their attendance[1-3,7,14]. Some of these measures are labor intensive with associated cost expenditures. Additionally, most patients attend their clinic appointment and in theory, don't require a reminder. If a select group of patients could be targeted then a limited reminder protocol might be considered. Before we embarked upon any campaign to decrease truancy rates, we felt it was critical to determine what factors were important in this area. Ideally, if we could isolate several factors, steps could be undertaken to improve the system and then re-evaluate after institution of an improved management strategy.

Another limitation of our study is the fact that it applies only to the dataset of our institution and our patients. Its general, applicability may be questioned; however, our site is very similar to many tertiary care centers. Many patients come directly to the endoscopy unit without prior consultation, procedures are performed in large numbers with rapid turnover, the endoscopic rooms and time are the critical elements to the efficiency of any unit. As a tertiary care center with a wide base of referrals, it would appear that our unit is, in fact, similar to many other endoscopic units throughout the world and therefore, our results could likely be replicated elsewhere.

A final limitation of the study is the fact that we have excluded patients who were transferred from other hospitals as well as those scheduled for ERCP and EUS. These patients are more complex with a myriad of other issues (including the acuity of illness) and we felt that the group we needed to concentrate on was those in whom we perform most of the standard, elective endoscopic examinations.

In summary, we found that patients with a non-urgent condition, those referred from a specialist and those who do not have a consult and procedure at the same time are more likely to be absent from their scheduled endoscopic procedure than those without these characteristics. With this information, endoscopy units can hopefully modify their clinical practices to reduce patient truancy. Studies aimed at improving

efficiency in endoscopy units should be aware of these "high-risk" patients to enhance appropriate resource utilization by decreasing absenteeism.

COMMENTS

Background

Patient absenteeism of outpatient procedures is a major problem for all ambulatory clinics.

Research frontiers

Identifying patients who may not present for scheduled endoscopy may help gastroenterologists to reduce patient truancy.

Innovations and breakthroughs

Previous studies have shown that patients at highest risk for truancy are those with a history of truancy; and methods to enhance patient attendance at clinics (i.e. telephone reminders, mailed reminders) have had mixed results.

Applications

Identifying patients who are not scheduled for same-day consult and endoscopy, those referred by a specialist rather than a family physician, and those with non-urgent reasons for referral may help gastroenterologists to reduce patient truancy.

Peer review

This manuscript is of enough interest and describes original work that merits its publication in WJG.

REFERENCES

1 **Lee CS**, McCormick PA. Telephone reminders to reduce non-attendance rate for endoscopy. *J R Soc Med* 2003; **96**: 547-548

2 **Downer SR**, Meara JG, Da Costa AC. Use of SMS text messaging to improve outpatient attendance. *Med J Aust* 2005; **183**: 366-368

3 **Denberg TD**, Coombes JM, Byers TE, Marcus AC, Feinberg LE, Steiner JF, Ahnen DJ. Effect of a mailed brochure on appointment-keeping for screening colonoscopy: a randomized trial. *Ann Intern Med* 2006; **145**: 895-900

4 **Denberg TD**, Melhado TV, Coombes JM, Beaty BL, Berman K, Byers TE, Marcus AC, Steiner JF, Ahnen DJ. Predictors of nonadherence to screening colonoscopy. *J Gen Intern Med* 2005; **20**: 989-995

5 **Turner BJ**, Weiner M, Yang C, TenHave T. Predicting adherence to colonoscopy or flexible sigmoidoscopy on the basis of physician appointment-keeping behavior. *Ann Intern Med* 2004; **140**: 528-532

6 **Takacs P**, Chakhtoura N, De Santis T. Video colposcopy improves adherence to follow-up compared to regular colposcopy: a randomized trial. *Arch Gynecol Obstet* 2004; **270**: 182-184

7 **Adams LA**, Pawlik J, Forbes GM. Nonattendance at outpatient endoscopy. *Endoscopy* 2004; **36**: 402-404

8 **Collins J**, Santamaria N, Clayton L. Why outpatients fail to attend their scheduled appointments: a prospective comparison of differences between attenders and non-attenders. *Aust Health Rev* 2003; **26**: 52-63

9 **Sawyer SM**, Zalan A, Bond LM. Telephone reminders improve adolescent clinic attendance: a randomized controlled trial. *J Paediatr Child Health* 2002; **38**: 79-83

10 **Bos A**, Hoogstraten J, Prahl-Andersen B. Failed appointments in an orthodontic clinic. *Am J Orthod Dentofacial Orthop* 2005; **127**: 355-357

11 **Maxwell S**, Maljanian R, Horowitz S, Pianka MA, Cabrera Y, Greene J. Effectiveness of reminder systems on appointment adherence rates. *J Health Care Poor Underserved* 2001; **12**: 504-514

12 **Murdock A**, Rodgers C, Lindsay H, Tham TC. Why do patients not keep their appointments? Prospective study in a gastroenterology outpatient clinic. *J R Soc Med* 2002; **95**: 284-286

13 **McClure RJ**, Newell SJ, Edwards S. Patient characteristics affecting attendance at general outpatient clinics. *Arch Dis Child* 1996; **74**: 121-125

14 **Abuksis G**, Mor M, Segal N, Shemesh I, Morad I, Plaut S, Weiss E, Sulkes J, Fraser G, Niv Y. A patient education program is cost-effective for preventing failure of endoscopic procedures in a gastroenterology department. *Am J Gastroenterol* 2001; **96**: 1786-1790

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2887

World J Gastroenterol 2009 June 21; 15(23): 2887-2892
World Journal of Gastroenterology ISSN 1007-9327

Lower *Bifidobacteria* counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients

Angèle PM Kerckhoffs, Melvin Samsom, Michel E van der Rest, Joris de Vogel, Jan Knol, Kaouther Ben-Amor, Louis MA Akkermans

Angèle PM Kerckhoffs, Melvin Samsom, Louis MA Akkermans, Gastrointestinal Research Unit, Department of Gastroenterology and Surgery, University Medical Center Utrecht, Heidelberglaan 100, F02.618, 3584CX, Utrecht, The Netherlands
Michel E van der Rest, Joris de Vogel, BioVisible BV, LJ Zielstraweg 1, 9713 GX, Groningen, The Netherlands
Jan Knol, Kaouther Ben-Amor, Danone Research-Centre for Specialized Nutrition, Bosrandweg 20, 6704PH, Wageningen, The Netherlands
Author contributions: Kerckhoffs APM, Samsom M, van der Rest ME, de Vogel J, Knol J, Ben-Amor K and Akkermans LMA contributed equally to this work.
Correspondence to: Angèle PM Kerckhoffs, MD, Department of Gastroenterology, Heidelberglaan 100, F02.618, 3584CX Utrecht, The Netherlands. angelekerckhoffs@hotmail.com
Telephone: +31-88-755-9812 Fax: +31-88-755-5533
Received: March 5, 2009 Revised: April 21, 2009
Accepted: April 28, 2009
Published online: June 21, 2009

Abstract

AIM: To determine the composition of both fecal and duodenal mucosa-associated microbiota in irritable bowel syndrome (IBS) patients and healthy subjects using molecular-based techniques.

METHODS: Fecal and duodenal mucosa brush samples were obtained from 41 IBS patients and 26 healthy subjects. Fecal samples were analyzed for the composition of the total microbiota using fluorescent *in situ* hybridization (FISH) and both fecal and duodenal brush samples were analyzed for the composition of bifidobacteria using real-time polymerase chain reaction.

RESULTS: The FISH analysis of fecal samples revealed a 2-fold decrease in the level of bifidobacteria (4.2 ± 1.3 *vs* 8.3 ± 1.9, $P < 0.01$) in IBS patients compared to healthy subjects, whereas no major differences in other bacterial groups were observed. At the species level, *Bifidobacterium catenulatum* levels were significantly lower (6 ± 0.6 *vs* 19 ± 2.5, $P < 0.001$) in the IBS patients in both fecal and duodenal brush samples than in healthy subjects.

CONCLUSION: Decreased bifidobacteria levels in both fecal and duodenal brush samples of IBS patients compared to healthy subjects indicate a role for microbiotic composition in IBS pathophysiology.

Key words: Irritable bowel syndrome; Gut microbiota; Bifidobacteria; *Bifidobacterium catenulatum*

Peer reviewer: Yehuda Ringel, MD, Assistant Professor of Medicine, Gastroenterology and Hepatology, University of North Carolina at Chapel Hill, 130 Mason Farm Road, CB 7080, 4107 Bioinformatics Building, Chapel Hill, NC 27599-7080, United States

Kerckhoffs APM, Samsom M, van der Rest ME, de Vogel J, Knol J, Ben-Amor K, Akkermans LMA. Lower *Bifidobacteria* counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. *World J Gastroenterol* 2009; 15(23): 2887-2892 Available from: URL: http://www.wjgnet.com/1007-9327/15/2887.asp DOI: http://dx.doi.org/10.3748/wjg.15.2887

INTRODUCTION

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by abdominal pain or discomfort and altered bowel function. Alterations in psychosomatic factors, gastrointestinal motility, visceral hypersensitivity and microbiotic composition have been suggested to play a role in the pathophysiology of IBS[1]. Alterations in fecal and small intestinal microbiotic composition in IBS patients have been reported and studies revealed a somewhat higher bacterial count in jejunal juice of IBS patients and lower numbers of fecal coliforms, lactobacilli and bifidobacteria than in healthy subjects[2,3]. More specifically, molecular-based methods showed that in IBS patients levels of members of the *Clostridium coccoides* subgroup, *Lactobacillus*, *Collinsella* and *Bifidobacterium catenulatum* groups are different from that of healthy subjects[4,5]. These differences in the fecal microbiotic composition may underlie

symptom generation by promoting abnormal colonic fermentation[6]. However, mucosa-associated bacteria might be more relevant in the symptom generation of IBS, since fecal and jejunal juice samples are only representing the composition of luminal microbiota. Alterations in luminal bacteria composition may change the commensal microbiota and affect the microbiota adhering to the mucosa. Microorganisms adhering to the intestinal wall are more likely to affect the host's immune, physiological or neuronal system or vice versa. The composition of luminal and mucosa-associated bacteria are not the same since the micro-environments are different at the surface of the intestinal epithelium and the lumen[7,8]. Therefore, we aimed to determine the composition of fecal luminal and mucosa-associated microbiota in IBS patients using molecular identification and quantification techniques.

MATERIALS AND METHODS

Subjects

Twelve male and 29 female IBS patients included in this study fulfilled the Rome Ⅱ criteria for IBS and were categorized as diarrhea predominant (IBS-D), constipation predominant (IBS-C) or alternating IBS subgroup (IBS-A)[9]. The IBS population consisted of 14 IBS-D subjects, 11 IBS-C subjects and 16 IBS-A subjects. The control group consisted of 8 male and 18 female healthy subjects from the general population, devoid of GI symptoms or major abdominal surgery. The healthy subjects were significantly ($P < 0.001$) younger (31 ± 2.06 years) than the group of IBS patients (42 ± 2.12 years). Subjects taking medication known to influence bacterial composition and gastrointestinal motility, especially antimicrobial medications and/or probiotics were excluded from the study. The Human Ethics Committee of the University Medical Centre Utrecht approved the study and all subjects gave written informed consent.

Sampling

To obtain small intestinal mucosa-associated material a sterile cytology brush (Uno-Brush, Prince Médical, Ercuis, France) sheathed in a sterile catheter was placed through the endoscope biopsy channel and advanced under direct vision out beyond the endoscope tip[10]. The duodenal mucosa was brushed three times and then pulled back into the sheath of the catheter. The catheter was removed and the brush was immediately cut off the catheter and placed into a sterile tube in liquid nitrogen and stored at -80℃ until analysis. Fecal samples, obtained before endoscopy, were collected and stored at -80℃ until further handling.

Fluorescent in situ hybridization (FISH) analysis of fecal samples

The total number of bacteria present in the fecal samples was determined with the EUB 338 probe which targets all bacteria[11]. FISH analysis was essentially performed as described previously with the genus-specific probes listed in Table 1[12]. Approximately 0.5 g of homogenized feces was suspended in 4 mL of 0.2 mm-pore-size-filtered PBS and 0.5 mL 37% formaldehyde and thoroughly mixed by vortexing for 3 min. After incubation for 4 h at 4℃ the suspension was vortexed again for 2 min. Debris was removed by a short spin at 80 *g* for 1 min. In an eppendorf tube, 300 μL of the supernatant was collected and the fixed cells were washed twice with PBS. For FISH analysis of the *Lactobacillus-Enterococcus* group the cells were first permeabilized by resuspending the pellet in 100 μL Proteinase-K solution (180 kU/L) (Sigma-Aldrich, Zwijndrecht, The Netherlands) and incubated for 10 min at 37℃. The cells were washed as described above and resuspended in 300 μL PBS/ethanol (1:1 v/v). After one hour of storage at -20℃, the cell suspension was diluted 1:10 in hybridization buffer at the required temperature for hybridization, and 5 ng labeled probe was added. Cells were hybridized for 16 h at the prescribed hybridization temperature. After resuspension in 4 mL washing buffer, cells were filtered on a 0.2 mm pore size Isopore polycarbonate membrane filter (Millipore Corporation) and washed with 5 mL of 50℃ washing buffer. Filters were mounted on microscope slides with Vectashield (Vector Laboratories, Burlingame, CA, USA), and hybridized cells were counted visually using an Olympus BX60 epifluorescence microscope using a FITC or Cy3-specific filter. All microscopic counts were determined in duplicate, with a minimum of 300 cells counted per assay.

Table 1 Genus-specific probes used for FISH analysis

Probe	Target group	Ref.
EUB 338	Total bacteria	[44]
Bac 303	*Bacteroides-Prevotella* group	[45,46]
Bif 164	*Bifidobacterium*	[12]
Erec 482	*Clostridium coccoides-Eubacterium rectale* group	[47]
Chis 150	*Clostridium histolyticum* group	[47]
Clit 135	*Clostridium lituseburense* group	[47]
Cld 73	*Clostridium difficile*	This study: CGCCGC TCTTTACCGAAGT
Fprau 645	*Faecalibacterium prausnitzii*	[46,48]
Lab 158	*Lactobacillus-Enterococcus* group	[49]

DNA extraction and polymerase chain reaction (PCR) amplification of fecal and duodenal brush samples

Brush and fecal samples were thawed on ice cooled water. DNA was extracted using the DNeasy Tissue kit (Qiagen, Venlo, The Netherlands) or the Fast DNA Spin kit (Qbiogene, Irvine, USA) from the brush and fecal samples respectively. The eluted DNA samples were stored at -20℃. The integrity of the isolated DNA was determined visually after electrophoresis on a 1.0% agarose gel containing ethidium bromide.

Real-time PCR

Quantification of *Lactobacilli* genera and species

specifically belonging to bifidobacteria was performed using a 5'nuclease (TaqMan) assay as described previously[13,14]. Briefly, a 20 μL PCR amplification mixture containing 10 μL TaqMan Fast Universal Master Mix (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands), optimized concentrations of primers and probes and 2.0 μL isolated DNA was prepared. The temperature profile for the amplification consisted of 20 s at 95℃ and 45 cycles of 1 s at 95℃ and 20 s at 60℃ (ABI 7900 HT Fast; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The percentages of the different bacteria were subsequently calculated as described by Liu *et al*[15,16].

Statistical analysis

FISH results with microbial numbers below the detection limit (8.3×10^5/g) were excluded from statistical analysis. Nonparametric FISH microbiota data were compared using Mann-Whitney tests or Kruskal-Wallis test for subgroup analysis. Independent samples *t*-test was used to compare differences in real-time PCR microbiota data between IBS patients and healthy subjects. One-way ANOVA with Bonferroni correction was used for analysis of microbiota in the IBS subgroups.

P-values less than 0.05 were considered statistically significant. All statistical analysis was performed using commercially available software (SPSS 12.0.1 for Microsoft Windows). Data are expressed as mean ± SE.

RESULTS

Characterization of the fecal microbiota of IBS patients and healthy subjects

The mean percentages of all bacterial groups measured are presented in Table 2. The results show that *F. prausnitzii*, *E. rectale*/*C. coccoides* and bifidobacteria are the most abundant groups in both IBS patients and healthy subjects. The levels of bifidobacteria were significantly lower ($P < 0.05$) in IBS patients (4.2% ± 1.3%) than in healthy subjects (8.3% ± 1.9%). No significant differences were observed between IBS-D, IBS-C and IBS-A subgroups. The *C. lituseburense* group was detected in significantly lower levels ($P < 0.01$) in IBS patients compared to healthy subjects; however, *C. lituseburense* reached the detection limit only in 14 healthy subjects and 18 IBS patients. The proportions of *Lactobacillus* spp, *C. coccoides*, *C. histolyticum*, *C. difficile*, *Bacteroides* and *F. prausnitzii* showed no differences between IBS patients and healthy subjects. This set of probes covered 44% and 32% of the total fecal microbiota in the healthy subjects and IBS patients, respectively. The low coverage is predominantly due to low counts in Bacteroides.

Characterization of the fecal bifidobacteria microbiota of IBS patients and healthy subjects

In healthy subjects, the proportion of bifidobacteria identified as *Bifidobacterium catenulatum* (19.31% ± 2.5%) was significantly ($P < 0.001$) higher compared to IBS patients (6.24% ± 0.6%). The low proportion of *B. catenulatum* was consistent in all IBS subgroups (Table 3, Figure 1A). The proportions of the other species (*Bifidobacterium adolescentis*, *Bifidobacterium bifidum* and *Bifidobacterium longum*) were not significantly different between healthy subjects, IBS patients and IBS subgroups. Low levels of *B. bifidum* were detected in fecal samples of all subjects as compared to the other *Bifidobacterium* species and as compared to the *B. bifidum* level in duodenal samples (Tables 3 and 4). The bifidobacterial species covered by these Q-PCR assays were only 43% and 29.5% of the total bifidobacteria population for healthy subjects and IBS patients, respectively.

Table 2 FISH analysis of the composition of the fecal microbiota of HS, IBS patients and IBS subgroups

Probe	HS	IBS	IBS-A	IBS-D	IBS-C
Fprau 645	12.0 ± 2.1	9.2 ± 0.80	10.6 ± 1.6	8.2 ± 1.4	8.6 ± 1.2
Erec 482	16.6 ± 5.4	11.7 ± 2.5	6.4 ± 1.3	7.1 ± 1.2	20.5 ± 5.8
Bif 164	8.3 ± 1.9	4.2 ± 1.3[a]	1.7 ± 0.63	7.9 ± 5.2	4.5 ± 0.94
Lab 158	4.7 ± 0.88	4.0 ± 0.78	2.0 ± 0.38	4.0 ± 1.7	6.0 ± 1.5
Chis 150	2.4 ± 0.43	2.1 ± 0.57	2.0 ± 0.61	1.6 ± 0.37	2.4 ± 1.4
Bac 303	1.5 ± 0.89	3.6 ± 1.5	0.41 ± 0.21	4.4 ± 3.3	5.7 ± 2.8
Cld73	0.88 ± 0.28	0.43 ± 0.09	0.61 ± 0.16	0.32 ± 0.15	0.21 ± 0.08
Clit 135	0.39 ± 0.10	0.09 ± 0.03[a]	0.06 ± 0.03	0.09 ± 0.04	0.11 ± 0.06
Sum	44.4 ± 6.85	32.1 ± 3.27	22.7 ± 2.30	28.3 ± 5.90	43.5 ± 5.89

[a]$P < 0.05$ *vs* HS. Data are expressed as percentage, mean ± SE.

Table 3 Real time PCR analysis of fecal bifidobacteria in HS, IBS patients and IBS subgroups

	HS	IBS	IBS-A	IBS-D	IBS-C
B. catenulatum	19.31 ± 2.5	6.24 ± 0.6[b]	6.57 ± 1.1[b]	5.67 ± 0.8[b]	6.49 ± 1.2[b]
B. adolescentis	17.05 ± 2.5	15.96 ± 1.6	16.86 ± 3.4	16.73 ± 1.8	14.06 ± 3.05
B. bifidum	2.1×10^{-4} ± 1.4×10^{-4}	9.1×10^{-4} ± 6.3×10^{-4}	3.3×10^{-4} ± 2.2×10^{-4}	1.9×10^{-3} ± 1.8×10^{-3}	4.5×10^{-4} ± 3.1×10^{-4}
B. longum	7.11 ± 1.4	7.30 ± 0.8	6.68 ± 1.4	8.71 ± 1.7	6.45 ± 1.33

[b]$P < 0.001$ *vs* HS. Data are expressed as percentage, mean ± SE.

Characterization of the duodenal microbiota of IBS patients and healthy subjects

In healthy subjects, *B. catenulatum* level as percentage of total bifidobacterial load (17.04% ± 2.3%) was significantly ($P < 0.001$) higher when compared to IBS patients (4.85% ± 0.5%). The significantly lower proportion of *B. catenulatum* was observed in all IBS subgroups (Table 4, Figure 1B). The levels of *B. adolescentis, B. bifidum* and *B. longum* as percentage of total bifidobacterial load were comparable between healthy subjects, IBS patients and IBS subgroups (Table 4). With the set of probes used, the total percentage of bifidobacteria of the bifidobacterial load which could be detected is 46% for healthy subjects and 31% for IBS patients.

Characterization of B. catenulatum in age-matched IBS patients and healthy subjects

Since the patients and healthy subjects were not matched, the age difference between the healthy subjects and IBS patients may be a confounding factor. In a subset of the subjects, 19 IBS patients (33 ± 2.8) matched for age

Table 4 Duodenal mucosa-associated bifidobacteria in HS, IBS patients and IBS subgroups

	HS	IBS	IBS-A	IBS-D	IBS-C
B. catenulatum	17.04 ± 2.3	4.85 ± 0.5[b]	5.26 ± 0.9[b]	4.26 ± 0.7[b]	5.04 ± 1.0[b]
B. adolescentis	15.68 ± 2.3	13.90 ± 1.4	14.66 ± 2.7	14.58 ± 1.7	12.21 ± 3.0
B. bifidum	6.09 ± 0.9	5.13 ± 0.8	5.36 ± 1.7	4.43 ± 1.1	5.68 ± 1.3
B. longum	6.88 ± 1.3	7.27 ± 0.8	5.84 ± 1.3	9.01 ± 1.6	6.88 ± 1.40

[b]$P < 0.001$ *vs* HS. Data are expressed as percentage, mean ± SE.

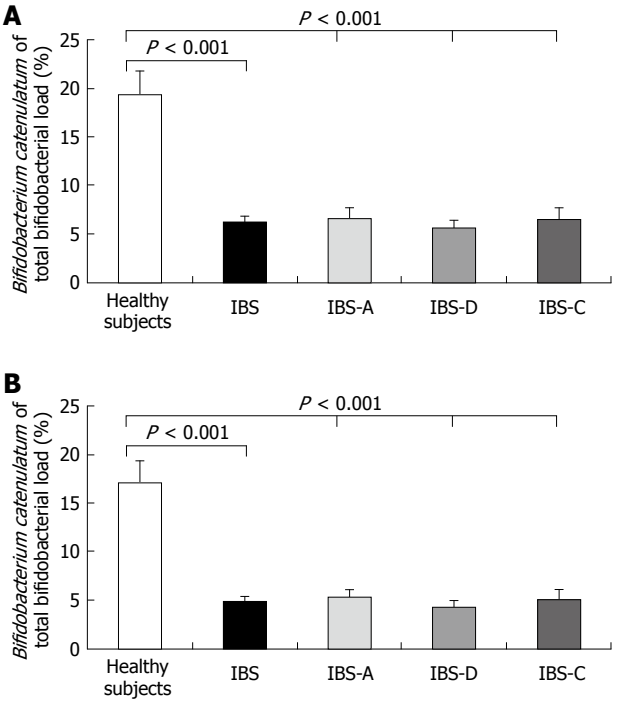

Figure 1 Percentage of *Bifidobacterium catenulatum* as percentage of total bifidobacterial load in fecal samples and duodenal samples of healthy subjects, IBS patients and IBS subgroups (mean ± SE). A: Fecal samples; B: Duodenal samples.

with 19 healthy subjects (33 ± 2.7), decreased levels of *B. catenulatum* were also shown in duodenal as well as in fecal samples of the IBS patients. The mean percentage of *B. catenulatum* of total bifidobacterial load in duodenal samples was significantly ($P < 0.001$) lower in IBS patients (5.48% ± 0.60%) compared to healthy subjects (17.19% ± 2.43%). Percentage of *B. catenulatum* of total bifidobacterial load in the fecal samples was significantly ($P < 0.001$) lower in IBS patients (6.98% ± 0.69%) compared to healthy subjects (19.50% ± 2.67%).

DISCUSSION

Composition of gastrointestinal microbiota is known to be relatively stable and composed of permanent, the so-called core phyla, and transient species which contribute to gastrointestinal health and disease[17-19]. The presence of beneficial microbes in the intestine prevents colonization by potentially pathogenic microbes, referred to as colonization resistance[20,21]. Imbalances in the microbiota are characterized by a decrease in beneficial anaerobic bacteria and increases in aerobic bacteria, fungi and harmful anaerobic bacteria[20].

In this study, we showed, using FISH analysis, that IBS patients have significantly lower fecal levels of bifidobacteria but no differences in the other major bacterial groups. Previous studies have also shown microbial alterations in fecal samples of IBS patients using both culturing and molecular-based techniques[2,4]. Using culturing techniques, Balsari *et al*[2] have already shown in 1982 a decrease in bifidobacteria, coliforms and lactobacilli in IBS patients. Using molecular-based techniques decreased *B. catenulatum*, *C. coccoides*, *Lactobacillus* and *Collinsella* counts in the fecal samples of IBS patients were found[4,5]. These studies were limited to the fecal flora. We broadened the study by examining bifidobacteria levels in duodenal mucosa-associated samples.

Differences in microbiotic composition between luminal and mucosa-associated bacteria have been shown[7,22-25]. The different micro-environment of the epithelium compared to the lumen might lead to a different microbiotic composition[7,26]. Bacteria that attach to the mucosa may exert greater influence on innate immune processes in the intestine[24,27]. In addition, the adhesion of non-pathogenic bacteria to the epithelial surface may contribute to the barrier that effects host resistance to pathogenic bacteria[24].

In this study, we showed that the percentages of *B. bifidum* of the total bifidobacterial counts were lower in the fecal samples than in the duodenal mucosa-associated samples in both IBS patients and controls. This might be due to high hydrophobicity of *B. bifidum* which is related to its ability to adhere to surfaces[28,29]. Furthermore, *B. catenulatum* counts were decreased in duodenal mucosa-associated samples as well as in fecal samples of IBS patients compared to controls. The effect of *B. catenulatum* on the health of the host is unknown. However as a group, bifidobacteria are considered beneficial for the host, as they produce lactic and acetic acids that decrease pH and inhibit the growth of potential pathogenic bacteria[30-32].

Moreover, *Bifidobacterium* spp prevent diarrhea and intestinal infections, alleviate constipation and stimulate the immune system[31]. The lower levels of bifidobacteria might be epiphenomenal or develop as a consequence of altered gastrointestinal motility or genetic makeup of IBS patients rather than being the cause of IBS symptoms[31].

Since the patients and healthy subjects were not matched, the age difference between the healthy subjects and IBS patients might have been a confounding factor. It was reported that elderly (> 65 years old) have lower fecal levels of *B. catenulatum*[33,34]. The elderly were not included in our study. The effect of the age difference between healthy subjects (mean age 32 years) and IBS patients (mean age 42 years) on *B. catenulatum* levels is not known. However, in age-matched IBS patients and healthy subjects statistically significantly decreased levels of *B. catenulatum* were also seen in duodenal as well as in fecal samples of the IBS patients.

An imbalanced microbiotic composition may lead to a different fermentation pattern, especially with an increased hydrogen production resulting in bloating[6,35].

Both antibiotics and probiotics have been shown to reduce IBS symptoms, which further suggests that microbial imbalance may underlie symptom generation in IBS patients[36-40]. Previously, a therapeutic trial suggested that particular *B. infantis* species were efficacious in the treatment of IBS symptoms[41]. No effects of *B. infantis* on stool consistency or frequency could be observed which implies that this therapeutic approach may be applicable to all IBS patients irrespective of their stool pattern[41]. *B. breve* in combination with *L. plantarum* has been shown to decrease pain and the severity of symptoms in IBS patients[42]. Prebiotics, oligofructose and inulin might reduce symptoms in IBS-C patients as they selectively stimulate bifidobacteria which results in increased stool frequency[43].

In conclusion, lower bifidobacteria levels were found both in duodenal mucosa-associated samples as well as in fecal samples of IBS patients when compared to healthy subjects. Specifically, *B. catenulatum* was found to be reduced in duodenal mucosa-associated bacteria as well as in the feces of IBS patients. The relevance of specific *Bifidobacterium* spp in relation to IBS symptoms is unknown; however modulation of the gut microbiota by means of prebiotics or bifidobacteria-containing probiotics to restore a balanced microbiotic composition may have a therapeutic role.

ACKNOWLEDGMENTS

The authors thank Monique Haarman and Eric Caldenhoven for their scientific contribution to the study.

COMMENTS

Background

Alterations in fecal and small intestinal microbiotic composition in irritable bowel syndrome (IBS) patients have been reported and studies revealed a somewhat higher bacterial count in jejunal juice of IBS patients and lower numbers of fecal coliforms, lactobacilli and bifidobacteria than in healthy subjects.

Research frontiers

The aim of the study was to determine the composition of both fecal and duodenal mucosa-associated microbiota in IBS patients and healthy subjects using molecular-based techniques.

Innovations and breakthroughs

Decreased bifidobacteria levels in both fecal and duodenal brush samples of IBS patients compared to healthy subjects indicate a role for microbiotic composition in IBS pathophysiology.

Peer review

The authors investigated the fecal and duodenal mucosal-associated microbiota in patients with IBS. This topic is of interest since, as the authors mentioned, alterations in intestinal flora have been suggested as a potential etiological factor in the pathogenesis of this disorder.

REFERENCES

1 **Talley NJ**, Spiller R. Irritable bowel syndrome: a little understood organic bowel disease? *Lancet* 2002; **360**: 555-564
2 **Balsari A**, Ceccarelli A, Dubini F, Fesce E, Poli G. The fecal microbial population in the irritable bowel syndrome. *Microbiologica* 1982; **5**: 185-194
3 **Posserud I**, Stotzer PO, Björnsson ES, Abrahamsson H, Simrén M. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* 2007; **56**: 802-808
4 **Malinen E**, Rinttilä T, Kajander K, Mättö J, Kassinen A, Krogius L, Saarela M, Korpela R, Palva A. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005; **100**: 373-382
5 **Kassinen A**, Krogius-Kurikka L, Mäkivuokko H, Rinttilä T, Paulin L, Corander J, Malinen E, Apajalahti J, Palva A. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007; **133**: 24-33
6 **King TS**, Elia M, Hunter JO. Abnormal colonic fermentation in irritable bowel syndrome. *Lancet* 1998; **352**: 1187-1189
7 **Zoetendal EG**, von Wright A, Vilpponen-Salmela T, Ben-Amor K, Akkermans AD, de Vos WM. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl Environ Microbiol* 2002; **68**: 3401-3407
8 **Lepage P**, Seksik P, Sutren M, de la Cochetière MF, Jian R, Marteau P, Doré J. Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. *Inflamm Bowel Dis* 2005; **11**: 473-480
9 **Thompson WG**, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Müller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45** Suppl 2: II43-II47
10 **León-Barúa R**, Gilman RH, Rodríguez C, Bonilla JJ, Yi A, Maúrtua D, Sack RB. Comparison of three methods to obtain upper small bowel contents for culture. *Am J Gastroenterol* 1993; **88**: 925-928
11 **Porter K**, Feig Y. The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 1980; **25**: 943-948
12 **Langendijk PS**, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH, Welling GW. Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl Environ Microbiol* 1995; **61**: 3069-3075
13 **Haarman M**, Knol J. Quantitative real-time PCR assays to identify and quantify fecal Bifidobacterium species in infants receiving a prebiotic infant formula. *Appl Environ Microbiol* 2005; **71**: 2318-2324
14 **Haarman M**, Knol J. Quantitative real-time PCR analysis of fecal Lactobacillus species in infants receiving a prebiotic infant formula. *Appl Environ Microbiol* 2006; **72**: 2359-2365
15 **Liu W**, Saint DA. Validation of a quantitative method for real time PCR kinetics. *Biochem Biophys Res Commun* 2002; **294**: 347-353
16 **Liu W**, Saint DA. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. *Anal Biochem* 2002; **302**: 52-59
17 **Savage DC**. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977; **31**: 107-133
18 **McCartney AL**, Wenzhi W, Tannock GW. Molecular analysis of the composition of the bifidobacterial and lactobacillus microflora of humans. *Appl Environ Microbiol* 1996; **62**: 4608-4613
19 **Guarner F**, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512-519
20 **Noverr MC**, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol* 2004; **12**: 562-568
21 **Sullivan A**, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infect Dis* 2001; **1**: 101-114
22 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638
23 **Mai V**, Morris JG Jr. Colonic bacterial flora: changing understandings in the molecular age. *J Nutr* 2004; **134**:

459-464
24 **Hooper LV**, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 2002; **22**: 283-307
25 **Bhat P**, Albert MJ, Rajan D, Ponniah J, Mathan VI, Baker SJ. Bacterial flora of the jejunum: a comparison of luminal aspirate and mucosal biopsy. *J Med Microbiol* 1980; **13**: 247-256
26 **Pryde SE**, Richardson AJ, Stewart CS, Flint HJ. Molecular analysis of the microbial diversity present in the colonic wall, colonic lumen, and cecal lumen of a pig. *Appl Environ Microbiol* 1999; **65**: 5372-5377
27 **Okada Y**, Setoyama H, Matsumoto S, Imaoka A, Nanno M, Kawaguchi M, Umesaki Y. Effects of fecal microorganisms and their chloroform-resistant variants derived from mice, rats, and humans on immunological and physiological characteristics of the intestines of ex-germfree mice. *Infect Immun* 1994; **62**: 5442-5446
28 **Mättö J**, Malinen E, Suihko ML, Alander M, Palva A, Saarela M. Genetic heterogeneity and functional properties of intestinal bifidobacteria. *J Appl Microbiol* 2004; **97**: 459-470
29 **Pérez PF**, Minnaard Y, Disalvo EA, De Antoni GL. Surface properties of bifidobacterial strains of human origin. *Appl Environ Microbiol* 1998; **64**: 21-26
30 **Wilhelm MP**, Lee DT, Rosenblatt JE. Bacterial interference by anaerobic species isolated from human feces. *Eur J Clin Microbiol* 1987; **6**: 266-270
31 **O'Sullivan DJ**, Kullen MJ. Tracking of probiotic bifidobacteria in the intestine. *Int Dairy J* 1998; **8**: 513-525
32 **Asahara T**, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing Escherichia coli O157: H7. *Infect Immun* 2004; **72**: 2240-2247
33 **Hopkins MJ**, Macfarlane GT. Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection. *J Med Microbiol* 2002; **51**: 448-454
34 **Woodmansey EJ**, McMurdo ME, Macfarlane GT, Macfarlane S. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. *Appl Environ Microbiol* 2004; **70**: 6113-6122
35 **Lin HC**. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA* 2004; **292**: 852-858
36 **Whorwell PJ**, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, Quigley EM. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol* 2006; **101**: 1581-1590
37 **Niedzielin K**, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2001; **13**: 1143-1147
38 **Nobaek S**, Johansson ML, Molin G, Ahrné S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 1231-1238
39 **Pimentel M**, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 3503-3506
40 **Pimentel M**, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003; **98**: 412-419
41 **O'Mahony L**, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541-551
42 **Saggioro A**. Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol* 2004; **38**: S104-S106
43 **Gibson GR**, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 1995; **108**: 975-982
44 **Amann RI**, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* 1990; **56**: 1919-1925
45 **Manz W**, Amann R, Ludwig W, Vancanneyt M, Schleifer KH. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* 1996; **142** (Pt 5): 1097-1106
46 **Lay C**, Sutren M, Rochet V, Saunier K, Doré J, Rigottier-Gois L. Design and validation of 16S rRNA probes to enumerate members of the Clostridium leptum subgroup in human faecal microbiota. *Environ Microbiol* 2005; **7**: 933-946
47 **Franks AH**, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; **64**: 3336-3345
48 **Suau A**, Rochet V, Sghir A, Gramet G, Brewaeys S, Sutren M, Rigottier-Gois L, Doré J. Fusobacterium prausnitzii and related species represent a dominant group within the human fecal flora. *Syst Appl Microbiol* 2001; **24**: 139-145
49 **Harmsen HJ**, Elfferich P, Schut F, Welling GW. A 16S rRNA-targeted Probe for detection of Lactobacilli and Enterococci in Faecal Samples by fluorescent In Situ Hybridization. *Microb Ecol Health Dis* 1999; **11**: 3-12

S- Editor Li LF **L- Editor** Logan S **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2893

World J Gastroenterol 2009 June 21; 15(23): 2893-2899
World Journal of Gastroenterology ISSN 1007-9327

Glutamine synthetase activity and glutamate uptake in hippocampus and frontal cortex in portal hypertensive rats

Gabriela Beatriz Acosta, María Alejandra Fernández, Diego Martín Roselló, María Luján Tomaro, Karina Balestrasse, Abraham Lemberg

Gabriela Beatriz Acosta, Institute of Pharmacological Research (ININFA), National Research Council of Argentina (CONICET) and University of Buenos Aires, Junín 956. 5th floor, C1113AAD, Buenos Aires, Argentina
María Alejandra Fernández, Diego Martín Roselló, Abraham Lemberg, Laboratory of Portal Hypertension, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956. 5th floor, C1113AAD, Buenos Aires, Argentina
María Luján Tomaro, Karina Balestrasse, Department of Biological Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 596. 5th floor, C1113AAD, Buenos Aires, Argentina
Author contributions: Acosta GB, Fernández MA, and Roselló DM contributed equally to this work; Acosta GB performed glutamate uptake, contributed new reagents and analyed data; Tomaro ML and Balestrasse K performed glutamine synthetase; Acosta GB and Lemberg A designed research and wrote the paper.
Supported by Grant B013 from the University of Buenos Aires, Argentina and PIP 5869 from National Research Council of Argentina
Correspondence to: Dr. Gabriela Beatriz Acosta, Institute of Pharmacological Research (ININFA), National Research Council of Argentina (CONICET) and University of Buenos Aires, Junín 956. 5th floor, C1113AAD, Buenos Aires, Argentina. gacosta@ffyb.uba.ar
Telephone: +54-11-49615949/6784 Fax: +54-11-49638593
Received: January 21, 2009 Revised: April 24, 2009
Accepted: May 1, 2009
Published online: June 21, 2009

Abstract

AIM: To study glutamine synthetase (GS) activity and glutamate uptake in the hippocampus and frontal cortex (FC) from rats with prehepatic portal vein hypertension.

METHODS: Male Wistar rats were divided into sham-operated group and a portal hypertension (PH) group with a regulated stricture of the portal vein. Animals were sacrificed by decapitation 14 d after portal vein stricture. GS activity was determined in the hippocampus and FC. Specific uptake of radiolabeled L-glutamate was studied using synaptosome-enriched fractions that were freshly prepared from both brain areas.

RESULTS: We observed that the activity of GS increased in the hippocampus of PH rats, as compared to control animals, and decreased in the FC. A significant decrease in glutamate uptake was found in both brain areas, and was more marked in the hippocampus. The decrease in glutamate uptake might have been caused by a deficient transport function, significantly and persistent increase in this excitatory neurotransmitter activity.

CONCLUSION: The presence of moderate ammonia blood levels may add to the toxicity of excitotoxic glutamate in the brain, which causes alterations in brain function. Portal vein stricture that causes portal hypertension modifies the normal function in some brain regions.

Key words: Portal hypertension; Glutamine synthetase; Glutamate uptake; Frontal cerebral cortex; Hippocampus; Rat

Peer reviewers: Dr. Vicente Felipo, Laboratory of Neurobiology, Fundación C.V. Centro de Investigacion Principe Felipe, Avda Autopista del Saler, 16, 46013 Valencia, Spain; Robert Flisiak, PhD, Department of Infectious Diseases, Medical University of Bialystok, 15-540 Bialystok, Zurawia str., 14, Poland

Acosta GB, Fernández MA, Roselló DM, Tomaro ML, Balestrasse K, Lemberg A. Glutamine synthetase activity and glutamate uptake in hippocampus and frontal cortex in portal hypertensive rats. *World J Gastroenterol* 2009; 15(23): 2893-2899 Available from: URL: http://www.wjgnet.com/1007-9327/15/2893.asp DOI: http://dx.doi.org/10.3748/wjg.15.2893

INTRODUCTION

Two major complications appear in severe liver failure: hepatic encephalopathy (HE) and portal hypertension (PH), but the mechanism involved in the production of brain damage is still unclear.

PH is found in patients with cirrhosis, and in portal vein thrombosis, it is characterized by an increase in splanchnic blood flow and pressure, caused by abdominal blood flow resistance, secondary to important liver parenchyma alterations (fibrosis or cirrhosis). Normally, the liver splanchnic blood must reach, through the liver, the hepatic veins and finally the vena cava[1]. As a result

of increased splanchnic blood flow, collateral vein shunts appear and abdominal circulation avoids the damaged liver parenchyma to reach the systemic circulation[2].

HE in acute and chronic liver disease is a complex syndrome, associated frequently with hyperammonemia. Increases in blood brain barrier (BBB) permeability present in PH, allow ammonia ions and other neurotoxic substances to penetrate brain tissue[3-6]. Hyperammonemia is caused by a defect in the liver parenchyma that forms urea from intestinal nitrogenic substances, and vein shunts from the splanchnic circulation carry it into the general circulation[7].

According to Erceg *et al*[8], chronic hyperammonemia, with or without liver failure, impairs the glutamate-nitric oxide-cGMP pathway in the brain and reduces extracellular cGMP in the brain. This function is associated with a decreased ability of rats to learn a Y maze conditional discrimination task. It has been suggested that a decrease in extracellular cGMP is involved in impaired learning ability and intellectual function.

Ammonia is a well-known toxic substance for the central nervous system (CNS), especially when levels exceed the antitoxic capacity of the brain cells. Glutamate plays an important role in cellular metabolism, and contributes to normal excitatory neurotransmission in the brain. When this function is not accomplished effectively, and either ammonia or glutamate are not sufficiently detoxified, their concentrations increase pathologically, neuron and astrocyte functions deteriorate, and damage and even cell death can result[9]. It has been shown clearly that acute ammonia toxicity and liver failure lead to excitotoxicity as a result of activation of N-methyl-D-aspartate (NMDA) receptors in the brain[10], and that blocking these receptors can lead to ammonia-induced death[11]. In contrast, chronic hyperammonemia leads to down-regulation of signal transduction pathways associated with these receptors, which contributes to cognitive impairment[8].

The glutamine/glutamate cycle participates in cell metabolism, and has important relevance in normal and pathological functions. When this cycle does not function adequately, CNS functional damage can appear, and even cellular death can be produced[12]. To accomplish the transformation to ammonia and glutamate into glutamine, the brain depends on the activity of the enzyme glutamine synthetase (GS) in astrocytes. This is associated with correct function of the glutamate transporters, to provide an adequate uptake, release and metabolism of ammonia and glutamate[13]. Therefore, the importance of correct function of the glutamine/glutamate cycle during this detoxifying step in brain is clear.

The aim of the present study was to analyze the participation of GS activity and glutamate uptake in the hippocampus and cerebral frontal cortex (FC), using a prehepatic PH rat model, with the intention to mimic the two major complications that appear in chronic liver failure. By using this model, it may be possible to understand more clearly the defense mechanism of the brain against the two toxic substances, ammonia and the excitatory neurotransmitter glutamate.

MATERIALS AND METHODS

Animals and surgical procedures

Male Wistar rats with an average weight of 240 g were used. The animals were placed in individual cages, with free access to food (standard laboratory rat chow) and water, and a 12-h light cycle: 8.00-20.00 h. Special care for perfect air renewal was taken. PH was obtained by calibrated stenosis of the portal vein, according to the method of Chojkier and Groszmann[14]. Rats were lightly anesthetized with ether and then a midline abdominal incision was made. The portal vein was located and isolated from the surrounding tissues. A ligature of 3.0 silk sutures was placed around the vein, and snugly tied to a 20-gauge blunt-end needle placed alongside the portal vein. The needle was subsequently removed to yield a calibrated stenosis of the portal vein. Fourteen days after portal vein ligation, animals exhibit an increase in portal pressure. Sham-operated rats underwent the same experimental procedure, except that the portal vein was isolated but not stenosed. Animals were placed in individual cages and allowed to recover from surgery. Animals were sacrificed by decapitation at 14 d after portal vein stricture.

Experiments were carried out in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication Nº 80-23/96) and local regulations. All efforts were made to minimize suffering of animals and to reduce the number of animals used.

Portal pressure measurement

Fourteen days after the corresponding operation, the rats were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). Portal pressure was measured through a needle placed in the splenic pulp, and maintained in place by cyanoacrylate gel. The needle was cannulated to a polyethylene catheter (50) filled with a heparinized saline solution (25 U/mL), and connected to a Statham Gould P23ID pressure transducer (Statham, Hato Rey, Puerto Rico), coupled to a Grass 79D polygraph (Grass Instruments, Quincy, MA, USA).

GS activity

GS activity was assessed as described by Rowe *et al*[15], with some minor modifications. Fourteen days after portal vein stricture, rats were sacrificed by decapitation and the FC and hippocampus were incubated in 500 μL HEPES-Tris buffer, which contained 140 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L HEPES, 10 mmol/L glucose (adjusted to pH 7.4 with Tris base) for 30 min at 37℃. After incubation, each brain region was homogenized in 200 μL 10 mmol/L potassium phosphate, pH 7.2. Reaction mixtures contained 150 μL brain region homogenate and 150 μL stock solution (100 mmol/L imidazole-HCl buffer, 40 mmol/L $MgCl_2$, 50 mmol/L β-mercaptoethanol, 20 mmol/L ATP, 100 mmol/L glutamate and 200 mmol/L hydroxylamine, adjusted to pH 7.2) Tubes were incubated at 37℃ for 15 min.

The reaction was stopped by adding 0.6 mL ferric chloride reagent (0.37 mol/L $FeCl_3$, 0.67 mol/L HCl and 0.20 mol/L trichloroacetic acid). Samples were placed for 5 min on ice. Precipitated proteins were removed by centrifugation at 10000 *g*, and the absorbance of the supernatants was read at 535 nm against a reagent blank. Under these conditions, 1 μmol γ-glutamylhydroxamic acid gave an absorbance of 0.340. GS specific activity was expressed as μmol γ-glutamylhydroxamate per hour per milligram of protein.

Preparation of tissue samples for glutamate uptake

As described for the GS activity assay, at 14 d after portal vein stricture, animals were killed by decapitation. The brain was removed from the cranial cavity, and the FC and hippocampus were dissected onto a Petri dish at 0℃, according to the method of Glowinski and Iversen[16], and homogenized with a glass-PTFE homogenizer in 15 volumes of 0.32 mol/L sucrose. The homogenates were centrifuged at 800 *g* for 10 min, the pellet was discarded, and the supernatant was centrifuged at 20000 *g* for 20 min. The pellet (P2 = crude synaptosomal fraction) was suspended with a glass-PTFE homogenizer in fresh 0.32 mol/L sucrose, and again centrifuged at 20000 *g* for 20 min. The procedure was repeated three times, and the resulting pellet was suspended and used in uptake experiments within 5 h after preparation.

Glutamate uptake procedure

Uptake experiments were carried out using fresh synaptosomal fractions that originated from 20 mg of tissue of FC and hippocampus (wet weight) per 1 mL incubation medium. This consisted of 125.0 mmol/L NaCl, 3.5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.25 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 10 mmol/L HEPES and 10 mmol/L D-glucose, pH adjusted to 7.4. The tissue was first incubated for 5 min at 30℃, as described by Takarada *et al*[17], followed by the addition of 10 nmol/L radiolabeled substrate of [^{3}H] L-glutamate, and subsequent incubation for 5-30 min (time course study). The incubation was terminated by vacuum-filtration through Whatman glass fiber-filters (type D) and rapid washing, three times, with isotonic saline solution (at 2-4℃). The radioactivity on the filters was measured using liquid scintillation counting. Parallel experiments were always performed without incubation as time zero, to obtain radioactivity not specifically taken up into brain preparation for the radiolabeled substrate used in this experiment.

Protein content was estimated by the technique of Lowry *et al*[18] using bovine serum albumin as a standard.

Drugs, chemicals and radiolabeled compounds

[^{3}H] L-Glutamate (specific activity: 52.0 Ci/mmol) was from Perkin Elmer NEN Life Science Inc. (Boston, MA, USA). Plasma ammonia concentration was determinate by the Ammoniac Enzymatic UV kit (Biomerieux, France). All other chemicals and reagents were analytical grade and obtained through regular commercial sources.

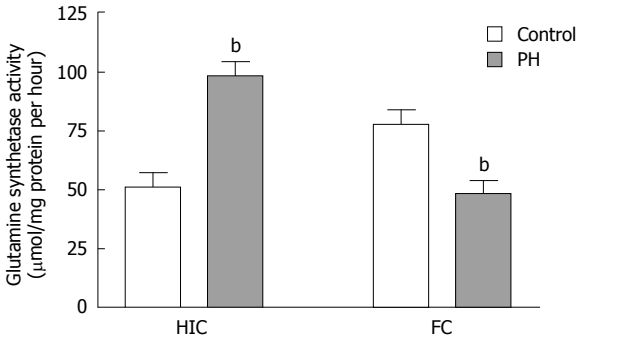

Figure 1 GS activity in the hippocampus and FC. Values are the mean ± SE in eight experiments. $^bP < 0.01$ compared with respective control group. HIC: Hippocampus.

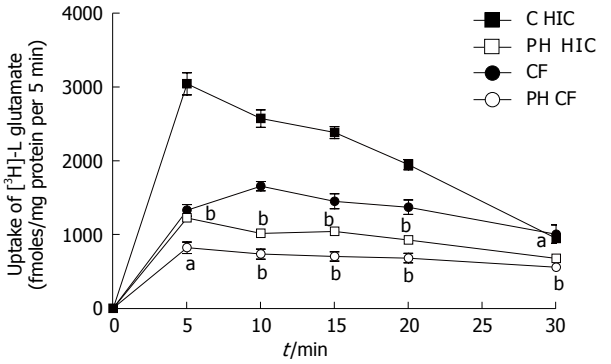

Figure 2 Time course of [^{3}H] L-glutamate uptake in FC and hippocampus. Synaptosomes were incubated with [^{3}H] L-glutamate at 10 nmol/L substrate concentration, for > 30 min, in the presence of 125 mmol/L NaCl at 30℃. Values are the mean ± SE in six or seven separate experiments done in triplicate. $^aP < 0.05$; $^bP < 0.01$ compared with respective control group. C: Control group; PH: Portal hypertension group.

Statistical analysis

The uptake was estimated with Graph Pad Prism 3.1 software (San Diego, CA, USA). Data of uptake activity were presented as the mean ± SE, and were analyzed by one-way analysis of variance, followed by Tukey test[19]. Student's *t* test was used to assess differences between paired groups. $P < 0.05$ was considered as significant.

RESULTS

In these experiments using prehepatic portal hypertensive animals, with almost normal liver function (2-2.5 times normal values), we found a significant increase in hippocampal GS activity, as compared to sham-operated animals ($P < 0.001$) (Figure 1). On the contrary, in FC, a significant decrease in the enzyme activity was documented ($P < 0.001$), in comparison with control rat brains (Figure 1).

The uptake of [^{3}H] glutamate occurred in a temperature-dependent manner and increased with incubation time up to 5 min, with a plateau thereafter at 30 min. Glutamate uptake in the hippocampus and FC showed that, at 30℃, [^{3}H] L-glutamate was taken up into synaptosomes in a time-dependent manner. Its level reached a plateau after 20 min in the presence of Na^+ ions (Figure 2), and remained linear with time for about 2-3 min in the hippocampus and FC, in PH and

sham-operated rats. The uptake of [^{3}H] L-glutamate was temperature- and Na^+-dependent in both regions studied. Time course experiments verified that the uptake of this excitatory amino acid was essentially linear up to 2-3 min at the respective incubation temperature (Figure 2).

The portal hypertensive rat, with almost normal liver function (only moderately increased aspartate aminotransferase and alanine aminotransferase activity and blood ammonia), was shown to have morphological and functional alterations of GS and glutamate uptake in astrocytes and neurons.

There was a significant decrease in glutamate uptake in the portal hypertensive group, as compared to the sham-operated rats, in the hippocampus ($P < 0.001$) and FC ($P < 0.05$) (Figure 2). We found a more pronounced decrease in glutamate uptake in the hippocampus. [^{3}H] L-Glutamate uptake was concentration-dependent at 30℃ and was dramatically suppressed at 2℃ (data not shown). At 30 min, we observed a decline in [^{3}H] L-glutamate uptake in the two regions, which might have been caused by the metabolic changes in the glutamine/glutamate cycle.

Liver homogenates obtained from both groups of animals showed no biochemical alterations and no histological damage (data not shown).

Portal pressure was 8.5 ± 0.5 mmHg in the sham-operated group and 12.5 ± 0.8 mmHg in the PH group ($P < 0.01$).

Plasma ammonium level was 23 ± 9 μmol/L in the control group and 79 ± 15 μmol/L in the PH group, which corresponded to an increase of 243% ($P < 0.01$).

DISCUSSION

PH is responsible for severe circulatory derangements in the splanchnic and systemic circulation, and brain damage results from HE, as a consequence of acute and chronic liver failure. In particular, HE is responsible for severe and often lethal outcomes in these diseases. The HE can be characterized by a wide spectrum of neuropsychiatric abnormalities.

In the present study of prehepatic portal hypertensive rats, we demonstrated different activities of GS and glutamate uptake in the hippocampus and FC. We observed that the activity of GS increased in the hippocampus of PH rats compared to control animals, while there was a decrease in the FC. There was a moderate increase in ammonia concentration.

Using fresh synaptosomal fractions from the FC and hippocampus, we found that glutamate uptake was decreased significantly in PH rats, with a more marked decrease in the hippocampus. In the presence of 125 mmol/L NaCl, uptake of this radiolabeled amino acid occurred in a temperature-dependent manner, and increased with incubation time up to 2-3 min, with a plateau thereafter at 30 min. These results suggest that there are biochemical differences between the brain regions, possibly caused by the toxic metabolic action of ammonia, glutamate, and perhaps glutamine, on the rat brain.

The morphological alterations in the liver parenchyma in chronic disease and in portal vein thrombosis create collateral veins that shunt splanchnic blood flow to the systemic circulation, in an attempt to overcome the increased pressure of portal vein flow. This phenomenon modifies the normal physiology of several organs, including the CNS, and transports intestinal toxins directly to the brain.

In previous experiments with this rat model, alterations in the CNS have been documented, including BBB permeability modifications[5,20]. The impact of PH on the BBB has been demonstrated in rats, in which, 40 d after portal vein stricture, the BBB recovers its function associated to normalize portal pressure and the impermeability to dyes[20].

Glutamate is a major excitatory neurotransmitter, and any alteration in the glutaminergic pathway must modify brain function. Normally, glutamate is synthesized in brain tissue from glucose.

Butterworth *et al*[21], studied the brain in liver failure, glutamate, some other amino acids and neurotransmitters, such as serotonin and dopamine, that are involved in the development of HE. Glutamate uptake and transport are important steps in protecting CNS cells from glutamate excitotoxicity[22,23].

As the removal of ammonia in the brain is linked to the metabolism of glutamate in astrocytes, damage to these cells has been described in PH animals, which involves glutamate uptake and clearance[24].

Rapid clearance of neurotransmitters released from synapses, especially glutamate, acts to limit its signaling and prevents its harmful over-stimulation[25]. It has been well established for several decades that hyperammonemia leads to reduced glutamate uptake, which is caused by a reduced amount and function of glutamate transporters[26-28]. Moreover, it has been shown recently that reduced glutamate transporters and increased extracellular glutamate are responsible for hypokinesia in rats with HE and hyperammonemia[29].

GS plays a central role in the metabolic regulation of the excitatory neurotransmitter glutamate and in the detoxification of ammonia. GS is located mainly in astrocytes. It has been suggested that glutamate can regulate GS brain distribution[30]. This enzyme is responsible for the protection of neurons against excess ammonia and glutamate, by metabolizing both substances into glutamine. Approximately 85% of ammonia is converted to glutamine[31,32]. In addition, glutamate from neurons can be reconverted into glutamine[33]. Astrocytes play a key role in the pathogenesis of ammonia-induced neurotoxicity and HE. Schliess *et al*[34] have found that ammonia induces protein tyrosine nitration in cultured rat astrocytes, which is sensitive to the NMDA receptor antagonist MK-801. Actually, the production of reactive nitrogen intermediates and protein tyrosine nitration may alter astrocyte function and contribute to ammonia neurotoxicity[34].

Acute intoxication with large doses of ammonia leads to CNS cell damage. The main mechanism for ammonia elimination in the brain is its reaction with glutamate to form glutamine. This reaction is catalyzed by GS and consumes ATP[35]. It has observed that GS activity and

glutamine content in the brain are modulated by NMDA receptors and nitric oxide.

There are two main types of hyperammonemia: (1) chronic moderate hyperammonemia, as occurs in liver cirrhosis, which leads to altered cerebral function, and is responsible for the neurological alterations in different hyperammonemic states, and also for some of the neurological alterations in liver disease and HE; and (2) acute intoxication with large doses of ammonia, which may lead to rapid death of animals or patients. This situation can occur in acute liver failure.

Direct toxic effects of glutamine on CNS cells have been demonstrated in isolated mitochondria, which shows that elevated accumulation of glutamine has injurious effects on these cells[36].

Isolated rat cerebral mitochondria treated with high glutamine concentrations (5 mmol/L), as present in acute hyperammonemic rats, show swelling and mitochondrial permeability transition (MPT)[37]. Murthy *et al*[38] have shown that ammonia alone does not induce MPT, but that its metabolism to glutamine is necessary to produce this alteration. Mitochondrial swelling, as a result of the presence of high levels of glutamine and ammonia, is known to stimulate the uptake of glutamine[39].

The addition of glutamine to cultured astrocytes induces MPT development and the formation of free radicals[40]. Hence, glutamine synthesis, from ammonia and glutamate in astrocytes, represents an important process in brain ammonia detoxification, but also can produce negative effects.

The increased activity of GS in the FC, as seen in this PH rat model, may represent a response to moderate increases in ammonia. However, in the hippocampus, we observed decreased activity of GS, which may correspond to an increase in glutamate, caused by decreased uptake and a prolonged toxic effect.

The astrocyte is the brain cell that is directly involved in HE. It participates in chronic porto-systemic encephalopathy, with ultrastructural alterations in the brain. Astrocyte mitochondria are included in this pathophysiological mechanism in the hippocampus[41] and alterations in its respiratory chain, and show ultrastructural damage. By using proton magnetic resonance, Häussinger[42] has shown that brains from HE patients have an increase in glutamine/glutamate signaling. Structural and functional alterations in cultured astrocytes, when their exposure to different ammonia concentrations is modified, suggest the possibility that HE may constitute a primary gliopathy[43].

The mechanisms that produce brain damage in chronic encephalopathy may include the following. (1) Increased ammonia in the brain, which is associated with an increase in glutamate, caused by its reduced uptake, which leads to altered metabolic pathways and functional and morphological damage to the mitochondria in the hippocampus and FC; and (2) The hippocampus possesses a fundamental function in behavioral patterns on memory and spatial mechanisms among other functions. Therefore, it is possible that hippocampus suffers more readily from toxicity than the FC, which also participates in this process, but with minor involvement.

It is possible that the results obtained in the hippocampus in PH rats are indicative of its plasticity, which provokes, during toxic insult, changes in the expression of its enzymes, which maintain the functional equilibrium during this pathological situation. In the mammalian brain, this region is fundamental for the encoding of recent and past experience, including spatial and non-spatial information[41]. The FC has an efficient adaptative mechanism, not described in the hippocampus; its structure perhaps suffers more damage when toxic substances, such as ammonia, glutamate and glutamine are not detoxified efficiently. It may be that, in patients with cirrhosis, these mechanisms can participate in memory processes and behavioral alterations. It might be that the extracellular glutamate that arises from impairment of the glutamate/glutamine cycle in PH participates in the pathogenesis of HE. Furthermore, in PH, increases in ammonia and glutamate and/or glutamine may participate in oxidative stress that is induced by a glutamate-mediated pathway.

Warren and Schenker[44] have demonstrated that inhibition of GS by methionine sulfoximine decreases the death rate in rats intoxicated with ammonia. They found fewer metabolic alterations in HE, including brain edema, less astrocyte swelling, and a decrease in ammonia-induced reactive oxygen species. These experiments demonstrate the negative role that glutamine can play in the pathophysiology of HE[39,45-47]. In liver failure, brain edema, intracranial hypertension, neurotransmitter derangements and neurological symptoms represent the cerebral repercussions of distant liver parenchymal toxicity.

Our results from a PH rat model showed that two different brain regions had different responses in terms of GS activity and glutamate uptake. The BBB in these animals showed an increase in its permeability, apparently as a result of increased portal pressure, because when pressure returned to normal, the BBB recovered its permeability[20]. Also, an increase in astrocyte number, associated with an increase in endothelial cells (angiogenesis), has been documented previously[48].

Finally, it is clear that a partial stricture of the portal vein is capable of modifying functions in two brain regions, the hippocampus and FC. The different changes observed are difficult to explain. Perhaps, further experiments with PH models, using blockers and antagonist of glutamate uptake are needed to explain these results.

COMMENTS

Background

To analyze the participation of glutamine synthetase (GS) activity and glutamate uptake in the hippocampus and cerebral frontal cortex (FC), using a rat model of prehepatic portal hypertension (PH), with the intention of mimicking the two major complications that appear in chronic liver failure: hepatic encephalopathy (HE) and PH.

Research frontiers

In this field, the research hotspots are how to use prehepatic PH rats and how to study the different activities of GS and glutamate uptake in the hippocampus and FC.

Innovations and breakthroughs

This study determinate the uptake of [^{3}H] L-glutamate by synaptosomes of rat cerebral cortex. That provides accurate and reproducible data and can be employed to measure kinetic parameters, and this study determinate GS activity to maintain a stable glutamate/glutamine ratio in the brain.

Applications

Using the PH model, it is possible to understand more clearly the mechanism of toxicity and defense of the brain against two toxic substances: ammonia and the excitatory neurotransmitter glutamate.

Terminology

The extracellular actions of glutamate are limited by glutamate-specific Na^+-dependent transporters that remove glutamate, mostly by taking it up into astrocytes. Astrocytes convert glutamate into glutamine and pass it back to neurons, where it can be converted into glutamate and used to replenish the neurotransmitter stores in synaptic vesicles.

Peer review

The authors have used a rat model of PH and have analyzed the activity of GS and the uptake of glutamate by synaptosomes in the hippocampus and FC 14 d after surgery. The paper seems to be important for understanding HE. The whole paper is relatively easy to read and understand.

REFERENCES

1 **Schölmerich J**, Holstege A. Aetiology and pathophysiology of chronic liver disorders. *Drugs* 1990; **40** Suppl 3: 3-22
2 **Rodríguez-Vilarrupla A**, Fernández M, Bosch J, García-Pagán JC. Current concepts on the pathophysiology of portal hypertension. *Ann Hepatol* 2007; **6**: 28-36
3 **Albrecht J**. Roles of neuroactive amino acids in ammonia neurotoxicity. *J Neurosci Res* 1998; **51**: 133-138
4 **Norenberg MD**. Astrocytic-ammonia interactions in hepatic encephalopathy. *Semin Liver Dis* 1996; **16**: 245-253
5 **Scorticati C**, Prestifilippo JP, Eizayaga FX, Castro JL, Romay S, Fernández MA, Lemberg A, Perazzo JC. Hyperammonemia, brain edema and blood-brain barrier alterations in prehepatic portal hypertensive rats and paracetamol intoxication. *World J Gastroenterol* 2004; **10**: 1321-1324
6 **Albrecht J**, Sonnewald U, Waagepetersen HS, Schousboe A. Glutamine in the central nervous system: function and dysfunction. *Front Biosci* 2007; **12**: 332-343
7 **Felipo V**, Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol* 2002; **67**: 259-279
8 **Erceg S**, Monfort P, Hernandez-Viadel M, Llansola M, Montoliu C, Felipo V. Restoration of learning ability in hyperammonemic rats by increasing extracellular cGMP in brain. *Brain Res* 2005; **1036**: 115-121
9 **Ankarcrona M**, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* 1995; **15**: 961-973
10 **Hermenegildo C**, Monfort P, Felipo V. Activation of N-methyl-D-aspartate receptors in rat brain in vivo following acute ammonia intoxication: characterization by in vivo brain microdialysis. *Hepatology* 2000; **31**: 709-715
11 **Hermenegildo C**, Marcaida G, Montoliu C, Grisolía S, Miñana MD, Felipo V. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochem Res* 1996; **21**: 1237-1244
12 **Hansson E**, Rönnbäck L. Astrocytes in glutamate neurotransmission. *FASEB J* 1995; **9**: 343-350
13 **Suárez I**, Bodega G, Fernández B. Glutamine synthetase in brain: effect of ammonia. *Neurochem Int* 2002; **41**: 123-142
14 **Chojkier M**, Groszmann RJ. Measurement of portal-systemic shunting in the rat by using gamma-labeled microspheres. *Am J Physiol* 1981; **240**: G371-G375
15 **Rowe WB**, Ronzio RA, Wellner VP, Meister A. Glutamine synthetase (Sheep Brain). *Methods Enzymol* 1970; **17**: 900-910
16 **Glowinski J**, Iversen LL. Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. *J Neurochem* 1966; **13**: 655-669
17 **Takarada T**, Balcar VJ, Baba K, Takamoto A, Acosta GB, Takano K, Yoneda Y. Uptake of [3H]L-serine in rat brain synaptosomal fractions. *Brain Res* 2003; **983**: 36-47
18 **Lowry OH**, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275
19 **Winner BJ**. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill, 1971: 79–84
20 **Eizayaga F**, Scorticati C, Prestifilippo JP, Romay S, Fernandez MA, Castro JL, Lemberg A, Perazzo JC. Altered blood-brain barrier permeability in rats with prehepatic portal hypertension turns to normal when portal pressure is lowered. *World J Gastroenterol* 2006; **12**: 1367-1372
21 **Butterworth RF**. Hepatic encephalopathy: a neuropsychiatric disorder involving multiple neurotransmitter systems. *Curr Opin Neurol* 2000; **13**: 721-727
22 **Tanaka J**, Toku K, Zhang B, Ishihara K, Sakanaka M, Maeda N. Astrocytes prevent neuronal death induced by reactive oxygen and nitrogen species. *Glia* 1999; **28**: 85-96
23 **Wang GJ**, Chung HJ, Schnuer J, Lea E, Robinson MB, Potthoff WK, Aizenman E, Rosenberg PA. Dihydrokainate-sensitive neuronal glutamate transport is required for protection of rat cortical neurons in culture against synaptically released glutamate. *Eur J Neurosci* 1998; **10**: 2523-2531
24 **Ohtsuki S**. New aspects of the blood-brain barrier transporters; its physiological roles in the central nervous system. *Biol Pharm Bull* 2004; **27**: 1489-1496
25 **Clements JD**, Lester RA, Tong G, Jahr CE, Westbrook GL. The time course of glutamate in the synaptic cleft. *Science* 1992; **258**: 1498-1501
26 **Mena EE**, Cotman CW. Pathologic concentrations of ammonium ions block L-glutamate uptake. *Exp Neurol* 1985; **89**: 259-263
27 **Norenberg MD**, Huo Z, Neary JT, Roig-Cantesano A. The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. *Glia* 1997; **21**: 124-133
28 **Monfort P**, Kosenko E, Erceg S, Canales JJ, Felipo V. Molecular mechanism of acute ammonia toxicity: role of NMDA receptors. *Neurochem Int* 2002; **41**: 95-102
29 **Cauli O**, Llansola M, Erceg S, Felipo V. Hypolocomotion in rats with chronic liver failure is due to increased glutamate and activation of metabotropic glutamate receptors in substantia nigra. *J Hepatol* 2006; **45**: 654-661
30 **Bender AS**, Norenberg MD. Effects of ammonia on L-glutamate uptake in cultured astrocytes. *Neurochem Res* 1996; **21**: 567-573
31 **Yudkoff M**, Nissim I, Pleasure D. Astrocyte metabolism of [15N]glutamine: implications for the glutamine-glutamate cycle. *J Neurochem* 1988; **51**: 843-850
32 **Sonnewald U**, Westergaard N, Jones P, Taylor A, Bachelard HS, Schousboe A. Metabolism of [U-13C5] glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling. *J Neurochem* 1996; **67**: 2566-2572
33 **Hertz L**, Swanson RA, Newman GC, Marrif H, Juurlink BH, Peng L. Can experimental conditions explain the discrepancy over glutamate stimulation of aerobic glycolysis? *Dev Neurosci* 1998; **20**: 339-347
34 **Schliess F**, Görg B, Fischer R, Desjardins P, Bidmon HJ, Herrmann A, Butterworth RF, Zilles K, Häussinger D. Ammonia induces MK-801-sensitive nitration and phosphorylation of protein tyrosine residues in rat astrocytes. *FASEB J* 2002; **16**: 739-741
35 **Kosenko E**, Llansola M, Montoliu C, Monfort P, Rodrigo R, Hernandez-Viadel M, Erceg S, Sánchez-Perez AM, Felipo V. Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. *Neurochem Int* 2003; **43**: 493-499
36 **Willard-Mack CL**, Koehler RC, Hirata T, Cork LC,

Takahashi H, Traystman RJ, Brusilow SW. Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience* 1996; **71**: 589-599

37 **Ziemińska E**, Dolińska M, Lazarewicz JW, Albrecht J. Induction of permeability transition and swelling of rat brain mitochondria by glutamine. *Neurotoxicology* 2000; **21**: 295-300

38 **Murthy CR**, Rama Rao KV, Bai G, Norenberg MD. Ammonia-induced production of free radicals in primary cultures of rat astrocytes. *J Neurosci Res* 2001; **66**: 282-288

39 **Dolińska M**, Hilgier W, Albrecht J. Ammonia stimulates glutamine uptake to the cerebral non-synaptic mitochondria of the rat. *Neurosci Lett* 1996; **213**: 45-48

40 **Rama Rao KV**, Jayakumar AR, Norenberg MD. Induction of the mitochondrial permeability transition in cultured astrocytes by glutamine. *Neurochem Int* 2003; **43**: 517-523

41 **Lores-Arnaiz S**, Perazzo JC, Prestifilippo JP, Lago N, D'Amico G, Czerniczyniec A, Bustamante J, Boveris A, Lemberg A. Hippocampal mitochondrial dysfunction with decreased mtNOS activity in prehepatic portal hypertensive rats. *Neurochem Int* 2005; **47**: 362-368

42 **Häussinger D**. Regulation of hepatic ammonia metabolism: the intercellular glutamine cycle. *Adv Enzyme Regul* 1986; **25**: 159-180

43 **Rama Rao KV**, Jayakumar AR, Norenberg DM. Ammonia neurotoxicity: role of the mitochondrial permeability transition. *Metab Brain Dis* 2003; **18**: 113-127

44 **Warren KS**, Schenker S. Effect of an inhibitor of glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. *J Lab Clin Med* 1964; **64**: 442-449

45 **Blei AT**, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. *Hepatology* 1994; **19**: 1437-1444

46 **Takahashi H**, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am J Physiol* 1991; **261**: H825-H829

47 **Jayakumar AR**, Rama Rao KV, Schousboe A, Norenberg MD. Glutamine-induced free radical production in cultured astrocytes. *Glia* 2004; **46**: 296-301

48 **Perazzo JC**, Canessa O, Ferrini M, Franchino MA, Bengochea A, Ghanem C, Lemberg A. Alteraciones morfológicas de Astrocitos en cerebros de ratas hipertensas portales. *Medicina* (Buenos Aires) 1998; **58**: 582 (Abst)

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2900

World J Gastroenterol 2009 June 21; 15(23): 2900-2903
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Diagnostic value of maximal-outer-diameter and maximal-mural-thickness in use of ultrasound for acute appendicitis in children

Bo-Kyung Je, Sung-Bum Kim, Seung Hwa Lee, Ki Yeol Lee, Sang Hoon Cha

Bo-Kyung Je, Seung Hwa Lee, Ki Yeol Lee, Sang Hoon Cha, Department of Radiology, College of Medicine, Korea University, #516, Gojan1-Dong, Danwon-Gu, Ansan-City, Gyeonggi-Do 425-707, South Korea
Sung-Bum Kim, Department of Internal Medicine, Dongshin Hospital, #430, Hongeundong, Seodaemungu, Seoul 120-100, South Korea
Author contributions: Je BK, Lee SH, Lee KY and Cha SH performed the US examinations; Kim SB analyzed the data and was also involved in editing the manuscript; Je BK designed the study and wrote the manuscript.
Supported by A grant from Korea University
Correspondence to: Dr. Bo-Kyung Je, Department of Radiology, College of Medicine, Korea University, #516, Gojan1-Dong, Danwon-Gu, Ansan-City, Gyeonggi-Do 425-707, South Korea. radje@korea.ac.kr
Telephone: +82-31-4125227 Fax: +82-31-4125224
Received: March 10, 2009 Revised: April 14, 2009
Accepted: April 21, 2009
Published online: June 21, 2009

Abstract

AIM: To evaluate the maximal-outer-diameter (MOD) and the maximal-mural-thickness (MMT) of the appendix in children with acute appendicitis and to determine their optimal cut-off values to diagnose acute appendicitis.

METHODS: In total, 164 appendixes from 160 children between 1 and 17 years old (84 males, 76 females; mean age, 7.38 years) were examined by high-resolution abdominal ultrasound for acute abdominal pain and the suspicion of acute appendicitis. We measured the MOD and the MMT at the thickest point of the appendix. Patients were categorized into two groups according to their medical records: patients who had surgery (surgical appendix group) and patients who did not have surgery (non-surgical appendix group). Data were analyzed by MedCalc v.9.3. The rank sum test (Mann-Whitney test) was used to evaluate the difference in the MOD and the MMT between the two groups. ROC curve analysis was used to determine the optimal cut-off value of the MOD and the MMT on diagnosis of acute appendicitis.

RESULTS: There were 121 appendixes (73.8%) in the non-surgical appendix group and 43 appendixes (26.2%) in the surgical appendix group. The median MOD differed significantly between the two groups (0.37 cm *vs* 0.76 cm, $P < 0.0001$), and the median MMT also differed (0.15 cm *vs* 0.33 cm, $P < 0.0001$). The optimal cut-off value of the MOD and the MMT for diagnosis of acute appendicitis in children was > 0.57 cm (sensitivity 95.4%, specificity 93.4%) and > 0.22 cm (sensitivity 90.7%, specificity 79.3%), respectively.

CONCLUSION: The MOD and the MMT are reliable criteria to diagnose acute appendicitis in children. An MOD > 0.57 cm and an MMT > 0.22 cm are the optimal criteria.

Key words: Appendicitis; Ultrasonography; Pediatrics; Diagnosis; ROC curve

Peer reviewers: Kenji Miki, MD, Department of Surgery, Showa General Hospital, 2-450 Tenjin-cho, Kodaira, Tokyo 187-8510, Japan; Frank I Tovey, OBE, ChM, FRCS, Honorary Research Fellow, Department of Surgery, University College London, London, United Kingdom

Je BK, Kim SB, Lee SH, Lee KY, Cha SH. Diagnostic value of maximal-outer-diameter and maximal-mural-thickness in use of ultrasound for acute appendicitis in children. *World J Gastroenterol* 2009; 15(23): 2900-2903 Available from: URL: http://www.wjgnet.com/1007-9327/15/2900.asp DOI: http://dx.doi.org/10.3748/wjg.15.2900

INTRODUCTION

Since Puylaert[1] described the role of ultrasound (US) as a diagnostic method for acute appendicitis in 1986, the diagnosis of acute appendicitis has become more dependent on the use of US, especially in the doubtful cases for the clinicians. Because of the smaller trunks and thinner subcutaneous fat layer in children compared to adults, US evaluation of the appendix is easier in children. According to previous reports[1-4], radiologists have used several US findings to diagnose acute appendicitis. Among these findings, the maximal outer diameter (MOD) of the appendix was regarded as the most reliable measurement. When the MOD is > 0.6 cm, radiologists suggest the presence of acute appendicitis is indicated.

However, the MOD may be larger than 0.6 cm without acute inflammation. The concern is that the

MOD may be exaggerated by the presence of intra-luminal materials such as gas, feces and fluid[5-7]. To decrease the false positive rate of the MOD criterion, some radiologists have recently attempted to determine another size criterion, the maximal mural thickness (MMT) of the appendix[8-10].

The purpose of this study was to evaluate the diagnostic value of the MOD and the MMT measurements of the appendix in children with clinical suspicion of acute appendicitis and to determine the optimal cut-off values of these measurements in diagnosis of acute appendicitis.

MATERIALS AND METHODS

Among the abdominal US of the children who visited our institute for acute abdominal pain and the suspicion of acute appendicitis between July 2004 and November 2008, we selected 160 children who had a visible appendix on US. These children were aged 1-17 years (84 males, 76 females; mean age, 7.38 years).

After receiving informed consent, the children were examined by experienced radiologists with three different US units (iU22, Philips Medical Systems, Andover, MA, USA; HDI 5000 SonoCT, Philips Medical Systems, Best, The Netherlands; ATL HDI 5000, Philips Medical Systems, Andover, MA, USA). The appendix was scanned from the base to the tip under graded compression using a high resolution transducer (9-12 MHz linear transducer or a 5-8 MHz sector transducer). Then the MOD and the MMT were measured at the thickest point in the cross-sectional image (Figure 1). The MOD was defined as the distance between the outer hyperechoic borders of the the appendix, and the MMT was defined as the distance from the hyperechoic luminal interface to the outer hyperechoic border. Intra-luminal contents including fluid, gas, feces, stones or nothing were recorded.

The medical records of the patients were traced till the symptoms were resolved. We categorized the patients into two groups: patients who had surgery (surgical appendix group) and patients who recovered without surgery (non-surgical appendix group). Data were analyzed by MedCalc v.9.3 software (MedCalc, Mariakerke, Belgium). We used the rank sum test (Mann-Whitney test) to evaluate the difference in the MOD and the MMT between the two groups and used ROC curve analysis to determine the optimal cut-off values of the MOD and the MMT for diagnosing acute appendicitis.

RESULTS

The MOD and the MMT of 164 appendixes in 160 children were included in this study. Forty-four children underwent an appendectomy. The pathological diagnoses were 15 cases of acute appendicitis (without any comment), six cases of acute early appendicitis, 13 cases of acute suppurative appendicitis, six cases of acute gangrenous appendicitis, two cases of acute gangrenous appendicitis with perforation, one case of acute necrotizing appendicitis and one case of congestion. As congestion did not contain any inflammatory cells, the case of congestion was re-classified in the non-surgical appendix group. The case of congestion was finally diagnosed with Crohn's disease in the ileum and cecum. As a result, there were 121 appendixes (73.8%) in the non-surgical appendix group and 43 appendixes (26.2%) in the surgical appendix group.

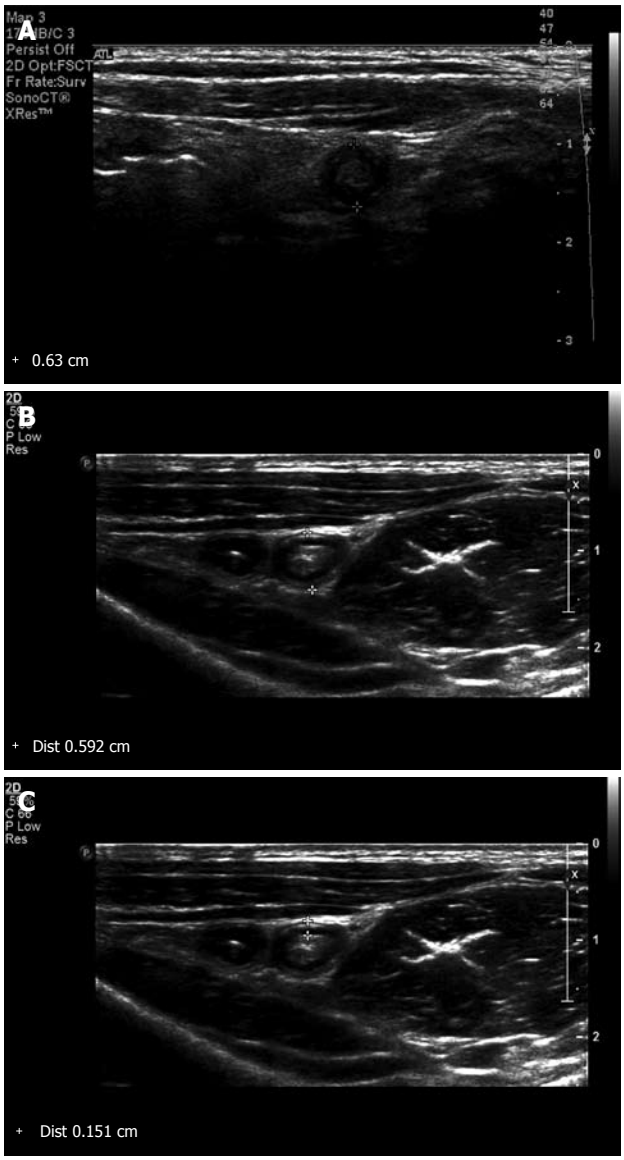

Figure 1 Measurement of MOD and MMT of an appendix in the cross-sectional image. The MOD (A) of the appendix of a 10-year-old boy who had surgery for acute appendicitis. The MOD (B) and the MMT (C) of the appendix of a 7-year-old boy who had a normal appendix in spite of acute abdominal pain.

Because each reference interval of the MOD and the MMT rejected normality ($P < 0.0001$ in each case), the Mann-Whitney test was used for the statistical analysis.

The range of the MOD was 0.20-1.45 cm in all patients, 0.20-0.69 cm in the non-surgical appendix group and 0.42-1.45 cm in the surgical appendix group. The median MOD of 0.37 cm (95% CI: 0.29-0.45 cm) in the non-surgical appendix group was significantly different ($P < 0.0001$) from the median MOD of 0.76 cm (95% CI: 0.69-0.90 cm) in the surgical appendix group. The data comparison graphs between the two groups were presented in Figure 2A.

The range of the MMT was 0.08-0.58 cm in all patients, 0.08-0.49 cm in the non-surgical appendix group

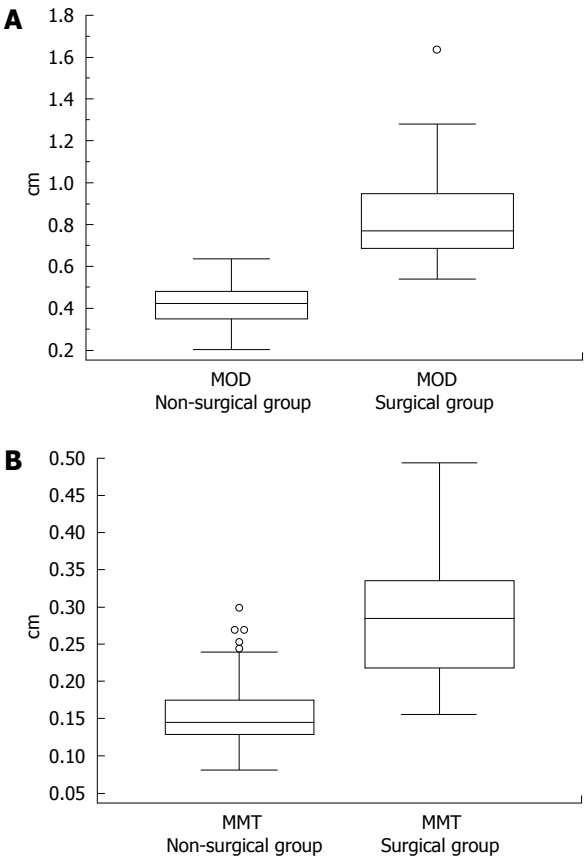

Figure 2 Data comparison graphs of MOD (A) and MMT (B) in box-and-whisker plots. The median MOD and the median MMT were significantly different ($P < 0.0001$) between two groups.

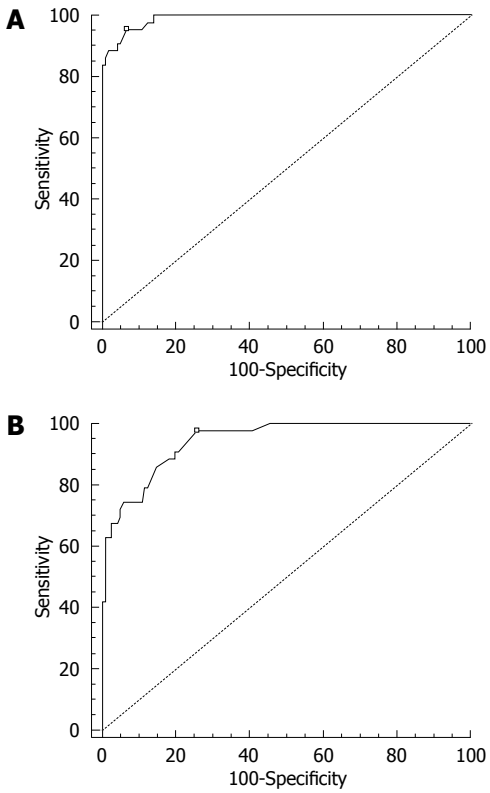

Figure 3 ROC curves of MOD (A) and MMT (B). The optimal cut-off points are marked as white square boxes on the graphs.

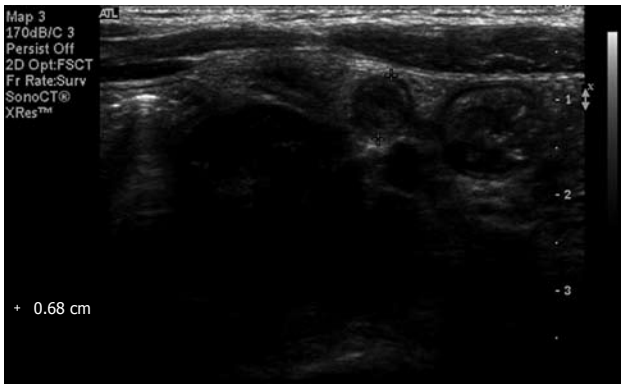

Figure 4 Cross-sectional image of a distended normal appendix in a 7-year-old boy. The MOD of the appendix was 0.65 cm. The intra-luminal hyperechogenicity was due to gas and fecal materials. We diagnosed this as normal appendix, and the symptoms spontaneously resolved.

and 0.10-0.58 cm in the surgical appendix group. The median MMT of 0.15 cm (95% CI: 0.13-0.17 cm) in the non-surgical appendix group was significantly different ($P < 0.0001$) from the median MMT of 0.33 cm (95% CI: 0.30-0.38 cm) in the surgical appendix group. The data comparison graphs between the two groups were presented in Figure 2B.

By ROC curve analysis, the optimal cut-off MOD was 0.57 cm with 89.6% sensitivity, 93.2% specificity and a 13.1 positive likelihood ratio (Figure 3A). The optimal cut-off MMT was 0.22 cm with 84.6% sensitivity, 95.8% specificity and a 20.1 positive likelihood ratio (Figure 3B).

DISCUSSION

According to previous reports[1-4,11], the diagnosis of acute appendicitis by US is based on the following findings; the MOD of the appendix > 0.6 cm; the appendix cannot be compressed with manual pressure by the examiner; the cross-sectional shape of the appendix is round rather than oval; there is an absence of gas in the appendiceal lumen; and there is hyperperfusion of the appendiceal wall on a Doppler study. However, the most credible criterion, the MOD, may be exaggerated and inaccurate in certain conditions (Figure 4)[5-7].

Earlier, Park *et al*[10] suggested that the MMT may have a role as a useful adjunctive measurement, especially for patients with fecal-impacted, non-inflammatory appendixes. As well as this study, there have been several research studies measuring the MMT in children. Simonovský[8] reported that the difference in the normal appendiceal MMT between a group of young children and adolescents and an adult group was marginally significant ($P = 0.042$). In addition, the investigator stated that an MMT < 3 mm should be regarded as normal in children aged six years or younger. Wiersma *et al*[9] also reported the sizes of the MOD and the MMT of a normal appendix in children as 0.21-0.64 cm and 0.11-0.27 cm, respectively. However, they studied only normal appendixes.

In our study, we examined data for both diseased and disease-free appendixes on a large scale, and we compared the MOD and MMT between the surgical appendix and the non-surgical appendix. In a statistical

analysis, both the MMT and the MOD had diagnostic value for acute appendicitis in children.

In addition, we were able to obtain the optimal cut-off MOD and MMT values for diagnosing acute appendicitis in pediatric patients. By ROC curve analysis, the optimal cut-off MOD was 0.57 cm with 89.6% sensitivity, 93.2% specificity and a 13.1 positive likelihood ratio. The optimal cut-off MMT was 0.22 cm with 84.6% sensitivity, 95.8% specificity and a 20.1 positive likelihood ratio. Considering that literature during the last three years has reported that the sensitivity and the specificity for diagnosis of acute appendicitis with US were 80%-100% and 86.5%-100%[10,12-19], our study showed acceptable sensitivity and specificity. While we expected that the MMT would be more sensitive than the MOD, the MMT was less sensitive but more specific than the MOD. We presumed that the MMT could only decrease the false positive ratio of the MOD and could not affect the false negative ratio as the MOD was more sensitive. Therefore, in children, an MOD > 0.57 cm suggests the presence of acute appendicitis and an MMT > 0.22 cm enhances the possibility of having acute appendicitis.

There were several limitations to this study. At first, because our data were obtained only from visible appendixes on US, cases of perforated appendicitis were excluded. Secondly, we categorized patients according to the results of surgery, and therefore cases of chronic or abortive appendicitis may be categorized in the non-surgical appendix group. Thirdly, because the examiner was not one radiologist, inter-observer variance still exists.

In conclusion, the MOD and the MMT are reliable criteria for diagnosis of acute appendicitis in children. An MOD > 0.57 cm and an MMT > 0.22 cm are the optimal criteria.

COMMENTS

Background

US became a reliable modality for diagnosing acute appendicitis, especially in doubtful cases for clinicians. When the maximal outer diameter (MOD) of the appendix is larger than 0.6 cm, radiologists suggest the presence of acute appendicitis. However, because the MOD may be larger than 0.6 cm without acute inflammation, the maximal mural thickness (MMT) of the appendix has been evaluated as another size criterion.

Research frontiers

Dr. Wiersma and colleagues in Juliana Children's hospital in The Netherlands reported the sizes of the MOD and the MMT in the normal appendix. Dr. Park and colleagues in Kwandong University Myongji Hospital in South Korea suggested that the MMT may play a role as a useful measurement for the fecal-impacted, non-inflammatory appendix. However, they included only normal appendixes. The authors examined data from both diseased and disease-free appendixes on a large scale to evaluate the MMT and the MOD and to determine the optimal cut-off values of these measurements in the diagnosis of acute appendicitis in children.

Applications

The MOD and the MMT are reliable criteria for diagnosing acute appendicitis in children. An MOD > 0.57 cm and an MMT > 0.22 cm are the optimal criteria.

Peer review

The authors demonstrated the usefulness of measurements of MOD and MMT in diagnosing pediatric acute appendicitis. They compared the MOD and MMT between surgical and non-surgical appendix groups and showed significant differences between the two groups. They also determined optimal cut-off value of MOD and MMT for diagnosis of acute appendicitis. The manuscript was well organized and well written.

REFERENCES

1 **Puylaert JB**. Acute appendicitis: US evaluation using graded compression. *Radiology* 1986; **158**: 355-360
2 **Jeffrey RB Jr**, Laing FC, Townsend RR. Acute appendicitis: sonographic criteria based on 250 cases. *Radiology* 1988; **167**: 327-329
3 **Rettenbacher T**, Hollerweger A, Macheiner P, Gritzmann N, Daniaux M, Schwamberger K, Ulmer H, zur Nedden D. Ovoid shape of the vermiform appendix: a criterion to exclude acute appendicitis--evaluation with US. *Radiology* 2003; **226**: 95-100
4 **Rettenbacher T**, Hollerweger A, Macheiner P, Rettenbacher L, Tomaselli F, Schneider B, Gritzmann N. Outer diameter of the vermiform appendix as a sign of acute appendicitis: evaluation at US. *Radiology* 2001; **218**: 757-762
5 **Hahn HB**, Hoepner FU, Kalle T, Macdonald EB, Prantl F, Spitzer IM, Faerber DR. Sonography of acute appendicitis in children: 7 years experience. *Pediatr Radiol* 1998; **28**: 147-151
6 **Rioux M**. Sonographic detection of the normal and abnormal appendix. *AJR Am J Roentgenol* 1992; **158**: 773-778
7 **Simonovský V**. Sonographic detection of normal and abnormal appendix. *Clin Radiol* 1999; **54**: 533-539
8 **Simonovský V**. Normal appendix: is there any significant difference in the maximal mural thickness at US between pediatric and adult populations? *Radiology* 2002; **224**: 333-337
9 **Wiersma F**, Srámek A, Holscher HC. US features of the normal appendix and surrounding area in children. *Radiology* 2005; **235**: 1018-1022
10 **Park NH**, Park CS, Lee EJ, Kim MS, Ryu JA, Bae JM, Song JS. Ultrasonographic findings identifying the faecal-impacted appendix: differential findings with acute appendicitis. *Br J Radiol* 2007; **80**: 872-877
11 **Rettenbacher T**, Hollerweger A, Macheiner P, Rettenbacher L, Frass R, Schneider B, Gritzmann N. Presence or absence of gas in the appendix: additional criteria to rule out or confirm acute appendicitis--evaluation with US. *Radiology* 2000; **214**: 183-187
12 **Yu SH**, Kim CB, Park JW, Kim MS, Radosevich DM. Ultrasonography in the diagnosis of appendicitis: evaluation by meta-analysis. *Korean J Radiol* 2005; **6**: 267-277
13 **Assefa G**, Meseret S, Nigussie Y. The role of ultrasound in diagnosing acute appendicictis. *Ethiop Med J* 2006; **44**: 67-74
14 **Doria AS**, Moineddin R, Kellenberger CJ, Epelman M, Beyene J, Schuh S, Babyn PS, Dick PT. US or CT for Diagnosis of Appendicitis in Children and Adults? A Meta-Analysis. *Radiology* 2006; **241**: 83-94
15 **Peletti AB**, Baldisserotto M. Optimizing US examination to detect the normal and abnormal appendix in children. *Pediatr Radiol* 2006; **36**: 1171-1176
16 **Chang YJ**, Kong MS, Hsia SH, Wu CT, Lai MW, Yan DC, Chao HC, Chen CC, Chen SY. Usefulness of ultrasonography in acute appendicitis in early childhood. *J Pediatr Gastroenterol Nutr* 2007; **44**: 592-595
17 **Johansson EP**, Rydh A, Riklund KA. Ultrasound, computed tomography, and laboratory findings in the diagnosis of appendicitis. *Acta Radiol* 2007; **48**: 267-273
18 **Mardan MA**, Mufti TS, Khattak IU, Chilkunda N, Alshayeb AA, Mohammad AM, ur Rehman Z. Role of ultrasound in acute appendicitis. *J Ayub Med Coll Abbottabad* 2007; **19**: 72-79
19 **Khanal BR**, Ansari MA, Pradhan S. Accuracy of ultrasonography in the diagnosis of acute appendicitis. *Kathmandu Univ Med J* (KUMJ) 2008; **6**: 70-74

S- Editor Cheng JX **L- Editor** O'Neill M **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2904

World J Gastroenterol 2009 June 21; 15(23): 2904-2907
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Experience of limited pancreatic head resection for management of branch duct intraductal papillary mucinous neoplasm in a single center

Kwang Yeol Paik, Seong Ho Choi

Kwang Yeol Paik, Department of Surgery, Kepco Medical Foundation, Hanil General Hospital, #388-1 Ssangmoon-Dong, Dobong-Gu, Seoul 132-033, South Korea
Seong Ho Choi, Department of Surgery, Samsung Medical Center, College of Medicine, Sungkyunkwan University, #50 Irwon-Dong, Gangnam-Gu, Seoul 135-710, South Korea
Author contributions: Paik KY wrote the paper; Choi SH designed the research; Paik KY and Choi SH performed the research; Paik KY provided new reagents and analytical tools and analyzed data.
Supported by IN-SUNG Foundation for Medical Research # CA98111
Correspondence to: Seong Ho Choi, Department of Surgery, Samsung Medical Center, College of Medicine, Sungkyunkwan University, #50 Irwon-Dong, Gangnam-Gu, Seoul 135-710, South Korea. pancreas@skku.edu
Telephone: + 82-2-34101669 Fax: +82-2-34100090
Received: March 19, 2009 Revised: May 13, 2009
Accepted: May 20, 2009
Published online: June 21, 2009

Abstract

AIM: To share our surgical experience and the outcome of limited pancreatic head resection for the management of branch duct intraductal papillary mucinous neoplasm (IPMN).

METHODS: Between May 2005 and February 2008, nine limited pancreatic head resections (LPHR) were performed for IPMN of the pancreatic head. We reviewed the nine patients, retrospectively.

RESULTS: Tumor was located in the uncinate process of the pancreas in all nine patients. Three patients had stents inserted in the main pancreatic duct due to injury. The mean size of tumor was 28.4 mm. Postoperative complications were found in five patients: 3 pancreatic leakages, a pancreatitis, and a duodenal stricture. Pancreatic leakages were improved by external drainage. No perioperative mortality was observed and all patients are recorded alive during the mean follow-up period of 17.2 mo.

CONCLUSION: In selected patients after careful evaluation, LPHR can be used for the treatment of branch duct type IPMN. In order to avoid pancreatic ductal injury, pre- and intra-operative definite localization and careful operative techniques are required.

Key words: Limited pancreatic head resection; Branch duct type; Intraductal papillary mucinous neoplasm

Peer reviewer: Ian C Roberts-Thomson, Professor, Department of Gastroenterology and Hepatology, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South 5011, Australia

Paik KY, Choi SH. Experience of limited pancreatic head resection for management of branch duct intraductal papillary mucinous neoplasm in a single center. *World J Gastroenterol* 2009; 15(23): 2904-2907 Available from: URL: http://www.wjgnet.com/1007-9327/15/2904.asp DOI: http://dx.doi.org/10.3748/wjg.15.2904

INTRODUCTION

Branch duct type intraductal papillary mucinous neoplasm (IPMN) has a low malignant potential and is more frequently located in the head of the pancreas[1-4]. When this lesion is located in the pancreatic head, the conventional treatment for IPMN has been pancreaticoduodenectomy (PD). Also, partial pancreatic resection is feasible as a treatment for small branch duct type IPMN, which shows less aggressive behavior.

In recent years, some surgeons have advocated limited pancreatectomy for management of benign IPMN and regarding this the following procedures have been reported in several papers: inferior head resection of the pancreas[5], partial pancreatic head resection[6], uncinate process resection[7,8], single branch resection of the pancreas[9], and ductal branch oriented minimal pancreatectomy[10]. It had been expected that there would be advantages of minimal pancreatic parenchymal loss and prevention of functional insufficiency by performing limited pancreatectomy. However, recommendations and reports of postoperative complications and clinical outcomes following these procedures have been limited. Therefore we report a single center surgical experience and short-term outcome of limited pancreatic head resection (LPHR) for the management of branch duct type IPMN.

MATERIALS AND METHODS

From May 2005 to February 2008, a retrospective review

Table 1 Clinical aspect of patients undergoing LPHR

Case No.	F/U (mo)	Complication	Pathology	Size (mm)	Preop Dx	Pancreatogram	Age	Gender
1	24	P-leakage	Adenoma	30	MCN	ERCP	69	F
2	20	Pancreatitis	Adenoma	25	Cystic.n	ERCP	67	M
3	22	P-leakage	Adenoma	20	IPMN	MRCP	71	M
4	22	D-stricture	Adenoma	22	Cystic.n	ERCP	72	M
5	19	-	Adenoma	25	MCN	ERCP	50	F
6	14	-	Adenoma	42	IPMN	ERCP	69	M
7	12	-	Adenoma	27	IPMN	MRCP	60	M
8	12	P-leakage	Adenoma	30	IPMN	MRCP	42	M
9	10	-	Adenoma	35	IPMN	MRCP	67	F

ERCP: Endoscopic retrograde cholangiopancreatogram; MRCP: Magnetic resonance cholangiopancreatogram; MCN: Mucinous cystic neoplasm; Cystic.n: Cystic neoplasm of pancreas; IPMN: Intraductal papillary mucinous neoplasm; P-leakage: Pancreatic leakage; D-stricture: Duodenal stricture.

was undertaken of 12 patients who underwent partial pancreatectomy for IPMN at our institution, among whom, nine patients underwent LPHR. In-hospital clinical course and method of operation, postoperative complications and follow-up data were analyzed.

Assessment of the tumor location was carried out before surgery using computed tomography (CT) in all nine cases, upon suspicion of IPMN.

Additional evaluations were routinely performed with endoscopic retrograde cholagiopancreatography (ERCP) or magnetic resonance cholagiopancreatography (MRCP) for evaluating the pancreatic duct, which were confirmed during surgery through direct visualization of lesion by dissection of pancreas head and intraoperative ultrasonography (IOUSG). All pancreatectomies were performed by a single surgeon. Pancreatic parenchymal control was performed using bipolar coagulator for fine dissection and easy bleeding control. All nine resected tumors were examined by a single pathologist with regard to resection margin and tumor characteristics during operation. The definition of postoperative pancreatic leakage was in accordance with the International Study Group on Pancreatic Fistula (ISGPF) definition[11].

Indication for limited pancreatectomy

Prior to 2005, PD was the standard treatment for IPMN of the pancreas head. Sugiyama *et al*[12] reported that size > 30 mm and presence of mural nodules were the strongest predictors of malignancy in branch duct IPMN. But our previous study revealed that many of < 30 mm resected branch duct IPMN were malignant[13]. For these reason, under 30 mm-sized branch duct IPMN were resected at our center, and < 20 mm lesions were observed with close follow-up. Since 2005, LPHR procedure was tried for those lesions > 20 mm, mainly located in the uncinate process in which the main pancreatic duct was intact on imaging study, and branched IPMN was suspected on pancreatogram.

Operative procedure

Pancreatic head was exposed by omentectomy and superior mesenteric vein (SMV) branch ligation such as in PD. After the exposure, exploration of the lesion and main pancreatic duct was carried out using IOUSG. Preoperative or intraoperative pancreatic stents were not used before resection. Bipolar coagulator was used for fine dissection of pancreas head.

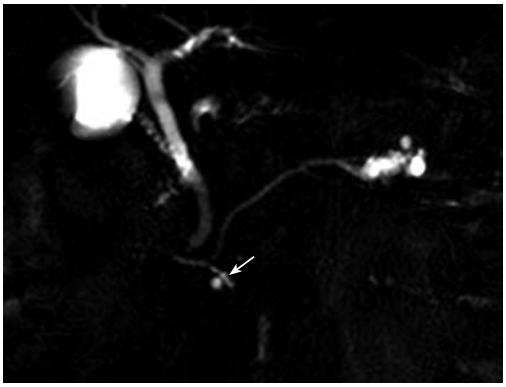

Figure 1 MRCP showing connection between main pancreatic duct and cystic lesion in uncinate process (arrow).

RESULTS

Clinical findings

The mean age of the patients was 63 years (range: 42-72 years), with six male patients. Six patients presented clinical symptoms and incidental lesions were found in three patients.

All nine patients underwent multidetector abdominal CT, four of nine patients underwent MRCP, and another five patients underwent ERCP. All nine tumors were located in the uncinate process, and two patients had another lesion in the tail portion of the pancreas. Five patients were confirmed IPMN by imaging studies, another four patients were suspected IPMN or other cystic neoplasm including mucinous cystic neoplasm (MCN). Clinical profiles are summarized in Table 1.

MRCP finding

Three quarters of the cases of MRCP showed connection between the main pancreatic duct and the cystic lesion in the uncinate process (Figure 1). These findings aroused suspicion of branched type of IPMN, and LPHR was planned.

LPHR

Uncinate process resection or ductal branch-oriented

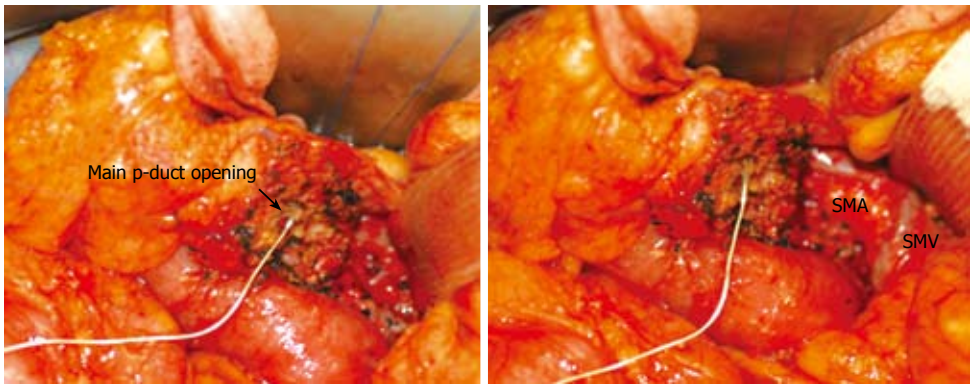

Figure 2 Main pancreatic duct was torn during uncinate process resection (arrow).

minimal pancreatectomy[7,10] were performed in six patients. Single-branch resection[9] was performed in three patients. Two of nine patients underwent additional distal pancreatectomy for pancreatic tail IPMN. The Wirsung duct was damaged in three of the six patients who underwent uncinate process resection or ductal branch-oriented minimal pancreatectomy in which internal silastic stents were inserted and primary repair was carried out (Figure 2). After pancreatectomy, we confirmed the pathologic diagnosis with resection margin.

Pathology

The mean tumor size was 28.4 mm (range 20-42 mm). All nine tumors were confirmed as intraductal papillary mucinous adenoma and resection margins were free of tumor.

Postoperative course

The mean number of hospital days was 21.1 d (range: 8-48 d). There was no mortality and five morbidities. Pancreatic leakage occurred in three patients, two of which involved injured pancreatic duct during operation and an inserted silastic internal pancreatic stent. The third ductal injury was not detected during operation. Pancreatic leakage was detected on postoperative days 4 and 5 respectively. One patient was discharged on postoperative day 32 with a Jackson-Pratt (JP) drain which was removed on day 62 after drain amylase was normalized. Another patient with re-exploration at diagnosis of leakage showed severe inflammation, because of which an external drain (sump drain) was added. Both patients were discharged at postoperative day 43 after removal of the drain. In the case of non-injury of the pancreatic duct, drain amylase increased after operation but normalized at postoperative day 15. One pancreatitis and one duodenal stricture were observed. Duodenal stricture was improved after gastrojejunostomy. The mean follow-up time was 17.2 mo during which there were no recurrences or metastases.

DISCUSSION

Despite the small number of cases included in this study, to our knowledge this study and evaluation of the nine cases of LPHR for branched IPMN is so far the largest amongst related studies. It can be said that partial pancreatic head resection can be a better treatment option than conventional PD for branch duct IPMN on pancreas head under free resection margin. PD may present as surgical overkill for benign and low-grade malignant tumors such as branch duct type IPMN of the pancreatic head. Such procedures result in a significant loss of normal pancreatic parenchyma with subsequent impairment of exocrine and endocrine pancreatic functions[14-16]. Resection of the distal bile duct in patients undergoing PD requires a bilio-enteric anastomosis, which increases the risk of ascending cholangitis and subsequent intrahepatic abscesses[17]. Following PD, the incidence of diabetes mellitus varies between 15% and 40%[18,19]. It can be noted in reports of recent papers that there is unchanged endocrine and exocrine pancreatic function following segmental pancreatic resection[20-22].

Ventral pancreatectomy was performed for management of cystic tumor as limited pancreatic head resection in 1993[23]. This procedure resected too great a width of normal pancreas for a small lesion and reconstructed pancreatic duct and bile duct by entric-anastomosis. Thereafter, pancreatic duct preserving procedures were reported, such as inferior pancreatic head resection, and uncinate process resection[5,7,8]. More minimal pancreatic head resections were performed such as single branch resection of the pancreas, and ductal branch-oriented minimal pancreatectomy[9,10].

Pancreatic leakage is one of the most frustrating complications after limited pancreatic resection. Although this study showed no mortality, three cases of pancreatic leakages occurred. Three pancreatic injuries were found during pancreatectomy. Firstly, the main pancreatic duct was injured after pancreatic dissection from the superior mesenteric vein (SMV) where mucin leaked from a small ductal opening in which a stent was inserted. Secondly, due to ductal tearing, the main pancreatic duct opening was widened during which a stent was inserted through the opening site. Lastly, minutely injured duct was repaired without stent insertion, without leakage after operation. Sata *et al*[9] experienced pancreatic leakages which were managed by the insertion of an endoscopic naso-pancreatic drainage tube. In segmental pancreas resection, pancreatic leakage rates reach up to 40%[20-22].

Generally, benign branch duct type IPMN in the pancreas head, especially the uncinate process, does not involve the main pancreatic duct. However IPMN too close to the main duct, or a large mass, need to be carefully evaluated. For safe dissection during operation without injuring the main pancreatic duct, pre- or intra-

operative pancreatic duct evaluation is crucial. IOUSG or MRCP were insufficient for detecting the pancreatic duct or distance of duct to mass during operation. Although all nine patients had IOUSG performed, three pancreatic injuries occurred. Takada *et al*[7] applied preoperative pancreatic duct stents guided by ERCP, during which the patients did not experience pancreatic leakage. They mentioned that the purpose of the stent was intraoperative identification of pancreatic duct with protection against iatrogenic injury, and postoperative drainage for minimizing pancreatic fistula. Yamaguchi *et al*[10] proposed the placement of a main duct tube for preventing transient stenosis of pancreatic duct. However in this study, preoperative stents were not applied. We speculated that the pancreatic drainage tube produced a pancreatitis.

LPHR does not necessitate anastomosis between the pancreatic duct, bile duct and the bowel. A disadvantage of LPHR was the higher rate of leakage (33.3%). In order to avoid pancreatic ductal injury, preoperative or intraoperative definite localization and careful surgical techniques were important. If the pancreatic duct was injured during operation, internal drainage procedure was necessary. If the disadvantage of ductal injury can be overcome, LPHR can be a useful procedure for the treatment of branch duct type IPMN in selected patients.

COMMENTS

Background

Branch duct type intraductal papillary mucinous neoplasm (IPMN) has a low malignant potential. Limited pancreatectomy is advocated for those lesions, thereby reducing the risk of functional insufficiency and morbidity in extensive resection.

Innovations and breakthroughs

Recommendations and reports of clinical outcomes following limited pancreatic head resection procedures were limited. Several case reports have been published. Despite the small number of cases included in this study, evaluation of the nine cases of limited pancreatic head resections (LPHR) for branched IPMN is so far the largest amongst related studies.

Applications

Disadvantage of LPHR was the higher rate of leakage. If the disadvantage of ductal injury can be overcome, LPHR can be a useful procedure for the treatment of branch duct type IPMN in selected patients.

Peer review

This interesting paper explores the possibility of limited resections for branch duct-type IPMN. But, three of 9 patients also developed a pancreatic fistula; a rate that is also higher than after pancreaticoduodenectomy.

REFERENCES

1 **Terris B**, Ponsot P, Paye F, Hammel P, Sauvanet A, Molas G, Bernades P, Belghiti J, Ruszniewski P, Flejou JF. Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less aggressive pathologic features as compared with those involving the main pancreatic duct. *Am J Surg Pathol* 2000; **24**: 1372-1377

2 **Sugiyama M**, Atomi Y. Intraductal papillary mucinous tumors of the pancreas: imaging studies and treatment strategies. *Ann Surg* 1998; **228**: 685-691

3 **Tanaka M**. Intraductal papillary mucinous neoplasm of the pancreas: diagnosis and treatment. *Pancreas* 2004; **28**: 282-288

4 **Kobari M**, Egawa S, Shibuya K, Shimamura H, Sunamura M, Takeda K, Matsuno S, Furukawa T. Intraductal papillary mucinous tumors of the pancreas comprise 2 clinical subtypes: differences in clinical characteristics and surgical management. *Arch Surg* 1999; **134**: 1131-1136

5 **Nakagohri T**, Kenmochi T, Kainuma O, Tokoro Y, Kobayashi S, Asano T. Inferior head resection of the pancreas for intraductal papillary mucinous tumors. *Am J Surg* 2000; **179**: 482-484

6 **Nakagohri T**, Konishi M, Inoue K, Izuishi K, Kinoshita T. Partial pancreatic head resection for intraductal papillary mucinous carcinoma originating in a branch of the duct of santorini. *Eur Surg Res* 2002; **34**: 437-440

7 **Takada T**, Amano H, Ammori BJ. A novel technique for multiple pancreatectomies: removal of unicinate process of the pancreas combined with medial pancreatectomy. *J Hepatobiliary Pancreat Surg* 2000; **7**: 49-52

8 **Sharma MS**, Brams DM, Birkett DH, Munson JL. Uncinatectomy: a novel surgical option for the management of intraductal papillary mucinous tumors of the pancreas. *Dig Surg* 2006; **23**: 121-124

9 **Sata N**, Koizumi M, Tsukahara M, Yoshizawa K, Kurihara K, Nagai H. Single-branch resection of the pancreas. *J Hepatobiliary Pancreat Surg* 2005; **12**: 71-75

10 **Yamaguchi K**, Shimizu S, Yokohata K, Noshiro H, Chijiiwa K, Tanaka M. Ductal branch-oriented minimal pancreatectomy: two cases of successful treatment. *J Hepatobiliary Pancreat Surg* 1999; **6**: 69-73

11 **Bassi C**, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005; **138**: 8-13

12 **Sugiyama M**, Izumisato Y, Abe N, Masaki T, Mori T, Atomi Y. Predictive factors for malignancy in intraductal papillary-mucinous tumours of the pancreas. *Br J Surg* 2003; **90**: 1244-1249

13 **Chung JC**, Jo SH, Choi SH, Choi DW, Kim YI. Surgical management for intraductal papillary mucinous tumor of pancreas confined to branch duct. *J Korean Surg Soc* 2006; **70**: 288-293

14 **Sakorafas GH**, Farnell MB, Nagorney DM, Sarr MG, Rowland CM. Pancreatoduodenectomy for chronic pancreatitis: long-term results in 105 patients. *Arch Surg* 2000; **135**: 517-523; discussion 523-524

15 **Tran K**, Van Eijck C, Di Carlo V, Hop WC, Zerbi A, Balzano G, Jeekel H. Occlusion of the pancreatic duct versus pancreaticojejunostomy: a prospective randomized trial. *Ann Surg* 2002; **236**: 422-428; discussion 428

16 **Rault A**, SaCunha A, Klopfenstein D, Larroude D, Epoy FN, Collet D, Masson B. Pancreaticojejunal anastomosis is preferable to pancreaticogastrostomy after pancreaticoduodenectomy for longterm outcomes of pancreatic exocrine function. *J Am Coll Surg* 2005; **201**: 239-244

17 **Yamaguchi K**, Tanaka M, Chijiiwa K, Nagakawa T, Imamura M, Takada T. Early and late complications of pylorus-preserving pancreatoduodenectomy in Japan 1998. *J Hepatobiliary Pancreat Surg* 1999; **6**: 303-311

18 **Beger HG**, Buchler M, Bittner RR, Oettinger W, Roscher R. Duodenum-preserving resection of the head of the pancreas in severe chronic pancreatitis. Early and late results. *Ann Surg* 1989; **209**: 273-278

19 **Martin RF**, Rossi RL, Leslie KA. Long-term results of pylorus-preserving pancreatoduodenectomy for chronic pancreatitis. *Arch Surg* 1996; **131**: 247-252

20 **Rotman N**, Sastre B, Fagniez PL. Medial pancreatectomy for tumors of the neck of the pancreas. *Surgery* 1993; **113**: 532-535

21 **Sperti C**, Pasquali C, Ferronato A, Pedrazzoli S. Median pancreatectomy for tumors of the neck and body of the pancreas. *J Am Coll Surg* 2000; **190**: 711-716

22 **Warshaw AL**, Rattner DW, Fernandez-del Castillo C, Z'graggen K. Middle segment pancreatectomy: a novel technique for conserving pancreatic tissue. *Arch Surg* 1998; **133**: 327-331

23 **Takada T**. Ventral pancreatectomy: resection of the ventral segment of the pancreas. *J Hepatobiliary Pancreat Surg* 1993; **1**: 36-40

S- Editor Tian L **L- Editor** Logan S **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2908

World J Gastroenterol 2009 June 21; 15(23): 2908-2912
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Ampullary carcinoma: Effect of preoperative biliary drainage on surgical outcome

Sheikh Anwar Abdullah, Tarun Gupta, Khairul Azhar Jaafar, Yaw Fui Alexander Chung, London Lucien Peng Jin Ooi, Steven Joseph Mesenas

Sheikh Anwar Abdullah, Tarun Gupta, Khairul Azhar Jaafar, Steven Joseph Mesenas, Department of Gastroenterology and Hepatology, Singapore General Hospital, 169608, Singapore
Yaw Fui Alexander Chung, London Lucien Peng Jin Ooi, Department of Surgery, Singapore General Hospital, 169608, Singapore
Author contributions: Abdullah SA, Gupta T, Jaafar KA, Mesenas SJ performed data collection and analysis; Mesenas SJ, Chung YFA and Ooi LLPJ performed surgical and endoscopic procedures.
Correspondence to: Dr. Steven Joseph Mesenas, Department of Gastroenterology and Hepatology, Singapore General Hospital, Outram Road 169608, Singapore. steven.mesenas@sgh.com.sg
Telephone: +65-81253452 Fax: +65-62273625
Received: February 13, 2009 Revised: April 29, 2009
Accepted: May 6, 2009
Published online: June 21, 2009

Abstract

AIM: To evaluate the influence of preoperative biliary drainage on morbidity and mortality after surgical resection for ampullary carcinoma.

METHODS: We analyzed retrospectively data for 82 patients who underwent potentially curative surgery for ampullary carcinoma between September 1993 and July 2007 at the Singapore General Hospital, a tertiary referral hospital. Diagnosis of ampullary carcinoma was confirmed histologically. Thirty-five patients underwent preoperative biliary drainage (PBD group), and 47 were not drained (non-PBD group). The mode of biliary drainage was endoscopic retrograde cholangiopancreatography (n = 33) or percutaneous biliary drainage (n = 2). The following parameters were analyzed: wound infection, intra-abdominal abscess, intra-abdominal or gastrointestinal bleeding, septicemia, biliary or pancreatic leakage, pancreatitis, gastroparesis, and re-operation rate. Mortality was assessed at 30 d (hospital mortality) and also long-term. The statistical endpoint of this study was patient survival after surgery.

RESULTS: The groups were well matched for demographic criteria, clinical presentation and operative characteristics, except for lower hemoglobin in the non-PBD group (10.9 ± 1.6 *vs* 11.8 ± 1.6 in the PBD group). Of the parameters assessing postoperative morbidity, incidence of wound infection was significantly less in the PBD than the non-PBD group [1 (2.9%) *vs* 12 (25.5%)]. However, the rest of the parameters did not differ significantly between the groups, i.e. sepsis [10 (28.6%) *vs* 14 (29.8%)], intra-abdominal bleeding [1 (2.9%) *vs* 5 (10.6%)], intra-abdominal abscess [1 (2.9%) *vs* 8 (17%)], gastrointestinal bleeding [3 (8.6%) *vs* 5 (10.6%)], pancreatic leakage [2 (5.7%) *vs* 3 (6.4%)], biliary leakage [2 (5.7%) *vs* 3 (6.4%)], pancreatitis [2 (5.7%) *vs* 2 (4.3%)], gastroparesis [6 (17.1%) *vs* 10 (21.3%)], need for blood transfusion [10 (28.6%) *vs* 17 (36.2%)] and re-operation rate [1 (2.9%) *vs* 5 (10.6%)]. There was no early mortality in either group. Median survival was 44 mo (95% CI: 34.2-53.8) in the PBD group and 41 mo (95% CI: 27.7-54.3; P = 0.86) in the non-PBD group.

CONCLUSION: Biliary drainage before surgery for ampullary cancer significantly reduced postoperative wound infection. Overall mortality was not influenced by preoperative drainage.

Key words: Ampullary carcinoma; Preoperative biliary drainage; Postoperative complications

Peer reviewer: Michael A Fink, MBBS FRACS, Department of Surgery, The University of Melbourne, Austin Hospital, Melbourne, Victoria 3084, Australia

Abdullah SA, Gupta T, Jaafar KA, Chung YFA, Ooi LLPJ, Mesenas SJ. Ampullary carcinoma: Effect of preoperative biliary drainage on surgical outcome. *World J Gastroenterol* 2009; 15(23): 2908-2912 Available from: URL: http://www.wjgnet.com/1007-9327/15/2908.asp DOI: http://dx.doi.org/10.3748/wjg.15.2908

INTRODUCTION

In patients with ampullary cancer who undergo surgical resection, obstructive jaundice is associated with a higher risk of postoperative complications than in non-jaundiced patients[1]. The impact of jaundice on postoperative morbidity and mortality is well known. However the routine use of preoperative biliary

drainage (PBD) remains controversial. The potential advantages of preoperative stenting include improved nutritional, metabolic and immune function and the possibility of reduced postoperative morbidity and mortality[1]. Opponents of PBD argue that it increases infective complications and morbidity[2,3]. However, there are certain clinical situations such as acute suppurative cholangitis and severe malnutrition in which urgent biliary drainage is indicated and can be life-saving[4]. It is not clear whether the procedure itself or its complications influence the morbidity after surgical resection. The optimal duration of preoperative drainage also remains unknown. Although several reports have been published, there are still no clear guidelines regarding the use of PBD in these patients. We analyzed our patients to assess the influence of PBD on postoperative outcome following pancreaticoduodenectomy (PD) for ampullary tumors.

MATERIALS AND METHODS

We reviewed retrospectively the records of all patients who underwent definitive surgery for carcinoma involving the ampulla of Vater between and September 1993 and July 2007 at the Singapore General Hospital, a tertiary care referral and teaching hospital.

Ampullary cancer was defined as a tumor arising from the ampulla, with evidence of invasion and histopathological signs of neoplasia. In the case of large tumors with involvement of adjacent duodenum or pancreas, the tumor was classified as ampullary if it was centered on the ampulla. Cancers of pancreatic, duodenal and choledochal origin were excluded.

Details of patients who underwent PD (Whipple's operation) or pylorus-preserving pancreaticoduodenectomy (PPPD) for ampullary tumors were entered into a database that included patient characteristics, details of biliary stenting, procedure-related infective complications, surgery, morbidity and mortality. The operations were performed in the Department of Surgery, Singapore General Hospital.

Morbidity

Pancreatic leakage was diagnosed when > 50 mL of drainage fluid, with a serum amylase concentration > 3 times the upper limit of normal was obtained on or after postoperative day 5, or when pancreatic anastomotic disruption was demonstrated radiologically[3]. Wound infection was defined as spontaneous or surgically released purulent discharge that was positive for bacterial growth on culture. Bile leakage was defined as a bilirubin concentration in the drainage fluid that exceeded that in the serum, which resulted in a change of clinical management or the occurrence of a bilioma that required drainage. Infectious morbidity was defined as any complication with evidence of associated localized or systemic infection indicated by fever, leukocytosis and positive culture.

Intra-abdominal or gastrointestinal bleeding was defined as bleeding with either hemodynamic instability or patients who required > 2 U blood transfusion or who required re-operation. Intra-abdominal abscess was defined as purulent discharge with positive cultures from abdominal drains placed at surgery, or as fluid collection that required a drainage procedure. Delayed gastric emptying was defined as inability to tolerate a regular diet for more than seven postoperative days, the need for nasogastric tube drainage for seven or more days postoperatively, or the need for tube reinsertion after removal.

Mortality

Hospital deaths were defined as those that occurred within 30 d of operation or as a direct result of postoperative complications. Late mortality was defined as mortality after 30 d during the follow-up period.

Pathological data

Pathological data were obtained from the patients' medical records and the surgical pathology files. Histological grade, type and the presence of malignant change were noted.

Statistical analysis

Results were expressed as medians and ranges or as numbers and percentages of patients. Two-tailed *t* test and χ^2 test were used for data analysis. SPSS version 13.0 statistical software was used (Chicago, IL, USA) for analysis. Differences were considered statistically significant at $P < 0.05$. The major statistical endpoint of this study was patient survival. Event time distributions for this endpoint were estimated using the method of Kaplan and Meier[5] and compared using the log-rank statistic.

RESULTS

Eighty-two patients were included in the study. Seventy-nine had Whipple's operation and three had PPPD for ampullary tumors. Forty patients (48.8%) were male and 42 (51.2%) were female. Thirty-five (42.7%) patients underwent preoperative biliary plastic stent insertion. There were no inherent differences between the two groups with regard to the decision to proceed with PBD stent insertion. Demographics of the 82 patients with ampullary cancer are summarized in Table 1. The two groups were comparable with regard to sex, age, stage and grading of the ampullary cancer, and diabetes mellitus. Patients who underwent PBD had a significantly higher level of hemoglobin (11.8 ± 1.5 g/dL) on preoperative laboratory evaluation compared with non-stented patients (10.9 ± 1.6 g/dL) ($P < 0.05$). The majority of biliary stents were placed endoscopically, with two patients having percutaneous transhepatic biliary drainage. Numbers were too small to undergo statistical analysis between the types of biliary decompression; thus, "stents" included all patients who underwent PBD. The time interval between biliary drainage and surgery was 39 d (range: 10-89 d). There was a decrease in bilirubin in all patients, with the mean reduction being 47.6 μmol/L. All patients were given intravenous antibiotics at the time of surgery.

Table 1 Patient variables in the PBD and non-PBD groups

	PBD	Non-PBD	Significance
Patients	35	47	
Median age (yr)	65 (23-84)	62 (38-84)	0.91
Sex ratio (M/F)	14/21	26/21	0.28
Mean serum bilirubin	112.4 ± 116.1	91.6 ± 110.2	0.39
Mean hemoglobin	11.8 ± 1.5	10.9 ± 1.6	0.03
Diabetes mellitus	7	12	0.66
Mean albumin	30.5 ± 5.7	31.1 ± 6.2	0.77

Table 2 Morbidity and mortality in the two groups

Morbidity	PBD	Non-PBD	Significance
Sepsis	10	14	0.91
Wound infection	1	12	0.01
Intra-abdominal bleeding	1	5	0.36
Intra-abdominal abscess	1	8	0.09
Gastrointestinal bleeding	3	5	0.95
Bile leakage	2	3	0.73
Pancreatic leakage	2	2	0.73
Delayed gastric emptying	6	10	0.85
Reoperation	1	5	0.36
Hospital death	0	0	0.00
Late mortality	10	13	0.15

All postoperative complications and subpopulation analyses between PBD and non-PBD groups are shown in Table 2. Postoperative sepsis occurred in 29.3% (24/82) of the patients. Six patients had postoperative bleeding that required re-exploration. There were 13 (15.7%) patients with wound infection (one in the PBD and 12 in the non-PBD group), and analysis demonstrated a significantly higher occurrence of wound infection in the non-PBD group ($P = 0.01$).

Tumor grading and histology

Of the 82 infiltrating carcinomas, 21 (25.6%) were well-differentiated, 49 (59.8%) were moderately differentiated, and 12 (14.6%) were poorly differentiated. The most common histological type was intestinal (52/82, 63.4%), followed by pancreaticobiliary (22/82, 26.8%) and colloid (3/82, 3.7%). Five carcinomas had a histological type that was classified as "other".

Mortality and survival

There were no hospital deaths in either group. Twenty-three patients died (28%) during the study period. Median survival was 44 mo (95% CI: 34.2-53.8) in the PBD group and 41 mo (95% CI: 27.7-54.3; $P = 0.86$) in the non-PBD group. There was no significant difference in terms of survival between the groups (Figure 1).

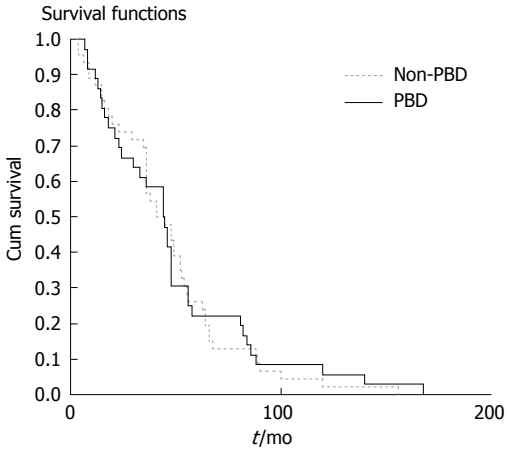

Figure 1 Survival functions for PBD and non-PBD groups.

DISCUSSION

Ampullary tumors share a similar clinical presentation in which jaundice is the predominant symptom. The main initial objectives in these patients are to obtain a precise diagnosis and resolution of jaundice. In an attempt to reach these goals, many treatment modalities have been proposed and applied in recent years. All have been oriented to the necessity of reducing jaundice preoperatively and to preventing perioperative complications, in most cases caused by cholestasis. We found an increase in the rate of wound infection in the perioperative period in the non-PBD group. Malnourishment and malignant obstructive jaundice predispose a patient to wound dehiscence by slowing healing, and increasing the rate of wound infection. A study by Irvin *et al*[6] has suggested that malignant disease may be an important factor in the pathogenesis of wound complications in patients with jaundice. Wound dehiscence or incisional hernia occurred in 59.1% of patients with obstructive jaundice that resulted from malignant disease, but patients with jaundice caused by biliary stones or benign pathology did not develop these complications.

Other factors have been confirmed as having a significant effect on the development of postoperative wound infection, in terms of patient characteristics including diabetes and anemia[7]. Patients with diabetes are more susceptible to wound infection because of impaired neutrophil chemotaxis and phagocytosis. In our series, 25% of non-PBD patients had diabetes compared to 20% in the PBD group, which, although not statistically significant, may have influenced the outcome of surgery. Furthermore the non-PBD group had a hemoglobin level that was significantly lower than that in the PBD group. Impaired wound healing in patients with obstructive jaundice has also been postulated to be caused by an altered immune response, with elevated tumor necrosis factor α activity secondary to circulating endotoxemia[8].

Grande *et al*[9] have compared wound healing in the presence and absence of obstructive jaundice. They used prolyl hydroxylase activity as a marker for collagen synthesis, and found it to be significantly elevated in patients who underwent biliary drainage for benign or malignant biliary obstruction. These patients had better wound healing.

Three common methods of PBD are percutaneous, endoscopic and surgical instrumentation. The endoscopic insertion of a stent through the papilla is a method of draining an obstructed biliary system during endoscopic retrograde cholangiopancreatography (ERCP). In animal studies comparing internal and external biliary drainage, animals undergoing internal drainage experienced

increased survival[10,11], decreased sepsis[12], renal failure[11], and more rapid recovery of immune function[13,14], compared with those undergoing external drainage. Endoscopic biliary drainage is now considered an effective, if not the preferable, treatment for the palliation of malignant biliary tract obstruction[15,16].

To the best of our knowledge, this is the first study to analyze ampullary cancer in the context of PBD. Previous studies have looked at peri-ampullary cancer. Trede and Schwall's[17] retrospective analysis of 150 patients with jaundice undergoing partial or total pancreatectomy revealed a complication rate of 31% and four deaths in 68 patients with no drainage, compared with 17% complications and one death in 82 patients with PBD. Evidence in support of preoperative biliary drainage comes from a prospective randomized trial by Lygidakis *et al*[18], which assigned 38 patients to either PBD (15 ± 2 d) by endoscopic internal stenting, or no PBD. The authors reported a 16% complication rate and no deaths in 19 patients undergoing PBD, compared with 70% complications and two deaths in 19 patients without drainage. Although this difference was highly significant, the investigators scored positive intraoperative blood and bile cultures as a complication. The clinical relevance of such findings is not clear. Smith *et al*[19] have identified prospectively 155 patients who underwent partial PD and found no survival difference between PBD and non-PBD groups. However, the authors concluded that the presence of jaundice at the time of resection had an adverse impact on early postoperative survival. Therefore, preoperative resolution of jaundice following biliary stenting predicted more favorable early survival outcome.

In contrast, a prospective randomized study by Lai *et al*[20] revealed no significant benefit in patients undergoing PBD, and included a wide variety of pathologies, but details of specific complications were not delineated clearly. Choi *et al*[21] have shown that PBD compromised hepatic excretory function, as represented by a slow rate of decrease in serum bilirubin. Limongelli *et al*[22] have revealed that PBD predisposes to a positive intraoperative bile culture, which increases the risk of developing infectious complications and wound infection after pancreatic surgery. Povoski *et al*[23] have reviewed retrospectively 240 consecutive cases of PD. Postoperative morbidity and mortality rates were higher in the PBD group, and they have suggested that PBD should be avoided whenever possible in patients with potentially resectable pancreatic and peripancreatic lesions. Therefore, based on data that, at best, provide mixed results, why should the patients still undergo PBD? First, most patients with ampullary cancer at the time of diagnosis have symptomatic jaundice with pruritus, and some degree of abdominal pain. To some extent, it is hoped that stenting will provide symptomatic improvement. Second, patients who present with acute cholangitis require prior PBD before undergoing definitive surgery.

In contrast, endoscopic biliary drainage before surgery is not a widely accepted procedure among pancreatic surgeons. Potential disadvantages of PBD include those inherent to ERCP such as pancreatitis, bleeding, cholangitis and duodenal perforation. In addition, endoscopic biliary stenting has been shown to generate a severe inflammatory reaction in the bile duct[24], which may make surgical resection more difficult. This was not substantiated by our study, with its comparable mortality and significantly reduced wound infection in the PBD group.

In conclusion, our experience confirms that PBD in patients with obstructive jaundice due to ampullary cancer results in a reduction in wound infection following surgical resection compared with patients not undergoing PBD. Morbidity other than wound infection and mortality were similar with or without PBD. We believe that a detailed prospective randomized trial including a cost analysis in a well-defined and well-matched group of patients undergoing operation for ampullary cancer is warranted, to further evaluate the effectiveness of routine preoperative PBD.

COMMENTS

Background

The effectiveness of preoperative biliary drainage (PBD) in pancreaticoduodenectomy is still extensively debated because of the various conflicting postoperative outcomes, which include benign or malignant, pancreatic or peripancreatic, and ampullary or periampullary lesions.

Research frontiers

The impact of jaundice on postoperative complications is well recognized. However, whether PBD with improvement of jaundice affects surgical outcomes remains controversial.

Innovations and breakthroughs

This is believed to be the first study to investigate the impact of PBD in resectable ampullary cancer patients. Other studies have shown mixed results with regard to postoperative morbidity and mortality amongst ampullary and periampullary cancer.

Applications

The authors conducted the study amongst 82 patients with ampullary cancer and 35 of them had PBD, with a significant reduction in postoperative wound infection rate. A further randomized prospective control study should be conducted in resectable ampullary cancer patients undergoing PBD to look into this positive outcome.

Peer review

This paper addresses an important clinical issue: whether preoperative biliary drainage influences the outcome of resectional surgery for ampullary carcinoma. The results are presented reasonably clearly. The discussion compares the outcomes of this study with other relevant studies and explores the possible reasons for the findings.

REFERENCES

1 **Gundry SR**, Strodel WE, Knol JA, Eckhauser FE, Thompson NW. Efficacy of preoperative biliary tract decompression in patients with obstructive jaundice. *Arch Surg* 1984; **119**: 703-708

2 **Povoski SP**, Karpeh MS Jr, Conlon KC, Blumgart LH, Brennan MF. Preoperative biliary drainage: impact on intraoperative bile cultures and infectious morbidity and mortality after pancreaticoduodenectomy. *J Gastrointest Surg* 1999; **3**: 496-505

3 **Sohn TA**, Yeo CJ, Cameron JL, Pitt HA, Lillemoe KD. Do preoperative biliary stents increase postpancreaticoduode nectomy complications? *J Gastrointest Surg* 2000; **4**: 258-267; discussion 267-268

4 **Kumar R**, Sharma BC, Singh J, Sarin SK. Endoscopic biliary drainage for severe acute cholangitis in biliary obstruction as a result of malignant and benign diseases. *J Gastroenterol*

Hepatol 2004; **19**: 994-997
5 **Kaplan EL**, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457-481
6 **Irvin TT**, Vassilakis JS, Chattopadhyay DK, Greaney MG. Abdominal wound healing in jaundiced patients. *Br J Surg* 1978; **65**: 521-522
7 **Lilienfeld DE**, Vlahov D, Tenney JH, McLaughlin JS. Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. *Am J Infect Control* 1988; **16**: 3-6
8 **Dawiskiba J**, Kwiatkowska D, Zimecki M, Kornafel P, Tyran W, Czapińska E, Woźniak Z. The impairment of wound healing process is correlated with abnormalities of TNF-alpha production by peritoneal exudate cells in obstructive jaundiced rats. *HPB Surg* 2000; **11**: 311-318
9 **Grande L**, Garcia-Valdecasas JC, Fuster J, Visa J, Pera C. Obstructive jaundice and wound healing. *Br J Surg* 1990; **77**: 440-442
10 **Gouma DJ**, Coelho JC, Schlegel JF, Li YF, Moody FG. The effect of preoperative internal and external biliary drainage on mortality of jaundiced rats. *Arch Surg* 1987; **122**: 731-734
11 **Greve JW**, Maessen JG, Tiebosch T, Buurman WA, Gouma DJ. Prevention of postoperative complications in jaundiced rats. Internal biliary drainage versus oral lactulose. *Ann Surg* 1990; **212**: 221-227
12 **Gouma DJ**, Coelho JC, Fisher JD, Schlegel JF, Li YF, Moody FG. Endotoxemia after relief of biliary obstruction by internal and external drainage in rats. *Am J Surg* 1986; **151**: 476-479
13 **Megison SM**, Dunn CW, Horton JW, Chao H. Effects of relief of biliary obstruction on mononuclear phagocyte system function and cell mediated immunity. *Br J Surg* 1991; **78**: 568-571
14 **Thompson RL**, Hoper M, Diamond T, Rowlands BJ. Development and reversibility of T lymphocyte dysfunction in experimental obstructive jaundice. *Br J Surg* 1990; **77**: 1229-1232
15 **Smith AC**, Dowsett JF, Russell RC, Hatfield AR, Cotton PB. Randomised trial of endoscopic stenting versus surgical bypass in malignant low bileduct obstruction. *Lancet* 1994; **344**: 1655-1660
16 **Andersen JR**, Sørensen SM, Kruse A, Rokkjaer M, Matzen P. Randomised trial of endoscopic endoprosthesis versus operative bypass in malignant obstructive jaundice. *Gut* 1989; **30**: 1132-1135
17 **Trede M**, Schwall G. The complications of pancreatectomy. *Ann Surg* 1988; **207**: 39-47
18 **Lygidakis NJ**, van der Heyde MN, Lubbers MJ. Evaluation of preoperative biliary drainage in the surgical management of pancreatic head carcinoma. *Acta Chir Scand* 1987; **153**: 665-668
19 **Smith RA**, Dajani K, Dodd S, Whelan P, Raraty M, Sutton R, Campbell F, Neoptolemos JP, Ghaneh P. Preoperative resolution of jaundice following biliary stenting predicts more favourable early survival in resected pancreatic ductal adenocarcinoma. *Ann Surg Oncol* 2008; **15**: 3138-3146
20 **Lai EC**, Mok FP, Fan ST, Lo CM, Chu KM, Liu CL, Wong J. Preoperative endoscopic drainage for malignant obstructive jaundice. *Br J Surg* 1994; **81**: 1195-1198
21 **Choi YM**, Cho EH, Lee KY, Ahn SI, Choi SK, Kim SJ, Hur YS, Cho YU, Hong KC, Shin SH, Kim KR, Woo ZH. Effect of preoperative biliary drainage on surgical results after pancreaticoduodenectomy in patients with distal common bile duct cancer: focused on the rate of decrease in serum bilirubin. *World J Gastroenterol* 2008; **14**: 1102-1107
22 **Limongelli P**, Pai M, Bansi D, Thiallinagram A, Tait P, Jackson J, Habib NA, Williamson RC, Jiao LR. Correlation between preoperative biliary drainage, bile duct contamination, and postoperative outcomes for pancreatic surgery. *Surgery* 2007; **142**: 313-318
23 **Povoski SP**, Karpeh MS Jr, Conlon KC, Blumgart LH, Brennan MF. Association of preoperative biliary drainage with postoperative outcome following pancreaticoduodenectomy. *Ann Surg* 1999; **230**: 131-142
24 **Karsten TM**, Coene PP, van Gulik TM, Bosma A, van Marle J, James J, Lygidakis NJ, Klopper PJ, van der Heyde MN. Morphologic changes of extrahepatic bile ducts during obstruction and subsequent decompression by endoprosthesis. *Surgery* 1992; **111**: 562-568

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2913

World J Gastroenterol 2009 June 21; 15(23): 2913-2917
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Microproteinuria for detecting calcineurin inhibitor-related nephrotoxicity after liver transplantation

Jing Li, Bin Liu, Lu-Nan Yan, Lan-Lan Wang, Wan Y Lau, Bo Li, Wen-Tao Wang, Ming-Qing Xu, Jia-Yin Yang, Fu-Gui Li

Jing Li, Department of Anesthesiology and Critical Care Medicine, West-China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Bin Liu, Lu-Nan Yan, Bo Li, Wen-Tao Wang, Ming-Qing Xu, Jia-Yin Yang, Fu-Gui Li, Division of Liver Transplantation, West-China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Lan-Lan Wang, Department of Clinical Immunological Laboratory, West-China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Wan Y Lau, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China
Author contributions: Li J and Liu B took care of the patients, designed the study, collected and analyzed the data, and wrote the manuscript; Yan LN designed the study, collected and analyzed the data, and wrote the manuscript; Wang LL collected and analyzed the data, coordinated the work group and contributed to the discussion; Lau WY contributed to the discussion; Li B, Wang WT, Xu MQ, Yang JY, Li FG took care of the patients, collected and analyzed the data.
Correspondence to: Lu-Nan Yan, Division of Liver Transplantation, West China Hospital, Sichuan University, Chengdu 610041, China. surgeryliubin@163.com
Telephone: +86-28-85422867 Fax: +86-28-85423724
Received: March 19, 2009 Revised: April 27, 2009
Accepted: May 4, 2009
Published online: June 21, 2009

Abstract

AIM: To investigate whether microproteinuria could be used as an early and sensitive indicator to detect calcineurin inhibitor (CNI)-related nephrotoxicity after liver transplantation.

METHODS: All liver transplant recipients with normal serum creatinine (SCr) and detectable microproteinuria at baseline were included in this study. The renal function was monitored by the blood clearance of ^{99m}Tc-diethylenetriaminepentaacetic acid every 6 mo. Microproteinuria, SCr and blood urea nitrogen (BUN) were measured at entry and at subsequent follow-up visits. The patients were divided into different groups according to the mean values of glomerular filtration rate (GFR) at the follow-up time points: Group 1, GFR decreased from baseline by 0%-10%; Group 2, GFR decreased from baseline by 11%-20%; Group 3, GFR decreased from baseline by 21%-40%; Group 4, GFR decreased from baseline by > 40% and/or SCr was increasing.

RESULTS: A total of 143 patients were enrolled into this study (23 females and 120 males). The mean follow-up was 32 mo (range 16-36 mo). Downward trends in renal function over time were observed in the study groups. SCr and BUN increased significantly only in Group 4 patients ($P < 0.001$). β2-microglobulin (β2m) and α1-microglobulin (α1m) significantly increased with the subtle change of renal function in recipients who were exposed to CNI-based immunosuppression regimens. The reductions in GFR were closely correlated with elevated α1m ($r^2 = -0.728$, $P < 0.001$) and β2m ($r^2 = -0.787$, $P < 0.001$).

CONCLUSION: β2m and α1m could be useful as early and sensitive indicators of CNI-induced nephrotoxicity.

Key words: Microproteinuria; Liver transplantation; Calcineurin inhibitors; Nephrotoxicity

Peer reviewers: Dr. Shoji Kubo, Hepato-Billiary-Pancreatic Surgery, Osaka City University Graduate, School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan; Yogesh K Chawla, Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Li J, Liu B, Yan LN, Wang LL, Lau WY, Li B, Wang WT, Xu MQ, Yang JY, Li FG. Microproteinuria for detecting calcineurin inhibitor-related nephrotoxicity after liver transplantation. *World J Gastroenterol* 2009; 15(23): 2913-2917 Available from: URL: http://www.wjgnet.com/1007-9327/15/2913.asp DOI: http://dx.doi.org/10.3748/wjg.15.2913

INTRODUCTION

Calcineurin inhibitors (CNIs) have improved survival significantly after liver transplantation, but nephrotoxicity is an adverse effects common to both cyclosporine and tacrolimus[1-3]. Deterioration of renal function with CNI therapy has been widely reported in liver transplant

recipients[4-6]. A recent analysis of 36 849 liver transplant recipients performed in the United States between 1990 and 2000 revealed an 18.1% incidence of chronic renal failure after 5 years, with CNI dose and duration of exposure correlated with subsequent renal damage[7].

The monitoring of transplant patients, however, is still dependent on somewhat old methodologies: serum creatinine (SCr) levels, total urine output, blood urea nitrogen (BUN), or calculated glomerular filtration rate (GFR). However, these tests are particularly problematic as they do not have sufficient specificity, sensitivity, or accuracy to allow appropriate and timely interventions[8]. Additionally, CNIs are characterized by a narrow therapeutic index, so renal damage is often irreversible when these indices become abnormal, or the indices can still fluctuate in the normal range while renal insufficiency is obvious. Given these limitations, more and more transplant specialists are looking to emerging fields, such as proteomics and metabolism, to improve the current situation. Microproteinuria, as a hallmark of reflecting early changes in the glomeruli and proximal tubular function, can be used as an accurate predictor to monitor early changes in renal function[9,10].

The aim of this prospective study was to find out whether microproteinuria could be used as an early and sensitive indicator to monitor CNI-related nephrotoxicity in liver transplant recipients. It was approved by the Ethics Committee at the West China Hospital, Sichuan University.

MATERIALS AND METHODS

From April 2005 to December 2008, 423 adult patients underwent liver transplantation in our hospital. CNI-based immunosuppression regimens comprising a CNI (cyclosporine A or tacrolimus) + azathioprine + prednisone were delivered to recipients after transplantation. All patients who received a CNI-based regimen for immunosuppression after liver transplantation were potential candidates for this study. Recipients receiving CNI therapy without interruption after liver transplantation, with normal SCr at baseline and detectable microproteinuria in fresh urine were all included in this study. If death occurred within 3 mo posttransplantation or renal dysfunction was caused by non-CNI drugs (such as antibiotics or antivirals), or by other means during follow-up period, the recipients were excluded from the study.

Follow-up protocols were performed once every month for the first 6 mo after liver transplantation, once every 2 mo during months 7-12, and once every 6 mo after 1 year. At each time point, the patient contributed a urine sample which was correlated with the measurements from blood samples. Midstream fresh urine samples were collected and not centrifuged. Measurements of microproteinuria including α1-microglobulin (α1m), β2-microglobulin (β2m), immunoglobulin, microalbumin and transferrin were performed immediately with a Dade Behring array nephelometry system (Dade Behring Inc, USA). SCr (picric acid method) was measured by a Modula clinical chemistry analyzer (Roche Diagnostics, Roche, Switzerland). GFR was measured every 6 mo by the Gates method (PHILIPS Helix SPX-6D SPECT, Holland) which analyzed the blood clearance of ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) (5 mCi, from the China Institute of Atomic Energy, radiochemical purity 95%). As consent for a renal biopsy was difficult to obtain, this was performed only when clinically indicated, especially in patients with increasing SCr (an indication of progressive deterioration in renal function).

The actual GFR is considered to be the best overall index of renal function in health and disease[11].Therefore, we chose the actual GFR as the criteria for renal function in this study. The patients were divided into 4 groups according to the mean values of GFR at every 6-mo follow-up: Group 1, GFR declined from baseline by 0%-10%; Group 2, GFR declined from baseline by 11%-20%; Group 3, GFR declined from baseline by 21%-40%; Group 4, GFR declined from baseline by > 40% and/or SCr was increasing.

The normal concentrations of individual proteins present in the urine are illustrated by maximum values[12,13], and are significantly different between different laboratories[12-14]. The normal reference values of microproteinuria provided by Dade Behring, Inc. are: microalbumin < 30 mg/L, α1m < 12.0 mg/L, β2m < 0.20 mg/L, immunoglobulin < 9.6 mg/L, and transferrin < 1.9 mg/L. According to the values determined in 500 Chinese healthy individuals in our laboratory, the normal values used in this study for microalbumin were < 19 mg/L, α1m < 12.5 mg/L, β2m < 0.22 mg/L, immunoglobulin < 20.0 mg/L, and transferrin < 2.2 mg/L.

Statistical analysis was performed by SPSS 13.0 (SPSS Inc., Chicago, IL). Differences in microproteinuria, serum creatinine and BUN were tested with the one way ANOVA test for multiple comparisons. Data were expressed as mean ± SD, or median (range). The Kruskal-Wallis rank sum test (individual comparisons were done by the Wilcoxon rank sum test) and correlation analyses were used in this study. A *P*-value less than 0.05 indicated statistical significance.

RESULTS

A total of 143 liver transplant patients were recruited into this study. Of these, 16 withdrew during the follow-up period. The reasons for withdrawal were infection in 3 patients, acute rejection in 2, use of non-CNI drugs in 4, uncontrolled hypertension in 3, abnormal liver function in 2, and 2 patients died. There were 23 females and 120 male, aged 21-68 years. The grafts and recipients were blood group identical in 127 cases and compatible in 16 cases. The mean follow-up was 32 mo (range 16-36 mo). The primary disease of the 143 recipients included diffuse ischemic intrahepatic biliary stenosis in 8, Caroli disease in 7, Budd-Chiari syndrome with liver cirrhosis in 4, liver cirrhosis after hepatitis B in 56, postoperative liver failure after right lobe hepatectomy caused by hepatic trauma in 4, Wilson disease in 11, α1-antitrypsin deficiency in 2, echinococcus disease of the liver in

Table 1 Demographic data of patients at the point of entry into this study (mean ± SD)

Variable (*n* = 143)	Group 1 (*n* = 102)	Group 2 (*n* = 35)	Group 3 (*n* = 6)	Group 4 (*n* = 0)
Age (yr), median(range)	48.6 (28-66)	49.2 (33-67)	48.5 (21-68)	0
Body mass index (BMI) (kg/m^2)	22.0 ± 2.8	20.5 ± 2.4	21.8 ± 2.9	0
Pre-transplant MELD score	17.8 ± 7.6	16.2 ± 8.4	15.7 ± 9.7	0
BUN (mmol/L)	5.6 ± 2.3	6.0 ± 2.5	5.9 ± 2.8	0
SCr (μmol/L)	54.7 ± 15.8	60.5 ± 20.1	68.7 ± 17.4	0
GFR (mL/min per 1.73 m^2)	105.4 ± 20.5	99.6 ± 15.2	103.3 ± 23.1	0
Dosage (mg/kg per day)				
Cyclosporine A	4.0 ± 1.1	4.6 ± 1.5	5.1 ± 1.2	0
Tacrolimus	0.055 ± 0.03	0.061 ± 0.07	0.057 ± 0.05[a]	0
Trough levels (ng/mL)				
Cyclosporine A	212.4 ± 45.8	243.7 ± 56.2	251.3 ± 61.6	0
Tacrolimus	6.3 ± 0.6	6.7 ± 0.7	7.5 ± 0.9[a]	0
Duration of previous treatment (mo)				
Cyclosporine A	5.8 ± 3.4	6.7 ± 4.6	6.9 ± 3.9	0
Tacrolimus	5.3 ± 2.8	6.2 ± 3.5	6.7 ± 2.5	0
Time post-transplant	7.5 ± 2.2	8.8 ± 2.6	8.2 ± 3.1	0
Microproteinuria (mg/L)				
β2m	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.3[a]	0
α1m	14.2 ± 10.6	18.5 ± 17.3	24.8 ± 20.1[a]	0
Microalbumin	28.6 ± 19.3	37.7 ± 28.1	40.1 ± 30.5	0
Immunoglobulin	27.4 ± 8.3	25.1 ± 11.4	29.3 ± 15.6	0
Transferrin	2.8 ± 0.7	2.4 ± 1.6	3.1 ± 1.4[a]	0

[a]$P < 0.05$, *vs* group 1. Group 1: GFR declined from baseline by 0%-10%; Group 2: GFR declined from baseline by 11%-20%; Group 3: GFR declined from baseline by 21%-40%; Group 4: GFR declined from baseline by > 40% and/or SCr was increasing.

Table 2 Mean values of every follow-up time point in the study groups (mean ± SD)

Variable (*n* = 143)	Group 1 (*n* = 73)	Group 2 (*n* = 40)	Group 3 (*n* = 30)	Group 4 (*n* = 5)
GFR (mL/min per 1.73 m^2)	97.4 ± 12.7	85.6 ± 17.9	62.3 ± 20.5	49.6 ± 20.2
The declining percentage of GFR from baseline (%)	7.3 ± 2.6	16.7 ± 10.1[a,c]	32.5 ± 12.9[a,c]	52.4 ± 20.8[a,c]
SCr (μmol/L)	76.3 ± 16.2	83.7 ± 15.4	90.3 ± 19.8	173.7 ± 28.5[a,c]
BUN (mmol/L)	5.8 ± 1.7	6.3 ± 1.2	6.9 ± 1.5	11.2 ± 2.6[a,c]
Dosage (mg/kg per day)				
Cyclosporine A	2.5 ± 0.8	3.0 ± 1.0	3.3 ± 0.9	3.8 ± 1.1[a]
Tacrolimus	0.049 ± 0.03	0.052 ± 0.07	0.054 ± 0.05	0.058 ± 0.03[a]
Trough levels				
Cyclosporine A (ng/mL)	145.2 ± 30.5	154.8 ± 25.6	165.3 ± 39.4	183.2 ± 31.2[a]
Tacrolimus (ng/mL)	5.1 ± 0.8	5.5 ± 1.2	6.2 ± 1.0[a]	6.8 ± 1.3[a,c]
Diabetes (%)	9.2	10.5	9.3	11.1
Hypertension (%)	38.5	34.2	37.2	33.3
Follow-up time, mean (range, mo)	35.6 (23-36)	33.7 (24-36)	30.4 (19-36)	27.8 (16-36)
Microproteinuria				
β2m (mg/L)	0.2 ± 0.1	0.4 ± 0.2[a]	1.0 ± 1.3[a,c]	4.2 ± 2.5[a,c]
α1m (mg/L)	22.6 ± 21.1	38.9 ± 25.4[a]	42.3 ± 35.9[a,c]	65.1 ± 30.4[a,c]
Microalbumin (mg/L)	46.9 ± 26.2	83.6 ± 55.5[a]	70.9 ± 33.8	45.4 ± 32.9
Immunoglobulin (mg/L)	23.9 ± 14.6	21.5 ± 29.1	37.3 ± 26.4	33.5 ± 29.1
Transferrin (mg/L)	3.2 ± 10.2	10.8 ± 27.8	8.2 ± 15.9	8.1 ± 12.4

[a]$P < 0.05$, *vs* group 1; [c]$P < 0.05$, *vs* group 2. Group 1: GFR declined from baseline by 0%-10%; Group 2: GFR declined from baseline by 11%-20%; Group 3: GFR declined from baseline by 21%-40%; Group 4: GFR declined from baseline by > 40% and/or SCr was increasing.

4, hepatolithiasis in 3, hepatocellular carcinoma in 38, and cholangiocarcinoma in 6. All hepatocellular cancer patients met the UCSF criteria (a single tumor ⩽ 6.5 cm in diameter, or 2 or 3 tumors, none exceeding 4.5 cm in diameter and whose sum of tumor diameters did not exceed 8 cm). The demographic data of these patients are shown in Table 1. There were 102 (71.3%) recipients in Group 1, 35 (24.5%) in Group 2, 6 (4.2%) in Group 3, and none in Group 4 at baseline. At entry into this study, all recipients had normal levels of BUN, SCr, and GFR with detectable microproteinuria in fresh urine. There were no significant differences in body mass index, or pre-transplant MELD score.

Through measurements of GFR by the blood clearance of ^{99m}Tc-DTPA at entry into the study and at the follow-up visits, we found there was a downward trend in renal function over time, and the reductions in GFR were significantly different across all groups (Table 2). The value of GFR was 97.4 ± 12.7 mL/min per 1.73 m^2 in Group 1 (decreased 7.3% ± 2.6% from baseline), and 85.6 ± 17.9 mL/min per 1.73 m^2 in Group 2 ($P < 0.001$ *vs* Group 1) (decreased 16.7% ± 10.1% from baseline), and

62.3 ± 20.5 mL/min per 1.73 m^2 in Group 3 ($P < 0.001$ *vs* Group 1; $P < 0.001$ *vs* Group 2) (decreased 32.5% ± 12.9% from baseline). In Group 4, the value of GFR was 49.6 ± 13.2 mL/min per 1.73 m^2 ($P < 0.001$ *vs* Group 1; $P < 0.001$ *vs* Group 2; $P = 0.002$ *vs* Group 3) (decreased 52.4% ± 20.8% from baseline). The SCr significantly increased only in Group 4 (173.7 ± 28.5 umol/L) when compared with the other groups ($P = 0.017$ *vs* Group 1, $P = 0.021$ *vs* Group 2, $P = 0.035$ *vs* Group 3). Similar trends were found for BUN. However, the urinary β2m ($P < 0.01$) and α1m ($P = 0.043$) were significantly different between the 4 groups of patients. This study also indicated that microalbumin, immunoglobulin and transferrin had no significant differences in the 4 groups of patients.

In Group 1, the mean level of urinary α1m at entry and at subsequent follow-up visits was 22.6 ± 21.1 mg/L, which was significantly lower than that in Group 2 (38.9 ± 25.4 mg/L, $P < 0.001$), and in Group 3 (42.3 ± 35.9 mg/L, $P < 0.001$). In Group 4, the level of urinary α1m (65.1 ± 30.4 mg/L) was significantly higher than that of all the other groups ($P < 0.001$ *vs* Group 1; $P < 0.001$ *vs* Group 2; $P = 0.002$ *vs* Group 3). The findings in this study suggested that the increase in urinary α1m occurred long before the elevation of SCr or BUN, and the reductions in GFR were closely correlated with the increases in α1m ($r^2 = -0.728$, $P < 0.001$). The urinary α1m, as a marker of tubular damage, was sensitive in assessing subtle changes in renal function caused by CNI nephrotoxicity.

The mean level of urinary β2m during follow-up visits was 0.2 ± 0.1 mg/L in Group 1, 0.4 ± 0.2 mg/L in Group 2 ($P = 0.041$ *vs* Group1) and 1.0 ± 1.3 mg/L in Group 3 patients ($P < 0.001$ *vs* Group 1; $P < 0.001$ *vs* Group 2). The level of urinary β2m in Group 4 patients (4.2 ± 2.5 mg/L) was further increased compared to other subgroups ($P < 0.001$ *vs* Group 1; $P < 0.001$ *vs* Group 2; $P < 0.001$ *vs* Group 3). The urinary β2m concentration gradually increased from Group 1 to Group 4. The results of this study showed that the increase in urinary β2m, as a marker of tubular epithelial cell dysfunction, occurred long before the elevation of SCr or BUN, and was also closely correlated with the reduction in GFR ($r^2 = -0.787$, $P < 0.001$). The urinary β2m was also sensitive for detecting subtle changes in renal function in patients exposed to CNI-based immunosuppression regimens. Additionally, monitoring both β2m and α1m could improve the sensitivity and specificity of measurement of CNI-related nephrotoxicity.

DISCUSSION

The tremendous success of CNIs in reducing acute rejection episodes and early immunologic graft injury has not been accompanied by a benefit in long-term recipient survival[15,16]. CNI nephrotoxicity in liver transplantation is a significant concern and appears to be progressive over time when CNI exposure is maintained[17]. Microproteinuria has been used as an early marker of nephrotoxicity to detect small changes in the function of tubular epithelial cells in many pathological conditions[10]. The persistence of microproteinuria may result from drug toxicity or pretransplant renal diseases after liver transplantation. Therefore, recipients with normal serum creatinine at baseline and detectable microproteinuria were selected as subjects in this study.

Follow-up data of this study demonstrated that there was a downward trend in renal function over time, with the persistence of microproteinuria. The urinary concentration of β2m and α1m significantly increased with the subtle change in renal function in all study groups, but the levels of SCr and BUN significantly increased only when renal function was severely reduced by CNI nephrotoxicity (in Group 4, renal function declined 52.4% ± 20.8 % from baseline). A similar study also found microproteinuria occurred long before the elevation of SCr[18]. The subsequent reductions in GFR were closely correlated with elevated α1m ($r^2 = -0.728$, $P < 0.001$) and β2m ($r^2 = -0.787$, $P < 0.001$) in the study groups. The results of this study were similar to another report[19], indicating that tubular epithelial dysfunction defined by elevation of tubular injury biomarkers (β2m or α1m) was very common when CNI exposure was maintained. Additionally, as β2m is unstable in fresh urine, fewer patients were found to have β2m in the urine than α1m in this study. This problem can partly be overcome by maintaining the urine pH value (by adding basic buffer to the urine) to prevent the degradation of β2m. This study suggested that urinary β2m and α1m are sensitive urinary markers for detecting CNI-related nephrotoxicity in liver transplant recipients.

In conclusion, monitoring of patients with SCr requires a higher laboratory effort and the use of gender-specific cut-off values. Measurement of microproteinuria is easily available, non-expensive, and convenient in daily clinical practice. The urinary β2m or α1m can be used as an early, sensitive and simple diagnostic indicator for detecting CNI-related renal dysfunction. Furthermore, it should be used as the screening method after liver transplantation to prevent the progressive deterioration of subclinical renal dysfunction.

ACKNOWLEDGMENTS

The authors are grateful to the staff of the Liver Transplantation Division for collection of the clinical materials, and to Mrs. Fang Liu, Mrs. Yang-Juan Bai and Mr. Jiang-Tao Tang for their skillful technical assistance. Special thanks to the staff of the Department of Clinical Laboratory for processing the urine samples and to staff of the Department of Nuclear Medicine for performing the GFR measurements.

COMMENTS

Background

Deterioration of renal function with calcineurin inhibitor (CNI) therapy has been widely reported in liver transplant recipients. Monitoring of renal function, however, is still dependent on somewhat old technologies: serum creatinine (SCr), blood urea nitrogen (BUN), total urine output. Whether microproteinuria could be used as an early and sensitive indicator to monitor CNI-related nephrotoxicity in liver transplant recipients has not been unequivocally addressed.

Research frontiers

Measurement of SCr, BUN, and total urine output are particularly problematic, as they do not have sufficient specificity, sensitivity, or accuracy to allow appropriate and timely prevention of the deterioration of renal function. In this field, the research goal is to identify more sensitive indicators in the early diagnosis of CNI-related nephrotoxicity in liver transplant recipients.

Innovations and breakthroughs

Recent reports have highlighted that nephrotoxicity of CNIs contributes to renal function deterioration in liver transplant recipients. This is the first study to investigate whether microproteinuria could be used as an early and sensitive indicator to monitor CNI-related nephrotoxicity in liver transplant recipients.

Applications

By using β2-microglobulin (β2m) and α1-microglobulin (α1m) as markers for early diagnosis of CNI-related nephrotoxicity, this study may present a future screening method for preventing the progression of CNI-related renal dysfunction in liver transplant recipients.

Terminology

Microproteinuria: it is a hallmark of the early changes in glomerular and proximal tubular function and includes α1m, β2m, immunoglobulin, microalbumin and transferrin.

Peer review

Microproteinuria was studied in these study groups at entry and at subsequent follow-up visits and was correlated with glomerular filtration rate. It revealed that microproteinuria could be used as an early and sensitive indicator to monitor CNI-related nephrotoxicity in liver transplant recipients. This manuscript is very interesting and may present a future screening method to prevent the progression of CNI-related subclinical renal dysfunction after liver transplantation.

REFERENCES

1 **Orlando G**, Baiocchi L, Cardillo A, Iaria G, De Liguori Carino N, De Luca L, Ielpo B, Tariciotti L, Angelico M, Tisone G. Switch to 1.5 grams MMF monotherapy for CNI-related toxicity in liver transplantation is safe and improves renal function, dyslipidemia, and hypertension. *Liver Transpl* 2007; **13**: 46-54

2 **Cohen AJ**, Stegall MD, Rosen CB, Wiesner RH, Leung N, Kremers WK, Zein NN. Chronic renal dysfunction late after liver transplantation. *Liver Transpl* 2002; **8**: 916-921

3 **Ojo AO**, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931-940

4 **Tönshoff B**, Höcker B. Treatment strategies in pediatric solid organ transplant recipients with calcineurin inhibitor-induced nephrotoxicity. *Pediatr Transplant* 2006; **10**: 721-729

5 **Moreno Planas JM**, Cuervas-Mons Martinez V, Rubio Gonzalez E, Gomez Cruz A, Lopez-Monclus J, Sánchez-Turrion V, Lucena Poza JL, Jimenez Garrido M, Millan I. Mycophenolate mofetil can be used as monotherapy late after liver transplantation. *Am J Transplant* 2004; **4**: 1650-1655

6 **Shenoy S**, Hardinger KL, Crippin J, Desai N, Korenblat K, Lisker-Melman M, Lowell JA, Chapman W. Sirolimus conversion in liver transplant recipients with renal dysfunction: a prospective, randomized, single-center trial. *Transplantation* 2007; **83**: 1389-1392

7 **Nair S**, Verma S, Thuluvath PJ. Pretransplant renal function predicts survival in patients undergoing orthotopic liver transplantation. *Hepatology* 2002; **35**: 1179-1185

8 **Herzog D**, Martin S, Turpin S, Alvarez F. Normal glomerular filtration rate in long-term follow-up of children after orthotopic liver transplantation. *Transplantation* 2006; **81**: 672-677

9 **Franchini I**, Alinovi R, Bergamaschi E, Mutti A. Contribution of studies on renal effects of heavy metals and selected organic compounds to our understanding of the progression of chronic nephropathies towards renal failure. *Acta Biomed* 2005; **76** Suppl 2: 58-67

10 **Radermacher J**, Mengel M, Ellis S, Stuht S, Hiss M, Schwarz A, Eisenberger U, Burg M, Luft FC, Gwinner W, Haller H. The renal arterial resistance index and renal allograft survival. *N Engl J Med* 2003; **349**: 115-124

11 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470

12 **Otu HH**, Can H, Spentzos D, Nelson RG, Hanson RL, Looker HC, Knowler WC, Monroy M, Libermann TA, Karumanchi SA, Thadhani R. Prediction of diabetic nephropathy using urine proteomic profiling 10 years prior to development of nephropathy. *Diabetes Care* 2007; **30**: 638-643

13 **Price CP**, Newman DJ, Blirup-Jensen S, Guder WG, Grubb A, Itoh Y, Johnson M, Lammers M, Packer S, Seymour D. First International Reference Preparation for Individual Proteins in Urine. IFCC Working Group on Urine Proteins. International Federation of Clinical Chemistry. *Clin Biochem* 1998; **31**: 467-474

14 **Schultz CJ**, Dalton RN, Turner C, Neil HA, Dunger DB. Freezing method affects the concentration and variability of urine proteins and the interpretation of data on microalbuminuria. The Oxford Regional Prospective Study Group. *Diabet Med* 2000; **17**: 7-14

15 **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326-2333

16 **Sandborn WJ**, Hay JE, Porayko MK, Gores GJ, Steers JL, Krom RA, Wiesner RH. Cyclosporine withdrawal for nephrotoxicity in liver transplant recipients does not result in sustained improvement in kidney function and causes cellular and ductopenic rejection. *Hepatology* 1994; **19**: 925-932

17 **Flechner SM**, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. *Clin Transplant* 2008; **22**: 1-15

18 **D'Amico G**, Bazzi C. Urinary protein and enzyme excretion as markers of tubular damage. *Curr Opin Nephrol Hypertens* 2003; **12**: 639-643

19 **Schaub S**, Mayr M, Hönger G, Bestland J, Steiger J, Regeniter A, Mihatsch MJ, Wilkins JA, Rush D, Nickerson P. Detection of subclinical tubular injury after renal transplantation: comparison of urine protein analysis with allograft histopathology. *Transplantation* 2007; **84**: 104-112

S- Editor Li LF **L- Editor** Cant MR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2918

World J Gastroenterol 2009 June 21; 15(23): 2918-2919
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Surgical treatment of locally advanced anal cancer after male-to-female sex reassignment surgery

Marco Caricato, Fabio Ausania, Giovanni Francesco Marangi, Ilaria Cipollone, Gerardo Flammia, Paolo Persichetti, Lucio Trodella, Roberto Coppola

Marco Caricato, Fabio Ausania, Ilaria Cipollone, Gerardo Flammia, Roberto Coppola, Department of General Surgery, Campus Bio Medico University of Rome, Rome 00139, Italy
Giovanni Francesco Marangi, Paolo Persichetti, Plastic and Reconstructive Surgery, Campus Bio Medico University of Rome, Rome 00139, Italy
Lucio Trodella, Radiation Oncology, Campus Bio Medico University of Rome, Rome 00139, Italy
Author contributions: Caricato M had full access to all of the data in the study and takes responsibility for the integrity of the data; the study concept and design were devised by Ausania F; the acquisition of data was done by Marangi GF, Cipollone I, Flammia G; Critical revision of the manuscript was done by Coppola R, Trodella L, Persichetti P.
Correspondence to: Fabio Ausania, MD, Department of General Surgery, Campus Bio Medico University of Rome, Via Alvaro del Portillo, 20000128, Rome 00139, Italy. f.ausania@googlemail.com
Telephone: +39-32-88850527
Received: March 8, 2009 Revised: May 15, 2009
Accepted: May 22, 2009
Published online: June 21, 2009

Abstract

We present a case of a transsexual patient who underwent a partial pelvectomy and genital reconstruction for anal cancer after chemoradiation. This is the first case in literature reporting on the occurrence of anal cancer after male-to-female sex reassignment surgery. We describe the surgical approach presenting our technique to avoid postoperative complications and preserve the sexual reassignment.

Key words: Sex reassignment; Pelvic surgery; Anal cancer

Peer reviewers: Dr. Ronan A Cahill, Department of General Surgery, Waterford Regional Hospital, Waterford, Cork, Ireland; Otto Schiueh-Tzang Lin, MD, C3-Gas, Gastroenterology Section, Virginia Mason Medical Center, 1100 Ninth Avenue, Seattle, WA 98101, United States

Caricato M, Ausania F, Marangi GF, Cipollone I, Flammia G, Persichetti P, Trodella L, Coppola R. Surgical treatment of locally advanced anal cancer after male-to-female sex reassignment surgery. *World J Gastroenterol* 2009; 15(23): 2918-2919 Available from: URL: http://www.wjgnet.com/1007-9327/15/2918.asp
DOI: http://dx.doi.org/10.3748/wjg.15.2918

INTRODUCTION

Pelvic surgery after male to female sex reassignment implies a particular surgical approach: firstly, anatomic variations during resection can be considerable; secondly, although genital reconstruction is an essential target for patients, it is a difficult operation especially after chemoradiation. We present a case of a transsexual patient who underwent a partial pelvectomy and genital reconstruction for anal cancer.

CASE REPORT

A 63 year-old patient presented with rectal bleeding and anal pain. In 1982, she was submitted to male-to-female sex reassignment surgery according to the technique described by Meyer *et al*[1] consisting of penile disassembly followed by vaginoplasty using all penile components except the corpora cavernosa.

Physical examination on January 2006 revealed an anal ulcer and a biopsy showed evidence of a squamous carcinoma. Clinical staging at diagnosis indicated an involvement of the inguinal lymph nodes (T2N2M0, stage Ⅲ B anal cancer[2]).

Concurrent chemoradiation was administered according to the following scheme: the radiation field included the primary tumour and inguinal lymph nodes; a total of 45 Gy in 25 fractions was administered at the primary tumour and a total of 30.6 Gy at the inguinal fields. Each chemotherapy cycle lasted 38 d and consisted of oxaliplatin 180 mg/m^2 on days 1, 19 and 38 and xeloda 1300 mg/m^2 concurrently to radiotherapy. A radiotherapy boost was also administered either on the primary tumour or the inguinal lymph nodes.

Clinical evaluation six weeks after the completion of chemoradiation showed a complete clinical response. At one year follow-up, CT scan showed local recurrence involving the anal sphincter, neo-vagina and bladder neck. A surgical approach was planned in collaboration with reconstructive surgery in order to preserve sex reassignment.

The surgical approach consisted of two parts.

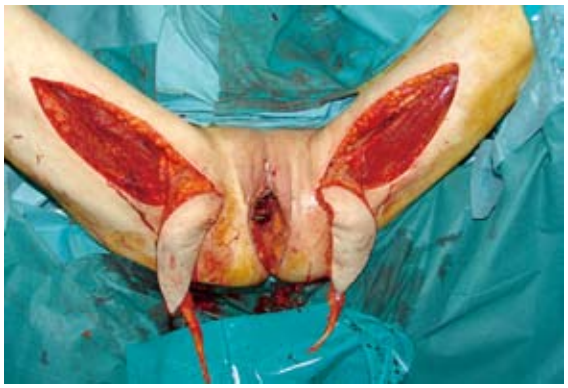

Figure 1 Elliptical skin islands and flaps preparation.

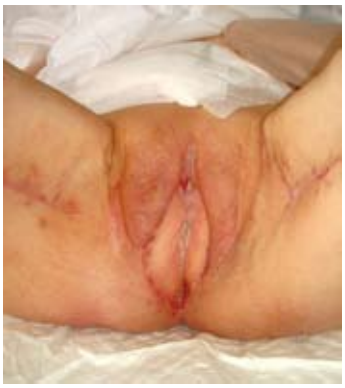

Figure 2 Reconstruction after one month.

During the abdominal portion of the procedure, the rectum was mobilized, and the posterior bladder wall and the seminal vesicles were exposed. During the pelvic portion of the procedure the excision was completed. The perineal skin incision included the neo-vagina and urethra. The rectum and the anus were removed en bloc with the bladder neck, neo-vagina, seminal vesicles and prostate. The bladder neck was sutured, and sovrapubic cystostomy and the colostomy were performed.

Following the excision, a loss of substance of 20 cm × 15 cm resulted. An elliptical skin island up to 15 cm × 6 cm was outlined over the proximal two-thirds of the gracilis muscle of each thigh, according to a technique previously described by our group[3] (Figure 1). The anterior margin of the incision lay on a line drawn between the pubic tubercle and the semitendinosus tendon. The gracilis tendon was identified distally, and the tendinosus insertion divided. The posterior incision was made down to the muscle. The flap was then elevated from distal to proximal on the thigh. The vascular supply of the flap was the medial femoral circumflex artery, which enters the gracilis muscle 7-10 cm below the pubic tubercle. Once the pedicle was identified and preserved, the proximal muscle was dissected. The entire myocutaneous flap was tunnelled through the subcutaneous skin bridge into the vaginal defect and exteriorized through the introitus. The bilateral flaps were sutured to each other at the midline. The neovagina was shaped into a pouch and then inserted into the pelvic space that left after the exenteration. The proximal end of the neovagina was sutured to the introitus. The result after one month is presented in Figure 2.

The postoperative course was complicated by the occurrence of urinary leak, successfully treated with bilateral temporary nephrostomy.

The histology confirmed a locally advanced squamous carcinoma of the anus. Surgical margins were involved. The patient is free of disease at 8 mo follow-up.

DISCUSSION

This is the first case in literature reporting on the occurrence of anal cancer after male-to-female sex reassignment surgery. We tried to achieve two important targets: (1) to avoid postoperative complications of extended surgery after chemoradiation. In fact, perineal reconstruction with gracilis muscle flaps proved very useful in preventing the primary postactinic healing delay. Immediate perineal reconstruction is helpful in avoiding delayed complications due to radiotherapy, namely tissue sclerosis and irreversible damage to irradiated tissues. Gracilis flap transposition ensures a persistent vascular contribution to the irradiated perineal tissues, increasing local oxygenation and leading to more rapid recovery of the surgical wound. In addition to the vascular advantages, the gracilis muscular flap, fixed to the pelvic floor, prevents dead spaces and thus reduces the incidence of seromas. Extended perineal surgery can subvert the architecture of the pelvic floor reducing the sustaining forces and leading to herniation into the perineal space. We would like to point out that the muscular gracilis flap is crucial to compensate for the lack of perineal support after this operation. Once the defect is closed, no further procedure is needed to treat the above mentioned complications in unreconstructed patients, allowing patients to recover quickly and possibly receive adjuvant therapies. (2) to reconstruct the sexual reassignment. This point may not be essential for the surgeon who often assumes that radical surgery is the only target of the operation, but it remains an important issue for patients.

In conclusion, this is the first case in literature reporting on surgical treatment of locally advanced anal cancer after male-to-female sex reassignment surgery. Surgical reconstruction is a reasonable and helpful option to avoid severe complications and preserve sexual reassignment.

REFERENCES

1 **Meyer R**, Kesselring UK. One-stage reconstruction of the vagina with penile skin as an island flap in male transsexuals. *Plast Reconstr Surg* 1980; **66**: 401-406
2 **Greene FL**, Page DL, Fleming ID, Fritz AG, Balch CM, editors. AJCC Cancer Staging Handbook. 6th ed. New York: Springer-Verlag, 2002
3 **Persichetti P**, Cogliandro A, Marangi GF, Simone P, Ripetti V, Vitelli CE, Coppola R. Pelvic and perineal reconstruction following abdominoperineal resection: the role of gracilis flap. *Ann Plast Surg* 2007; **59**: 168-172

S- Editor Li LF **L- Editor** O'Neill M **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2920

World J Gastroenterol 2009 June 21; 15(23): 2920-2922
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Anabolic steroid-induced cardiomyopathy underlying acute liver failure in a young bodybuilder

Miguel Bispo, Ana Valente, Rosário Maldonado, Rui Palma, Helena Glória, João Nóbrega, Paula Alexandrino

Miguel Bispo, Ana Valente, Rosário Maldonado, Rui Palma, Helena Glória, Paula Alexandrino, Intensive Care Unit of Gastroenterology and Hepatology, Santa Maria University Hospital, 1069-166 Lisbon, Portugal
Miguel Bispo, Department of Gastroenterology, Egas Moniz Hospital, 1349-019 Lisbon, Portugal
João Nóbrega, Coronary Care Unit, Santa Maria University Hospital, 1069-166 Lisbon, Portugal
Author contributions: Bispo M carried out the main role in reviewing the literature and writing the article. All authors contributed substantially to analyzing the patient's data and revising the article.
Correspondence to: Miguel Bispo, MD, Department of Gastroenterology, Egas Moniz Hospital, Rua da Junqueira 126, 1349-019, Lisbon,
Portugal. gastregas@hegasmoniz.min-saude.pt
Telephone: +351-919-002599 Fax: +351-213-624139
Received: March 15, 2009 Revised: May 11, 2009
Accepted: May 18, 2009
Published online: June 21, 2009

Abstract

Heart failure may lead to subclinical circulatory disturbances and remain an unrecognized cause of ischemic liver injury. We present the case of a previously healthy 40-year-old bodybuilder, referred to our Intensive-Care Unit of Hepatology for treatment of severe acute liver failure, with the suspicion of toxic hepatitis associated with anabolic steroid abuse. Despite the absence of symptoms and signs of congestive heart failure at admission, an anabolic steroid-induced dilated cardiomyopathy with a large thrombus in both ventricles was found to be the underlying cause of the liver injury. Treatment for the initially unrecognized heart failure rapidly restored liver function to normal. To our knowledge, this is the first reported case of severe acute liver failure due to an unrecognized anabolic steroid-induced cardiomyopathy. Awareness of this unique presentation will allow for prompt treatment of this potentially fatal cause of liver failure.

Key words: Acute liver failure; Anabolic steroids; Bodybuilder; Cardiomyopathy; Hypoxic hepatitis

Peer reviewer: Masahiro Arai, MD, PhD, Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashi-ooi, Shinagawa-ku, Tokyo 140-8522, Japan

Bispo M, Valente A, Maldonado R, Palma R, Glória H, Nóbrega J, Alexandrino P. Anabolic steroid-induced cardiomyopathy underlying acute liver failure in a young bodybuilder. *World J Gastroenterol* 2009; 15(23): 2920-2922 Available from: URL: http://www.wjgnet.com/1007-9327/15/2920.asp DOI: http://dx.doi.org/10.3748/wjg.15.2920

INTRODUCTION

In the absence of a sudden and profound reduction in systemic blood pressure, heart failure is frequently forgotten in the differential diagnosis of acute hepatitis[1]. However, it has long been recognized that ischemic liver injury may follow subclinical circulatory disturbances, particularly in patients with pre-existing passive hepatic congestion, and present as acute hepatitis[2]. Here, we report the first case of severe acute liver failure due to an unrecognized anabolic steroid-induced cardiomyopathy.

CASE REPORT

A 40-year-old male bodybuilder was transferred to our Intensive-Care Unit of Hepatology for treatment of severe acute liver failure. The patient had a history of anabolic steroid abuse over the last 10 years, self-administered in cycles of 6-10 wk, with a 2-3 wk suspension period between cycles. The most frequently used anabolic steroids were: methandrostenolone, stanozolol and oxymetholone (oral); and nandrolone decanoate, testosterone enanthate and trenbolone enanthate (intramuscular). Notably, he used massive doses of all anabolic steroids, including trenbolone enanthate (500-700 mg per week). There was no history of alcohol abuse or acetaminophen intake. He had no family history or past personal history of liver or cardiovascular diseases. The patient was in good physical condition until approximately one month prior to admission, when he experienced increasing fatigue, decreased exercise tolerance and general malaise. Although he stopped exercising and self-administrating the drugs, these symptoms continued to progress and he subsequently developed anorexia, recurrent vomiting, right upper-quadrant abdominal pain and progressive jaundice in the 4 d prior to admission. No history of dyspnea, orthopnea, paroxysmal nocturnal dyspnea or edema could be elicited. At initial evaluation in the patient's local hospital, laboratory

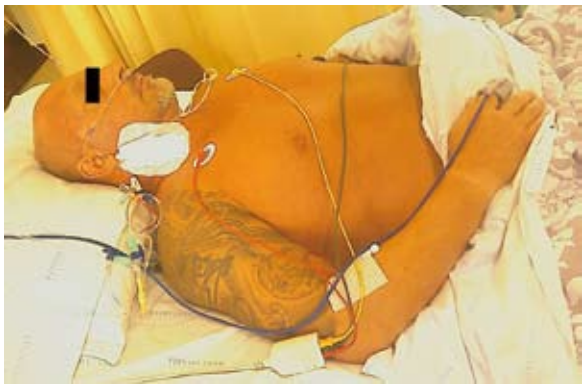

Figure 1 The patient was an athletic young male, with significant jaundice, in no acute distress and able to lie flat.

testing revealed an increase in serum transaminases (aspartate aminotransferase, 7897 IU/L; alanine aminotransferase, 7125 IU/L), coagulopathy (international normalized ratio, 3.3; factor V, 15%), hyperbilirubinemia (total bilirubin 6.8 mg/dL), high ammonia levels (73 umol/L) (normal, 11-32), acute renal failure (creatinine, 3.8 mg/dL), hyponatremia (126 mmol/L) and high lactate dehydrogenase (LDH) level (7140 IU/L). An abdominal ultrasound revealed hepatomegaly and patent flow of the inferior vena cava and hepatic veins. By the end of the first day, the patient was transferred to our Intensive-Care Unit of Hepatology, with the suspicion of anabolic steroid-induced toxic hepatitis.

On transfer, the patient appeared to be an athletic young male in no acute distress and was able to lie flat (Figure 1). Blood pressure was 96/60 mmHg, pulse was 120 beats/min, and respiratory rate was 26 breaths/min. He was conscious with no signs of hepatic encephalopathy. Additional findings on physical examination included generalized jaundice, no evidence of jugular venous distension, clear lung fields and distant heart sounds. He had an enlarged tender liver and no stigmata of chronic liver disease. His extremities were warm, with slight pretibial edema. Laboratory data were similar to those on admission, with markedly depressed values of factor V (15%) and factor Ⅶ (6%). Free testosterone and delta 4-androstenedione concentrations were elevated. Acetaminophen level was undetectable. Serology of known hepatotropic viruses was negative. Antinuclear antibodies were undetectable and serum copper and ceruloplasmin were normal. On chest X-ray, the cardiothoracic index was augmented with clear lung fields. The case was presented to our liver transplantation centre. After correction of hyponatremia with saline, in an attempt to obtain a mildly hyperosmotic state and prevent cerebral edema[3], symptoms and signs of congestive heart failure became evident, with pulmonary congestion and elevated central venous pressure. Echocardiogram showed a dilated cardiomyopathy with an estimated ejection fraction of 15% and a large thrombus in both ventricles. A diagnosis of severe toxic cardiomyopathy associated with anabolic steroids was made after ruling out other causes of non-ischemic dilated cardiomyopathy, including infectious, autoimmune and metabolic causes. With aggressive therapy for cardiac failure with dopamine, dobutamine and watchful diuresis, the patient's liver function improved dramatically. There was a rapid fall in serum transaminases and LDH levels. Four days after admission, the international normalized ratio was < 2.0 and low-molecular weight heparin therapy was started. Serial echocardiograms showed the disappearance of the intraventricular thrombus and improvement in left ventricular function (the fractional shortening increased up to 25%). Coronary angiography disclosed no lesions. After a 16-d hospitalization, the patient was discharged with oral anticoagulation. He his presently in the New York Heart Association functional class Ⅱ, working as a nightclub security agent. However, not enough time has elapsed since treatment to assess full recovery of pathological changes and heart performance.

DISCUSSION

Although the diagnosis of ischemic liver injury can be straightforward in the presence of a clinically evident hemodynamic insult, heart failure may also lead to subclinical circulatory disturbances and remain an unrecognized cause of liver injury[1,2]. Remarkably, in a recent report only around one-half of the patients with ischemic liver injury had been in a state of shock[4]. Isolated cases of unrecognized cardiomyopathy as the cause of severe ischemic liver injury have been reported in the recent literature[5-7]. In these cases, as in our patient, a cardiomyopathy lacking many of the symptoms and signs of congestive heart failure was the cause of the ischemic liver injury, with striking clinical and laboratory evidence of severe liver failure, leading to the possibility of liver transplantation as a rescue therapy. Our patient was an athlete without a history or clinical evidence of heart disease on presentation, and was referred to our Intensive Care Unit of Hepatology for treatment of severe acute liver failure. Several features led to a delay in the diagnosis of the underlying heart disease. Firstly, he was an athletic young male with none of the classic cardiovascular risk factors. Secondly, anabolic steroids are known hepatotoxic drugs[8,9], with only a few reports of severe cardiac toxicity in the literature[10,11]. Thirdly, for unclear reasons, symptoms of congestive heart failure were initially absent; theoretically, the severity of his right-sided heart failure in combination with a low output state may account for this[6]. Finally, cardiac and hepatic diseases share many similar clinical features, such as fatigue, decreased exercise tolerance, hepatomegaly and edema. This case highlights the fact that ischemic injury of the liver should always be considered in the differential diagnosis of acute hepatitis[1]. Retrospectively, some features may have suggested an ischemic rather than a viral or toxic cause of liver injury, including an early massive rise in LDH levels-a ratio of serum alanine aminotransferase to LDH of less than 1.5 may suggest ischemic injury[12]; and a concomitant early rise in the serum creatinine level (additional evidence of end-organ hypoperfusion)[13]. Chest X-ray also revealed

cardiomegaly, despite no evident pulmonary congestion. The rapid fall in serum transaminases after therapy for cardiac failure is characteristic of ischemic liver injury[13].

The medical history of this patient was significant for chronic self-administration of massive doses of anabolic steroids, including trenbolone enanthate, a strictly underground long-active steroid. Although anabolic steroids are associated with significant liver toxicity[8], toxic hepatitis induced by anabolic steroids with predominantly hepatocellular injury is extremely rare[9]. More frequently reported hepatotoxic effects include cholestatic liver injury[8], development of hepatic adenomas[14] and peliosis hepatis[15]. There have been a few reports of severe cardiovascular events associated with anabolic steroid abuse, including cases of severe dilated cardiomyopathy in otherwise young healthy patients[10,11]. Experimental studies[16,17] and non-invasive imaging studies in bodybuilders[18] have demonstrated a dose-dependent impairment of myocardial function after long-term anabolic steroid therapy. It has been proposed that increased fibrosis of the myocardium may be mediated by aldosterone-like effects[10]. The reversibility of such myocardial effects after discontinuation of the drugs is still unknown[18]. A partial recovery of left ventricular function a few months after cessation of anabolic steroid abuse has been reported in two body builders[10].

Anabolic steroid consumption is becoming more widespread and their adverse effects, including cardiovascular and hepatic toxicity, are expected to increase in the years to come[8,10,11]. To the best of our knowledge, this is the first reported case of severe liver failure due to an unrecognized anabolic steroid-induced cardiomyopathy. Awareness of this unique presentation will allow for prompt treatment of this potentially fatal cause of liver failure.

REFERENCES

1 **Seeto RK**, Fenn B, Rockey DC. Ischemic hepatitis: clinical presentation and pathogenesis. *Am J Med* 2000; **109**: 109-113
2 **Cohen JA**, Kaplan MM. Left-sided heart failure presenting as hepatitis. *Gastroenterology* 1978; **74**: 583-587
3 **Stravitz RT**, Kramer AH, Davern T, Shaikh AO, Caldwell SH, Mehta RL, Blei AT, Fontana RJ, McGuire BM, Rossaro L, Smith AD, Lee WM. Intensive care of patients with acute liver failure: recommendations of the U.S. Acute Liver Failure Study Group. *Crit Care Med* 2007; **35**: 2498-2508
4 **Henrion J**, Schapira M, Luwaert R, Colin L, Delannoy A, Heller FR. Hypoxic hepatitis: clinical and hemodynamic study in 142 consecutive cases. *Medicine* (Baltimore) 2003; **82**: 392-406
5 **Fussell KM**, Awad JA, Ware LB. Case of fulminant hepatic failure due to unrecognized peripartum cardiomyopathy. *Crit Care Med* 2005; **33**: 891-893
6 **Hoffman BJ**, Pate MB, Marsh WH, Lee WM. Cardiomyopathy unrecognized as a cause of hepatic failure. *J Clin Gastroenterol* 1990; **12**: 306-309
7 **Wiesen S**, Reddy KR, Jeffers LJ, Schiff ER. Fulminant hepatic failure secondary to previously unrecognized cardiomyopathy. *Dig Dis* 1995; **13**: 199-204
8 **Sánchez-Osorio M**, Duarte-Rojo A, Martínez-Benítez B, Torre A, Uribe M. Anabolic-androgenic steroids and liver injury. *Liver Int* 2008; **28**: 278-282
9 **Stimac D**, Milić S, Dintinjana RD, Kovac D, Ristić S. Androgenic/Anabolic steroid-induced toxic hepatitis. *J Clin Gastroenterol* 2002; **35**: 350-352
10 **Nieminen MS**, Rämö MP, Viitasalo M, Heikkilä P, Karjalainen J, Mäntysaari M, Heikkilä J. Serious cardiovascular side effects of large doses of anabolic steroids in weight lifters. *Eur Heart J* 1996; **17**: 1576-1583
11 **Ferrera PC**, Putnam DL, Verdile VP. Anabolic steroid use as the possible precipitant of dilated cardiomyopathy. *Cardiology* 1997; **88**: 218-220
12 **Cassidy WM**, Reynolds TB. Serum lactic dehydrogenase in the differential diagnosis of acute hepatocellular injury. *J Clin Gastroenterol* 1994; **19**: 118-121
13 **Gitlin N**, Serio KM. Ischemic hepatitis: widening horizons. *Am J Gastroenterol* 1992; **87**: 831-836
14 **Martin NM**, Abu Dayyeh BK, Chung RT. Anabolic steroid abuse causing recurrent hepatic adenomas and hemorrhage. *World J Gastroenterol* 2008; **14**: 4573-4575
15 **Cabasso A**. Peliosis hepatis in a young adult bodybuilder. *Med Sci Sports Exerc* 1994; **26**: 2-4
16 **Karhunen MK**, Rämö MP, Kettunen R. Anabolic steroids alter the haemodynamic effects of endurance training and deconditioning in rats. *Acta Physiol Scand* 1988; **133**: 297-306
17 **Takala TE**, Rämö P, Kiviluoma K, Vihko V, Kainulainen H, Kettunen R. Effects of training and anabolic steroids on collagen synthesis in dog heart. *Eur J Appl Physiol Occup Physiol* 1991; **62**: 1-6
18 **D'Andrea A**, Caso P, Salerno G, Scarafile R, De Corato G, Mita C, Di Salvo G, Severino S, Cuomo S, Liccardo B, Esposito N, Calabrò R. Left ventricular early myocardial dysfunction after chronic misuse of anabolic androgenic steroids: a Doppler myocardial and strain imaging analysis. *Br J Sports Med* 2007; **41**: 149-155

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2923

World J Gastroenterol 2009 June 21; 15(23): 2923-2926
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Laparoscopic resection of an adrenal pseudocyst mimicking a retroperitoneal mucinous cystic neoplasm

Bum-Soo Kim, Sun-Hyung Joo, Sung-Il Choi, Jeong-Yoon Song

Bum-Soo Kim, Sun-Hyung Joo, Sung-Il Choi, Jeong-Yoon Song, Division of Hepatobiliary Surgery, Department of Surgery, Kyung Hee University College of Medicine and East-West Neo Medical Center, 149 Sangil-dong, Gangdong-gu, Seoul, 134-727, South Korea

Author contributions: Kim BS wrote the paper; Kim BS and Joo SH contributed equally to the paper; Choi SI designed the paper; Song JY edited the figures.

Correspondence to: Sun-Hyung Joo, MD, Division of Hepatobiliary Surgery, Department of Surgery, Kyung Hee University College of Medicine and East-West Neo Medical Center, 149 Sangil-dong, Gangdong-gu, Seoul, 134-727, South Korea. sunhyung@chol.com

Telephone: +82-2-4406135 Fax: +82-2-4406295

Received: March 9, 2009 Revised: May 15, 2009
Accepted: May 22, 2009
Published online: June 21, 2009

Abstract

Adrenal pseudocysts are rare cystic masses that arise within the adrenal gland and are usually non-functional and asymptomatic. Adrenal pseudocysts consist of a fibrous wall without a cellular lining. We report a patient with a 9 cm, left-sided suprarenal cystic mass who presented with abdominal discomfort of 2 years' duration. A 38-year-old woman was referred to our service for evaluation of abdominal discomfort and gastrointestinal symptoms. Routine laboratory tests were within normal limits. An abdominal computed tomography scan showed a 9 cm × 8 cm × 8 cm well-defined cystic lesion displacing the left kidney. Magnetic resonance imaging showed a cystic lesion with low signal intensity on the T1-weighted image and high signal intensity on the T2-weighted image. A laparoscopic left adrenalectomy was performed to diagnose the lesion. The final pathology showed an adrenal pseudocyst without a cellular lining. The patient had no postoperative complications and she was discharged four days after surgery.

Key words: Adrenal gland; Pseudocyst; Laparoscopy; Adrenalectomy

Peer reviewer: Keiji Hirata, MD, Surgery 1, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan

Kim BS, Joo SH, Choi SI, Song JY. Laparoscopic resection of an adrenal pseudocyst mimicking a retroperitoneal mucinous cystic neoplasm. *World J Gastroenterol* 2009; 15(23): 2923-2926 Available from: URL: http://www.wjgnet.com/1007-9327/15/2923.asp DOI: http://dx.doi.org/10.3748/wjg.15.2923

INTRODUCTION

Adrenal pseudocysts are rare lesions that arise within the adrenal gland, most of which are non-functional and asymptomatic. The majority of adrenal pseudocysts are located in the suprarenal area. Preoperatively, larger cysts might be difficult to distinguish from cysts of renal or pancreatic origin. Adrenal pseudocysts consist of a fibrous wall without a cellular lining. We report a case of a patient with a 9 cm, left-sided suprarenal cystic mass who presented with abdominal discomfort of two years' duration.

CASE REPORT

A 38-year-old woman was admitted to our service with a two-year history of abdominal discomfort, anorexia, and nausea. She had no history of trauma and had undergone a laparoscopic unilateral salpingoophorectomy. A physical examination revealed no palpable abdominal masses and no tenderness in the abdomen. Routine laboratory tests were within normal limits. An abdominal computed tomography scan showed a 9 cm well-defined cystic lesion displacing the left kidney (Figure 1). The cystic lesion appeared to arise from the retroperitoneum. For further evaluation of the cystic lesion, magnetic resonance imaging was carried out and the cystic lesion showed low signal intensity on the T1-weighted image and high signal intensity on the T2-weighted image (Figure 2). There were no findings of suspected malignancy on the radiological image. We decided to perform a laparoscopic resection of the cystic lesion to relieve long-lasting gastrointestinal symptoms and to diagnose the lesion.

The patient was placed in the right lateral decubitus, flexed at the waist. A 12-mm trocar was inserted in the umbilical region *via* an open incision. Three other operating ports were placed along the left costal margin, as shown in Figure 3. The second 10-mm trocar was

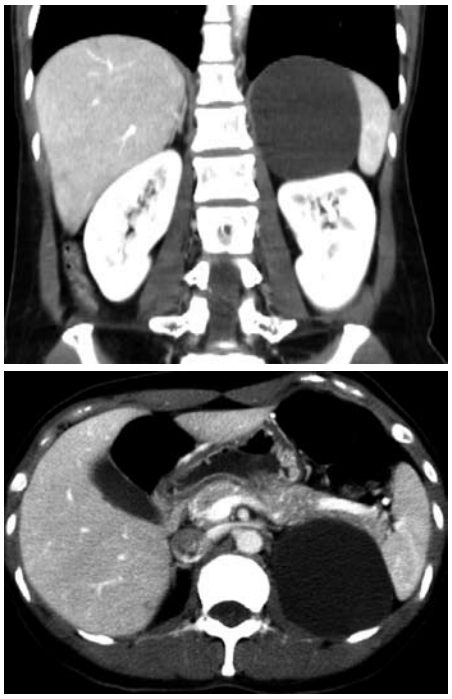

Figure 1 An abdominal computed tomography scan showing a 9 cm well-defined cystic lesion, which abuts the spleen, kidney and pancreas tail.

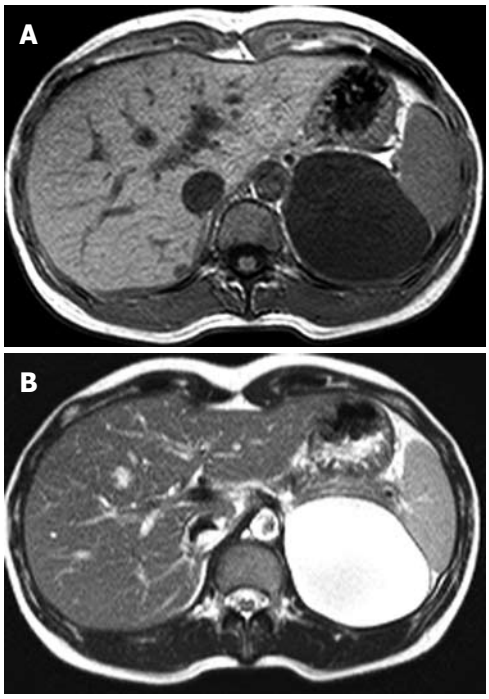

Figure 2 Magnetic resonance imaging of the abdomen. A low signal intensity cystic mass on the T1-weighted image (A) and a high signal intensity cystic mass on the T2-weighted image (B).

introduced on the anterior axillary line. The third 5-mm trocar was introduced on left side of the second trocar and placed under the costal margin. The fourth 5-mm trocar was introduced in the posterior axillary line and was used for a retractor. The splenic flexure of the colon was mobilized from the left paracolic gutter to the inferior pole of the spleen. The splenorenal ligament was divided to allow the spleen and tail of the pancreas to rotate medially, exposing the left retroperitoneum. At that time, a smooth, well-capsulated cystic mass, measuring 9 cm × 8 cm × 8 cm, arising from the left adrenal gland was found. The superior, medial and lateral border of the lesion was first dissected from neighboring tissue with a LigaSure (Valley Lab, Boulder, CO). The dissection proceeded inferiorly to expose and ligate the left adrenal vein. The adrenal vein was identified and divided with a 2.5-mm endoscopic vascular stapler (Ethicon). The adrenal cystic lesion, completely freed, was placed in a surgical bag (Endocatch; Ethicon). The cystic contents were aspirated with a needle and were a thin, yellowish fluid. The specimen was extracted *via* the 10-mm port side by enlarging the incision from 1 to 2.5 cm. The operative time was 110 min and the blood loss was 50 mL. Gross appearance showed a thin-walled, yellowish, 6 cm × 3 cm × 3 cm unilocular adrenal lesion (Figure 4). Histological examination showed an adrenal pseudocyst without an epithelial or endothelial lining (Figure 5). The patient had no postoperative complications and she was discharged four days after surgery. The abdominal pain and gastrointestinal symptoms resolved after surgery.

DISCUSSION

Adrenal cysts are classified as a cystic degeneration of adrenal neoplasms, true cysts, pseudocysts, and infectious cysts[1-3]. Among adrenal cysts, the most common types are epithelial cysts and pseudocysts. True cysts are lined with endothelial or mesothelial cells[4]; however, adrenal pseudocysts are devoid of an epithelial or endothelial lining, arise within the adrenal gland, and are surrounded by a fibrous tissue wall. Infectious cysts are most commonly caused by *Echinococcus*[5].

Adrenal pseudocysts are rare and account for 32% to 80% of all adrenal cysts[3,6-9]. Adrenal pseudocysts are often found incidentally on imaging studies or at the time of autopsy. The majority of adrenal pseudocysts are found because of their size-related symptoms[6-8]. Symptoms include gastrointestinal disturbance, early satiety, and a palpable abdominal mass. Patients can present with acute abdominal findings if intracystic hemorrhage or rupture occurs[10].

The etiology of adrenal pseudocysts is unknown; however, several mechanisms have been proposed to account for their occurrence, including cystic degeneration of a primary adrenal neoplasm, degeneration of a vascular neoplasm, and malformation and hemorrhage of adrenal veins into the adrenal gland[1-3,6,7,11]. The etiology of our patient's pseudocyst was indeterminate.

Due to the wide use of the diagnostic imaging modalities, the detection rate of adrenal cystic lesions is increasing. However, preoperative confirmatory diagnosis of a large adrenal cyst can be very difficult, because of the indistinct boundary with surrounding organs and adhesion to neighboring organs[12].

The differential diagnosis of adrenal pseudocysts includes splenic, hepatic, and renal cysts, as well as mesenteric or retroperitoneal cysts, urachal cysts, and

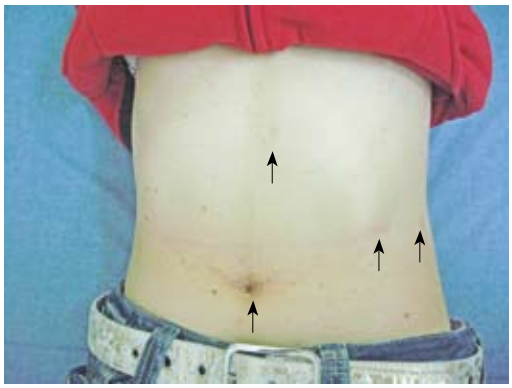

Figure 3 Placement of the laparoscopic trocar and operative ports. A 12-mm trocar was placed in the periumbilical region, and three other operating ports were placed in the abdomen (arrows). The photograph was taken eight months after surgery.

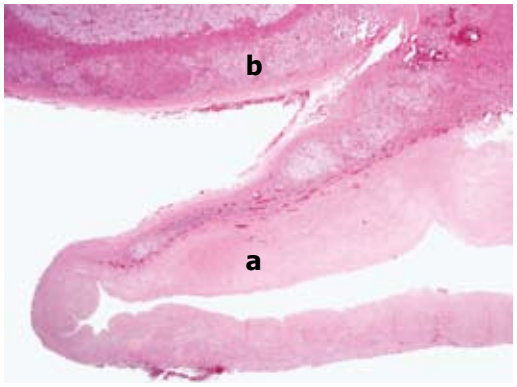

Figure 5 Histopathology showing an adrenal pseudocyst (a) and adrenal tissue remnants on the upper aspect (b). The cyst wall is composed of a thick layer of hyalinized connective tissue without an epithelial or endothelial lining (a). HE staining (Original magnification, × 40).

solid adrenal tumors[12,13]. Adrenal pseudocysts must be differentiated from other benign or malignant lesions originating from the adrenal gland or the kidney unilaterally[14-17]. In addition, an exact diagnosis is clinically important because an adrenal cyst > 6 cm carries an increased risk of adrenal malignancy. The reported incidence of malignancy in adrenal cystic lesions is approximately 7%[18,19].

Typically, radiological findings of adrenal cysts reveal thin walls filled with watery fluid and occasional calcifications[20,21]. Often, the inferior wall of the cyst is concave or flat, conforming to the shape of the upper pole of the renal contour.

On computed tomography scan, the present cyst could not be differentiated from a renal or adrenal lesion. The CT scan was not definitive, therefore an MRI was obtained. The adrenal gland was not visualized by the MRI scan and the cystic mass was thus considered to be of pancreatic rather than adrenal origin. The cystic mass could not be differentiated from a retroperitoneal mucinous cystic neoplasm. We chose the laparoscopic approach because of the time involved, the anticipated minimal blood loss, the acceptable rate of complications, and the excellent postoperative quality of life. There

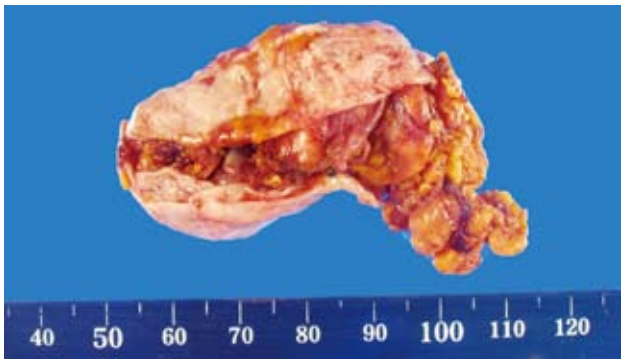

Figure 4 Gross appearance of the 6 cm × 3 cm × 3 cm adrenal cystic lesions. The cystic lesion adheres to adrenal gland tissue.

are few reports of a case of retroperitoneal mucinous cystadenomas treated by laparoscopic surgery[22-24].

During the laparoscopic procedure, we found that the cystic mass had arisen from the left adrenal gland, not the pancreas. We believed that this cystic mass was an adrenal cyst and removed it completely by laparoscopic surgery.

The choice of treatment for adrenal pseudocysts depends on several factors, including endocrine function, symptoms, size, and correct differentiation from an adrenal cyst.

Surgical excision is indicated in the presence of symptoms, suspicion of malignancy, an increase in size, the occurrence of complications, or detection of a functioning adrenal cyst, and can be managed by open surgery or a laparoscopic approach[12,25-27]. Kalady *et al*[28] recommended that adrenal cysts > 6 cm should be approached using an open procedure because of concerns about potential malignancy. In the case of giant pseudocysts, most authors prefer an open adrenalectomy, because the laparoscopic approach is not sufficient to control such large masses with active internal bleeding[29]. However, several reports demonstrate that laparoscopic adrenalectomy for large (> 6 cm) adrenal lesions is surgically feasible and can be applied for any adrenal disease, including benign and potentially malignant lesions[25,30-32].

This case was sufficiently instructive to point out that adrenal cystic lesions can be radiologically mistaken for renal cysts or cystic neoplasms.

This is a rare report of an adrenal pseudocyst mimicking a retroperitoneal mucinous cystic neoplasm, which was treated by laparoscopic resection. Although adrenal pseudocysts might be misdiagnosed as retroperitoneal mucinous cystic neoplasms, a laparoscopic approach to cystic lesions in the suprarenal area should be the initial choice.

REFERENCES

1 **Medeiros LJ**, Lewandrowski KB, Vickery AL Jr. Adrenal pseudocyst: a clinical and pathologic study of eight cases. *Hum Pathol* 1989; **20**: 660-665
2 **Gaffey MJ**, Mills SE, Fechner RE, Bertholf MF, Allen MS Jr. Vascular adrenal cysts. A clinicopathologic and

immunohistochemical study of endothelial and hemorrhagic (pseudocystic) variants. *Am J Surg Pathol* 1989; **13**: 740-747
3 **Neri LM**, Nance FC. Management of adrenal cysts. *Am Surg* 1999; **65**: 151-163
4 **Fukushima N**, Oonishi T, Yamaguchi K, Fukayama M. Mesothelial cyst of the adrenal gland. *Pathol Int* 1995; **45**: 156-159
5 **Fitzgerald EJ**. Hydatid disease of the adrenal gland. *Ir J Med Sci* 1987; **156**: 366-367
6 **Erickson LA**, Lloyd RV, Hartman R, Thompson G. Cystic adrenal neoplasms. *Cancer* 2004; **101**: 1537-1544
7 **Karayiannakis AJ**, Polychronidis A, Simopoulos C. Giant adrenal pseudocyst presenting with gastric outlet obstruction and hypertension. *Urology* 2002; **59**: 946
8 **Bellantone R**, Ferrante A, Raffaelli M, Boscherini M, Lombardi CP, Crucitti F. Adrenal cystic lesions: report of 12 surgically treated cases and review of the literature. *J Endocrinol Invest* 1998; **21**: 109-114
9 **Barzon L**, Boscaro M. Diagnosis and management of adrenal incidentalomas. *J Urol* 2000; **163**: 398-407
10 **Papaziogas B**, Katsikas B, Psaralexis K, Makris J, Chatzimavroudis G, Tsiaousis R, Dragoumis D, Radopoulos K, Panagiotopoulou K, Atmatzidis K. Adrenal pseudocyst presenting as acute abdomen during pregnancy. *Acta Chir Belg* 2006; **106**: 722-725
11 **Groben PA**, Roberson JB Jr, Anger SR, Askin FB, Price WG, Siegal GP. Immunohistochemical evidence for the vascular origin of primary adrenal pseudocysts. *Arch Pathol Lab Med* 1986; **110**: 121-123
12 **Sroujieh AS**, Farah GR, Haddad MJ, Abu-Khalaf MM. Adrenal cysts: diagnosis and management. *Br J Urol* 1990; **65**: 570-575
13 **Fan F**, Pietrow P, Wilson LA, Romanas M, Tawfik OW. Adrenal pseudocyst: a unique case with adrenal renal fusion, mimicking a cystic renal mass. *Ann Diagn Pathol* 2004; **8**: 87-90
14 **Mohan H**, Aggarwal R, Tahlan A, Bawa AS, Ahluwalia M. Giant adrenal pseudocyst mimicking a malignant lesion. *Can J Surg* 2003; **46**: 474
15 **Demir A**, Tanidir Y, Kaya H, Turkeri LN. A giant adrenal pseudocyst: case report and review of the literature. *Int Urol Nephrol* 2006; **38**: 167-169
16 **Ghandur-Mnaymneh L**, Slim M, Muakassa K. Adrenal cysts: pathogenesis and histological identification with a report of 6 cases. *J Urol* 1979; **122**: 87-91
17 **Levin SE**, Collins DL, Kaplan GW, Weller MH. Neonatal adrenal pseudocyst mimicking metastatic disease. *Ann Surg* 1974; **179**: 186-189
18 **Khoda J**, Hertzanu Y, Sebbag G, Lantsberg L, Barky Y. Adrenal cysts: diagnosis and therapeutic approach. *Int Surg* 1993; **78**: 239-242
19 **Outwater E**, Bankoff MS. Clinically significant adrenal hemorrhage secondary to metastases. Computed tomography observations. *Clin Imaging* 1989; **13**: 195-200
20 **Foster DG**. Adrenal cysts. Review of literature and report of case. *Arch Surg* 1966; **92**: 131-143
21 **Lockhart ME**, Smith JK, Kenney PJ. Imaging of adrenal masses. *Eur J Radiol* 2002; **41**: 95-112
22 **Chen JS**, Lee WJ, Chang YJ, Wu MZ, Chiu KM. Laparoscopic resection of a primary retroperitoneal mucinous cystadenoma: report of a case. *Surg Today* 1998; **28**: 343-345
23 **Cadeddu MO**, Mamazza J, Schlachta CM, Seshadri PA, Poulin EC. Laparoscopic excision of retroperitoneal tumors: technique and review of the laparoscopic experience. *Surg Laparosc Endosc Percutan Tech* 2001; **11**: 144-147
24 **Ishikawa K**, Hirashita T, Araki K, Kitano M, Matsuo S, Matsumata T, Kitano S. A case of retroperitoneal mucinous cystadenoma treated successfully by laparoscopic excision. *Surg Laparosc Endosc Percutan Tech* 2008; **18**: 516-519
25 **Kar M**, Pucci E, Brody F. Laparoscopic resection of an adrenal pseudocyst. *J Laparoendosc Adv Surg Tech A* 2006; **16**: 478-481
26 **Ulusoy E**, Adsan O, Güner E, Cetinkaya M, Ataman T, Seçkin S. Giant adrenal cyst: preoperative diagnosis and management. *Urol Int* 1997; **58**: 186-188
27 **Amarillo HA**, Bruzoni M, Loto M, Castagneto GH, Mihura ME. Hemorrhagic adrenal pseudocyst: laparoscopic treatment. *Surg Endosc* 2004; **18**: 1539
28 **Kalady MF**, McKinlay R, Olson JA Jr, Pinheiro J, Lagoo S, Park A, Eubanks WS. Laparoscopic adrenalectomy for pheochromocytoma. A comparison to aldosteronoma and incidentaloma. *Surg Endosc* 2004; **18**: 621-625
29 **Stimac G**, Katusic J, Sucic M, Ledinsky M, Kruslin B, Trnski D. A giant hemorrhagic adrenal pseudocyst: case report. *Med Princ Pract* 2008; **17**: 419-421
30 **Ramacciato G**, Mercantini P, La Torre M, Di Benedetto F, Ercolani G, Ravaioli M, Piccoli M, Melotti G. Is laparoscopic adrenalectomy safe and effective for adrenal masses larger than 7 cm? *Surg Endosc* 2008; **22**: 516-521
31 **Novitsky YW**, Czerniach DR, Kercher KW, Perugini RA, Kelly JJ, Litwin DE. Feasibility of laparoscopic adrenalectomy for large adrenal masses. *Surg Laparosc Endosc Percutan Tech* 2003; **13**: 106-110
32 **Henry JF**, Sebag F, Iacobone M, Mirallie E. Results of laparoscopic adrenalectomy for large and potentially malignant tumors. *World J Surg* 2002; **26**: 1043-1047

S- Editor Tian L **L- Editor** Stewart GJ **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2927

World J Gastroenterol 2009 June 21; 15(23): 2927-2929
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Severe acute cholestatic hepatitis of unknown etiology successfully treated with the Chinese herbal medicine Inchinko-to (TJ-135)

Susumu Ohwada, Isao Kobayashi, Nobuo Harasawa, Kyoichiro Tsuda, Yosikatsu Inui

Susumu Ohwada, Isao Kobayashi, Nobuo Harasawa, Kyoichiro Tsuda, Kanetsu Chuo Hospital, Kitaharacho 71, Takasaki, Gunma, 370-3513, Japan
Yosikatsu Inui, Inui Clinic of Internal Medicine, Shimokobanamachi Takasaki, Gunma, 370-0076, Japan
Author contributions: Ohwada S organized and wrote the manuscript; Kobayashi I, Harasawa N, Tsuda K, Inui Y provided patient's data, contributed to manuscript writing, and approved the final manuscript.
Correspondence to: Susumu Ohwada, Kanetsu Chuo Hospital, Kitaharacho 71, Takasaki, 370-3513, Gunma, Japan. gogoohwada@yahoo.co.jp
Telephone: +81-27-3435115 Fax: +81-27-3432260
Received: November 11, 2008 Revised: March 21, 2009
Accepted: March 28, 2009
Published online: June 21, 2009

Abstract

Severe acute hepatitis of unknown etiology is difficult to treat and often progresses to subacute fulminant hepatitis or late-onset hepatic failure. A 45-year-old well-nourished, healthy man had progressive fatigue and his liver function tests showed severe liver dysfunction. The etiology of sever acute cholestatic hepatitis was unknown. The liver function tests normalized gradually, which excluded high persistent total bilirubin after starting on predonine. A liver biopsy showed chronic active hepatitis with mild fibrosis (A2, F1). Oral Inchinko-to, a Chinese herbal medicine, at 7.5 g daily was prescribed. The treatment was effective with no adverse effects. We present a successfully treated case and discuss hepatoprotective and choleretic effects of Inchinko-to.

Key words: Acute cholestatic hepatitis; Etiology; Inchinko-to; Herbal medicine

Peer reviewer: Jose JG Marin, Professor, Head of the Departamento Physiology and Pharmacology, University of Salamanca, CIBERehd, Campus Miguel de Unamuno, ED-S09, Salamanca 37007, Spain

Ohwada S, Kobayashi I, Harasawa N, Tsuda K, Inui Y. Severe acute cholestatic hepatitis of unknown etiology successfully treated with the Chinese herbal medicine Inchinko-to (TJ-135). *World J Gastroenterol* 2009; 15(23): 2927-2929 Available from: URL: http://www.wjgnet.com/1007-9327/15/2927.asp DOI: http://dx.doi.org/10.3748/wjg.15.2927

INTRODUCTION

Treatment of severe acute hepatitis of unknown etiology is difficult, and the disease often progresses to subacute fulminant hepatitis or late-onset hepatic failure[1]. Several studies have reported on the effectiveness of Inchinko-to, a Chinese herbal medicine used to treat liver disorders and jaundice in Japan[2-5]. Successful treatment of acute hepatic failure of unknown etiology with Inchinko-to has been reported[6].

We treated a patient who suffered from severe acute cholestatic hepatitis of unknown etiology with Inchinko-to. The treatment was effective with no adverse effects. We report and discuss the case.

CASE REPORT

The patient was a 45-year-old man, who had fatigue, vomiting, slight fever, and noticed that his urine had been darker than usual for 1 mo before visiting the clinic. He began to suffer from severe fatigue and loss of appetite, and thus visited the clinic. On physical examination, his skin was dark yellow, without a palpable liver. Liver function tests revealed severe liver dysfunction (Table 1). He was a well-nourished, healthy man with a history of duodenal ulcer. He did not drink alcohol and had no history of blood or blood products transfusion or the use of any drugs, including herbal medicine. He was exposed to no known environmental hazards. His older brother had non-alcoholic steatohepatitis and underwent surgery for rectal carcinoma. Abdominal ultrasonography showed a severely swollen gallbladder and normal intra- and extrahepatic bile ducts; the liver shape and architecture were normal. Upper endoscopy showed an edematous narrowing at the post-bulbar portion of the duodenum and a duodenal ulcer scar in the duodenal bulb.

He was admitted to the hospital. Ursodeoxycholic acid (UDCA) was started at 600 mg daily. Tests for hepatitis A, B, C, and E viruses, Epstein-Barr virus and cytomegalovirus were all negative. Autoantibodies were negative, including anti-mitochondrial, smooth muscle, antinuclear, and perinuclear anti-neutrophil cytoplasmic antibody. Gamma globulin was within the normal limits. The hemolytic complement activity (CH50) was elevated slightly. Abdominal computed tomography showed a severely swollen gallbladder, normal intra- and extrahepatic bile ducts, and normal liver shape and architecture (Figure 1, top). Magnetic resonance imaging showed an edematous,

Table 1 Laboratory data on admission

White blood cells (/μL)	4600	Alfa1 globulin (%)	4.2
Red blood cells (/μL)	493	Alfa2 globulin (%)	7.2
Hemoglobin (g/dL)	15.1	Beta globulin (%)	11.2
Hematocrit (%)	46	Gamma globulin (%)	15.3
Platelets (/μL)	16.7×10^4	CH50 (OU/mL)	52.4
Total protein (g/dL)	5.8	Anti-nuclear antibody	-
Albumin (g/dL)	3.5		
Total bilirubin (mg/dL)	6	P-ANCA	-
Direct bilirubin (mg/dL)	4.9	Anti-mitochondrial	-
AST (IU/L)	810	antibody	
ALT (IU/L)	1239	HA-IgM	-
LDH (IU/L)	418	HBs Ag	-
Alkaline phosphatase	687	HBs Ab	-
(IU/L)		HBe Ag	-
rGTP (IU/L)	305	HCV Ab	-
Prothrombin time (%)	92.9	HEV-IgM	-
Activated partial	29.2	HEV-IgG	-
thromboplastin time (s)		EBV-VCM	-
C-reactive protein (mg/dL)	1	CMV-IgM	-
NH_3 (mcg/dL)	70		

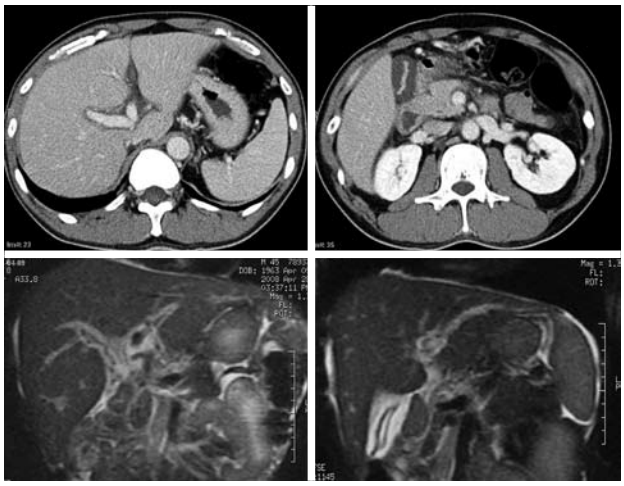

Figure 1 Abdominal CTs and MRIs on admission. Abdominal CTs showing normal liver shape and architecture, normal intra-and extrahepatic bile ducts, and a severely swollen gallbladder (top); MRIs showing a normal liver, bile duct, an edematous, thickened gallbladder wall, and periportal edema (bottom).

thickened gallbladder wall, periportal edema, slight ascites, and a normal liver, bile duct, and pancreatic duct (Figure 1, bottom). Based on these findings, the patient was diagnosed with severe acute cholestatic hepatitis of unknown etiology. The total bilirubin level continued to rise, and so the patient was started on predonine 20 mg daily on 7 May 2008. The serum aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels normalized gradually, while the total bilirubin remained high. Oral Inchinko-to at 7.5 g daily was prescribed on 17 May 2008. He was weaned from predonine. Subsequently, the total bilirubin level began to decrease (Figure 2). A liver biopsy showed chronic active hepatitis with mild fibrosis (A2, F1).

The patient was discharged. He is currently well at 12 mo after UDCA and Inchinko-to treatment.

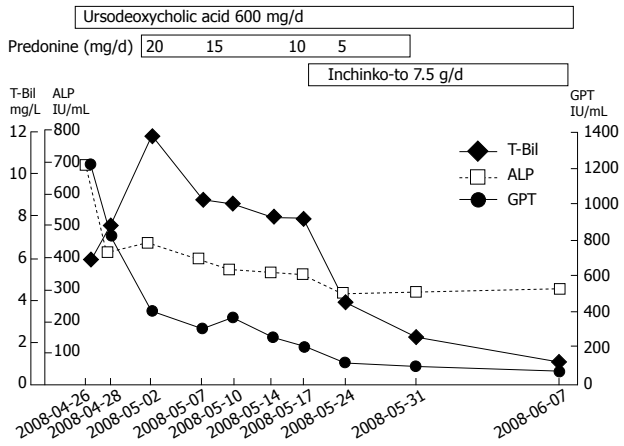

Figure 2 Clinical course of the patient.

DISCUSSION

Without evidence of a virus infection, autoimmune disease, alcohol or drug use, blood or blood products administration, and environmental hazards, the patient was considered to have severe acute cholestatic hepatitis of unknown etiology. Initially, predonine was prescribed for suspected autoimmune hepatitis with a high total bilirubin level[7]. The increasing total bilirubin was thought to suggest progression to subacute fulminant hepatitis[1]. Inchinko-to, a Chinese herbal medicine, has been used clinically for various liver diseases in Japan and China[2-5].

Recent experimental studies have clarified the molecular mechanism of hepatoprotective and choleretic effects of Inchinko-to and its ingredients, and have provided a good rationale for its clinical application to a wide variety of liver diseases, although there is no sound evidence with prospective randomized clinical studies[8-13]. The ingredients of Inchinko-to are genipin, 6,7-dimethylesculetin, capillin, capillene, and capillarisin[2,14,15]. Therefore, we decided to give Inchinko-to to our patient who suffered from severe acute cholestatic hepatitis of unknown etiology. The treatment was effective with no adverse effects.

The death of liver cells in hepatitis is thought to involve apoptosis[16]. Fas/FasL-mediated cytotoxicity is critical to hepatic injury, particularly fulminant hepatitis[17]. Genipin markedly suppressed liver apoptosis/injury in a lethal fulminant hepatitis model[9] and improved acute liver dysfunction by the suppression of tumor necrosis factor-α production[10]. In addition, capillin and capillene inhibit liver cell apoptosis induced by transforming growth factor[8].

Cholestasis results in hepatic and systemic accumulation of potentially toxic bile acids, resulting in liver damage and jaundice[18]. The altered expression of specific hepatocellular transport systems and profound changes in the cytoskeleton of the hepatocytes are associated with cholestatic liver disease[18]. In particular, the impairment of canalicular transport systems has a major role in the pathogenesis of acquired forms of intrahepatic cholestasis[18]. Inchinko-to exerts potent choleretic effects *via* a bile-acid-independent mechanism of multidrug-resistance-associated protein 2 mediation and glutathione content modulation[11-13] by genipin, and of constitutive

androstane receptor activation by 6,7-dimethylesculetin[12]. The marked reduction in bilirubin level after Inchinko-to administration in our case suggested that Inchinko-to and its ingredients stimulated and restored the impaired canalicular transport systems.

UDCA was also used in this case and it has been reported to be effective in cases of chronic liver disease[19] and prolonged cholestasis of acute hepatitis[20]. The therapeutic benefit of UDCA in the treatment of cholestasis may result from a combination of cytoprotective, antiapoptotic, immunomodulatory, and choleretic effects[21]. The choleretic effects of UDCA are medicated by up-regulation of canalicular transporter protein levels[22], and inhibit apoptosis by modulating mitochondrial function[23]. No apparent effect was observed on liver function tests in our case soon after the administration of UDCA. Therefore, the combination of Inchinko-to and UDCA possibly worked synergistically in improving the acute cholestatic hepatitis in this case.

Finally, our success in treating a patient with acute cholestatic hepatitis of unknown etiology supports a previous study[6], and suggests its efficacy in treating such liver disease. Our findings warrant a further clinical trial.

ACKNOWLEDGMENTS

We thank Associate Professor Hitoshi Takagi, Gunma University Graduate School of Medicine and Assistant Director Yoichi Kon, Gunma Cancer Center, Ota, Gunma, Japan, for helpful advice regarding the diagnosis and treatment.

REFERENCES

1 **Fujiwara K**, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; **38**: 646-657
2 **Aburada M**, Sasaki H, Harada M. Pharmacological studies of Gardeniae Fructus II. Contribution of the constituent crude drugs to choleretic activity of "Inchiko-to" in rats. *Yakugaku Zasshi* 1976; **96**: 147-153
3 **Kobayashi H**, Horikoshi K, Yamataka A, Lane GJ, Yamamoto M, Miyano T. Beneficial effect of a traditional herbal medicine (inchin-ko-to) in postoperative biliary atresia patients. *Pediatr Surg Int* 2001; **17**: 386-389
4 **Iinuma Y**, Kubota M, Yagi M, Kanada S, Yamazaki S, Kinoshita Y. Effects of the herbal medicine Inchinko-to on liver function in postoperative patients with biliary atresia--a pilot study. *J Pediatr Surg* 2003; **38**: 1607-1611
5 **Kaiho T**, Tsuchiya S, Yanagisawa S, Takeuchi O, Togawa A, Okamoto R, Saigusa N, Miyazaki M. Effect of the herbal medicine Inchin-Ko-To for serum bilirubin in hepatectomized patients. *Hepatogastroenterology* 2008; **55**: 150-154
6 **Arai M**, Yokosuka O, Fukai K, Kanda T, Kojima H, Kawai S, Imazeki F, Hirasawa H, Saisho H. A case of severe acute hepatitis of unknown etiology treated with the Chinese herbal medicine Inchinko-to. *Hepatol Res* 2004; **28**: 161-165
7 **Tang CP**, Shiau YT, Huang YH, Tsay SH, Huo TI, Wu JC, Lin HC, Lee SD. Cholestatic jaundice as the predominant presentation in a patient with autoimmune hepatitis. *J Chin Med Assoc* 2008; **71**: 45-48
8 **Yamamoto M**, Ogawa K, Morita M, Fukuda K, Komatsu Y. The herbal medicine Inchin-ko-to inhibits liver cell apoptosis induced by transforming growth factor beta 1. *Hepatology* 1996; **23**: 552-559
9 **Yamamoto M**, Miura N, Ohtake N, Amagaya S, Ishige A, Sasaki H, Komatsu Y, Fukuda K, Ito T, Terasawa K. Genipin, a metabolite derived from the herbal medicine Inchin-ko-to, and suppression of Fas-induced lethal liver apoptosis in mice. *Gastroenterology* 2000; **118**: 380-389
10 **Takeuchi S**, Goto T, Mikami K, Miura K, Ohshima S, Yoneyama K, Sato M, Shibuya T, Watanabe D, Kataoka E, Segawa D, Endo A, Sato W, Yoshino R, Watanabe S. Genipin prevents fulminant hepatic failure resulting in reduction of lethality through the suppression of TNF-alpha production. *Hepatol Res* 2005; **33**: 298-305
11 **Shoda J**, Miura T, Utsunomiya H, Oda K, Yamamoto M, Kano M, Ikegami T, Tanaka N, Akita H, Ito K, Suzuki H, Sugiyama Y. Genipin enhances Mrp2 (Abcc2)-mediated bile formation and organic anion transport in rat liver. *Hepatology* 2004; **39**: 167-178
12 **Huang W**, Zhang J, Moore DD. A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest* 2004; **113**: 137-143
13 **Okada K**, Shoda J, Kano M, Suzuki S, Ohtake N, Yamamoto M, Takahashi H, Utsunomiya H, Oda K, Sato K, Watanabe A, Ishii T, Itoh K, Yamamoto M, Yokoi T, Yoshizato K, Sugiyama Y, Suzuki H. Inchinkoto, a herbal medicine, and its ingredients dually exert Mrp2/MRP2-mediated choleresis and Nrf2-mediated antioxidative action in rat livers. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1450-G1463
14 **Takeda S**, Yuasa K, Endo T, Aburada M. Pharmacological studies on iridoid compounds. II. Relationship between structures and choleretic actions of iridoid compound. *J Pharmacobiodyn* 1980; **3**: 485-492
15 **Sakagami Y**, Mizoguchi Y, Seki S, Miyajima K, Kobayashi K, Shin T, Takeda H, Morisawa S. Effect of Inchin-ko-to treatment on intrahepatic cholestasis and acute severe hepatitis [English abstract]. *J Med Pharm Soc Wakan-Yaku* 1987; **4**: 124-129
16 **Kondo T**, Suda T, Fukuyama H, Adachi M, Nagata S. Essential roles of the Fas ligand in the development of hepatitis. *Nat Med* 1997; **3**: 409-413
17 **Ogasawara J**, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S. Lethal effect of the anti-Fas antibody in mice. *Nature* 1993; **364**: 806-809
18 **Trauner M**, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 1998; **339**: 1217-1227
19 **Takano S**, Ito Y, Yokosuka O, Ohto M, Uchiumi K, Hirota K, Omata M. A multicenter randomized controlled dose study of ursodeoxycholic acid for chronic hepatitis C. *Hepatology* 1994; **20**: 558-564
20 **Kadayifci A**, Savas MC, Arslan S, Güllü IH. Ursodeoxycholic acid in the management of prolonged cholestasis of acute hepatitis B. *J Clin Gastroenterol* 1997; **24**: 125-126
21 **Beuers U**, Boyer JL, Paumgartner G. Ursodeoxycholic acid in cholestasis: potential mechanisms of action and therapeutic applications. *Hepatology* 1998; **28**: 1449-1453
22 **Fickert P**, Zollner G, Fuchsbichler A, Stumptner C, Pojer C, Zenz R, Lammert F, Stieger B, Meier PJ, Zatloukal K, Denk H, Trauner M. Effects of ursodeoxycholic and cholic acid feeding on hepatocellular transporter expression in mouse liver. *Gastroenterology* 2001; **121**: 170-183
23 **Rodrigues CM**, Fan G, Ma X, Kren BT, Steer CJ. A novel role for ursodeoxycholic acid in inhibiting apoptosis by modulating mitochondrial membrane perturbation. *J Clin Invest* 1998; **101**: 2790-2799

S- Editor Li LF **L- Editor** Kerr C **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2930

World J Gastroenterol 2009 June 21; 15(23): 2930-2932
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

A case of hepatic angiomyolipoma difficult to distinguish from hepatocellular carcinoma

Masahiro Takahara, Yasuhiro Miyake, Kazuyuki Matsumoto, Daisuke Kawai, Eisuke Kaji, Tatsuya Toyokawa, Morihito Nakatsu, Masaharu Ando, Mamoru Hirohata

Masahiro Takahara, Kazuyuki Matsumoto, Daisuke Kawai, Eisuke Kaji, Tatsuya Toyokawa, Morihito Nakatsu, Masaharu Ando, Mamoru Hirohata, Department of Internal Medicine, Mitoyo General Hospital, Kagawa 769-1695, Japan
Yasuhiro Miyake, Department of Molecular Hepatology and Department of Gastroenterology & Hepatology, University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan
Author contributions: Takahara M and Miyake Y wrote the paper; Matsumoto K, Kawai D, Kaji E, Toyokawa T, Nakatsu M and Ando M performed the patient's care; Hirohata M supervised and approved the final manuscript.
Correspondence to: Yasuhiro Miyake, MD, Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan. miyakeyasuhiro@hotmail.com
Telephone: +81-86-2357219 Fax: +81-86-2255991
Received: March 11, 2009 Revised: May 23, 2009
Accepted: May 30, 2009
Published online: June 21, 2009

Abstract

We report a case of hepatic angiomyolipoma with uncommon clinical features. A 56-year-old man presented with a hepatic tumor in the caudate lobe. The tumor was hypoechoic on ultrasonography, showed early-phase hyperattenuation on enhanced computed tomography and did not absorb iron on superparamagnetic iron oxide-enhanced magnetic resonance imaging. Hepatocellular carcinoma was highly suspected, and the patient underwent hepatic resection. Histologically, the tumor was mainly composed of smooth muscle cells and contained small amounts of adipose cells and blood vessels. On immunohistochemical staining, the smooth muscle cells were positive for a melanocytic cell-specific monoclonal antibody. In cases with uncommon features of angiomyolipoma, it is quite difficult to distinguish angiomyolipoma from hepatocellular carcinoma.

Key words: Adipose cell; Hepatic angiomyolipoma; Hepatocellular carcinoma; HMB-45; Smooth muscle cell

Peer reviewer: Hisato Nakajima, MD, Department of Gastroenterology and Hepatology, The Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan

Takahara M, Miyake Y, Matsumoto K, Kawai D, Kaji E, Toyokawa T, Nakatsu M, Ando M, Hirohata M. A case of hepatic angiomyolipoma difficult to distinguish from hepatocellular carcinoma. *World J Gastroenterol* 2009; 15(23): 2930-2932 Available from: URL: http://www.wjgnet.com/1007-9327/15/2930.asp DOI: http://dx.doi.org/10.3748/wjg.15.2930

INTRODUCTION

Hepatic angiomyolipoma (HAML) was first reported by Ishak[1]. It is a rare benign tumor which is composed of a heterogeneous mixture of adipose cells, smooth muscle cells and vessels, and can be treated conservatively if spontaneous hemorrhage or malignant change does not occur[2-4]. The radiological features of HAML depend on the relative proportions of adipose cells[5]. For this reason, preoperative diagnosis of HAML is occasionally difficult, and it is not easy to differentiate from hepatocellular carcinoma (HCC). Herein, we report a case of HAML which was difficult to differentiate from HCC.

CASE REPORT

A 56-year-old Japanese man was admitted to our hospital for further examination of a liver tumor in the caudate lobe. He had no history of liver disease or hepatitis and did not drink heavily. Hepatitis B surface antigen and anti-hepatitis C antibody were negative. Serum levels of transaminase, α-fetoprotein and des-γ-carboxy prothrombin were within the normal range. On ultrasonography, the tumor was hypoechoic (Figure 1A). Enhanced computed tomography (CT) showed a hepatic mass with early-phase hyperattenuation and late-phase hypoattenuation, measuring 4.2 cm × 4.0 cm in the caudate lobe (Figure 1B and C). Magnetic resonance imaging (MRI) revealed hypointensity on T1-weighted images, hyperintensity on T2-weighted images and hyperintensity on diffusion weighted images (Figure 1D-F). The tumor did not absorb iron on superparamagnetic iron oxide-enhanced (SPIO) MRI

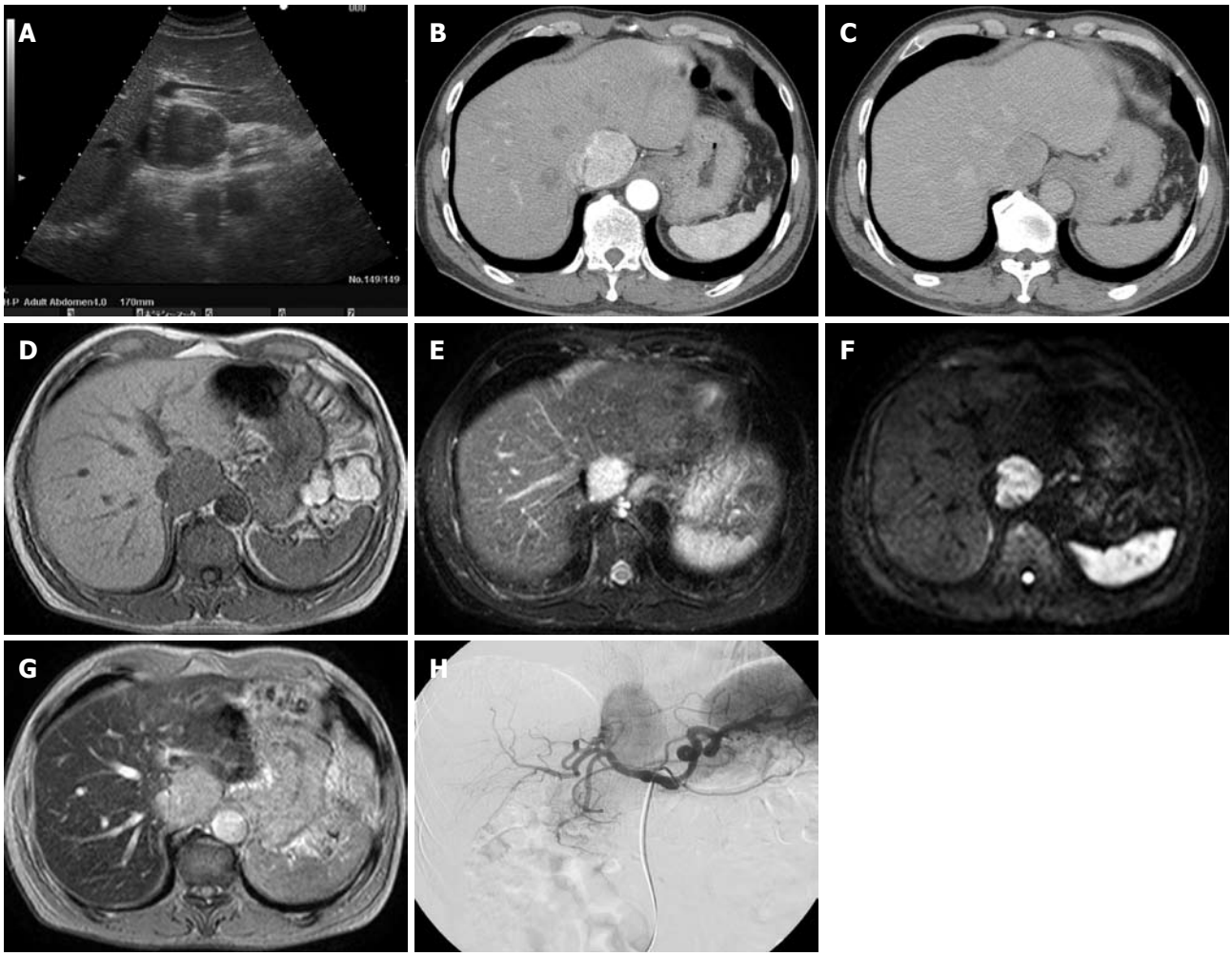

Figure 1 Images. A: The tumor was hypoechoic on ultrasonography, measuring 4.2 cm × 4.0 cm; B-C: Enhanced computed tomography showed a tumor with early-phase hyperattenuation and late-phase hypoattenuation; D-F: Magnetic resonance imaging showed a tumor with hypointensity on T1-weighted, hyperintensity on T2-weighted images and hyperintensity on diffusion weighted images; G: The tumor did not absorb iron on superparamagnetic iron oxide-enhanced MRI; H: On angiography, the tumor was shown as a circumscribed hypervascular mass.

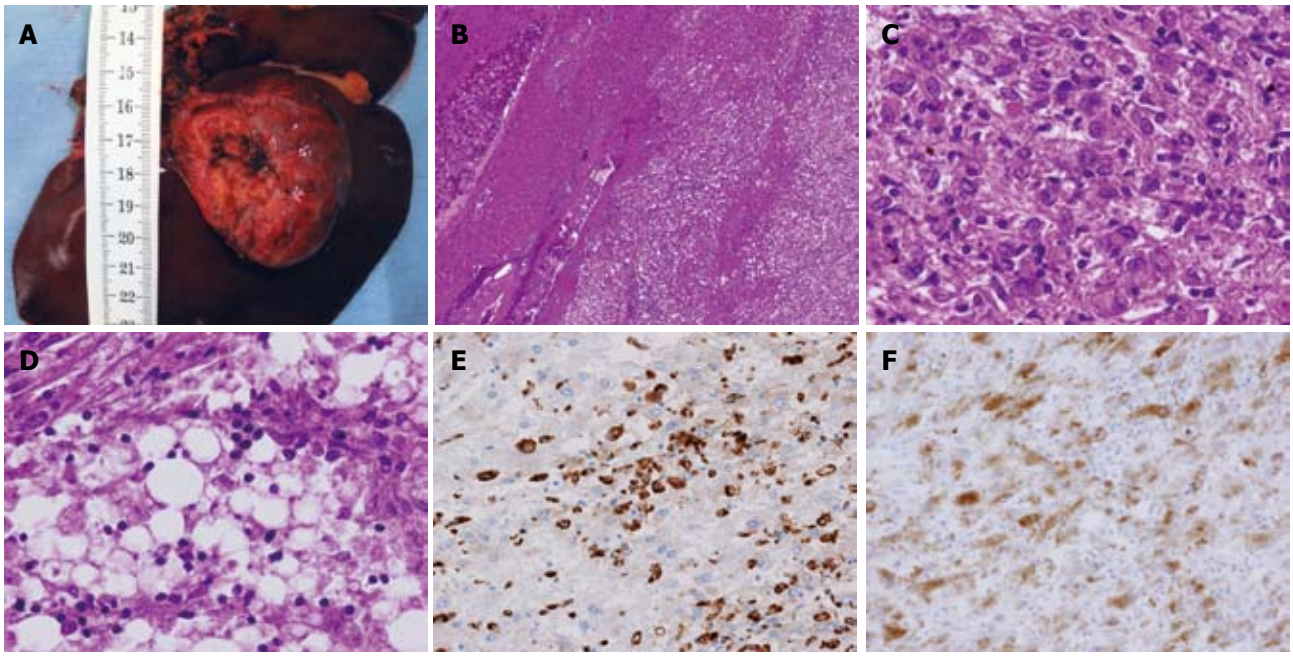

Figure 2 HAML. A: The tumor occupied a large area of the caudate lobe; B-D: The histological features of the tumor showed that it was mainly composed of smooth muscle cells (B: HE stain, × 4; C: HE stain, × 40) and a small number of adipose cells (D: HE stain, × 40); E, F: Immunohistochemically, the tumor was positive for CD68 (E: CD68, × 20), and HMB-45 (F: HMB-45, × 100).

(Figure 1G). On angiography, the tumor was shown as a circumscribed hypervascular mass (Figure 1H). HCC was highly suspected on radiological imaging. Resection of the left lobe with the tumor in the caudate lobe was performed. The tumor measured 6.0 cm in diameter, and the surface of the tumor was gray and white. Histologically, the tumor was mainly composed of smooth muscle cells and contained small amounts of adipose cells and blood vessels (Figure 2A-D). On immunohistochemical staining, the tumor was negative for desmin and S-100, but positive for actin and CD68, and the smooth muscle cells were positive for a melanocytic cell-specific monoclonal antibody (HMB-45) (Figure 2E and F). This tumor was diagnosed as HAML.

DISCUSSION

Recently, the concept of perivascular epithelioid cell tumor (PEComa) which was proposed by Bonetti et al in 1992 has gained wide acceptance[6]. PEComa is defined as a mesenchymal tumor composed of histologically and immunohistochemically distinctive perivascular epithelioid cells. Immunohistochemically, the tumors are consistently immunoreactive for HMB-45, a monoclonal antibody for melanoma. AML is considered a part of PEComa[7]. Smooth muscle cells of HAML stain positively for HMB-45. This finding is useful for the diagnosis of HAML, because liver tumors other than angiomyolipoma are negative for HMB-45[8].

In radiological diagnosis, HAML typically shows high echo on ultrasonography, early-phase hyperattenuation on enhanced computed tomography, hyperintensity on T2-weighted magnetic resonance imaging and a circumscribed hypervascular mass on angiography[9]. The soft tissue component (smooth muscle cells and vessels) is considered to be enhanced by the intravenous administration of contrast material[10]. However, the imaging features of HAML vary because of variations in the proportion of adipose cells, smooth muscle cells and vessels. In particular, the number of adipose cells varies between 10% and 90%[11]. HAML consisting of a small number of adipose cells shows low echo on ultrasonography and early-phase hyperattenuation on enhanced CT. These findings are similar to the imaging features of HCC[12]. On the other hand, there are no reports regarding the radiological features of SPIO MRI on HAML. SPIO contrast material, which is taken up by the reticuloendothelial system and depresses the signal of normal liver at T2-weighted imaging, is useful for the detection of hepatic tumors[13]. In our case, HAML was positive for CD68 stain, which is a Kupffer cell-related marker; however the signal at T2-weighted imaging on SPIO MRI was depressed similar to HCC. Thus, SPIO-MRI was not useful for differentiating HAML from HCC in our case.

HAML shows various histological patterns. According to the line of differentiation and the predominance of tissue components, the tumors are subcategorized into mixed, lipomatous ($\geqslant$ 70% fat), myomatous ($\leqslant$ 10% fat), and angiomatous types. The mixed type is the most common, but tumors with a small number of adipose cells such as the myomatous type, which are rare, show widely variable patterns in morphology[14]. In this study, we classified the HAML in our case as the myomatous type.

In conclusion, HAML is a benign tumor and requires no surgical treatment. However, the diagnosis is difficult because it has various histological patterns. In the myomatous type, the radiological findings including SPIO-MRO are similar to those of HCC. Hepatic tumors without obvious risk factors for HCC should be distinguished from HAML.

REFERENCES

1 **Ishak KG**. Mesenchymal tumors of the liver. In: Okuda K, Peters RL, eds. Hepatocellular carcinoma. New York: John Wiley & Sons, 1976: 247-307
2 **Nonomura A**, Mizukami Y, Kadoya M. Angiomyolipoma of the liver: a collective review. *J Gastroenterol* 1994; **29**: 95-105
3 **Guidi G**, Catalano O, Rotondo A. Spontaneous rupture of a hepatic angiomyolipoma: CT findings and literature review. *Eur Radiol* 1997; **7**: 335-337
4 **Yang CY**, Ho MC, Jeng YM, Hu RH, Wu YM, Lee PH. Management of hepatic angiomyolipoma. *J Gastrointest Surg* 2007; **11**: 452-457
5 **Takayama Y**, Moriura S, Nagata J, Hirano A, Ishiguro S, Tabata T, Matsumoto T, Sato T. Hepatic angiomyolipoma: radiologic and histopathologic correlation. *Abdom Imaging* 2002; **27**: 180-183
6 **Bonetti F**, Pea M, Martignoni G, Zamboni G. PEC and sugar. *Am J Surg Pathol* 1992; **16**: 307-308
7 **Pan CC**, Chung MY, Ng KF, Liu CY, Wang JS, Chai CY, Huang SH, Chen PC, Ho DM. Constant allelic alteration on chromosome 16p (TSC2 gene) in perivascular epithelioid cell tumour (PEComa): genetic evidence for the relationship of PEComa with angiomyolipoma. *J Pathol* 2008; **214**: 387-393
8 **Tsui WM**, Yuen AK, Ma KF, Tse CC. Hepatic angiomyolipomas with a deceptive trabecular pattern and HMB-45 reactivity. *Histopathology* 1992; **21**: 569-573
9 **Ren N**, Qin LX, Tang ZY, Wu ZQ, Fan J. Diagnosis and treatment of hepatic angiomyolipoma in 26 cases. *World J Gastroenterol* 2003; **9**: 1856-1858
10 **Horton KM**, Bluemke DA, Hruban RH, Soyer P, Fishman EK. CT and MR imaging of benign hepatic and biliary tumors. *Radiographics* 1999; **19**: 431-451
11 **Basaran C**, Karcaaltincaba M, Akata D, Karabulut N, Akinci D, Ozmen M, Akhan O. Fat-containing lesions of the liver: cross-sectional imaging findings with emphasis on MRI. *AJR Am J Roentgenol* 2005; **184**: 1103-1110
12 **Wang SN**, Tsai KB, Lee KT. Hepatic angiomyolipoma with trace amounts of fat: a case report and literature review. *J Clin Pathol* 2006; **59**: 1196-1199
13 **Ferrucci JT**, Stark DD. Iron oxide-enhanced MR imaging of the liver and spleen: review of the first 5 years. *AJR Am J Roentgenol* 1990; **155**: 943-950
14 **Tsui WM**, Colombari R, Portmann BC, Bonetti F, Thung SN, Ferrell LD, Nakanuma Y, Snover DC, Bioulac-Sage P, Dhillon AP. Hepatic angiomyolipoma: a clinicopathologic study of 30 cases and delineation of unusual morphologic variants. *Am J Surg Pathol* 1999; **23**: 34-48

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2933

World J Gastroenterol 2009 June 21; 15(23): 2933-2939
World Journal of Gastroenterology ISSN 1007-9327

Medline-based bibliometric analysis of gastroenterology journals between 2001 and 2007

Li-Fang Chou

Li-Fang Chou, Department of Public Finance, National Chengchi University, Taipei 11623, Taiwan, China
Author contributions: Chou LF conceived the study, performed the analysis and drafted the manuscript.
Correspondence to: Li-Fang Chou, Department of Public Finance, National Chengchi University, No. 64, Section 2, Chih-Nan Road, Taipei 11623, Taiwan, China. lifang@nccu.edu.tw
Telephone: +886-2-29387310 Fax: +886-2-29390074
Received: November 16, 2008 Revised: May 16, 2009
Accepted: May 23, 2009
Published online: June 21, 2009

Abstract

AIM: To analyze the MEDLINE-indexed publications in gastroenterology specialty journals from 2001 to 2007. Special attention was paid to specific types of articles, the number of publications for individual authors and the author count in each journal.

METHODS: The bibliographic entries of papers belonging to journals listed under the subject heading of "gastroenterology" were downloaded from MEDLINE on the PubMed web site. The analysis was limited to journal articles published between January 1, 2001 and December 31, 2007. The analytical dimensions of an article included journal, publication year, publication type, and author name (the last name and initials).

RESULTS: According to MEDLINE, 81561 articles were published in 91 gastroenterology journals from 2001 to 2007. The number of articles increased from 9447 in 2001 to 13340 in 2007. Only 12 journals had more than 2000 articles indexed in MEDLINE. The "*World Journal of Gastroenterology*" had the largest number of publications (5684 articles), followed by "*Hepato-Gastroenterology*" (3036) and "*Gastrointestinal Endoscopy*" (3005). Of all the articles published, reviews accounted for 17.2% and case reports for 15.4%. Only 3739 randomized controlled trials (4.6% of all articles) were published and their annual number increased from 442 in 2001 to 572 in 2007. Among 141741 author names appearing in the articles of gastroenterology journals, 92429 had published only in one journal, 22585 in two journals, 9996 in three journals, and 16731 in more than three journals. The "*World Journal of Gastroenterology*" had the greatest number of authors (17838), followed by "*Gastroenterology*" (12770), "*Digestive Diseases and Sciences*" (11395), "*American Journal of Gastroenterology*" (10889), and "*Hepatology*" (10588).

CONCLUSION: Global gastroenterology publications displayed a continuous growth in the new millennium. The change was most striking in certain journals. Regular bibliometric analyses on the trends and specific topics would help researchers publish more efficiently and allow editors to adjust the policy more accurately.

Key words: Bibliographic databases; Bibliometrics; Biomedical research; Gastroenterology; MEDLINE

Peer reviewers: Liang-Ping Hu, Professor, Consulting Center of Biomedical Statistics, Academy of Military Medical Sciences, Beijing 100850, China; Sheng-Li Ren, PhD, Department of Publication, National Natural Science Foundation of China, Beijing 100085, China

Chou LF. Medline-based bibliometric analysis of gastroenterology journals between 2001 and 2007. *World J Gastroenterol* 2009; 15(23): 2933-2939 Available from: URL: http://www.wjgnet.com/1007-9327/15/2933.asp DOI: http://dx.doi.org/10.3748/wjg.15.2933

INTRODUCTION

Gastroenterology is a highly competitive and productive field of medical research. Researchers in this field usually have a stronger need for bibliographic information than those in other fields. In the past few years, several bibliometric researches have been devoted to the global research trend[1], the research output in specific regions[2,3] and countries[4-6], and the share of author origins in specific journals[7-9] in gastroenterology. However, there still seemed to be a lack of a comparative overview of all gastroenterology specialty journals in the world.

The aim of the current study was to analyze the publications in gastroenterology journals in the new millennium, based on MEDLINE which is freely offered over the internet by the National Library of Medicine in the United States of America. Special attention was paid to specific types of articles, e.g. randomized controlled trials. Because researchers might not publish solely in

one journal during a period of several years, a new analytical method was also proposed to calculate the share of authors publishing in only one journal among all authors in each journal. This new indicator may serve as another dimension to author origins in a journal.

MATERIALS AND METHODS

Journal selection and data sources

The specialty journals of gastroenterology were limited to those listed under the subject heading of "gastroenterology" in MEDLINE. The master file of journals in MEDLINE was first downloaded (ftp://ftp.nlm.nih.gov/online/journals/lsi2008.xml, accessed on September 8, 2008) and a total of 141 gastroenterology journals were identified. Due to cessation of publication and name changes, not all journals were still available.

The bibliographic entries of papers belonging to selected journals were downloaded from MEDLINE on the PubMed web site (http://www.ncbi.nlm.nih.gov/sites/entrez/, accessed on September 8, 2008). The downloading consisted of journals with a Perl script. The retrieval was limited to papers published since 2001. The format type of the retrieval was MEDLINE.

Study design

One bibliographic record with the MEDLINE format contains pairs of tags and data, e.g. PMID-14647050 and AU-Lee SD, where PMID (PubMed unique identifier) and AU (author) are tags. Some types of tags, e.g. AU, PT (publication type) and MH (medical subject headings term), might appear more than once in one record. Some types of tags are not obligatory and might not be present in every record.

The downloaded datasets with MEDLINE format were merged and transformed into one single file with the structure of entity-attribute-value (EAV)[10] for further efficient processing, where entity stood for the PMID of a paper, attribute for the tag, and value for the data. For example, the pair "AU-Lee SD" in the paper of "PMID-14647050" would be converted into "14647050 [tab] AU [tab] Lee SD".

From the EAV file, the numbers of papers in each journal during the years of coverage were first calculated. The processing was limited to papers categorized as "journal article" in the publication type field. In addition, only papers published between January 1, 2001 and December 31, 2007 were included in this analysis.

The articles were also counted according to their publication type. An article might not contain only one publication type. For articles with the publication type of randomized controlled trial, their distribution in journals over the years was computed.

In computing the productivity of individual researchers in all gastroenterology journals, the methods of "total author counting"[11] was adopted. Each author of an article was recognized as having written one article, and then the number of articles authored or coauthored by each researcher during the 7-year period was counted.

Because an author's full name has been indexed in MEDLINE since 2002 and one fifth of the original publications did not contain the full author name[12], authors in the current study were identified according to the conventional author indexing of MEDLINE, i.e. last name, up to two initials of first and middle names, and/or a suffix abbreviation. Different authors with the same last name and initials would not be specifically differentiated in aggregate statistics. However, the name ambiguity would be considered in listing the most prolific authors.

For each journal, the total number of authors who had published in the journal during the 7-year period was calculated as the denominator and then the number of authors who had never published in other gastroenterology journals during the 7-year period was computed as the numerator. The fractional number for each journal might suggest the breadth of author origins.

Statistical analysis

The programming scripts with Perl version 5.10.0 (http://www.perl.com/) were written for downloading and computing. As a popular computer language since the internet era, Perl belongs to the open source software and can be freely downloaded and distributed for use. The National Library of Medicine also provides examples of Perl scripts for use of Entrez programming utilities from PubMed.

Only descriptive statistics, frequency in count and percentage, were displayed.

RESULTS

According to MEDLINE, 81 561 articles were published in 91 gastroenterology journals from 2001 to 2007 (Table 1). The number of articles increased from 9447 in 2001 to 13 340 in 2007. The "*World Journal of Gastroenterology*" had the largest number of publications (5684 articles), followed by "*Hepato-Gastroenterology*" (3036) and "*Gastrointestinal Endoscopy*" (3005). Only 12 journals had more than 2000 articles indexed in MEDLINE. By comparing the situations in 2001 and in 2007, the "*World Journal of Gastroenterology*" had the highest growth rate of publications (5.0-fold increase), followed by "*BMC Gastroenterology*" (2.3-fold), "*Revista de Gastroenterologia de Mexico*" (2.2-fold), "*International Journal of Colorectal Disease*" (2.0-fold), and "*Inflammatory Bowel Diseases*" (2.0-fold). The highest absolute growth was also claimed by "*World Journal of Gastroenterology*" (867 more articles), followed by "*Journal of Gastroenterology and Hepatology*" (208), "*Zhonghua Ganzangbing Zazhi* (*Chinese Journal of Hepatology*)" (200), and "*Digestive Diseases and Sciences*" (180). In contrast, 21 journals had fewer publications in 2007 than in 2001.

As to the publication type of these articles, reviews accounted for 17.2% (14 005 articles) of all articles from 2001 to 2007 and case reports 15.4% (12 539) (Table 2). There were only 3739 randomized controlled trials (4.6% of all articles) among 4627 clinical trials (5.7%). The annual number of randomized controlled trials increased from 442 in 2001 to 572 in 2007. Randomized controlled trials were most frequently published in "*Alimentary Pharmacology & Therapeutics*" (536 articles), followed by

Table 1 Publication trend of articles in gastroenterology journals, 2001-2007

Journal	2001	2002	2003	2004	2005	2006	2007	Total
Abdom Imaging	126	117	147	116	119	146	126	897
Acta Gastroenterol Belg	45	52	38	45	66	51	47	344
Acta Gastroenterol Latinoam	34	17	30	16	27	52	49	225
Aliment Pharmacol Ther	246	305	372	416	363	390	338	2430
Am J Gastroenterol	543	466	425	338	363	383	340	2858
Am J Physiol Gastrointest Liver Physiol	331	289	267	272	298	289	354	2100
Ann Hepatol		30	29	29	48	56	47	239
Arq Gastroenterol	44	42	45	49	46	61	63	350
Best Pract Res Clin Gastroenterol	64	67	66	90	66	68	67	488
BMC Gastroenterol	14	23	34	32	39	43	46	231
Can J Gastroenterol	98	79	98	95	66	85	97	618
Chin J Dig Dis				35	43	39		117
Clin Colorectal Cancer	27	31	34	43	60	54	49	298
Clin Gastroenterol Hepatol			62	153	219	231	275	940
Clin Liver Dis	52	56	53	49	44	48	50	352
Colorectal Dis	68	105	93	92	104	139	149	750
Curr Gastroenterol Rep	81	70	79	77	76	71	80	534
Curr Issues Intest Microbiol	5	5	8	7	7	10	5	47
Curr Opin Gastroenterol	91	85	66	78	86	81	78	565
Dig Dis	47	40	43	53	36	33	53	305
Dig Dis Sci	390	415	345	324	406	376	570	2826
Dig Liver Dis	129	172	174	166	153	176	200	1170
Dig Surg	95	96	78	70	60	65	78	542
Digestion	88	69	62	71	69	77	70	506
Dis Colon Rectum	263	245	255	270	301	246	257	1837
Dis Esophagus	63	68	75	66	80	91	92	535
Dysphagia	37	42	36	35	42	40	43	275
Eat Weight Disord	35	42	55	49	56	52	47	336
Eksp Klin Gastroenterol		145	120	94	96	81	119	655
Eur J Gastroenterol Hepatol	263	222	212	217	217	216	192	1539
Gastric Cancer	33	45	57	35	42	45	40	297
Gastroenterol Clin Biol	196	195	185	213	172	190	177	1328
Gastroenterol Clin North Am	54	74	54	59	46	49	51	387
Gastroenterol Hepatol	89	90	87	109	95	142	84	696
Gastroenterol Nurs	48	53	47	55	65	68	51	387
Gastroenterology	361	437	402	468	428	439	456	2991
Gastrointest Endosc	436	491	447	429	374	412	416	3005
Gastrointest Endosc Clin N Am	47	55	52	59	51	61	52	377
Gut	322	406	348	346	342	320	299	2383
Hepatobiliary Pancreat Dis Int		128	121	128	120	115	114	726
Hepatogastroenterology	414	422	580	443	426	209	542	3036
Hepatology	344	389	335	353	319	346	388	2474
Hernia	47	44	50	84	86	104	98	513
Indian J Gastroenterol	104	87	105	85	88	104	93	666
Inflamm Bowel Dis	66	70	65	156	160	147	200	864
Int J Colorectal Dis	63	64	86	89	74	123	191	690
Int J Gastrointest Cancer	43	46	43		51	23		206
Int J Pancreatol	25							25
J Clin Gastroenterol	185	180	156	177	177	177	157	1209
J Dig Dis							36	36
J Gastroenterol	125	206	192	170	156	147	172	1168
J Gastroenterol Hepatol	239	279	213	265	327	365	447	2135
J Gastrointest Surg	100	127	140	161	175	195	258	1156
J Gastrointestin Liver Dis						61	69	130
J Health Popul Nutr	32	45	43	47	44	57	56	324
J Hepatobiliary Pancreat Surg	88	107	76	84	91	97	98	641
J Hepatol	226	231	304	268	282	276	227	1814
J Pediatr Gastroenterol Nutr	256	268	207	234	273	244	219	1701
J Viral Hepat	62	64	71	80	90	117	126	610
JOP	55	18	24	68	69	79	87	400
Korean J Gastroenterol			81	103	136	144	133	597
Korean J Hepatol				41	51	57	59	208
Liver	58	87						145
Liver Int			75	96	153	159	172	655
Liver Transpl	184	186	234	256	225	297	281	1663
Minerva Gastroenterol Dietol	27	42	32	35	29	41	36	242
Nat Clin Pract Gastroenterol Hepatol				27	126	121	138	412
Neurogastroenterol Motil	55	64	64	114	104	105	128	634
Nippon Shokakibyo Gakkai Zasshi	127	155	141	112	130	120	144	929

Pancreas	128	134	154	147	143	116	131	953
Pancreatology	74	58	55	38	72	54	56	407
Rev Esp Enferm Dig	57	61	73	99	89	91	110	580
Rev Gastroenterol Disord	13	28	47	51	35	27	32	233
Rev Gastroenterol Mex	31	83	66	82	105	88	99	554
Rev Gastroenterol Peru	34	32	34	35	39	39	46	259
Rom J Gastroenterol		48	47	50	63			208
Ross Gastroenterol Zh	29							29
Scand J Gastroenterol	218	235	209	222	219	210	235	1548
Scand J Gastroenterol Suppl	16	18	30	15		25		104
Semin Gastrointest Dis	26	23	23					72
Semin Liver Dis	43	41	44	60	47	39	38	312
Surg Endosc	376	454	505	393	292	362	463	2845
Surg Laparosc Endosc Percutan Tech	86	89	90	80	92	107	144	688
Taehan Kan Hakhoe Chi		67	42					109
Tech Coloproctol	41	43	39	130	54	67	59	433
Trop Gastroenterol	65	66	59	55	56	43	51	395
Turk J Gastroenterol		48	59	58	53	65	49	332
World J Gastroenterol	172	236	632	812	1478	1315	1039	5684
Z Gastroenterol	134	156	127	115	90	90	93	805
Zhonghua Ganzangbing Zazhi	114	193	325	325	341	308	314	1920
Zhonghua Weichang Waike Zazhi					109	108	105	322
Total	9447	10663	11178	11663	12610	12660	13340	81561

"*American Journal of Gastroenterology*" (278), "*Gastrointestinal Endoscopy*" (186), "*Surgical Endoscopy*" (183), "*World Journal of Gastroenterology*" (176), and "*Gastroenterology*" (171). Among these journals, the "*World Journal of Gastroenterology*" had the greatest increase in randomized controlled trials: from 5 in 2001 to 34 in 2007 (detailed data not shown in tables).

If only the last name and initials of the authors were considered, 141 741 author names appeared in the articles of gastroenterology journals from 2001 to 2007. The "*World Journal of Gastroenterology*" had the greatest number of authors (17 838), followed by "*Gastroenterology*" (12 770), "*Digestive Diseases and Sciences*" (11 395), "*American Journal of Gastroenterology*" (10 889), and "*Hepatology*" (10 588) (Table 2). Among all authors, 82 174 had published only one article, 22 192 two articles, 10 672 three articles, and 26 703 more than three articles. On the other hand, 92 429 authors had published only in one journal, 22 585 in two journals, 9996 in three journals, and 16 731 in more than three journals. The share of authors publishing only in one journal among all authors of the journal was generally higher in journals with apparently narrower research fields or locality, e.g. "*Eksperimental'naia i Klinicheskaia Gastroenterologiia* (*Experimental & Clinical Gastroenterology*)" (95.5%), "*Revista de Gastroenterologia del Peru*" (84.0%), "*Eating and Weight Disorders*" (81.0%), "*Revista de Gastroenterologia de Mexico*" (79.4%), and "*Journal of Health, Population, and Nutrition*" (78.0%) (Table 2).

The top 10 prolific researchers in these gastroenterology journals are listed in Table 3. They were from seven institutions in six countries: three in the USA, four in Europe, and three in Asia. Only two of the top-ranked researchers were surgeons (Masatoshi Makuuchi and Markus W Buchler).

DISCUSSION

The current study demonstrated the most recent trend in publications from gastroenterology specialty journals worldwide. Gastroenterology publications have continued to prosper in the new millennium, not merely due to the expanded coverage of MEDLINE or the growth of a single journal. The number of randomized controlled trials has also increased, but their growth rate has slightly lagged behind that of other articles. Numerous researchers participated in gastroenterology publications; a substantial number of the authors were active in research and had multiple publications. Gastroenterology journals thus showed diverse authorships in which many authors of a journal also published in other specialty journals.

The current study chose MEDLINE as the data source because of its open access and international visibility. To compare the "quality" of scientific publications, people have adopted the controversial citation statistics and "impact factor" in recent years. Because the quantity of citations has increased tremendously and the databank of citations is not freely open to the public, the monopolized data from the black box cannot be extensively verified. Normally, most researchers just need a quick orientation in the field of interest, e.g. the features of journals, the most prolific authors or facilities, or the hottest subjects. Such requests can be easily satisfied by free MEDLINE after processing, without resorting to commercial databases which most individual researchers around the world can hardly afford.

Despite collective growth since 2001, the increases and decreases in individual gastroenterology journals could be observed. Among all journals, the "*World Journal of Gastroenterology*" was most striking. Not only had it published the greatest number of articles since 2003, but it had also attracted the most authors. Along with quantitative growth, the "*World Journal of Gastroenterology*" also had more randomized controlled trials. According to an earlier bibliometric analysis on the "*World Journal of Gastroenterology*", the author origins of the Journal had become more diverse by geographic distribution since 2003. From the analysis in the current study, the majority

Table 2 Articles of gastroenterology journals stratified by selected publication type and author type, 2001-2007

Journal	No. of all articles	Review	Case report	Clinical trial	Multicenter study	Randomized controlled trial	No. of authors	No. of exclusive authors[1]	Share of exclusive authors[1] (%)
Abdom Imaging	897	184	310	7	1	7	3094	1360	44.0
Acta Gastroenterol Belg	344	137	92	8	4	3	1113	435	39.1
Acta Gastroenterol Latinoam	225	36	52	5	4	2	815	542	66.5
Aliment Pharmacol Ther	2430	674	10	556	273	536	8177	2108	25.8
Am J Gastroenterol	2858	302	169	279	150	278	10889	2753	25.3
Am J Physiol Gastrointest Liver Physiol	2100	126		55		27	7045	2689	38.2
Ann Hepatol	239	89	58	10	2	7	705	332	47.1
Arq Gastroenterol	350	34	21	6	5	10	1067	689	64.6
Best Pract Res Clin Gastroenterol	488	459	6				1145	341	29.8
BMC Gastroenterol	231	6	47	12	6	13	934	164	17.6
Can J Gastroenterol	618	187	137	17	18	18	1579	556	35.2
Chin J Dig Dis	117	23	2	2		5	400	71	17.8
Clin Colorectal Cancer	298	130	25	25	17	14	910	552	60.7
Clin Gastroenterol Hepatol	940	153	198	64	76	89	4079	918	22.5
Clin Liver Dis	352	349	1				522	78	14.9
Colorectal Dis	750	91	11	32	14	33	2202	895	40.6
Curr Gastroenterol Rep	534	418	1	6	1	5	791	139	17.6
Curr Issues Intest Microbiol	47	24					133	81	60.9
Curr Opin Gastroenterol	565	245					735	144	19.6
Dig Dis	305	202	5	7	2	8	852	128	15.0
Dig Dis Sci	2826	183	649	148	58	135	11395	3418	30.0
Dig Liver Dis	1170	239	146	78	45	56	4634	1270	27.4
Dig Surg	542	96	148	9	3	13	1997	514	25.7
Digestion	506	106	45	49	28	41	2332	483	20.7
Dis Colon Rectum	1837	130	256	219	57	143	6506	2349	36.1
Dis Esophagus	535	71	145	21	7	15	2226	709	31.9
Dysphagia	275	16	33	13	1	6	850	579	68.1
Eat Weight Disord	336	35	21	17	5	14	1070	867	81.0
Eksp Klin Gastroenterol	655	138	23	76	1	18	984	940	95.5
Eur J Gastroenterol Hepatol	1539	315	378	83	93	87	6606	1966	29.8
Gastric Cancer	297	40	54	30	9	7	1301	249	19.1
Gastroenterol Clin Biol	1328	603	326	35	36	16	3188	1623	50.9
Gastroenterol Clin North Am	387	373	3				614	107	17.4
Gastroenterol Hepatol	696	229	184	12	3	3	2259	1235	54.7
Gastroenterol Nurs	387	114	29	12	1	9	430	293	68.1
Gastroenterology	2991	352	205	169	106	171	12770	4003	31.3
Gastrointest Endosc	3005	220	1275	230	100	186	8547	2426	28.4
Gastrointest Endosc Clin N Am	377	338	2				581	87	15.0
Gut	2383	343	227	132	139	144	9785	2612	26.7
Hepatobiliary Pancreat Dis Int	726	64	67	40	9	20	2128	464	21.8
Hepatogastroenterology	3036	145	654	155	31	100	9648	2952	30.6
Hepatology	2474	211	15	148	105	131	10588	3502	33.1
Hernia	513	60	151	20	18	32	1627	822	50.5
Indian J Gastroenterol	666	66	355	16	2	17	1791	893	49.9
Inflamm Bowel Dis	864	158	37	43	34	31	3266	854	26.1
Int J Colorectal Dis	690	63	74	57	21	40	3249	930	28.6
Int J Gastrointest Cancer	206	36	52	14	2		968	279	28.8
Int J Pancreatol	25	3	9				137	9	6.6
J Clin Gastroenterol	1209	320	241	59	31	56	4326	1192	27.6
J Dig Dis	36	6	3	2	1	1	160	29	18.1
J Gastroenterol	1168	219	299	45	24	29	4429	734	16.6
J Gastroenterol Hepatol	2135	274	351	93	37	91	7661	1672	21.8
J Gastrointest Surg	1156	114	132	23	16	24	4329	1476	34.1
J Gastrointestin Liver Dis	130	22	41	4	3	3	484	216	44.6
J Health Popul Nutr	324	16	1	8	2	16	1185	924	78.0
J Hepatobiliary Pancreat Surg	641	142	207	8	4	6	2345	427	18.2
J Hepatol	1814	259	114	120	56	81	8036	2200	27.4
J Pediatr Gastroenterol Nutr	1701	257	389	93	36	119	5894	3239	55.0
J Viral Hepat	610	74	16	81	48	56	3255	887	27.3
JOP	400	124	168	7		2	1396	473	33.9
Korean J Gastroenterol	597	65	128	7	4	8	1249	372	29.8
Korean J Hepatol	208	25	28	2		3	585	89	15.2
Liver	145	14	20	14	1	5	759	164	21.6
Liver Int	655	68	38	39	16	19	3613	751	20.8
Liver Transpl	1663	223	261	72	43	49	5517	2025	36.7
Minerva Gastroenterol Dietol	242	87	15	5	1	2	788	284	36.0
Nat Clin Pract Gastroenterol Hepatol	412	128	35	8	10	8	693	91	13.1

Neurogastroenterol Motil	634	111	5	52	5	39	1874	478	25.5
Nippon Shokakibyo Gakkai Zasshi	929	326	594	1	1	1	3464	856	24.7
Pancreas	953	60	109	35	16	9	3966	1144	28.8
Pancreatology	407	108	55	16	5	2	1627	399	24.5
Rev Esp Enferm Dig	580	80	145	28	5	13	2185	1234	56.5
Rev Gastroenterol Disord	233	154	4				124	10	8.1
Rev Gastroenterol Mex	554	204	112	15		8	1224	972	79.4
Rev Gastroenterol Peru	259	50	74	8		6	824	692	84.0
Rom J Gastroenterol	208	51	66	24	5	4	590	318	53.9
Ross Gastroenterol Zh	29	5	2	2			90	44	48.9
Scand J Gastroenterol	1548	80	128	151	65	120	6465	1817	28.1
Scand J Gastroenterol Suppl	104	92	2	1	1		270	28	10.4
Semin Gastrointest Dis	72	64	43				121	11	9.1
Semin Liver Dis	312	264	29	2			598	80	13.4
Surg Endosc	2845	229	430	203	73	183	9117	4404	48.3
Surg Laparosc Endosc Percutan Tech	688	45	318	23	4	24	2523	871	34.5
Taehan Kan Hakhoe Chi	109	4	30	4		2	417	61	14.6
Tech Coloproctol	433	53	82	26	14	23	1283	500	39.0
Trop Gastroenterol	395	84	166	11	1	7	929	440	47.4
Turk J Gastroenterol	332	11	87	7	4	6	1096	460	42.0
World J Gastroenterol	5684	584	637	338	46	176	17838	6839	38.3
Z Gastroenterol	805	198	193	39	14	14	1917	737	38.4
Zhonghua Ganzangbing Zazhi	1920	95	33	34	11	34	3693	1117	30.2
Zhonghua Weichang Waike Zazhi	322						1096	262	23.9
Total	81561	14005	12539	4627	2090	3739	141741	92429	65.2

[1]Exclusive authors denote the authors who had published in only one journal during the study period.

Table 3 The most prolific authors in gastroenterology journals, 2001-2007

Author	Affiliation	No. of articles
Nicholas J Talley	Mayo Clinic, Rochester, USA	205
Michael P Manns	Hannover Medical School, Germany	170
Peter Malfertheiner	University of Magdeburg, Germany	166
Todd H Baron	Mayo Clinic, Rochester, USA	165
Masatoshi Makuuchi	University of Tokyo, Japan	163
Markus W Buchler	University of Heidelberg, Germany	160
Shou-Dong Lee	Taipei Veterans General Hospital, Taiwan, China	155
Giovanni Gasbarrini	Catholic University of Rome, Italy	147
William J Sandborn	Mayo Clinic, Rochester, USA	141
Full-Young Chang	Taipei Veterans General Hospital, Taiwan, China	140

of authors in the "*World Journal of Gastroenterology*" did not only publish in this Journal during the study period. Both of these facts indicated that the "*World Journal of Gastroenterology*" had established its position in international gastroenterology publications.

The global research community has frequent fervent disputes about the quality of journal articles[13]. There appears to be a growing discontent about the misuse of the impact factor in hiring, promoting and grant-awarding. Researchers usually consider several factors when choosing a journal to publish their research results. A research article should be judged by its content, and not merely by the journal in which it was published. That is, the impact of an individual article should not be evaluated by the journal impact factor. The function of the academic journal as an effective platform for scientific communication can never be overestimated. Time will show which editorial team acts best.

The major limitation in the current study was the separation of distinct authors. The ambiguity of author names is an unresolved problem of bibliometric research[14]. Although MEDLINE started to index full author names in 2002, the problem still remains. Not every journal print author names in full. Besides, not every author spells their forename consistently in different articles. For example, Buchler MW appeared as Markus W Buchler, Markus-W Buchler, and Markus Wolfgang Buchler. Most cases of ambiguity existed in authors of East Asian origin. Because of homonymous features in Chinese/Japanese/Korean characters, a lot of distinct authors might share the same full name spelled in Latin letters. Therefore, the share of exclusive authors (never publishing in other journals) in each journal in the current study represented only the lowest estimate.

In conclusion, global gastroenterology publications demonstrated a continuous growth in the new millennium. The change was most striking in certain journals. Regular bibliometric analyses on the trends and specific topics would help researchers publish more efficiently and allow editors to adjust the policy more accurately.

ACKNOWLEDGMENTS

The author thanks Professor Tzeng-Ji Chen for his professional advice.

REFERENCES

1 **Lewison G**, Grant J, Jansen P. International gastroenterology research: subject areas, impact, and funding. *Gut* 2001; **49**: 295-302

2 **Sorrentino D**, De Biase F, Trevisi A, Bartoli E. Scientific publications in gastroenterology and hepatology in Western Europe, USA and Japan in the years 1992-1996: a global survey. *Digestion* 2000; **61**: 77-83

3 **Gao R**, Liao Z, Li ZS. Scientific publications in gastroenterology and hepatology journals from Chinese authors in various parts of North Asia: 10-year survey of

literature. *J Gastroenterol Hepatol* 2008; **23**: 374-378
4 **Lewison G**. Gastroenterology research in the United Kingdom: funding sources and impact. *Gut* 1998; **43**: 288-293
5 **Maeda K**, Rahman M, Fukui T. Japan's contribution to clinical research in gastroenterology and hepatology. *J Gastroenterol* 2003; **38**: 816-819
6 **Chen TJ**, Chen YC, Hwang SJ, Chou LF. The rise of China in gastroenterology? A bibliometric analysis of ISI and Medline databases. *Scientometrics* 2006; **69**: 539-549
7 **Hart PA**, Ibdah JA, Marshall JB. Internationalisation of high-impact gastroenterology journals, 1970-2005. *Gut* 2007; **56**: 895-896
8 **Yang H**, Zhao YY. Variations of author origins in World Journal of Gastroenterology during 2001-2007. *World J Gastroenterol* 2008; **14**: 3108-3111
9 **Cappell MS**, Davis M. A significant decline in the American domination of research in gastroenterology with increasing globalization from 1980 to 2005: an analysis of American authorship among 8,251 articles. *Am J Gastroenterol* 2008; **103**: 1065-1074
10 **Nadkarni PM**, Brandt C. Data extraction and ad hoc query of an entity-attribute-value database. *J Am Med Inform Assoc* 1998; **5**: 511-527
11 **Egghe L**, Rousseau R, van Hooydonk G. Methods for accrediting publications to authors or countries: Consequences for evaluation studies. *J Am Soc Inf Sci* 2000; **51**: 145-157
12 **Nahin AM**. Full author searching comes to PubMed®. *NLM Tech Bull* 2005; e4. Available from: URL: http://www.nlm.nih.gov/pubs/techbull/mj05/mj05_full_author.html
13 **Simons K**. The misused impact factor. *Science* 2008; **322**: 165
14 **Scoville CL**, Johnson ED, McConnell AL. When A. Rose is not A. Rose: the vagaries of author searching. *Med Ref Serv Q* 2003; **22**: 1-11

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2009 June 21; 15(23): 2940
World Journal of Gastroenterology ISSN 1007-9327

ACKNOWLEDGMENTS

Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Jamie S Barkin, MD, Professor of Medicine, Chief
Sinai Medical Center Division of Gastroenterology, Mt. Sinai Medical Center, University of Miami, School of Medicine, 4300 Alton Road, Miami Beach, FL 33140, United States

Tatjana Crnogorac-Jurcevic, MD, PhD
Cancer Research UK, Molecular Oncology Unit, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom

Benedicte Y De Winter, President, MD, PhD, Professor, Associate Professor, Chief
Laboratory of Gastroenterology, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, 2610 Antwerp, Belgium

Fabio Farinati, MD
Surgical and Gastroenterological Sciences, University of Padua, Via Giustiniani 2, Padua 35128, Italy

Valentin Fuhrmann, MD
Department of Internal Medicine 4, Intensive Care Unit, Medical University Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

Alfred Gangl, Professor
Department of Medicine 4, Medical University of Vienna, Allgemeines Krankenhaus, Waehringer Guertel 18-20, Vienna A-1090, Austria

Jacob George, Professor
C24 - Storr Liver Unit, Westmead Millennium Institute, University of Sydney, Westmead NSW 2145, Australia

James H Grendell, Professor of Medicine, Chief
Division of Gastroenterology, Hepatology, & Nutrition, Winthrop University Hospital, 222 Station Plaza N. #429, Mineola, New York 11501, United States

Salvatore Gruttadauria, MD, Assistant Professor
Abdominal Transplant Surgery, ISMETT, Via E. Tricomi, 190127 Palermo, Italy

Jin-Hong Kim, Professor
Department of Gastroenterology, Ajou University Hospital, San 5, Wonchon-dong, Yeongtong-gu, Suwon 442-721, South Korea

Elias A Kouroumalis, Professor
Department of Gastroenterology, University of Crete, Medical School, Department of Gastroenterology, University Hospital, PO Box 1352, Heraklion, Crete 71110, Greece

Guangbin Luo, PhD, Assistant Professor
Department of Genetics, School of Medicine, Case Western Reserve University, Biomedical Research Building 720, 2109 Adelbert Road, Cleveland, Ohio 44106-4955, United States

Yasuhiro Matsumura, MD, PhD
Investigative Treatment Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

Chris JJ Mulder, Professor
Department of Gastroenterology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands

Hiroaki Nagano, MD, PhD, Associate Professor
Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Department of Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka E-2, Suita 565-0871 Osaka, Japan

Amado S Peña, Professor
Department of Pathology, Immunogenetics, VU University Medical Centre, De Boelelaan 1117, PO Box 7057, Amsterdam 1007 MB, The Netherlands

CS Pitchumoni, Professor
Robert Wood Johnson School of Medicine, Robert Wood Johnson School of Medicine, New Brunswiuc NJ D8903, United States

Dr. Massimo Raimondo
Division of Gastroenterology and Hepatology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States

Chifumi Sato, Professor
Department of Analytical Health Science, Tokyo Medical and Dental University, Graduate School of Health Sciences, 1-5-45 Yushima, Bunkyoku, Tokyo 113-8519, Japan

Ala Sharara, MD, FACP Associate Professor of Medicine, Head
Division of Gastroenterology, Director, Endoscopy Unit, American University of Beirut Medical Center, Associate Consulting Professor, Duke University Medical Center, PO Box 11-0236, Riad El Solh 110 72020, Beirut, Lebanon

Yukihiro Shimizu, MD, PhD
Kyoto Katsura Hospital, 17 Yamada-Hirao, Nishikyo, Kyoto 615-8256, Japan

Ulrike S Stein, PhD, Assistant Professor
Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, 13125 Berlin, Germany

Sun-Lung Tsai, MD, PhD, Professor, Director
Hepatogastroenterology Section, Department of Internal Medicine and Liver Research Unit, Department of Medical Research, Chi Mei Medical Center, 901 Chung Hwa Road, Young-Kang City, Tainan County 710, Taiwan, China

Andrew Ukleja, MD, Assistant Professor, Clinical Assistant Professor of Medicine, Director of Nutrition Support Team, Director of Esophageal Motility Laboratory
Cleveland Clinic Florida, Department of Gastroenterology, 2950 Cleveland Clinic Blvd., Weston, FL 33331, United States

Dr. Karel van Erpecum
Department of Gastroenterology and Hepatology, University Hospital Utrecht, PO Box 855003508 GA, Utrecht, The Netherlands

Harry HX Xia, PhD, MD
Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ 07936-1080, United States

World J Gastroenterol 2009 June 21; 15(23): 2941
World Journal of Gastroenterology ISSN 1007-9327

Meetings

Events Calendar 2009

January 12-15, 2009
Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
http://www.easl.ch/

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
http://www.ddw.org

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
http://www.asge.org/index.aspx?id=5040

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists(IASGO)
http://iasgo2009.org/en/index.shtml

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
http://www.apdwcongress.org/2009/index.shtml

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
The Liver Meeting

November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
London, UK
Gastro 2009 UEGW/World Congress of Gastroenterology
www.gastro2009.org

Global Collaboration for Gastroenterology

For the first time in the history of gastroenterology, an international conference will take place which joins together the forces of four pre-eminent organisations: Gastro 2009, UEGW/WCOG London. The United European Gastroenterology Federation (UEGF) and the World Gastroenterology Organisation (WGO), together with the World Organisation of Digestive Endoscopy (OMED) and the British Society of Gastroenterology (BSG), are jointly organising a landmark meeting in London from November 21-25, 2009. This collaboration will ensure the perfect balance of basic science and clinical practice, will cover all disciplines in gastroenterology (endoscopy, digestive oncology, nutrition, digestive surgery, hepatology, gastroenterology) and ensure a truly global context; all presented in the exciting setting of the city of London. Attendance is expected to reach record heights as participants are provided with a compact "all-in-one" programme merging the best of several GI meetings. Faculty and participants from all corners of the earth will merge to provide a truly global environment conducive to the exchange of ideas and the forming of friendships and collaborations.

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2009 June 21; 15(23): 2942-2944
World Journal of Gastroenterology ISSN 1007-9327

Instructions to authors

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol* ISSN 1007-9327 CN 14-1219/R) is a weekly open-access (OA) peer-reviewed journal supported by an editorial board consisting of 1179 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of the value of the readers can be comprehended in two ways. First, the journal publishes articles that can be directly read or downloaded free of charge at any time, which attracts more readers. Second, the readers can apply the knowledge in clinical practice without delay after reading and understanding the information in their fields. In addition, the readers are encouraged to propose new ideas based on those of the authors, or to provide viewpoints that are different from those of the authors. Such discussions or debates among different schools of thought will definitely boost advancements and developments in the fields. Maximization of the value of the authors refers to the fact that these journals provide a platform that promotes the speed of propagation and communication to a maximum extent. This is also what the authors really need. Maximization of the value of the society refers to the maximal extent of the social influences and impacts produced by the high quality original articles published in the journal. This is also the main purpose of many journals around the world.

The major task of *WJG* is to rapidly report the most recent results in basic and clinical research on gastroenterology, hepatology, endoscopy and gastrointestinal surgery fields, specifically including autoimmune, cholestatic and biliary disease, esophageal, gastric and duodenal disorders, cirrhosis and its complications, celiac disease, dyspepsia, gastroesophageal reflux disease, esophageal and stomach cancers, carcinoma of the colon and rectum, gastrointestinal bleeding, gastrointestinal infection, intestinal inflammation, intestinal microflora and immunity, irritable bowel syndrome; liver biology/pathobiology, liver failure, growth and cancer; liver failure/cirrhosis/portal hypertension, liver fibrosis; *Helicobacter pylori*, hepatitis B and C virus, hepatology elsewhere; pancreatic disorders, pancreas and biliary tract disease, pancreatic cancer; transplantation, genetics, epidemiology, microbiology and inflammatory disorders, molecular and cell biology, nutrition; geriatric gastroenterology, pediatric gastroenterology, steatohepatitis and metabolic liver disease; diagnosis and screening, endoscopy, imaging and advanced technology.

The columns in the issues of *WJG* will be adjusted in 2009, which will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide Guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To systemically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (8) Original Articles: To originally report the innovative and valuable findings in gastroenterology and hepatology; (9) Brief Articles: To briefly report the novel and innovative findings in gastroenterology and hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; (13) Guidelines: To introduce Consensuses and Guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in gastroenterology and hepatology.

Indexed and abstracted in

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, Index Medicus, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, PubMed Central, Digital Object Identifier, CAB Abstracts and Global Health. ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

Published by

The WJG Press and Baishideng

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of The WJG Press and Baishideng, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials. gov sponsored by the United States National Library of Medicine, and we encourage all potential contributors to register with it. However, in the event that other registers become available, you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the corresponding author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://wjg.wjgnet.com/wjg. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/wjg/help/instructions.jsp) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to submission@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of

supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-59080039, Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words). Available from:http://www.wjgnet.com/wjg/help/8.doc

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/wjg/help/instructions.jsp.

Illustrations

Figures should be numbered as 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc*. It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension,

insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/wjg/help/14.doc.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H pylori*, *E coli*, *etc*.

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office
World Journal of Gastroenterology
Editorial Department: Room 903, Building D,
Ocean International Center,
No.62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-59080039
Fax: +86-10-85381893

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/wjg/help/10.doc.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/wjg/help/9.doc.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.