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WJG sets an example of internationalization for other Chinese academic journals

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CREATIVE PEOPLE DESERVE APPLAUSE

During the application period for the “Special Fund for Key Academic Journals” of the National Natural Science Foundation of China (NSFC) in early 2002, I came to know Professor Lian-Sheng Ma and *World Journal of Gastroenterology* (WJG), which he created. Since then, I have had close contact with him. As a successful entrepreneur and pharmacologist, he has devoted himself to the creation of academic journals by applying his past successful experience in the pharmaceutical industry to the academic publishing field. This shift carried a great personal risk but it has worked out well for the Chinese academic publishing industry. Professor Ma is always willing to listen to and incorporate other people’s opinions and suggestions, and can thereby, provide creative ideas. He likes making friends and therefore keeps close contact with a number of government employees, scientists and publishers. During regular telephone or face-to-face conversations, Professor Ma often pushes me to give an in-depth opinion on the feasibility of his ideas. When WJG was initially supported by the NSFC “Special Fund for Key Academic Journals” in 2002, it was published bimonthly. Within a few years, WJG has become a weekly journal. Such rapid development has far exceeded my expectations and is a marvel in the Chinese academic publishing industry. For this reason, I have full confidence in the rapid development of Chinese academic journals in the next few years.

According to the traditional view, the basic conditions for Professor Ma to create international English-language medical journals are not very good. However, he is very good at exploiting the strengths of the journals to compensate for their inadequacies. For this reason, he can adeptly manage the overall operation of the journal and perform better than many other scientists and pub-

Abstract

Supported by the “Special Fund for Key Academic Journals” of the National Natural Science Foundation of China, *World Journal of Gastroenterology* (WJG) has become a high-impact international clinical medical journal due to the great efforts of Professor Lian-Sheng Ma, Editor-in-Chief, and his team over several years. Now, WJG has successfully achieved a high degree of internationalization and sets a good example for other Chinese academic journals.

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Key words: China; Academic journals; Internationalization

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lishers. More importantly, he is not merely satisfied with issuing orders for the management of the journal operation. Instead, he always becomes personally involved in specific affairs, and therefore, has total control over the journal. He always participates in every aspect of journal production, whether the editing process or the design of the online version, constantly poses questions and suggests modifications, and immediately takes action. He not only has goals and ideas but also can find solutions to any problems that are encountered. These aspects constitute the basis for his success.

I was particularly touched by what Professor Ma did during the summer of 2003 while the severe acute respiratory syndrome epidemic was raging in Beijing. While many institutions came to a semi-standstill, Professor Ma launched the online version of *WJG* during that period. The overall outlook of online *WJG* is impressive and comparable to that of some world-renowned journals. At the beginning, thousands of visitors came to the site per day. Now, this figure has grown to tens of thousands, and *WJG* has become a leader among Chinese academic journals. Professor Ma's work style and creative spirit have rarely been seen in traditional Chinese publishers, because he has closely linked his personal interests (economic interests and social image) to the development of *WJG*.

The success of *WJG* has brought a fresh outlook to the Chinese academic publishing industry. Professor Ma should feel proud of his contributions, which have been made at a time when China is still not open to publishing privately funded academic journals. I hope that more successful and courageous Chinese entrepreneurs will become involved in the Chinese academic publishing industry; an area that remains to be developed, and one that can provide tremendous opportunities.

A JOURNAL REFLECTS A TYPE OF CULTURE

A journal not only reflects the experience and background of its editors/publishers, but also displays their unique personality and personal and cultural tastes. The readers can discover something beyond the contents of a journal. I have a habit of browsing world-renowned journals to capture changes in the overall trend and style of mainstream journals. I also look through *WJG*, from which I can glimpse inside Professor Ma's inner world and mind, and appreciate his efforts towards realizing his ambition to make *WJG* an excellent international journal.

With China's accession to the World Trade Organization and continuous rapid economic growth, its international status has been greatly improved. In recent years, a large number of foreign publishers and distributors have set up representative offices in China or have established cooperation with Chinese printers or publishers. I have had contact with more than 10 foreign publishers and therefore understand their primary intention and purpose. On many occasions, I have warned the publishers of

journals supported by the NSFC Special Fund for Key Academic Journals to develop such cooperation with prudence. *WJG* Press was not the first domestic publisher to establish cooperation with well-known foreign publishers, but it was the first that dared to discontinue such cooperation. In this way, Professor Ma seeks internationalization of *WJG* not to further his own reputation, but rather that of the journal.

The development of *WJG* over these years has not been smooth, and has had many difficulties. We have always been concerned about the overall situation of NSFC-funded journals, especially those like *WJG* that have incurred much controversy. Between 2004 and 2005, the self-citation rate of *WJG* was 20%, which exceeded the ISI standard, and this resulted in *WJG* being removed from Science Citation Index (SCI). To combat these arguments, Professor Zuo-Yan Zhu, the NSFC Vice President, pointed out at a meeting in January 2003 that the goal of Chinese academic journals should be to achieve internationalization, to be included in SCI, and to be a leader among international journals in the same field. There are many ways to achieve these goals. However, the most fundamental way is to improve the quality of the journals. Although many approaches are available to improve the impact factor of a journal in a short period, there is no doubt that a high-impact journal can be created only after its scientific quality is raised.

As mentioned above, in 2004, *WJG* drew wide criticism because of a high self-citation rate. I sensed that Professor Ma underwent tremendous pressure during that period. However, it is pleasing to know that he did not make any excuses or avoid problems, but spent a lot of time to establish the reasons for this and make improvements. Fortunately, these lessons have led to the subsequent rapid development of *WJG*.

Ms. Emilie Marcus, Editor-in-Chief of *Cell*, pointed out in a forum in 2006 that the presence of high-impact articles depends on strong inputs of financial and human resources. These words are easier said than done. How is financial investment achieved? Where should the funds be invested? How are talented scientists recruited and utilized? Coincidentally, in recent years, *WJG* has been practicing the above sentiments. This shows that great minds think alike.

Looking back on modern technological developments worldwide, it can be seen that the progress of all renowned journals has not been smooth, but full of ups and downs. To create a world-renowned journal, the sponsor has to spend a lot of time and effort, sometimes even for several generations. Such journals are more respected and their publishing model is worthy of being passed on to others. The history of *Science*, which was created by Thomas Edison, spans 125 years, while that of British *Nature* and *The Lancet* (created by the Wakely family) spans 137 and 183 years, respectively. Although these journals are not run by governments, they enjoy a great reputation worldwide. I believe that Professor Ma could have the confidence and courage to make *WJG* a world-renowned journal.

PRACTICAL EXPERIENCE OBTAINED DURING *WJG* DEVELOPMENT HAS GREAT SIGNIFICANCE

At the last meeting of the *WJG* working group, I mentioned that the reform of state-owned enterprises could provide a reference for the successful operation of academic journals. Here, I would like to discuss the implications of the rise of the Chinese Communist Party (CCP) for the reform of Chinese academic journals.

In recent years, many entrepreneurs in China have been keen to read about the developmental history of the CCP, to gain a better insight into business management. As the world's largest political party (with nearly 70 million members), the CCP has been in existence for 85 years and has ruled China for more than half a century. What are the fundamental reasons for its sustainability? This question has attracted the interest of many domestic and foreign politicians and entrepreneurs. The rapid growth of China's national economy since 1979 has led state-owned enterprises into a new era of development. All enterprises are in competition with their rivals for the throne. The rise of new outstanding enterprises is often coupled with the fall of some previously successful companies. The rapid turnover of businesses has become a feature of the development of the Chinese economy. Survival is the main theme of business management in our times.

Ten years ago, entrepreneurs might have been successful merely by having the courage to go into business. In more recent times, however, the survival of enterprises has been more dependent on management rather than luck or back-door dealing. For this reason, entrepreneurs have been anxious to seek better management approaches: some have learned from the West, some from Confucianism, and others from the developmental history of the CCP.

Western management style has its own advantages, but it may not be suitable for Chinese enterprises. Although Chinese entrepreneurs hope to derive the essence from Western management style, they tend to spend a lot of time dealing with superficial matters. The Western business community has a history of 200 years and is very mature. Many excellent Western academic journals also date back for more than a century. A mature business environment provides Western academic journals with a

sound management system. In contrast, the development of Chinese academic journals has occurred mainly after 1976. As a result of market, cultural and policy differences, there is no appropriate management system available for Chinese academic journals. This developmental stage provides a better opportunity for publishers to display their talents.

In terms of business management, an important criterion for entrepreneurs to judge the feasibility of a management theory is whether it is practical (in relation to the situation in China). The core issue with which entrepreneurs are concerned is how to realize Western management style in China. The ultimate goal of businesses is to achieve a profit, therefore, they advocate the same laws as the CCP: survival of the fittest and natural selection. How to guarantee basic living conditions is their common primary goal in response to external challenges. The development of enterprise management has about 100 years of history. As a result, various types of business management theories have been proposed. Despite diversity, their fundamental purpose is to answer the same question: how is efficiency improved? The CCP can organize many citizens in a fairly orderly manner and has an extremely high level of organization that is universally acknowledged. High efficiency and strong executive power are its biggest features.

The operation of academic journals has its own laws. If an academic journal is not run in accordance with market or economic rules, but managed in a very superficial manner, the journal will not be successful. *WJG* is aiming to implement Western management style in China by obeying market and economic rules. The success of *WJG* will make a significant contribution to Chinese scientific and cultural development. I hope Professor Ma can keep a diary to record his management practice, which could be a great asset to later generations.

Our generation, which is involved in the reform of China's academic journals, should not overlook the development of *WJG*, because its preliminary success has been achieved by overcoming many obstacles and fighting against the inherent snobbishness of the traditional publishing industry that has existed in China for decades. I am very pleased that some of my ideas for creating outstanding international academic journals have been adopted and gradually realized by Professor Ma. I am also happy that *WJG* sets an example of internationalization for other Chinese academic journals.

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Spices, herbal xenobiotics and the stomach: Friends or foes?

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Abstract

Spices and herbal remedies have been used since ancient times to treat a variety of disorders. It has been experimentally demonstrated that spices, herbs, and their extracts possess antimicrobial, anti-inflammatory, antirheumatic, lipid-lowering, hepatoprotective, nephroprotective, antimutagenic and anticancer activities, besides their gastroprotective and anti-ulcer activities. Despite a number of reports on the toxicity of herbs and spices, they are generally accepted as safer alternatives to conventional therapy against gastric ulcers. To this end, it is also believed, that excessive consumption of spices may favor the pathogenesis of gastric and duodenal ulcer and some studies have substantiated this common perception. Based on various *in vivo* experiments and clinical studies, on the effects of spices and herbs on gastric ulcers, it has indeed been shown that certain spices do possess remarkable anti-ulcer properties mediated by antisecretory, cytoprotective, antioxidant, and anti-*Helicobacter pylori* effects and mechanisms regulated by nitric oxide, prostaglandins, non-protein sulfhydryl molecules and epidermal growth factor expression. Accordingly, their consumption may attenuate and help prevent peptic ulcer disease. In the present review, the beneficial effects of spices and herbal nutritive components on the gastric mucosa are discussed against the paradigm of their deleterious potential.

INTRODUCTION

Herbs and spices are considered as an important additive to food in several parts of the world. They enhance aroma, piquancy, and impart flavor to food. For long, they have been regarded as the essential component of eastern cuisine and have also been adopted into western diets. Since ancient times, spices and herbs have also been used in traditional treatment of a number of diseases. Nowadays, several experimental studies and, to a lesser extent, clinical trials have also emphasized the role of herbs in the treatment of a variety of disorders^[1-4]. Several herbs and herbal extracts have been shown to possess antibacterial properties^[5-7]. For instance, onion, garlic, ginger, pepper and mustard have demonstrated antimicrobial activity against several types of bacteria^[8]. Tayel and El-Tras have recently reported a potent antibacterial activity of cinnamon and clove against several bacterial strains^[9]. Some spices possess antifungal activity^[10,11]. Beside their antifungal activity, herbs have also shown vermifugal, nematocidal and molluscicidal potential^[12-15]. In addition, gingerol, the active ingredient of ginger, and eugenol have shown anti-inflammatory

and antirheumatic activity^[16]. More recent studies have also demonstrated anti-inflammatory and antirheumatic properties of herbs^[17-19]. Furthermore, gingerol and curcumin have also shown lipid-lowering potential in experimental animals^[20-22] as well as in clinical trials^[23,24].

In addition, many studies have especially endorsed the experimental evidence of folkloric utilization of spices to treat gastrointestinal disorders, without gastric mucosal toxicity. Also clinical, video-endoscopic studies conducted by Graham *et al*^[25] have shown an adaptive cytoprotection. Furthermore, several experimental studies have also demonstrated cytoprotective activity of herbs^[26-29].

On the other hand, some spices are considered to be toxic to the gastric mucosa and may potentiate or induce gastric injuries. Gastric mucosal toxicity induced by some spices in experimental animals is related to their oxidative constituents such as phenylpropenes, safoles, methyl eugenol, 1'-hydroxyestragole, myristicin and elemicin^[30,31]. Meyers *et al*^[32] have reported deleterious effect of red pepper and black pepper on the stomach. Both have induced a significant enhancement in parietal secretion, pepsin secretion, and potassium loss, as well as a dose-dependent gastric cell exfoliation and mucosal micro-bleeding, which are comparable to those induced by aspirin. Other studies have also reported epigastric pain and dyspepsia. The mechanism of epigastric pain and dyspepsia induced by red and black pepper is not well-defined. However, it is believed to be a consequence of inhibition of gastric surface hydrophobicity, enhancement of surface wettability and activation of intramucosal pain receptors^[33]. Some spices may stimulate acid secretion and have deleterious effects on the gastric mucosal lining. Intragastric perfusion of albino rats with aqueous extracts of red pepper, fennel, omum/ajwain, cardamom, black pepper, cumin and coriander have stimulated a cholinergic response, and/or *via* other mechanism(s) have induced acid secretion with a respectively declining order. In injured stomach, cumin and coriander increase gastric secretion, and red pepper has an inhibitory effect^[34].

Herbs in general are believed to be safe, and several studies have found them to be non-detrimental and beneficial to gastric mucosa and have cytoprotective properties^[25,28]. In addition to their anti-ulcerogenic activity, it has been reported that spices contain protective factors that possess anticancer potential^[35-37]. The ulcerogenic and/or anti-ulcerogenic activity of herbs is likely to be due to the oxidative and anti oxidative action of their different phytoconstituents. The prominent mechanism of protective action is mediated by their antioxidant activity and the ability to scavenge reactive oxygen species (ROS). A variety of herbs such as clove, cinnamon, oregano, black pepper, turmeric, ginger and polygonum species are known to contain phytoconstituents with anti-oxidative potential^[38-46]. Antioxidative constituents of herbs are summarized in the Table 1.

The beneficial effects of herbs and plant extracts in the prevention of gastric injury have been indicated in several experimental studies. For example, various ex-

tracts of *Mammea americana* have significantly reduced the ulcer index and demonstrated a cytoprotective effect in experimentally induced ulcers^[47]. Further pharmacological and histopathological studies have demonstrated significant protection against ethanol-induced ulcers in rats by extracts of roots of *Asphodelus aestivus* and *Cichorium intybus*, herbs of *Equisetum palustre* and *Viscum album ssp. album* and fruits of *Laurus*^[48].

Clinical studies have also confirmed the gastric protection conferred by herbs. In a clinical study of 98 outpatients with chronic gastritis, who were randomly divided into a group treated with herbal pairs and a control group treated with *Banxia Xiexin Tang*, effectiveness was significantly higher in the treated group. The treatment has improved therapeutic effects, and minimized the adverse effects of gastritis^[49]. In another clinical study, 103 patients with duodenal ulcer received phytotherapy in the form of infusions and concoctions of medicinal plants. This resulted in ulcer scarring and a decrease in relapse^[50]. Furthermore, in a study of 170 patients with duodenal ulcer and gastroduodenitis treated with herbal combinations alone or with addition of antacids, pain and dyspeptic symptoms disappeared in > 85% of both treatment groups, with a similar rate of endoscopic healing^[51]. Hence, agents that possess antioxidative activity are expected to play a major role in the treatment of peptic ulcer disease.

EXPERIMENTAL INDUCTION OF ULCERS

Various noxious chemical agents are used to induce acute experimental gastric ulceration. Indomethacin and necrotizing agents including 80% ethanol, 0.2 mol/L NaOH, 25% NaCl are commonly used. Pyloric ligation is applied in antisecretory studies^[52-56]. Other universally accepted experimental ulcer models include stress induced by swimming^[55], aspirin^[56], ethanol/HCl, acetylsalicylic acid, cold-restraint^[57] and hypothermic restraint^[54]. In addition, gastric ulcers have also been induced by serosal application of acetic acid^[58].

FACTORS INVOLVED IN ULCER HEALING

Acid and other noxious agents such as bile acids, non-steroidal anti-inflammatory drugs (NSAIDs) and ethanol enhance the presence of mucosal barrier disruption, H⁺ back diffusion and ulcer susceptibility. Adequate mucosal flow and secretion of bicarbonate with formation of an alkaline buffer layer at the epithelial surface, is considered as a first line of mucosal defense. Prostaglandins (PGs) are involved in the ulcer healing process. Growth factors, some gut hormones (e.g. gastrin and cholecystokinin) and melatonin promote ulcer healing through generation of cyclooxygenase-2 (COX-2) and release of PGE2 in the ulcer margin^[59,60]. Similar action is achieved by application of antisecretory doses of exogenous PG^[61]. In addition to PGs, many other factors including growth factors, nitric oxide or calcitonin gene-related peptide, as well as some gut hormones such as gastrin and cholecystokinin, leptin,

Table 1 Antioxidant constituents of herbs

Antioxidant constituents	Herb	Ref.
Pimentol, biflorin	Clove, allspice	[38]
Phenolic constituents: phenolic acids, phenolic diterpenes, flavonoids, and volatile oils, phenolic volatile oils	Many herbs including clove, cinnamon and oregano	[39]
Piperine	Black pepper	[40]
Curcumin, methoxy phenols, dehydrogingerdione, bakuchiol	Turmeric, from Indian spices, ginger, <i>Psoralea corylifolia</i>	[41]
Phenolic compounds: flavonoids, phenolic acids and their derivatives, tannins, stilbenes, and anthraquinones	Polygonum species, spring onion, broccoli, orange, carrot and ginger	[42]
Phenolic diterpenes	<i>Rosmarinus officinalis</i> and <i>Salvia officinalis</i>	[43]
Phenolic, flavonoid, chlorogenic acid and neochlorogenic acid	Andean spice <i>Sanicula graveolens</i> (Apiaceae)	[44]
Phenylpropanoids (phenolic compounds)	Human diet, spices, aromas, essential oils, propolis and traditional medicine	[45]
Polyphenolic	<i>Piper umbellatum</i> , <i>Piper nigrum</i> , <i>Piper guineense</i>	[46]

ghrelin and gastrin-releasing peptide, are involved in gastroprotection. The protective action of gut hormones has been attributed to the release of PG or activation of sensory nerves^[62].

MECHANISMS OF PROTECTION RENDERED BY SPICES AND HERBS

The mechanism of herb-induced gastroprotection varies according to the nature and chemical constituents of the herbs. Three main functions including antisecretory, cytoprotective and antioxidant activities, isolated or in combination, are responsible for gastric mucosal protection.

Antisecretory activity

Several herbs and plants extracts have shown an anti-ulcer effect mainly due to their potent antisecretory action, which could be related to their flavonoid content. In experimental animals, *Cissus quadrangularis* extract and *Maytenus ilicifolia* have been shown to inhibit gastric secretions and ulcer index^[63,64]. Similarly, phytosphingosine hydrochloride has been shown to have antisecretory and anti-ulcer effects in animals with pyloric ligation^[65]. Methanolic extract of *Momordica charantia* prevents development of peptic ulcer and promotes healing of ulcers induced by acetic acid. Antisecretory studies in pylorus-ligated rats have demonstrated a significant reduction in acidity, pepsin content and ulcer index. On the other hand, the extract also amplifies the gastric mucosal content^[66]. A variety of herbs and plant extracts including standardized aqueous extract of *Cecropia glaziovii* Sneth (Cecropiaceae)^[67], alkaloid extract and 2-phenylquinoline obtained from the bark of *Galipea longiflora* (Rutaceae)^[68] and *Landolphia ovariensis* extracts^[69] have exhibited an anti-ulcer effect.

The inhibition of gastric acid and pepsin output in rats with intact or deactivated sensory nerves treated parenterally with capsaicin could contribute to the capsaicin-induced gastroprotection against acid-dependent mucosal lesions^[70].

Cytoprotective activity

In pylorus-ligated rats, methanolic extract of *M. charantia* L. fruit protects against peptic ulcer and promotes ulcer

healing *via* enhancing the gastric mucosal content and antisecretory activity in stress-, ethanol-, indomethacin- and cysteamine-induced ulcers^[66]. In both ethanol- and indomethacin-induced experimental ulcers, pretreatment with isopulegol, a monoterpene present in essential oils of several aromatic plants, has resulted in significant gastroprotective action, which is apparently mediated through its endogenous PGs and antioxidative properties^[71]. Moreover, Weikang (WK) decoction significantly protects against ethanol-induced gastric mucosal injury. This activity is mediated by enhancement of epidermal growth factor (EGF) content in gastric juice, nitric oxide (NO) in gastric tissue, PGE2 and superoxide dismutase (SOD) in plasma, inhibition of malondialdehyde (MDA) and endothelin in plasma, and an increase in mucosal thickness^[72]. In swim- and ethanol-stress-induced ulcers, extract of ginger rhizome (*Zingiber officinale*) normalizes antioxidant enzymes and protects against oxidative and gastric mucin damage^[55]. The gastroprotective effect of *Vanillosmopsis arborea* bark essential oil is likely mediated by α_2 -receptor activation^[73], whereas the anti-ulcerogenic effect of *Solanum torvum* Swartz (Solanaceae) aqueous and methanol extracts is probably due to cytoprotective mechanisms^[74]. Cytoprotective action of total carotenoid and astaxanthin esters depends on their mucin-related protective action as well as enhancing antioxidants enzymes level and H^+ , K^+ -ATPase inhibitory activity^[75]. Synthesis of cytoprotective PGs, increased resistance of gastric mucosa, and inhibition of leukotriene synthesis are possibly responsible for gastroprotection induced by boswellic acids (from *Boswellia*)^[57].

In various ulcer models in mice, the gastroprotective activity of *G. longiflora* (Rutaceae) is related to an increase in gastric mucus content and antisecretory activity. Additionally, NO is involved in mucosal protection, which could be attributed to their alkaloids, particularly 2-phenylquinoline^[68]. Pretreatment of albino rats with aqueous extract orally once daily for 2 wk significantly inhibited HCl/ethanol-induced ulcers and enhanced gastric mucus production. Additionally, it displayed antisecretory activity in pylorus-ligated rats. The results indicate that the leaf extracts of *Landolphia* possess anti-ulcer properties^[69].

A new flavonoid derivative, DA-6034, also prevents ulcers induced by ethanol, aspirin, indomethacin, stress, and acetic acid, and enhances endogenous PGE2 syn-

thesis and mucus content in the gel layer of the gastric mucosa. Promotion of the gastric defensive systems is the likely cause of its gastroprotective activities^[56].

Antioxidative activity

Oxidants are implicated in the pathophysiology of various diseases, including peptic ulcer disease, and antioxidants may therefore contribute to their prevention and treatment. NSAIDs account for some of the most commonly used drugs worldwide and may activate the oxidative process. For instance, treatment with indomethacin induces neutrophil activation with release of ROS and microvascular injuries, which is considered as the prime event in gastric mucosal damage^[76]. Therefore, investigators have continued to search for agents with antioxidative properties, which prevent or at least reduce ROS-induced mucosal damage. Such agents include isopulegol, which has shown significant gastroprotective effects in ethanol- and indomethacin-induced ulcer models. Gastroprotective action is probably mediated by its antioxidative properties, synthesis of endogenous PGs and K^+ (ATP) channel opening^[71]. Other herbs with antioxidative actions include carotenoid and astaxanthin esters, the herb collection Korniozil, and ginger rhizome. For instance, total carotenoid and astaxanthin esters have experimentally protected gastric mucin and increased levels of the antioxidant enzymes catalase, SOD, and glutathione peroxidase in gastric homogenate^[75]. In addition, Korniozil also protects against experimentally induced stress ulcers, with enhancement of gastric mucous coat regeneration, along with restoration of lipid peroxidation and antioxidative system function^[77]. Ginger rhizome extract has shown gastroprotective activity in animals with swim- and ethanol-stress-induced ulcers. This activity is based on gastric mucin generation, restoration of antioxidant enzymes and inhibition of *Helicobacter pylori* (*H. pylori*) growth^[55].

The gastroprotective effect of *Cissus sicyoides* extract administered orally in rodents treated with various ulcerogenic agents is also due to its antioxidative properties, increase of NO and SH groups, with enhancement of mucosal defense mechanisms and microcirculatory response. Therefore, the authors have supported its use for gastric ulcer treatment^[78].

Studies on piperine have demonstrated an antioxidative effect *in vitro*, lipid peroxide inhibition *in vivo*, and enhancement of the bioavailability of phytochemicals. The protective activity of piperine is linked to its antioxidative action and ability to inhibit ROS^[40]. Thymoquinone, the active constituent of *Nigella sativa*, has been shown to protect against gastric mucosal damage induced by ethanol. These effects can be ascribed to improvement in the antioxidant status, increased serum levels of serum glutathione, SOD, inhibition of radical oxygen species, and increased mucin content of the gastric mucosa^[29,79].

Other activities: PG-herb interaction

PG synthesis is important for gastric mucosal protection. PG has been used as an antisecretory drug to treat peptic ulcer disease. However, the use of a sufficient therapeutic

dose is associated with adverse effects, which limits its use, especially after the introduction of antisecretory drugs such as H₂-receptor antagonists and proton pump inhibitors. Several studies on herbs and plant extracts that activate PG synthesis have emphasized their role in gastroprotection and healing of gastric mucosal injuries. Silva *et al*^[71] have reported a significant dose-related protective effect of isopulegol, a monoterpene that is obtained from essential oils of several aromatic plants, against indomethacin- and ethanol-induced ulcers. This protective action is probably mediated by endogenous PGs, K^+ (ATP) channel opening, and antioxidant properties. Similarly, Alqasoumi *et al*^[54] have reported a PG and/or antisecretory, as well as antioxidant-mediated gastroprotective activity of Rocket extract. Boswellic acid, a triterpenoid used clinically to treat arthritis, has exhibited a mucosal protective action through PG synthesis stimulation and leukotriene synthesis inhibition^[57]. Endogenous PGs and PG EP1 receptors have an important role in the adaptive protection and functional responses in mice treated with sodium taurocholate^[80]. The active ingredient of chilli, capsaicin, which is believed to be ulcerogenic, on the contrary, prevents ulcer formation through its antisecretory action, as well as stimulation of mucus, alkali secretions and mucosal microcirculation. In addition, capsaicin stimulates afferent neurons in the stomach and transmits signals to the central nervous system, which trigger an anti-inflammatory response and gastroprotection^[81]. Treatment with small doses of topical capsaicin protects the gastric mucosa from damage by strong irritants^[69].

PGs have been found to reduce enhanced duodenal mucosal permeability induced by hydrochloric acid^[82]. In acute injury, small bowel mucosa function recovers with the restitution of epithelium, which is believed to be pivotal for epithelial repair, and occurs only in the presence of PG-mediated paracellular space closure^[83]. Hatazawa *et al*^[84] have studied the role of PGs/COX in the healing of indomethacin-induced small-intestinal ulcers in rats. They have reported ulcer healing by endogenous PGs, which is mediated by PG EP4 receptors, and involvement of COX-2 in the early stage and COX-1 in the late stage of healing. Bacterial lipopolysaccharide is involved in gastric mucosal protection in rats, *via* activation of COX and endogenous PG genes^[85]. In rats, *Teucrium polium* has demonstrated protection against indomethacin-induced gastric ulcer through PG synthesis, EGF receptor (EGFR) expression and modulation of mucin secretion, besides its antioxidative activity, and its lack of toxicity^[86].

EGF and effect of herbs

Ulcer preventive activity of herbs in experimentally induced gastric injury is thought to be mediated through their antisecretory and antioxidative properties, as well as PG generation. Various factors are involved in mucosal defense and repair, including gastrin, parietal cells and tumor necrosis factor (TNF)- α . Gastrin and parietal cells play an important role in the regulation of mucosal proliferation in response to gastric injury and inflammation^[87]. Similarly, TNF- α , which is released during gastric mucosal

injury, also contributes to epithelial cell repair in the gastric mucosa *via* its receptor and activation of the PG pathway^[88]. EGF and other growth factors are also pivotal for the process of mucosal healing. EGF and transforming growth factor (TGF)- α have a common receptor (EGFR). They promote ulcer healing through enhancement of cell proliferation, overexpression of growth factors, inhibition of gastric secretion and enhancement of blood flow at the ulcer margin^[89]. Treatment with EGF significantly induces extracellular signal-regulated kinase (ERK) activity, COX-2 and PGE2 generation, and cell proliferation. The EGF-induced proliferation of gastric epithelial cells is probably mediated by the ERK/COX-2 pathway^[90]. Gastric mucosa regeneration, with cell proliferation, migration, tissue injury repair and ulcer healing are controlled by activation of EGFR. Although TGF- α acts under normal circumstances and following acute injury, EGF acts mainly during the healing process of chronic ulcers. Both TGF- α and EGF, and other growth factors including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), promote restoration of connective tissue and angiogenesis in injured gastric mucosa. Further growth factors involved in regeneration include keratinocyte growth factor, hepatocyte growth factor and trefoil peptides^[91]. Regeneration of injured human gastric epithelial monolayers has been promoted by EGFR-dependent phosphoinositide 3-kinase activation^[92].

Besides its role in gastric inflammation and injury, EGF plays an important role in the regulation of cancer growth. Phosphorylation of EGFR and inhibition of heparin-binding EGF-like growth factor (HB-EGF) carboxyl-terminal fragment (CTF), and HB-EGF-CTF nuclear translocation are considered crucial in inhibitory regulation of cancer cell growth^[93]. Overexpression of EGF has often been found in gastric cancer. Growth factors alter the localization of tight-junction-associated proteins such as ZO-1 and occludin^[94]. Baek *et al*^[95] have also reported EGF overexpression and urokinase plasminogen activator receptor in human gastric cancers. In epithelial and mesenchymal cells, EGF binds to tyrosine kinase receptor and promotes malignant formation as well as tissue repair^[96].

Herb-induced growth factor enhancement may support treatment strategies for gastric ulcer and cancer. Capsaicin-sensitive nerves contribute to healing of acetic-acid-induced chronic gastric ulcer through stimulation of EGF expression in salivary glands, serum and gastric mucosa^[97]. Weitongning herb increases EGF and NO content in ulcer scars, which improves ulcer healing and reduces recurrence^[98]. Also, in ethanol-induced gastric mucosal injury, WK decoction has shown a significant gastroprotective effect that is mediated by increased levels of NO in gastric tissue, PGI2 and SOD in plasma, and EGF in gastric juice^[72]. Mexican tea herb and pilular adina herb have also demonstrated protection of gastric mucosa through stimulation of NO and EGF secretion and enhancement of EGFR expression^[99]. Wang *et al*^[100] have also reported increased expression of EGF and EGFR mRNA in experimental gastric ulcer in rats treated with Kuizhangping,

with possible promotion of ulcer healing and decreased ulcer recurrence. *Angelica* and *Chuanxiong* spices given to rats with myocardial infarction may affect VEGF expression and promote endothelial cell proliferation^[101]. This angiogenic effect may also be applied to the formation of new vessels in granulation tissue and restitution of injured gastric mucosa.

Through their effect on EGF, herbs may prove beneficial in the management of cancer. Constituents of ginger have inhibited EGF-induced cell transformation^[102]. Inhibition of EGFR tyrosine kinase proliferation and invasion of gastric cancer cells has also been achieved by curcumin^[103]. Molecular therapies that target growth factors EGF and VEGF and their receptors have shown promise against hepatocellular carcinoma in the absence of beneficial effects of chemotherapy^[104]. Recent data combining EGFR and VEGF inhibitors have suggested the superiority of targeting multiple pathways rather than a single pathway^[105]. The anticancer potential of curcumin has been demonstrated by inhibition of EGF-induced upregulation of aquaporin and ovarian cancer cell migration^[106].

Role of NO mediation by herbs

Tsai *et al*^[107] have evaluated the effect of some spices on NO overproduction generated by inducible NO synthase (iNOS), which is implicated in disease development. Rosemary, tarragon, oregano, basil, marjoram, allspice, and thyme have demonstrated poor to moderate activity. In contrast, cinnamon has excellent ability to scavenge NO. NO-scavenging activity of herbs is probably related to their high content of phenolic compounds, which scavenge NO or suppress iNOS. In another study, pretreatment with NOS inhibitor attenuated the gastroprotective effect induced by polyalthic acid^[108]. Moreover, plant-extract-induced gastroprotection is probably related to the enhancing effect on NOS inhibitor expression, gastric microcirculation and release of NO^[109]. Participation of NO, PGs and SH compounds may explain the anti-ulcerogenic action of diterpenoid from *Croton reflexifolius*^[108].

Non-protein-SH compounds and herbal involvement

Non-protein (NP)-SH compounds contribute to gastric mucosal defense. Depletion of NP-SH alters mucosal integrity. *Commiphora opobalsamum* (L.) Engl. (Balessan) protects against various models of experimental gastric ulcers in rats. It has been shown to protect in a dose-dependent manner against mucus and NP-SH depletion with a large margin of safety^[52]. Methanolic *C. sicyoides* extract given orally to rodents has been shown to inhibit experimental gastric injuries induced by necrotizing agents, *via* participation of NP-SH compounds and NO, and enhancement of the defense system^[78]. Maintaining adequate gastric mucus concentration and NP-SH levels is essential for gastric mucosa integrity and function. Other herbs and plant extracts also have the ability to preserve the defense system of the gastric mucosa. For instance, pretreatment with *Ginkgo biloba* extract has been shown to inhibit, in a dose-dependent manner, ethanol-induced NP-SH compound production, depletion of gastric wall mucus con-

centration, and lipid peroxidation, and preserve mucosal function^[110]. Similarly, anise aqueous suspension, and rocket and anise have significantly replenished ethanol-induced gastric wall mucus concentration and NP-SH depletion in experimental studies^[54,111].

***H. pylori* bactericidal activity of herbs**

The association between *H. pylori* and peptic ulcer disease is well-established and eradication is pivotal for ulcer healing and minimizing the relapse rate. Although the eradication rate of currently used regimens ranges between 80% and 90%, the problem of developing resistance is emerging. A number of investigators have evaluated the effect of herbs and plant extracts on *H. pylori*. Methylene chloride cinnamon extract has also shown an inhibitory effect on the free urease of *H. pylori*^[112]. Also curcumin and its methanolic extract have inhibited the growth of all strains of *H. pylori* *in vitro*^[113]. In addition, gingerol, a polyphenolic constituent of ginger root, has also demonstrated inhibitory activity on CagA+ strains of *H. pylori*^[114]. Moreover, eugenol and cinnamaldehyde prevented growth of *H. pylori* obtained from human gastric tissue, and inhibited the growth of all 30 tested *H. pylori* strains, with a lack of resistance^[115]. In a declining order, turmeric, cumin, ginger, chilli, borage, black caraway, oregano and liquorice have demonstrated partial bactericidal activity against *H. pylori*. *H. pylori* adhesion to the stomach has been inhibited by extracts of turmeric, borage and parsley^[116]. Zaidi *et al*^[117] have reported that > 50% of 50 commonly used Pakistani medicinal plants have inhibited the growth of eight *H. pylori* strains. *Curcuma amada* Roxb., *Mallotus philippines* (Lam) Muell., *Myristica fragrans* Houtt., and *Psoralea corylifolia* L. aqueous-ethanol extracts have demonstrated a potent anti-*H. pylori* activity and *Mal. philippines* (Lam) Muell. has exhibited potent bactericidal activity.

SAFETY AND TOXICITY OF SPICES

The majority of experimental studies have reported a lack or low levels of toxicity for most spices. However, there are many case reports, and *in vivo* and *in vitro* toxicological studies that have demonstrated the toxicity of certain herbs and their constituents^[118]. Some investigators have reported hepatotoxicity of curcumin and its derivatives^[119], as well as turmeric and its ethanolic extract in vulnerable mice^[120]. Longer treatment with high turmeric dose has been associated with a significant decline in body weight gain and alterations in liver weight^[121]. In contrast, chronic treatment with *Foeniculum vulgare* ethanolic extracts of fruit and *Ruta chalepensis* aerial parts have resulted in a significant weight gain in male mice^[122]. Changes in liver, spleen, lung or reproductive organs, along with a significant increase in sperm count and motility, and decreased hemoglobin have been reported in *Cinnamon zeylanicum*, *Piper longum* and *R. chalepensis*-treated animals^[122,123]. In an experimental model, piperine (10 and 20 mg/kg) decreased mating performance and fertility. Five days post-mating, oral treatment induced considerable anti-implantation activity. In addition, intrauterine injection of piperine has

caused loss of implants. However, piperine treatment has not produced any histopathological effect in the ovaries and uterus at the cellular level^[124]. Although initial reports on the safety of black pepper and its active ingredient piperine were controversial, the current literature has established its safety in animals. Moreover, piperine has shown antimutagenic and antitumor activities. Curcumin administered at higher doses has resulted in nuclear, genome and more extensive mitochondrial damage in human hepatoma G2 cells, mediated by the elevated concentration of ROS and lipid peroxidation. Curcumin also has caused genotoxicity in PC12 cells and has induced suicidal death of normal erythrocytes^[125-127]. At elevated concentrations, thymoquinone may be genotoxic and cytotoxic, induce programmed cell death in erythrocytes, and cause glutathione depletion and liver damage^[128,129].

CONCLUSION

It continues to be debatable whether spices and herbal xenobiotics are beneficial to gastric mucosal damage, and whether their toxicity outweighs any benefits. It is, however, clear that the consumption of certain common spices in food and the intake of herbal supplements may help the fight against peptic ulcer disease in humans. This review provides insights into the future use of herb- and spice-based drugs as alternative treatments for gastric ulcer. Dietary and nutritional practices in the oriental region and the consumption of spices in moderate quantities may be of benefit for the prevention of gastric ulcer. The review suggests the need for further investigations into the benefit of herbal xenobiotics and spices on the gastric mucosa, with in-depth *in vivo* studies, sustained clinical trials and cohort analyses. There is enough evidence to conclude that spices have a beneficial effect on gastric ulcers, despite various reports of their toxicity.

REFERENCES

1. Cai L, Wu CD. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J Nat Prod* 1996; **59**: 987-990
2. Nanji AA, Jokelainen K, Tipoe GL, Rahemtulla A, Thomas P, Dannenberg AJ. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G321-G327
3. Bengmark S. Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases. *JPEN J Parenter Enteral Nutr* 2006; **30**: 45-51
4. Nicoll R, Henein MY. Ginger (*Zingiber officinale* Roscoe): a hot remedy for cardiovascular disease? *Int J Cardiol* 2009; **131**: 408-409
5. Weseler A, Saller R, Reichling J. Comparative investigation of the antimicrobial activity of PADMA 28 and selected European herbal drugs. *Forsch Komplementarmed Klass Naturheilkd* 2002; **9**: 346-351
6. Liu CS, Cham TM, Yang CH, Chang HW, Chen CH, Chuang LY. Antibacterial properties of Chinese herbal medicines against nosocomial antibiotic resistant strains of *Pseudomonas aeruginosa* in Taiwan. *Am J Chin Med* 2007; **35**: 1047-1060

- 7 **Shan B**, Cai YZ, Brooks JD, Corke H. Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *J Agric Food Chem* 2007; **55**: 5484-5490
- 8 **Chen HC**, Chang MD, Chang TJ. [Antibacterial properties of some spice plants before and after heat treatment] *Zhonghua Minguo Weisheng Wujimian Yixue Zazhi* 1985; **18**: 190-195
- 9 **Tayel AA**, El-Tras WF. Possibility of fighting food borne bacteria by egyptian folk medicinal herbs and spices extracts. *J Egypt Public Health Assoc* 2009; **84**: 21-32
- 10 **Sekine T**, Sugano M, Majid A, Fujii Y. Antifungal effects of volatile compounds from black zira (*Bunium persicum*) and other spices and herbs. *J Chem Ecol* 2007; **33**: 2123-2132
- 11 **Seneviratne CJ**, Wong RW, Samaranayake LP. Potent antimicrobial activity of traditional Chinese medicine herbs against *Candida* species. *Mycoses* 2008; **51**: 30-34
- 12 **Hitokoto H**, Morozumi S, Wauke T, Sakai S, Kurata H. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Appl Environ Microbiol* 1980; **39**: 818-822
- 13 **Karapinar M**. Inhibitory effects of anethole and eugenol on the growth and toxin production of *Aspergillus parasiticus*. *Int J Food Microbiol* 1990; **10**: 193-199
- 14 **El Garhy MF**, Mahmoud LH. Anthelmintic efficacy of traditional herbs on *Ascaris lumbricoides*. *J Egypt Soc Parasitol* 2002; **32**: 893-900
- 15 **Kiuchi F**, Goto Y, Sugimoto N, Akao N, Kondo K, Tsuda Y. Nematocidal activity of turmeric: synergistic action of curcuminoids. *Chem Pharm Bull (Tokyo)* 1993; **41**: 1640-1643
- 16 **Sharma JN**, Srivastava KC, Gan EK. Suppressive effects of eugenol and ginger oil on arthritic rats. *Pharmacology* 1994; **49**: 314-318
- 17 **Setty AR**, Sigal LH. Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. *Semin Arthritis Rheum* 2005; **34**: 773-784
- 18 **Li EK**, Tam LS, Wong CK, Li WC, Lam CW, Wachtel-Galor S, Benzie IF, Bao YX, Leung PC, Tomlinson B. Safety and efficacy of *Ganoderma lucidum* (lingzhi) and San Miao San supplementation in patients with rheumatoid arthritis: a double-blind, randomized, placebo-controlled pilot trial. *Arthritis Rheum* 2007; **57**: 1143-1150
- 19 **Spiller F**, Alves MK, Vieira SM, Carvalho TA, Leite CE, Lunardelli A, Poloni JA, Cunha FQ, de Oliveira JR. Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan- and antigen-induced inflammation. *J Pharm Pharmacol* 2008; **60**: 473-478
- 20 **Al-Amin ZM**, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *Br J Nutr* 2006; **96**: 660-666
- 21 **Ejaz A**, Wu D, Kwan P, Meydani M. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. *J Nutr* 2009; **139**: 919-925
- 22 **Kang Q**, Chen A. Curcumin suppresses expression of low-density lipoprotein (LDL) receptor, leading to the inhibition of LDL-induced activation of hepatic stellate cells. *Br J Pharmacol* 2009; **157**: 1354-1367
- 23 **Alwi I**, Santoso T, Suyono S, Sutrisna B, Suyatna FD, Kresno SB, Ernie S. The effect of curcumin on lipid level in patients with acute coronary syndrome. *Acta Med Indones* 2008; **40**: 201-210
- 24 **Alizadeh-Navaei R**, Roostbeh F, Saravi M, Pouramir M, Jalali F, Moghadamnia AA. Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi Med J* 2008; **29**: 1280-1284
- 25 **Graham DY**, Smith JL, Opekun AR. Spicy food and the stomach. Evaluation by videoendoscopy. *JAMA* 1988; **260**: 3473-3475
- 26 **Al-Yahya MA**, Rafatullah S, Mossa JS, Ageel AM, Parmar NS, Tariq M. Gastroprotective activity of ginger *zingiber officinale* rosc., in albino rats. *Am J Chin Med* 1989; **17**: 51-56
- 27 **Rafatullah S**, Tariq M, Al-Yahya MA, Mossa JS, Ageel AM. Evaluation of turmeric (*Curcuma longa*) for gastric and duodenal antiulcer activity in rats. *J Ethnopharmacol* 1990; **29**: 25-34
- 28 **Marotta RB**, Floch MH. Diet and nutrition in ulcer disease. *Med Clin North Am* 1991; **75**: 967-979
- 29 **Al Mofleh IA**, Alhaider AA, Mossa JS, Al-Sohaibani MO, Al-Yahya MA, Rafatullah S, Shaik SA. Gastroprotective effect of an aqueous suspension of black cumin *Nigella sativa* on necrotizing agents-induced gastric injury in experimental animals. *Saudi J Gastroenterol* 2008; **14**: 128-134
- 30 **Miller EC**, Swanson AB, Phillips DH, Fletcher TL, Liem A, Miller JA. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res* 1983; **43**: 1124-1134
- 31 **Schiestl RH**, Chan WS, Gietz RD, Mehta RD, Hastings PJ. Safrole, eugenol and methyleugenol induce intrachromosomal recombination in yeast. *Mutat Res* 1989; **224**: 427-436
- 32 **Myers BM**, Smith JL, Graham DY. Effect of red pepper and black pepper on the stomach. *Am J Gastroenterol* 1987; **82**: 211-214
- 33 **Lichtenberger LM**, Romero JJ, Carryl OR, Illich PA, Walters ET. Effect of pepper and bismuth subsalicylate on gastric pain and surface hydrophobicity in the rat. *Aliment Pharmacol Ther* 1998; **12**: 483-490
- 34 **Vasudevan K**, Vembar S, Veeraraghavan K, Haranath PS. Influence of intragastric perfusion of aqueous spice extracts on acid secretion in anesthetized albino rats. *Indian J Gastroenterol* 2000; **19**: 53-56
- 35 **Tanida N**, Kawaura A, Takahashi A, Sawada K, Shimoyama T. Suppressive effect of wasabi (pungent Japanese spice) on gastric carcinogenesis induced by MNNG in rats. *Nutr Cancer* 1991; **16**: 53-58
- 36 **Lo YC**, Yang YC, Wu IC, Kuo FC, Liu CM, Wang HW, Kuo CH, Wu JY, Wu DC. Capsaicin-induced cell death in a human gastric adenocarcinoma cell line. *World J Gastroenterol* 2005; **11**: 6254-6257
- 37 **Mothana RA**, Gruenert R, Bednarski PJ, Lindequist U. Evaluation of the in vitro anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine. *Pharmazie* 2009; **64**: 260-268
- 38 **Oya T**, Osawa T, Kawakishi S. Spice constituents scavenging free radicals and inhibiting pentosidine formation in a model system. *Biosci Biotechnol Biochem* 1997; **61**: 263-266
- 39 **Shan B**, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J Agric Food Chem* 2005; **53**: 7749-7759
- 40 **Srinivasan K**. Black pepper and its pungent principle-piperine: a review of diverse physiological effects. *Crit Rev Food Sci Nutr* 2007; **47**: 735-748
- 41 **Adhikari S**, Indira Priyadarsini K, Mukherjee T. Physico-chemical studies on the evaluation of the antioxidant activity of herbal extracts and active principles of some Indian medicinal plants. *J Clin Biochem Nutr* 2007; **40**: 174-183
- 42 **Huang WY**, Cai YZ, Xing J, Corke H, Sun M. Comparative analysis of bioactivities of four *Polygonum* species. *Planta Med* 2008; **74**: 43-49
- 43 **Schwarz K**, Ternes W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. I. Determination of phenolic diterpenes with antioxidative activity amongst tocochromanols using HPLC. *Z Lebensm Unters Forsch* 1992; **195**: 95-98
- 44 **Cheel J**, Schmeda-Hirschmann G, Jordan M, Theoduloz C, Rodriguez JA, Gerth A, Wilken D. Free radical scavenging activity and secondary metabolites from in vitro cultures of *Sanicula graveolens*. *Z Naturforsch C* 2007; **62**: 555-562
- 45 **Korkina LG**. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. *Cell Mol Biol (Noisy-le-grand)* 2007; **53**: 15-25

- 46 **Agbor GA**, Vinson JA, Oben JE, Ngogang JY. In vitro anti-oxidant activity of three Piper species. *J Herb Pharmacother* 2007; **7**: 49-64
- 47 **Toma W**, Hiruma-Lima CA, Guerrero RO, Brito AR. Preliminary studies of *Mammea americana* L. (Guttiferae) bark/ latex extract point to an effective antiulcer effect on gastric ulcer models in mice. *Phytomedicine* 2005; **12**: 345-350
- 48 **Gürbüz I**, Ustün O, Yeşilada E, Sezik E, Akyürek N. In vivo gastroprotective effects of five Turkish folk remedies against ethanol-induced lesions. *J Ethnopharmacol* 2002; **83**: 241-244
- 49 **Xia J**. Medicinal herbs used in pairs for treatment of 98 cases of chronic gastritis. *J Tradit Chin Med* 2004; **24**: 208-209
- 50 **Chernomorets NN**, Seleznev AV, Revutskii BI, Alifanova RE, Kravchenko ZV, Cherkasskaia EP. [The differentiated phytotherapy of patients with duodenal peptic ulcer] *Lik Sprava* 1992; 112-115
- 51 **Chakürski I**, Matev M, Stefanov G, Koichev A, Angelova I. [Treatment of duodenal ulcers and gastroduodenitis with a herbal combination of *Symphitum officinalis* and *Calendula officinalis* with and without antacids] *Vutr Boles* 1981; **20**: 44-47
- 52 **Al-Howiriny T**, Al-Sohaibani M, Al-Said M, Al-Yahya M, El-Tahir K, Rafatullah S. Effect of *Commiphora opobalsamum* (L.) Engl. (Balessan) on experimental gastric ulcers and secretion in rats. *J Ethnopharmacol* 2005; **98**: 287-294
- 53 **Al-Mofleh IA**, Alhaider AA, Mossa IS, Al-Sohaibani MO, Rafatullah S, Qureshi S. Protection of gastric mucosal damage by *Coriandrum sativum* L. pretreatment in Wistar albino rats. *Environmental Toxicol Pharmacol* 2006; **22**: 64-69.
- 54 **Alqasoumi S**, Al-Sohaibani M, Al-Howiriny T, Al-Yahya M, Rafatullah S. Rocket "Eruca sativa": a salad herb with potential gastric anti-ulcer activity. *World J Gastroenterol* 2009; **15**: 1958-1965
- 55 **Nanjundaiah SM**, Annaiah HN, M Dharmesh S. Gastroprotective Effect of Ginger Rhizome (*Zingiber officinale*) Extract: Role of Gallic Acid and Cinnamic Acid in H⁺, K⁺-ATPase/H. pylori Inhibition and Anti-oxidative Mechanism. *Evid Based Complement Alternat Med* 2009; Epub ahead of print
- 56 **Choi SM**, Shin JH, Kang KK, Ahn BO, Yoo M. Gastroprotective effects of DA-6034, a new flavonoid derivative, in various gastric mucosal damage models. *Dig Dis Sci* 2007; **52**: 3075-3080
- 57 **Singh S**, Khajuria A, Taneja SC, Khajuria RK, Singh J, Johri RK, Qazi GN. The gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from *Boswellia serrata*, in rats. *Phytomedicine* 2008; **15**: 408-415
- 58 **Qian Y**, Si JM, Wu JG, Chen SJ, Zhu YF, Sun KK, Deng YY, Chen K, Wang LJ, Liu WL. [Effect of mucosal protective on the quality of gastric ulcer healing] *Zhejiang Daxue Xuebao Yixueban* 2007; **36**: 71-77
- 59 **Kivilaakso E**. Pathogenetic mechanisms in experimental gastric stress ulceration. *Scand J Gastroenterol Suppl* 1985; **110**: 57-62
- 60 **Nayeb-Hashemi H**, Kaunitz JD. Gastroduodenal mucosal defense. *Curr Opin Gastroenterol* 2009; **25**: 537-543
- 61 **Konturek SJ**, Konturek PC, Brzozowski T. Prostaglandins and ulcer healing. *J Physiol Pharmacol* 2005; **56** Suppl 5: 5-31
- 62 **Brzozowski T**, Konturek PC, Konturek SJ, Brzozowska I, Pawlik T. Role of prostaglandins in gastroprotection and gastric adaptation. *J Physiol Pharmacol* 2005; **56** Suppl 5: 33-55
- 63 **Jainu M**, Vijai Mohan K, Shyamala Devi CS. Gastroprotective effect of *Cissus quadrangularis* extract in rats with experimentally induced ulcer. *Indian J Med Res* 2006; **123**: 799-806
- 64 **Baggio CH**, Freitas CS, Otofuiji Gde M, Cipriani TR, Souza LM, Sasaki GL, Iacomini M, Marques MC, Mesia-Vela S. Flavonoid-rich fraction of *Maytenus ilicifolia* Mart. ex. Reiss protects the gastric mucosa of rodents through inhibition of both H⁺,K⁺-ATPase activity and formation of nitric oxide. *J Ethnopharmacol* 2007; **113**: 433-440
- 65 **Baek SW**, Kim NK, Jin HJ, Koh CW, Kim CK, Kwon OH, Kim JS, Cho MH, Park CK. Anti-ulcer actions of phyto-sphingosine hydrochloride in different experimental rat ulcer models. *Arzneimittelforschung* 2005; **55**: 461-465
- 66 **Alam S**, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of *Momordica charantia* L. in rats. *J Ethnopharmacol* 2009; **123**: 464-469
- 67 **Souccar C**, Cysneiros RM, Tanee MM, Torres LM, Lima-Landman MT, Lapa AJ. Inhibition of gastric acid secretion by a standardized aqueous extract of *Cecropia glaziovii* Sneth and underlying mechanism. *Phytomedicine* 2008; **15**: 462-469
- 68 **Zanatta F**, Gandolfi RB, Lemos M, Ticona JC, Gimenez A, Clasen BK, Cechinel Filho V, de Andrade SF. Gastroprotective activity of alkaloid extract and 2-phenylquinoline obtained from the bark of *Galipea longiflora* Krause (Rutaceae). *Chem Biol Interact* 2009; **180**: 312-317
- 69 **Olaleye SB**, Owoyele VB, Odukanmi AO. Antiulcer and gastric antisecretory effects of *Landolphia owariensis* extracts in rats. *Niger J Physiol Sci* 2008; **23**: 23-26
- 70 **Brzozowski T**, Konturek SJ, Sliwowski Z, Pytko-Polończyk J, Szlachcic A, Drozdowicz D. Role of capsaicin-sensitive sensory nerves in gastroprotection against acid-independent and acid-dependent ulcerogens. *Digestion* 1996; **57**: 424-432
- 71 **Silva MI**, Moura BA, Neto MR, Tomé Ada R, Rocha NF, de Carvalho AM, Macêdo DS, Vasconcelos SM, de Sousa DP, Viana GS, de Sousa FC. Gastroprotective activity of isopulegol on experimentally induced gastric lesions in mice: investigation of possible mechanisms of action. *Naunyn Schmiedeberg Arch Pharmacol* 2009; **380**: 233-245
- 72 **Fan TY**, Feng QQ, Jia CR, Fan Q, Li CA, Bai XL. Protective effect of Weikang decoction and partial ingredients on model rat with gastric mucosa ulcer. *World J Gastroenterol* 2005; **11**: 1204-1209
- 73 **de O Leite G**, da Penha AR, Fernandes CN, Souza HH, da Costa JG, Campos AR. Gastroprotective mechanism of *Vanillosmopsis arborea* bark essential oil. *Fitoterapia* 2009; **80**: 77-80
- 74 **Nguelefack TB**, Feumebo CB, Ateufack G, Watcho P, Tatsimo S, Atsamo AD, Tane P, Kamanyi A. Anti-ulcerogenic properties of the aqueous and methanol extracts from the leaves of *Solanum torvum* Swartz (Solanaceae) in rats. *J Ethnopharmacol* 2008; **119**: 135-140
- 75 **Kamath BS**, Srikanta BM, Dharmesh SM, Sarada R, Ravishankar GA. Ulcer preventive and antioxidative properties of astaxanthin from *Haematococcus pluvialis*. *Eur J Pharmacol* 2008; **590**: 387-395
- 76 **Naito Y**, Yoshikawa T. Oxidative stress involvement and gene expression in indomethacin-induced gastropathy. *Redox Rep* 2006; **11**: 243-253
- 77 **Bogdarin IuA**, Potekhin PP, Kozlov DV, Shirokova NIu. [Efficacy of the new collection of herbs at stressful experimental sharp ulcer defects of the gastroduodenal zone] *Eksp Klin Gastroenterol* 2005; 74-78, 102
- 78 **de Paula Ferreira M**, Nishijima CM, Seito LN, Dokkedal AL, Lopes-Ferreira M, Di Stasi LC, Vilegas W, Hiruma-Lima CA. Gastroprotective effect of *Cissus sicyoides* (Vitaceae): involvement of microcirculation, endogenous sulfhydryls and nitric oxide. *J Ethnopharmacol* 2008; **117**: 170-174
- 79 **Kanter M**, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of *Nigella sativa* L. oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. *World J Gastroenterol* 2005; **11**: 6662-6666
- 80 **Komoike Y**, Nakashima M, Nakagiri A, Takeuchi K. Prostaglandin E receptor EP1 subtype but not prostacyclin IP receptor involved in mucosal blood flow response of mouse stomachs following barrier disruption. *Digestion* 2003; **67**: 186-194
- 81 **Satyanarayana MN**. Capsaicin and gastric ulcers. *Crit Rev Food Sci Nutr* 2006; **46**: 275-328
- 82 **Nylander O**, Andersson H, Wilander E, Sababi M. Pros-

- taglandins reduce hydrochloric acid-induced increase in duodenal mucosal permeability by a mechanism not related to stimulation of alkaline secretion. *Acta Physiol Scand* 1995; **153**: 365-374
- 83 **Gookin JL**, Galanko JA, Blikslager AT, Argenzio RA. PG-mediated closure of paracellular pathway and not restitution is the primary determinant of barrier recovery in acutely injured porcine ileum. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G967-G979
- 84 **Hatazawa R**, Ohno R, Tanigami M, Tanaka A, Takeuchi K. Roles of endogenous prostaglandins and cyclooxygenase isozymes in healing of indomethacin-induced small intestinal lesions in rats. *J Pharmacol Exp Ther* 2006; **318**: 691-699
- 85 **Konturek PC**, Brzozowski T, Konturek SJ, Taut A, Kwiecien S, Pajdo R, Sliwowski Z, Hahn EG. Bacterial lipopolysaccharide protects gastric mucosa against acute injury in rats by activation of genes for cyclooxygenases and endogenous prostaglandins. *Digestion* 1998; **59**: 284-297
- 86 **Mehrabani D**, Rezaee A, Azarpira N, Fattahi MR, Amini M, Tanideh N, Panjehshahin MR, Saberi-Firouzi M. The healing effects of Teucrium polium in the repair of indomethacin-induced gastric ulcer in rats. *Saudi Med J* 2009; **30**: 494-499
- 87 **Beales IL**. Gastrin and interleukin-1 β stimulate growth factor secretion from cultured rabbit gastric parietal cells. *Life Sci* 2004; **75**: 2983-2995
- 88 **Luo JC**, Shin VY, Yang YH, Wu WK, Ye YN, So WH, Chang FY, Cho CH. Tumor necrosis factor- α stimulates gastric epithelial cell proliferation. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G32-G38
- 89 **Konturek PC**, Brzozowski T, Konturek SJ, Ernst H, Drozdowicz D, Pajdo R, Hahn EG. Expression of epidermal growth factor and transforming growth factor α during ulcer healing. Time sequence study. *Scand J Gastroenterol* 1997; **32**: 6-15
- 90 **Sasaki E**, Tominaga K, Watanabe T, Fujiwara Y, Oshitani N, Matsumoto T, Higuchi K, Tarnawski AS, Arakawa T. COX-2 is essential for EGF induction of cell proliferation in gastric RGM1 cells. *Dig Dis Sci* 2003; **48**: 2257-2262
- 91 **Jones MK**, Tomikawa M, Mohajer B, Tarnawski AS. Gastrointestinal mucosal regeneration: role of growth factors. *Front Biosci* 1999; **4**: D303-D309
- 92 **Tétreault MP**, Chailier P, Beaulieu JF, Rivard N, Ménard D. Specific signaling cascades involved in cell spreading during healing of micro-wounded gastric epithelial monolayers. *J Cell Biochem* 2008; **105**: 1240-1249
- 93 **Shimura T**, Kataoka H, Ogasawara N, Kubota E, Sasaki M, Tanida S, Joh T. Suppression of proHB-EGF carboxy-terminal fragment nuclear translocation: a new molecular target therapy for gastric cancer. *Clin Cancer Res* 2008; **14**: 3956-3965
- 94 **Yoshida K**, Kanaoka S, Takai T, Uezato T, Miura N, Kajimura M, Hishida A. EGF rapidly translocates tight junction proteins from the cytoplasm to the cell-cell contact via protein kinase C activation in TMK-1 gastric cancer cells. *Exp Cell Res* 2005; **309**: 397-409
- 95 **Baek MK**, Kim MH, Jang HJ, Park JS, Chung IJ, Shin BA, Ahn BW, Jung YD. EGF stimulates uPAR expression and cell invasiveness through ERK, AP-1, and NF- κ B signaling in human gastric carcinoma cells. *Oncol Rep* 2008; **20**: 1569-1575
- 96 **Berlanga-Acosta J**, Gavilondo-Cowley J, López-Saura P, González-López T, Castro-Santana MD, López-Mola E, Guillén-Nieto G, Herrera-Martínez L. Epidermal growth factor in clinical practice - a review of its biological actions, clinical indications and safety implications. *Int Wound J* 2009; **6**: 331-346
- 97 **Ma L**, Chow JY, Wong BC, Cho CH. Role of capsaicin sensory nerves and EGF in the healing of gastric ulcer in rats. *Life Sci* 2000; **66**: PL213-PL220
- 98 **Zheng XG**, Zhang JJ, Huang YC. [Study on the effect of weitonngning on epidermal growth factor and nitric oxide contents in tissue of stomach of rats with gastric ulcer] *Zhongguo Zhongxiyi Jiehe Zazhi* 2004; **24**: 549-551
- 99 **Cao MB**, Dong L, Chang XM, Zou BC, Qin B. Effect of Mexican tea herb and pilular adina herb on concrecence of gastric mucosa in experimental gastric ulcer rats. *Chin J Integr Med* 2007; **13**: 132-136
- 100 **Wang B**, Zhao HY, Zhou L, Wang YF, Cao J. Effect of Kuizhangping on expressions of EGF and EGFR mRNA in gastric mucosa in rats with experimental gastric ulcer. *Beijing Zhongyiyao Daxue Xuebao* 2008; **31**: Abstract
- 101 **Meng H**, Guo J, Sun JY, Pei JM, Wang YM, Zhu MZ, Huang C. Angiogenic effects of the extracts from Chinese herbs: Angelica and Chuanxiong. *Am J Chin Med* 2008; **36**: 541-554
- 102 **Bode AM**, Ma WY, Surh YJ, Dong Z. Inhibition of epidermal growth factor-induced cell transformation and activator protein 1 activation by [6]-gingerol. *Cancer Res* 2001; **61**: 850-853
- 103 **Cai XZ**, Wang J, Li XD, Wang GL, Liu FN, Cheng MS, Li F. Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. *Cancer Biol Ther* 2009; **8**: 1360-1368
- 104 **Marijon H**, Faivre S, Raymond E. [Targeted therapies in hepatocellular carcinomas: recent results and future development] *Bull Cancer* 2009; **96**: 553-561
- 105 **Kelly K**, Huang C. Biological agents in non-small cell lung cancer: a review of recent advances and clinical results with a focus on epidermal growth factor receptor and vascular endothelial growth factor. *J Thorac Oncol* 2008; **3**: 664-673
- 106 **Ji C**, Cao C, Lu S, Kivlin R, Amaral A, Kouttab N, Yang H, Chu W, Bi Z, Di W, Wan Y. Curcumin attenuates EGF-induced AQP3 up-regulation and cell migration in human ovarian cancer cells. *Cancer Chemother Pharmacol* 2008; **62**: 857-865
- 107 **Tsai PJ**, Tsai TH, Yu CH, Ho SC. Evaluation of NO-suppressing activity of several Mediterranean culinary spices. *Food Chem Toxicol* 2007; **45**: 440-447
- 108 **Reyes-Trejo B**, Sánchez-Mendoza ME, Becerra-García AA, Cedillo-Portugal E, Castillo-Henkel C, Arrieta J. Bioassay-guided isolation of an anti-ulcer diterpenoid from Croton reflexifolius: role of nitric oxide, prostaglandins and sulphydryls. *J Pharm Pharmacol* 2008; **60**: 931-936
- 109 **Zayachkivska OS**, Konturek SJ, Drozdowicz D, Brzozowski T, Gzhegotsky MR. Influence of plant-originated gastroprotective and antiulcer substances on gastric mucosal repair. *Fiziol Zh* 2004; **50**: 118-127
- 110 **Chen SH**, Liang YC, Chao JC, Tsai LH, Chang CC, Wang CC, Pan S. Protective effects of Ginkgo biloba extract on the ethanol-induced gastric ulcer in rats. *World J Gastroenterol* 2005; **11**: 3746-3750
- 111 **Al Mofleh IA**, Alhaider AA, Mossa JS, Al-Soohaibani MO, Rafatullah S. Aqueous suspension of anise "Pimpinella anisum" protects rats against chemically induced gastric ulcers. *World J Gastroenterol* 2007; **13**: 1112-1118
- 112 **Tabak M**, Armon R, Neeman I. Cinnamon extracts' inhibitory effect on Helicobacter pylori. *J Ethnopharmacol* 1999; **67**: 269-277
- 113 **Mahady GB**, Pendland SL, Yun G, Lu ZZ. Turmeric (Curcuma longa) and curcumin inhibit the growth of Helicobacter pylori, a group 1 carcinogen. *Anticancer Res* 2002; **22**: 4179-4181
- 114 **Mahady GB**, Pendland SL, Stoia A, Hamill FA, Fabricant D, Dietz BM, Chadwick LR. In vitro susceptibility of Helicobacter pylori to botanical extracts used traditionally for the treatment of gastrointestinal disorders. *Phytother Res* 2005; **19**: 988-991
- 115 **Ali SM**, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H, Rao LV, Habibullah CM, Sechi LA, Ahmed N. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. *Ann Clin Microbiol Antimicrob* 2005; **4**: 20
- 116 **O'Mahony R**, Al-Khtheeri H, Weerasekera D, Fernando N, Vaira D, Holton J, Basset C. Bactericidal and anti-adhesive properties of culinary and medicinal plants against Helico-

- bacter pylori. *World J Gastroenterol* 2005; **11**: 7499-7507
- 117 **Zaidi SF**, Yamada K, Kadowaki M, Usmanghani K, Sugiyama T. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. *J Ethnopharmacol* 2009; **121**: 286-291
- 118 **Asiri Y**, Al-Dhawali A, AlQasoumi S, Al-Yahya M, Rafatullah S. Pharmacovigilance in Herbal Medicine: A Paradigm to Drug Toxicity Monitoring In Conventional Health Care. *Hung Med J* 2008; **2**: 351-363
- 119 **Balaji S**, Chempakam B. Pharmacokinetics prediction and drugability assessment of diphenylheptanoids from turmeric (*Curcuma longa* L). *Med Chem* 2009; **5**: 130-138
- 120 **Kandarkar SV**, Sawant SS, Ingle AD, Deshpande SS, Maru GB. Subchronic oral hepatotoxicity of turmeric in mice--histopathological and ultrastructural studies. *Indian J Exp Biol* 1998; **36**: 675-679
- 121 **Deshpande SS**, Lalitha VS, Ingle AD, Raste AS, Gadre SG, Maru GB. Subchronic oral toxicity of turmeric and ethanolic turmeric extract in female mice and rats. *Toxicol Lett* 1998; **95**: 183-193
- 122 **Shah AH**, Qureshi S, Ageel AM. Toxicity studies in mice of ethanol extracts of *Foeniculum vulgare* fruit and *Ruta chalepensis* aerial parts. *J Ethnopharmacol* 1991; **34**: 167-172
- 123 **Shah AH**, Al-Shareef AH, Ageel AM, Qureshi S. Toxicity studies in mice of common spices, *Cinnamomum zeylanicum* bark and *Piper longum* fruits. *Plant Foods Hum Nutr* 1998; **52**: 231-239
- 124 **Daware MB**, Mujumdar AM, Ghaskadbi S. Reproductive toxicity of piperine in Swiss albino mice. *Planta Med* 2000; **66**: 231-236
- 125 **Cao J**, Jia L, Zhou HM, Liu Y, Zhong LF. Mitochondrial and nuclear DNA damage induced by curcumin in human hepatoma G2 cells. *Toxicol Sci* 2006; **91**: 476-483
- 126 **Mendonça LM**, Dos Santos GC, Antonucci GA, Dos Santos AC, Bianchi Mde L, Antunes LM. Evaluation of the cytotoxicity and genotoxicity of curcumin in PC12 cells. *Mutat Res* 2009; **675**: 29-34
- 127 **Bentzen PJ**, Lang E, Lang F. Curcumin induced suicidal erythrocyte death. *Cell Physiol Biochem* 2007; **19**: 153-164
- 128 **Khader M**, Bresgen N, Eckl PM. In vitro toxicological properties of thymoquinone. *Food Chem Toxicol* 2009; **47**: 129-133
- 129 **Qadri SM**, Mahmud H, Föller M, Lang F. Thymoquinone-induced suicidal erythrocyte death. *Food Chem Toxicol* 2009; **47**: 1545-1549

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Treatment of iron deficiency anemia associated with gastrointestinal tract diseases

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Abstract

The gastrointestinal (GI) tract is a common site of bleeding that may lead to iron deficiency anemia (IDA). Treatment of IDA depends on severity and acuity of patients' signs and symptoms. While red blood cell transfusions may be required in hemodynamically unstable patients, transfusions should be avoided in chronically anemic patients due to their potential side effects and cost. Iron studies need to be performed after episodes of GI bleeding and stores need to be replenished before anemia develops. Oral iron preparations are efficacious but poorly tolerated due to non-absorbed iron-mediated GI side effects. However, oral iron dose may be reduced with no effect on its efficacy while decreasing side effects and patient discontinuation rates. Parenteral iron therapy replenishes iron stores quicker and is better tolerated than oral therapy. Serious hypersensitive reactions are very rare with new intravenous preparations. While data on worsening of inflammatory bowel disease (IBD) activity by oral iron therapy are not conclusive, parenteral iron therapy still seems to be advantageous in the treatment of IDA in patients with IBD, because oral iron may not be sufficient to overcome the chronic blood loss and GI side effects of oral iron which may mimic IBD exacerbation. Finally, we believe the choice

of oral vs parenteral iron therapy in patients with IBD should primarily depend on acuity and severity of patients' signs and symptoms.

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Key words: Anemia; Inflammatory bowel disease; Intravenous iron; Iron deficiency; Oral iron

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INTRODUCTION

Iron deficiency anemia (IDA) is a consequence of depletion of body iron stores due to decreased iron uptake or increased iron loss/use. Body iron content is imposed by controlling its entrance into the body through the gastrointestinal (GI) system rather than by controlling its excretion. GI diseases are among the most common etiologies of IDA because the GI tract is a common site of blood loss and GI diseases may cause malabsorption of iron. Treatment of IDA may be especially cumbersome in patients with GI diseases due to relatively poor tolerability of oral iron preparations and decreased GI iron absorption. The goal of this review is to increase awareness of parenteral iron therapies in the treatment of IDA associated with GI diseases, particularly inflammatory bowel diseases (IBDs).

IRON DEFICIENCY ANEMIA

Clinical findings

Iron deficiency causes anemia through impaired heme synthesis in maturing erythrocyte precursors. In addition to typical symptoms of anemia, patients may present with pica (appetite for bizarre foods), atrophy of upper GI mucosa (glossitis, stomatitis, achlorhydria), and spooning of the nails (koilonychia). Achlorhydria may further worsen anemia by decreasing iron absorption through the GI tract. IDA is also associated with cervical esophageal webs (Plummer-Vinson syndrome), especially in elderly women^[1]. Furthermore, a high prevalence of iron deficiency with or without anemia has been reported among patients with restless legs syndrome^[2].

Etiology

The etiology of IDA can be broadly categorized into two: increased iron loss/use (acute/chronic blood loss, menses, blood donation, rapid growth during childhood, pregnancy) and decreased iron uptake (inadequate diet, malabsorption due to GI disease or surgery). Common etiologies of IDA stemming from GI diseases are shown in Table 1. Any hemorrhagic lesion within the GI tract may result in blood loss and iron deficiency. IDA is often the first sign of an occult GI malignancy, celiac disease, and gastritis^[3,4].

Diagnosis

Although patients with IDA generally present with microcytic, hypochromic anemia, early IDA has normocytic, normochromic indices. Reticulocyte count (corresponding to red cell production) and ferritin level (corresponding to stored iron amount) are typically low in uncomplicated IDA. Serum iron levels are not helpful by themselves because they vary with time of the day^[5] and due to various systemic insults. Serum ferritin concentration is an excellent indicator of body iron stores, however, ferritin is an acute phase reactant and its level may be elevated in chronic inflammatory states. Virtually all patients with serum ferritin concentrations less than 15 ng/mL are iron deficient, with a sensitivity and specificity of 59% and 99%, respectively^[6]. A cutoff limit of 30 ng/mL may increase its sensitivity to 92%^[7]. Transferrin iron binding capacity (ratio of iron-unbound transferrin to total transferrin) is a helpful marker in the diagnosis of IDA and is generally lower than 16% in patients with IDA. Several conditions may complicate the diagnosis of IDA such as infections, malignancies, chronic renal failure, and inflammatory conditions by falsely elevating ferritin levels. Newer assays such as soluble transferrin receptor level and reticulocyte hemoglobin (Hb) content may be helpful, although lack of reliable standards prevented these assays from becoming a routinely available test in clinical practice^[8]. Although examination of the bone marrow is considered to be the best method for evaluation of body iron status, it is invasive, expensive, and operator-dependent. In addition, recent papers questioned its accuracy^[9].

TREATMENT

Keeping the diverse etiology of IDA in mind, clinicians should not consider it as a simple ailment or deficiency but a sign of a potentially life-threatening disease. Therefore, the mainstay of IDA management should be identification and correction of the underlying pathology. The search for etiology should start with history, primarily directed to assess blood loss from the GI tract and due to obstetrical causes. Potentially curable GI malignancies can be found more commonly using colonoscopy than upper endoscopy^[10]. However, the overall prevalence of upper GI lesions is higher than that of lower GI lesions in patients with IDA^[11,12]. The initial diagnostic test (colonoscopy *vs* upper endoscopy) can be chosen based on findings in the history.

Red blood cell transfusions are required in hemodynamically unstable patients primarily due to acute GI bleeding. On the other hand, transfusions should be avoided in chronically anemic, hemodynamically stable patients without cardiac or pulmonary comorbidities unless the Hb level is < 7 g/dL^[13] due to the potentially life-threatening side effects of transfusions as well as their cost. In case of urgency, anemia can be corrected rapidly with parenteral iron therapy in patients with uncomplicated IDA.

Furthermore, iron studies should be performed after GI bleeding resolves even in non-anemic patients since frequent bleeding may deplete body iron stores without causing explicit anemia. Iron is essential for all cells in the body; and iron replacement in non-anemic but iron deficient patients may improve quality of life^[14] and cognitive function^[15]; may also delay/prevent the development of IDA in patients with frequent episodes of GI bleeding such as those with IBD and angiodysplasias. Clinicians should take side effects of oral iron therapy and the frequency of patients' bleeding episodes into consideration when deciding on iron supplementation in non-anemic iron deficient patients.

Oral iron replacement

Iron stores can be replenished through oral and parenteral therapy. In asymptomatic and mildly symptomatic patients with IDA, oral iron replacement therapy has been the mainstay therapy. Various iron salts have been used, ferrous sulfate being the most common. Use of oral iron is primarily limited by its GI side effects that are mediated by non-absorbed iron. Although newer preparations were claimed to have less side effects, ferrous sulfate is still the most commonly used oral iron preparation. Accordingly, no difference in efficacy and side effect profile were found between ferrous sulfate, ferrous gluconate, and ferrous fumarate in a randomized, double blind study^[16]. However, controlled-release iron preparations and polysaccharide-iron complexes were found to have fewer GI side effects than ferrous sulfate in a few randomized trials^[17,18].

Ferrous sulfate is 20% elemental iron so that a 325 mg tablet contains 65 mg iron. Although conventional wisdom

Table 1 Causes of iron deficiency anemia stemming from the GI tract

Decreased iron uptake	Increased iron loss (bleeding into GI tract)
Celiac sprue	Esophagus (esophagitis and cancer)
Giardiasis	Stomach (ulcer, gastritis, cancer, vascular lesions)
Achlorhydria (due to atrophic gastritis, <i>H. pylori</i> infection...)	Small bowel (vascular lesions)
Gastrojejunostomy and other surgical techniques bypassing the duodenum where iron absorption is maximal	Large bowel (cancer, angioectasias, adenoma, colitis)
Short bowel syndrome	

GI: Gastrointestinal; *H. pylori*: *Helicobacter pylori*.

Table 2 Pros and cons of parenteral iron over oral iron therapy

Pros
Assured repletion of iron stores regardless of factors affecting iron absorption
Rapid reversal of iron deficiency
Certain or at least assessable adherence to therapy
Infrequent side effects
One-time administration (FeCarb and LMWID)
Cons
Rare lethal drug-related adverse events
Expensive
Requires facilities/staff for administration
Simply taking tablets may be more convenient for some patients

FeCarb: Ferric carboxymaltose; LMWID: Low-molecular weight iron dextran.

dictates administration of 200 mg elemental iron daily for correction of IDA, there is no rationale for using such a high dose of oral iron. Iron absorption from the GI tract is highly efficient but saturable^[19]. Accordingly, Rimón *et al*^[20] demonstrated that oral iron preparations at doses as low as 15 mg/d could be used to correct iron deficiency.

To avoid GI side effects and consequent non-compliance, oral iron should be started at a low dose once daily after meals then the dose can be increased at the clinician's discretion. If well tolerated, patients should attempt to take iron preparations on an empty stomach to increase iron absorption. Within 7-14 d of therapy, an increase in reticulocyte count would be expected and within 2 mo Hb level should return to normal. Oral iron replacement should be continued to replenish iron stores, usually for an additional 4 to 6 mo after Hb normalization.

Parenteral iron replacement

The parenteral route should be used for iron replacement in patients who cannot be treated adequately with oral iron supplements due to severe GI side effects, inadequate absorption, and anemia that requires urgent correction. Many clinicians have been reluctant to use parenteral iron formulations due to the infrequent, random, but rarely lethal hypersensitive reactions to high molecular weight iron dextran (HMWID) infusions. However, the incidence of severe adverse effects is lower with low molecular weight iron dextran (LMWID) and newer parenteral iron complexes. Rates of life threatening adverse drug events were reported as 0.6, 0.9, 3.3, and 11.3 per

million doses of iron sucrose, iron gluconate, LMWID, and HMWID, respectively^[21]. The pros and cons of parenteral over oral iron replacement are listed in Table 2.

All parenteral iron complexes consist of a ferric iron core and a stabilizing carbohydrate shell. Table 3 demonstrates the selected characteristics, administration guidelines, and side effect profiles of four parenteral iron complexes^[22,23]. Following parenteral administration, the iron-carbohydrate complex is separated by the reticulo-endothelial system and iron is gradually released into the circulation combining with transferrin for transport to the liver, spleen, and bone marrow. Parenteral iron is administered at a dose to restore the total iron deficit (TID) in the body. The TID is traditionally calculated by Ganzoni's formula^[24]: $(2.4 \times \text{body weight (kg)} \times [\text{target Hb (g/dL)} - \text{observed Hb (g/dL)}]) + 500 \text{ mg (iron depot)}$. However, this formula may underestimate the iron depot in males which is estimated to be 700-900 mg^[25].

Iron gluconate and iron sucrose are weaker complexes than iron dextran and ferric carboxymaltose (FeCarb). Therefore, iron is released into circulation quicker and becomes readily available for erythropoiesis^[26]. While early release of iron may cause a quicker response in Hb levels, it increases the risk of acute adverse events because non-bound labile iron may cause transient capillary leak syndrome leading to nausea, hypotension, tachycardia, dyspnea, and edema mimicking anaphylaxis^[27]. Hence, iron gluconate and iron sucrose have lower maximal recommended single doses.

Due to the higher incidence of anaphylactic reactions associated with iron dextran, a test dose of 25 mg as slow intravenous (IV) push is required before LMWID administration. On the other hand, LMWID can be administered at higher doses enabling physicians to administer the total iron dose at one infusion. Furthermore, LMWID is less expensive compared to the other parenteral iron complexes. Iron dextran can also be administered intramuscularly, however, this is not recommended due to the incomplete absorption from the injection site and pain on injection.

FeCarb is a novel parenteral iron complex with a favorable side effect profile that can be applied at single doses up to 1000 mg per week at a high infusion speed (1000 mg IV over 15 min)^[28]. However, the safety information on FeCarb is not mature and various phase III trials are underway evaluating its efficacy and safety. As of December 2009, FeCarb has not been approved by the USA Food and Drug Administration.

Table 3 Selected characteristics and dosage guidelines of parenteral iron complexes

	LMWID	Iron gluconate	Iron sucrose	FeCarb
Concentration	50 mg/mL (2 mL vial)	12.5 mg/mL (5 mL ampule)	20 mg/mL (5 mL vial)	50 mg/mL (2 mL vial)
IV injection dose	100 mg over 2-5 min	125 mg over 10 min	100 mg over 5 min	100 mg over 2 min
Direct iron donation to transferrin	1-2	5-6	4-5	1-2
Test dose required	Yes, 25 mg slow IV push	No	No	No
Maximal single dose for IV infusion	Not limited	125	500	1000
Total dose infusion	Yes, in NS over 1-6 h	No	No	No
Pregnancy category	C	B	B	N/A
Life threatening ADEs (per 10 ⁶ doses)	3.3	0.9	0.6	N/A

LMWID: Low molecular weight iron dextran; IV: Intravenous; ADE: Adverse drug event; NS: Normal saline; N/A: Not available.

Table 4 Clinical studies comparing parenteral and oral iron therapy

Author	Intervention	Study method	n	Efficacy	P
Schröder <i>et al</i> ^[233]	Elemental iron 100-200 mg/d × 6 wk	Randomized	24	¹ RR: 53%	0.85
	Iron sucrose 7 mg/kg × one dose then 200 mg once-twice/wk × 5 wk		22	¹ RR: 55%	
Erichsen <i>et al</i> ^[234]	Elemental iron 120 mg/d × 14 d	Crossover trial with a washout period of > 6 wk	17	Mean increase in Hb: 0.2	< 0.05
	Iron sucrose 200 mg on days 1, 5, 10		17	Mean increase in Hb: 0.7	
Kulnigg <i>et al</i> ^[235]	Elemental iron 200 mg/d × 12 wk	2:1 (FeCarb:oral) randomization	136	Median increase in Hb: 2.8	NS
	FeCarb 1000 mg (max) weekly (× 1-3 wk)		60	Median increase in Hb: 3.7	
Gisbert <i>et al</i> ^[236]	Elemental iron 106 mg/d × 3-6 mo	Hb ≥ 10.0 g/dL	78	² RR: 89%	NS
	Iron sucrose 200 mg twice/wk × 3-6 mo	Hb < 10.0 g/dL	22	² RR: 77%	
Lindgren <i>et al</i> ^[236]	Elemental iron 200 mg/d × 20 wk	Randomized	46	¹ RR: 47%	0.07
	Iron sucrose 200 mg weekly until calculated dose reached		45	¹ RR: 66%	

¹Increase in Hb ≥ 2.0 g/dL; ²Complete normalization of Hb. RR: Response rate.

IRON DEFICIENCY IN INFLAMMATORY BOWEL DISEASE

IDA in IBD requires further attention because of its complex etiology and the current debate on its treatment. Anemia is thought to be the most common extraintestinal manifestation of IBD with a prevalence of 6%-74% in different study populations (higher in hospitalized patients)^[29]. Anemia in IBD is multifactorial in origin and is frequently due to the combination of iron deficiency (primary cause) and anemia of chronic disease (ACD). In some cases, anemia may be drug induced (mesalazine, sulfasalazine, azathioprine, mercaptopurine) or due to folate/vitamin B12 deficiency. Iron deficiency in IBD is primarily caused by chronic blood loss through the GI tract, however, decreased iron absorption may also play a role in patients with Crohn's disease affecting the proximal small bowel. The prevalence of iron deficiency in IBD ranges from 36% to 90% depending on the definition of iron deficiency and cohort selection^[30-32].

The frequent mixture of IDA and ACD in patients with IBD creates a diagnostic dilemma. Chronic inflammation increases serum ferritin and decreases serum transferrin level. As a result, the sensitivities of ferritin and iron saturation for IDA are decreased in patients with IBD. In addition to serum ferritin level and iron saturation, clinicians may use novel tests such as soluble transferrin level or a therapeutic trial of iron replacement therapy in anemic IBD patients.

The current debate on treatment of IDA in patients with IBD centers on the choice of the iron replacement route. The advantages of parenteral over oral iron replacement are thought to be:

Parenteral therapy may be more efficacious than oral supplementation because of low iron absorption from the GI tract in patients with IBD and lower compliance to the oral therapy. However, currently available data are not sufficient to reach such a conclusion. A few studies comparing the efficacy of oral and parenteral iron therapy have been published with conflicting results^[28,33-36] (Table 4). Although Lindgren *et al*^[36] recently concluded that IV iron therapy was more efficacious in the treatment of IDA in IBD after an intent-to-treat analysis, this study was confounded by a high discontinuation rate in the oral therapy arm (11 of 46 patients).

Persistent blood loss may exceed the capacity of intestinal absorption of iron in some patients^[37]. The maximal iron absorption from intestines depends primarily on the level of body iron store and iron intake. Iron-deficient patients receiving 100 mg elemental iron/d can absorb at most 25-37.5 mg iron/d^[38,39]. Since whole blood contains 0.5 mg elemental iron/mL^[40], oral iron therapy may replace the iron lost in 50-75 mL of blood in the best circumstances. Therefore, clinicians should not expect oral iron therapy to correct IDA and replenish body iron stores in patients losing more than 20-30 mL blood daily. Consequently, parenteral therapy may be more useful in patients with moderate-severe active IBD.

Parenteral therapy causes an earlier increase in Hb levels^[28,34]. For that reason, it is more valuable in situations where rapid correction of anemia is required such as in hemodynamically stable patients with moderately severe symptoms of anemia or those requiring an elective procedure within a few weeks.

Oral iron therapy has a high incidence of GI side effects such as epigastric pain and diarrhea that lead to discontinuation of therapy in up to 21% of patients^[29]. Additionally, it is extremely difficult to differentiate these side effects from IBD exacerbation. Blackened stools by non-absorbed iron may also mimic melena and lead to unnecessary tests. On the other hand, lower doses of iron may be better tolerated and as efficacious as higher doses. Gisbert *et al.*^[35] recently reported a discontinuation rate of 5% and normalization of Hb concentration in 89% of IBD patients treated with 106 mg of oral elemental iron daily.

In addition to its GI side effects, oral iron may worsen IBD as a result of non-absorbed iron-mediated toxic reactive oxygen species. Accordingly, oral iron therapy was demonstrated to worsen disease activity in several rodent models at doses 100- to 1000-fold higher than the therapeutic iron dose^[41-44]. In the only animal study in which the clinically therapeutic dose in humans was used, oral iron was found to increase histologic colitis scores in DSS-induced colitis in rats^[45]. Furthermore, oral iron therapy was found to worsen IBD activity indices in one clinical study^[34] while it improved or had no effect in other studies^[35,36,46]. Irrespectively, disease activity questionnaires in IBD patients being treated with oral iron may not reflect the true disease activity but may be confounded with the GI side effects of iron.

On the other hand, oral iron therapy is less expensive, does not require additional infusion staff and infusion facilities, does not have life-threatening side effects, and may be more convenient for some patients because of logistics. We believe parenteral iron therapy is still advantageous over oral therapy in patients with IBD due to a quicker response, efficacy in severe cases, and the GI side effects of oral therapy.

Another pressing issue on iron replacement is the duration of therapy. Kulnigg *et al.*^[47] recently reported that anemia recurred within 10 mo of therapy cessation in 50% of anemic IBD patients whose Hb levels had normalized after IV iron sucrose and erythropoietin therapy, indicating a need for continuous iron maintenance therapy. In addition, patients with a ferritin level > 400 mg/dL at the end of replacement therapy had a longer period until they become iron deficient. Therefore, after replenishing TID, clinicians may opt to continue iron therapy at a less frequent maintenance dose or to switch to low-dose oral therapy.

Today, the widely recommended indications for use of IV iron in IBD patients with IDA are: severe anemia (defined as Hb level < 10 g/dL), need of quick recovery, intolerance to oral iron, and failure of oral iron. We agree with all of these indications with the exception of setting a definite Hb threshold. Considering the multifactorial

etiology of anemia in IBD and the widely different tolerances of people to anemia, we believe that the choice of the iron administration route should primarily depend on the severity and acuity of patients' signs and symptoms. Concentrating only on the Hb level and overlooking the factors that may affect patients' tolerance to anemia such as age, cardiac and pulmonary comorbidities may blur the bigger picture and lead to over- or under-treatment. We believe symptoms such as dyspnea on mild exertion, tachycardia, severe fatigue, and ongoing gross blood loss from the GI tract should urge the clinicians to recommend first-line parenteral iron therapy to their patients with IBD and IDA.

CONCLUSION

GI diseases are one of the most common etiologies of IDA. Iron replacement should be initiated in patients with IDA and iron deficient non-anemic patients with recurrent episodes of GI bleeding. Novel parenteral iron preparations are safer than high molecular weight iron dextran and are more useful in patients with IBD primarily due to GI side effects of oral iron therapy. Finally, regardless of the route, iron therapy needs to be continued until iron stores are completely replenished. The need for maintenance therapy should be assessed in prospective trials.

REFERENCES

- 1 Novacek G. Plummer-Vinson syndrome. *Orphanet J Rare Dis* 2006; **1**: 36
- 2 Earley CJ. Clinical practice. Restless legs syndrome. *N Engl J Med* 2003; **348**: 2103-2109
- 3 Baccini F, Spiriti MA, Vannella L, Monarca B, Delle Fave G, Annibale B. Unawareness of gastrointestinal symptomatology in adult coeliac patients with unexplained iron-deficiency anaemia presentation. *Aliment Pharmacol Ther* 2006; **23**: 915-921
- 4 Hershko C, Ianculovich M, Souroujon M. A hematologist's view of unexplained iron deficiency anemia in males: impact of *Helicobacter pylori* eradication. *Blood Cells Mol Dis* 2007; **38**: 45-53
- 5 Favier A, Ruffieux D. Physiological variations of serum levels of copper, zinc, iron and manganese. *Biomed Pharmacother* 1983; **37**: 462-466
- 6 Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med* 1992; **7**: 145-153
- 7 Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clin Chem* 1998; **44**: 45-51
- 8 Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. *Clin Lab Haematol* 2006; **28**: 303-308
- 9 Ganti AK, Moazzam N, Laroia S, Tendulkar K, Potti A, Mehdi SA. Predictive value of absent bone marrow iron stores in the clinical diagnosis of iron deficiency anemia. *In Vivo* 2003; **17**: 389-392
- 10 Stephens MR, Hopper AN, White SR, Jugool S, Stratford R, Lewis WG, Allison MC. Colonoscopy first for iron-deficiency anaemia: a Numbers Needed to Investigate approach. *QJM* 2006; **99**: 389-395
- 11 Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron-deficiency anemia. *N Engl J Med* 1993;

- 329: 1691-1695
- 12 **Kepczyk T**, Kadakia SC. Prospective evaluation of gastrointestinal tract in patients with iron-deficiency anemia. *Dig Dis Sci* 1995; **40**: 1283-1289
- 13 **Hébert PC**, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med* 1999; **340**: 409-417
- 14 **Verdon F**, Burnand B, Stubi CL, Bonard C, Graff M, Michaud A, Bischoff T, de Vevey M, Studer JP, Herzig L, Chapuis C, Tissot J, Pécoud A, Favrat B. Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *BMJ* 2003; **326**: 1124
- 15 **Bruner AB**, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* 1996; **348**: 992-996
- 16 **Hallberg L**, Ryttinger L, Sölvell L. Side-effects of oral iron therapy. A double-blind study of different iron compounds in tablet form. *Acta Med Scand Suppl* 1966; **459**: 3-10
- 17 **Brick C**, Curry H, Hanna C, Knipfer M, Taylor L. Adverse effects of iron supplementation a comparative trial of a wax-matrix iron preparation and conventional ferrous sulfate tablets. *Clin Ther* 1985; **7**: 568-573
- 18 **Jacobs P**, Fransman D, Coghlan P. Comparative bioavailability of ferric polymaltose and ferrous sulphate in iron-deficient blood donors. *J Clin Apher* 1993; **8**: 89-95
- 19 **Rockey DC**. Treatment of iron deficiency. *Gastroenterology* 2006; **130**: 1367-1368
- 20 **Rimon E**, Kagansky N, Kagansky M, Mechnick L, Mashiah T, Namir M, Levy S. Are we giving too much iron? Low-dose iron therapy is effective in octogenarians. *Am J Med* 2005; **118**: 1142-1147
- 21 **Chertow GM**, Mason PD, Vaage-Nilsen O, Ahlmén J. Update on adverse drug events associated with parenteral iron. *Nephrol Dial Transplant* 2006; **21**: 378-382
- 22 **Silverstein SB**, Rodgers GM. Parenteral iron therapy options. *Am J Hematol* 2004; **76**: 74-78
- 23 **Muñoz M**, Gómez-Ramírez S, García-Erce JA. Intravenous iron in inflammatory bowel disease. *World J Gastroenterol* 2009; **15**: 4666-4674
- 24 **Ganzoni AM**. [Intravenous iron-dextran: therapeutic and experimental possibilities] *Schweiz Med Wochenschr* 1970; **100**: 301-303
- 25 **Walters GO**, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 1973; **26**: 770-772
- 26 **Yee J**, Besarab A. Iron sucrose: the oldest iron therapy becomes new. *Am J Kidney Dis* 2002; **40**: 1111-1121
- 27 **Van Wyck DB**. Labile iron: manifestations and clinical implications. *J Am Soc Nephrol* 2004; **15** Suppl 2: S107-S111
- 28 **Kulnigg S**, Stoinov S, Simanenkova V, Dudar LV, Karnafel W, Garcia LC, Sambuelli AM, D'Haens G, Gasche C. A novel intravenous iron formulation for treatment of anemia in inflammatory bowel disease: the ferric carboxymaltose (FERINJECT) randomized controlled trial. *Am J Gastroenterol* 2008; **103**: 1182-1192
- 29 **Kulnigg S**, Gasche C. Systematic review: managing anaemia in Crohn's disease. *Aliment Pharmacol Ther* 2006; **24**: 1507-1523
- 30 **Gasché C**, Reinisch W, Lochs H, Parsaei B, Bakos S, Wyatt J, Fueger GF, Gangl A. Anemia in Crohn's disease. Importance of inadequate erythropoietin production and iron deficiency. *Dig Dis Sci* 1994; **39**: 1930-1934
- 31 **Ormerod TP**. Anaemia in ulcerative colitis. *Proc R Soc Med* 1968; **61**: 931
- 32 **Gomollón F**, Gisbert JP. Anemia and inflammatory bowel diseases. *World J Gastroenterol* 2009; **15**: 4659-4665
- 33 **Schröder O**, Mickisch O, Seidler U, de Weerth A, Dignass AU, Herfarth H, Reinshagen M, Schreiber S, Junge U, Schrott M, Stein J. Intravenous iron sucrose versus oral iron supplementation for the treatment of iron deficiency anemia in patients with inflammatory bowel disease—a randomized, controlled, open-label, multicenter study. *Am J Gastroenterol* 2005; **100**: 2503-2509
- 34 **Erichsen K**, Ulvik RJ, Nysaeter G, Johansen J, Ostborg J, Berstad A, Berge RK, Hausken T. Oral ferrous fumarate or intravenous iron sucrose for patients with inflammatory bowel disease. *Scand J Gastroenterol* 2005; **40**: 1058-1065
- 35 **Gisbert JP**, Bermejo F, Pajares R, Pérez-Calle JL, Rodríguez M, Algaba A, Mancenido N, de la Morena F, Carneros JA, McNicholl AG, González-Lama Y, Maté J. Oral and intravenous iron treatment in inflammatory bowel disease: hematological response and quality of life improvement. *Inflamm Bowel Dis* 2009; **15**: 1485-1491
- 36 **Lindgren S**, Wikman O, Befrits R, Blom H, Eriksson A, Grännö C, Ung KA, Hjortswang H, Lindgren A, Unge P. Intravenous iron sucrose is superior to oral iron sulphate for correcting anaemia and restoring iron stores in IBD patients: A randomized, controlled, evaluator-blind, multicentre study. *Scand J Gastroenterol* 2009; **44**: 838-845
- 37 **Allen LH**. Iron supplements: scientific issues concerning efficacy and implications for research and programs. *J Nutr* 2002; **132**: 813S-819S
- 38 **Smith MD**, Pannacciulli IM. Absorption of inorganic iron from graded doses: its significance in relation to iron absorption tests and mucosal block theory. *Br J Haematol* 1958; **4**: 428-434
- 39 **Werner E**, Kaltwasser JP, Ihm P. [Oral iron treatment: intestinal absorption and the influence of a meal (author's transl)] *Dtsch Med Wochenschr* 1977; **102**: 1061-1064
- 40 **Helmer OM**, Emerson CP. The iron content of the whole blood of normal individuals. *J Biol Chem* 1934; **104**: 157-161
- 41 **Carrier J**, Aghdassi E, Platt I, Cullen J, Allard JP. Effect of oral iron supplementation on oxidative stress and colonic inflammation in rats with induced colitis. *Aliment Pharmacol Ther* 2001; **15**: 1989-1999
- 42 **Carrier J**, Aghdassi E, Cullen J, Allard JP. Iron supplementation increases disease activity and vitamin E ameliorates the effect in rats with dextran sulfate sodium-induced colitis. *J Nutr* 2002; **132**: 3146-3150
- 43 **Seril DN**, Liao J, Ho KL, Warsi A, Yang CS, Yang GY. Dietary iron supplementation enhances DSS-induced colitis and associated colorectal carcinoma development in mice. *Dig Dis Sci* 2002; **47**: 1266-1278
- 44 **Reifen R**, Matas Z, Zeidel L, Berkovitch Z, Bujanover Y. Iron supplementation may aggravate inflammatory status of colitis in a rat model. *Dig Dis Sci* 2000; **45**: 394-397
- 45 **Erichsen K**, Milde AM, Arslan G, Helgeland L, Gudbrandsen OA, Ulvik RJ, Berge RK, Hausken T, Berstad A. Low-dose oral ferrous fumarate aggravated intestinal inflammation in rats with DSS-induced colitis. *Inflamm Bowel Dis* 2005; **11**: 744-748
- 46 **de Silva AD**, Tsironi E, Feakins RM, Rampton DS. Efficacy and tolerability of oral iron therapy in inflammatory bowel disease: a prospective, comparative trial. *Aliment Pharmacol Ther* 2005; **22**: 1097-1105
- 47 **Kulnigg S**, Teischinger L, Dejaco C, Waldhör T, Gasche C. Rapid recurrence of IBD-associated anemia and iron deficiency after intravenous iron sucrose and erythropoietin treatment. *Am J Gastroenterol* 2009; **104**: 1460-1467

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Molecular basis and management of gastrointestinal stromal tumors

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Abstract

Molecularly targeted agents have dramatically impacted the management of several cancers. Targeting KIT has led to a new treatment paradigm in gastrointestinal stromal tumors (GISTs). KIT is a cell surface receptor with tyrosine kinases that, upon binding of its ligand, stem cell factor, activates various signaling pathways. Imatinib and sunitinib, both tyrosine kinase inhibitors directed to KIT, were approved for first- and second-line treatment of metastatic and unresectable GISTs. In this article, we will review the molecular pathogenesis of GISTs followed by a discussion of imatinib and sunitinib's role in the treatment of GISTs. Finally, we will introduce novel therapeutic options for imatinib- and sunitinib-resistant GISTs.

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Key words: Gastrointestinal stromal tumor; KIT; Imatinib; Sunitinib; Nilotinib

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INTRODUCTION

The gastrointestinal stromal tumor (GIST) is the most common (80%) mesenchymal neoplasm of the gastrointestinal tract^[1] and represents about 5% of all sarcomas^[2]. The annual incidence of GISTs is estimated to be 14.5 per million and prevalence 129 per million^[3], with as many as 6000 new cases per year in the United States^[4].

GISTs represent a morphological and biological gamut from incidentally discovered, < 1 cm nodules of benign appearance to large sarcomas, although all GISTs are currently accepted as potentially malignant. GISTs most commonly involve the stomach (50%) and small bowel (25%) (Figure 1); however, they may occur anywhere in the GI tract including the mesentery and omentum^[5,6]. Approximately half of the patients present with metastatic disease and nearly two-thirds of those have liver metastases. Extra-abdominal and lymph node metastases at presentation are rare, obviating the need for lymph node dissection unless they are directly involved^[2].

Until the 1980s, stromal tumors arising from gastrointestinal tract were classified as various entities such as leiomyosarcoma, leiomyoblastoma and bizarre leiomyoma. With the advent of immunohistochemistry, it was realized

that these tumors lacked immunophenotypic characteristics of smooth muscle. Mazur and Clark showed that a proportion of these tumors stained positively for S-100 and suggested the myenteric nervous system as the cells of origin, introducing the more generic term, “stromal tumor”^[7]. In 1998, Hirota and colleagues demonstrated that almost all GISTs expressed KIT and had activating c-kit mutations. They also showed that interstitial cells of Cajal (ICC) were positive for both KIT and CD34 suggesting ICC as the cells of origin of GISTs^[8].

KIT

KIT encoded by the *c-Kit* gene (mapped to chromosome 4q12) belongs to the type III receptor tyrosine kinase family and is structurally similar to platelet-derived growth factor receptors (PDGFRs), colony-stimulating factor-1 receptor, and fms-like tyrosine kinase-3 (FLT-3)^[9,10]. KIT consists of an extracellular (EC) domain with 5 immunoglobulin-like loops, a transmembrane region, and a cytoplasmic domain with juxtamembrane (JM) region and a split tyrosine kinase (TK) domain. The latter is divided into an adenosine triphosphate (ATP) binding region (TK1) and a phosphotransferase region (TK2) by a hydrophilic kinase insert (KI) (Figure 2).

Normally, KIT is activated by stem cell factor. Ligand binding to the EC domain results in the dimerization of receptors and phosphorylation of tyrosine in the cytoplasmic TK domains. This leads to a phosphorylation cascade and activation of signal transduction pathways including Ras/MAP kinase, Rac/Rho-JNK, PI3K/AKT and SFK/STAT signaling networks^[11]. Signaling by KIT plays an important role in erythropoiesis, lymphopoiesis, mast cell development and function, megakaryopoiesis, gametogenesis and melanogenesis.

KIT is expressed in more than 95% of GISTs, including tumors with wild-type KIT and most PDGFR-A mutant GISTs. However, in the latter group, KIT expression may be weaker. KIT mutation in GISTs does not cause KIT expression, but modifies KIT function^[12]. About 65%-85% of GISTs have KIT mutations^[13-15]. Based on the location, these mutations could be divided into 2 categories: mutations of the receptor regulatory domain (EC and JM) and mutations of the enzymatic domain (TK1 and TK2)^[16]. Most involve the JM domain (exon 11) and consist mostly of deletions or point mutations. Mutations in the JM domain affect KIT's autoregulatory function and promote spontaneous kinase activation^[17]. Exon 9 (EC domain) mutations are the second most common mutations followed by exon 13 (TK1 domain) and exon 17 (TK2 domain) mutations.

In approximately 10% of GISTs, no c-kit mutations are found even when the whole coding region is examined. In 2003, Heinrich *et al.*^[18] and Hirota *et al.*^[19] found gain-of-function mutations of the *PDGFR-A* gene in about half of the GISTs lacking c-kit mutations. A majority of PDGFR-A mutations affect the TK2 domain (exon 18). These mutations change the activation loop, which regulates the ATP-binding pocket and leads to kinase activation^[6]. Ac-



Figure 1 Endoscopic appearance of a jejunal gastrointestinal stromal tumor (GIST).

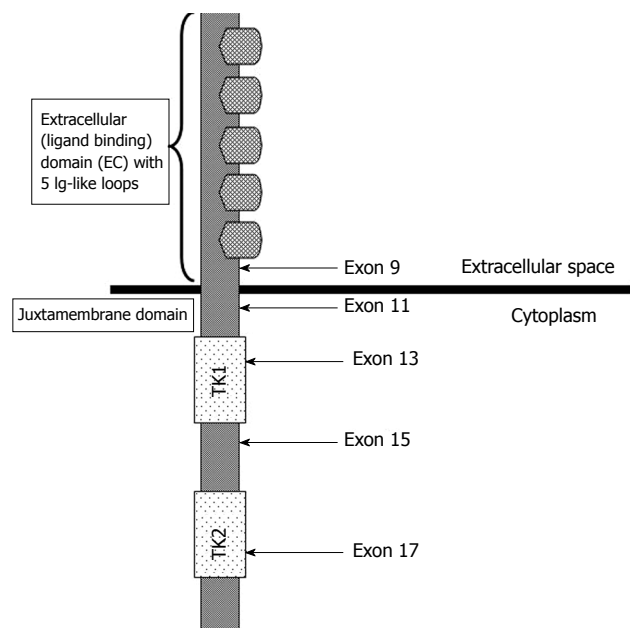


Figure 2 KIT structure and localization of common KIT mutations. Ig: Immunoglobulin.

tivated PDGFR-A activates the same signaling pathways that KIT activates, leading to GIST.

IMATINIB

Imatinib mesylate (henceforth referred to as imatinib) is a small-molecule TK inhibitor. It works by binding to ATP binding sites of KIT, PDGFR-A, and bcr-abl fusion product, consequently inhibiting their activity^[20].

Imatinib is rapidly absorbed in the GI tract, reaching maximum plasma concentrations 2-4 h after oral intake. Absolute bioavailability of imatinib is 98% and food does not affect its absorption^[21]. Imatinib is metabolized mainly by the cytochrome P450 (CYP) 3A4 enzymes in the liver, with minor contributions from other CYP enzymes and is eliminated mainly via the feces, mostly in the form of metabolites^[22]. As a result of intrinsic variability of CYP enzyme activity, there is a high inter-patient variability for imatinib exposure. Accordingly, imatinib trough levels at

day 29 were recently reported to be associated with clinical outcome^[23]. Concomitant administration of CYP3A4 inhibitors (e.g. ketoconazole) may increase and CYP3A4 inducers (e.g. rifampin) may decrease imatinib systemic exposure. Conversely, imatinib may inhibit the metabolism of CYP3A4 substrates (e.g. simvastatin), thereby increasing their exposure. Imatinib may also increase systemic exposure to paracetamol by inhibiting its O-glucuronidation^[21,24].

In May 2001, the US Food and Drug Administration (FDA) approved imatinib for chronic myelogenous leukemia (CML) based on excellent responses as first- and second-line treatments^[25]. Following demonstration of its activity against KIT and successful treatment of a patient with a GIST^[26], imatinib was evaluated in an open label, randomized, multicenter trial. Of 147 patients with unresectable or metastatic GISTs who were randomly assigned to receive either 400 or 600 mg imatinib daily, 79 (54%) had a partial response and 41 (28%) had stable disease. None of the patients had a complete response. There were no significant differences in the rate or duration of responses between the 2 dose levels of imatinib that were tested. The estimated 1-year overall survival rate was 88% for all patients^[27]. As a result of this study, the FDA approved the use of imatinib in advanced GISTs in 2002. Recently, a long-term follow-up analysis of the same trial demonstrated that median duration of response was 27 mo and median overall survival was 58 mo^[28].

In the same phase II trial, the 18F-fluoro-2-deoxy-D-glucose uptake in the tumors was found to be decreased markedly from baseline as early as 24 h after a single dose of imatinib. The median time to an objective response was 13 wk^[27]. Accordingly, in the Euro-Australasian phase III trial, the median time to best response was 15 wk, with most responses occurring within the first 9 mo. However, best responses were occasionally observed after as long as 2 years of treatment suggesting that responses to imatinib can be seen late in the treatment^[29].

Blay *et al.*^[30] investigated whether imatinib could be stopped after 1 year in patients who responded to imatinib and concluded that treatment with imatinib should continue until disease progression, patient intolerance or refusal. In this study, 58 patients with advanced GISTs were randomized to continuous and intermittent treatment arms. Twenty-six (81%) of the 32 patients in the intermittent arm had progressed and were restarted on imatinib. Of these 26 patients, 24 had a response or stable disease after imatinib was restarted. At the time of the final analysis, there was no difference in overall survival between continuous and intermittent treatment arms^[30].

Adjuvant and neoadjuvant therapy with imatinib

Imatinib showed promising results in adjuvant therapy of GISTs. However, there is an ongoing debate on the duration, dose, and the selection of patients for adjuvant imatinib therapy. It is assumed that GIST patients with a relatively high risk of recurrence would benefit the most from adjuvant treatment. Several models were constructed to estimate the recurrence risk of resected GISTs.

Table 1 Risk stratification of primary GISTs

Tumor feature		Risk of tumor progression	
Mitotic index	Size (cm)	Stomach	Small bowel ¹
< 5/50 HPF	≤ 2	Very low	Very low
	> 2 ≤ 5	Very low	Low
	> 5 ≤ 10	Low	Moderate
	> 10	Moderate	High
≥ 5/50 HPF	≤ 2	Very low	Moderate
	> 2 ≤ 5	Moderate	High
	> 5 ≤ 10	High	High
	> 10	High	High

Modified from Hornick *et al.*^[33] Copyrighted permission from Elsevier Inc.
¹GISTs arising from other sites should probably be stratified as small bowel tumors. GISTs: Gastrointestinal stromal tumors; HPF: High power fields.

Parameters commonly used were tumor size and mitotic rate^[4,31]. Based on the reports of a higher recurrence risk of small bowel GISTs compared to gastric ones^[32], a new risk stratification model incorporating tumor location was proposed^[12,33] (Table 1). In addition to estimation of the recurrence risk, in the near future, mutation analyses from tumor specimens may help practitioners to select different agents according to different genotypes, customize both the duration and the dose of imatinib or other effective agents in the adjuvant setting.

After a phase II trial (ACOSOG Z9000) demonstrated high tolerability and promising efficacy in high risk (tumor size > 10 cm, tumor rupture, or multifocal disease) GIST patients^[34,35], a phase III trial (ACOSOG Z9001) was initiated to compare 400 mg imatinib daily for 1 year with placebo in patients with KIT-expressing GISTs measuring at least 3 cm that were grossly resected. Accrual to the trial was halted early because of the better relapse-free survival (RFS) in the treatment arm in the interim analysis, and these results were presented at the 2007 ASCO meeting leading the FDA to approve imatinib for adjuvant treatment of GISTs in the USA^[36]. Final results showed a 1-year RFS of 97% and 83% in imatinib and placebo arms, respectively, with a hazard ratio (HR) of 0.35 (95% confidence interval, 0.22-0.53)^[37]. However, the risk reduction in patients with a tumor diameter between 3 and 6 cm was nearly zero. Secondly, there was no difference in overall survival between the 2 groups. It remains unclear whether imatinib prevents GIST recurrence or merely delays it. While 2 phase II trials from Korea and China confirmed the improved RFS in intermediate- and high-risk GIST patients treated with imatinib in an adjuvant setting^[38,39], 2 other phase III trials are still ongoing: EORTC 62024 comparing imatinib for 2 years *vs* placebo, and SSG XVIII /AIO trial comparing imatinib for 3 years *vs* 1 year. Long-term follow-up results will be needed to demonstrate the adjuvant treatment effect on overall survival, the subsets of patients that would benefit, and the optimal duration of the treatment. Table 2 demonstrates the adjuvant/neoadjuvant trials registered by the National Cancer Institute (USA).

On the neoadjuvant side, early results from the phase II RTOG 0132 trial established the feasibility of the ap-

Table 2 Adjuvant and neoadjuvant trials of imatinib in patients with GISTs

Trial	Accrual	Eligibility	Therapy (n)	End points
Phase II study of adjuvant imatinib mesylate in patients with completely resected high-risk primary GIST (ACOSOG-Z9000)	Closed	Diameter > 10 cm or tumor rupture or multifocal	Imatinib 400 mg daily for 1 year (107)	2-year OS: 97%, 2-year RFS: 73% ^[33]
Phase III randomized study of adjuvant imatinib mesylate in patients with resected primary GIST (ACOSOG-Z9001)	Closed	Diameter > 3 cm	Imatinib 400 mg daily for 1 year (359) Placebo (354)	1-year RFS: 98% ^[36] 1-year RFS: 83%
EORTC soft tissue and bone sarcoma group (EORTC-62024) randomized phase III trial	Closed	Diameter > 5 cm or mitotic rate > 5/50 HPF	Imatinib 400 mg daily for 2 years Observation (Total projected 750)	Primary: OS Secondary: RFS and safety
Scandinavian sarcoma group trial SSGXV III	Closed	Diameter > 10 cm or mitotic rate > 10/50 HPF or > 5 cm and > 5/50 HPF or tumor rupture	Imatinib 400 mg daily for 36 mo Imatinib 400 mg daily for 12 mo (Total projected 280)	Primary: RFS Secondary: OS, safety
Phase II study of neoadjuvant and adjuvant imatinib mesylate in patients with primary or recurrent potentially resectable malignant GIST (RTOG-S0132)	Closed	Locally advanced or metastatic/recurrent	Imatinib 600 mg daily for 6-8 wk followed by debulking/resection (52)	2-year PFS: 80%, objective response rate: 6%, R0 resection in 65% ^[39]
Five year adjuvant imatinib mesylate in GIST (Phase II)	Open	Diameter > 2 cm and mitotic rate > 5/50 HPF or non-gastric GIST > 5 cm	Imatinib 400 mg daily for 5 years (Projected 133)	Primary: Time to recurrence Secondary: Safety
Phase II study of neoadjuvant imatinib mesylate in patients with locally advanced gastrointestinal stromal tumor (Germany/Austria)	Open	Locally advanced, KIT expressing, histologically confirmed GIST	Imatinib 400 mg daily/BID (Projected 40)	Primary: ORR Secondary: R0-resectability and organ-preserving resectability

OS: Overall survival; RFS: Relapse-free survival; PFS: Progression-free survival.

proach in locally advanced and metastatic GIST patients^[40]. Fiore *et al*^[41] recently reported that all of the 15 patients who received preoperative imatinib benefited from neoadjuvant treatment: one patient had a complete response; 3 patients who were initially considered to have unresectable disease underwent complete resection; 7 patients with initial indication for extensive surgery were more conservatively operated on; 4 patients initially deemed at high perioperative risk underwent safe surgery^[41]. For patients that do not have access to the ongoing neoadjuvant trials (Table 2), we consider preoperative imatinib as an option for those with unresectable GISTs or in whom surgical morbidity would be improved by reducing the size of the tumor preoperatively. Positron emission tomography (PET) scans within 2-4 wk of therapy could be a predictive test separating responding from resistant patients. Non-responding patients, if surgically resectable, would be taken to surgery. Patients that are not surgical candidates could be considered for second-line therapy.

Toxicity

Almost all patients in clinical trials with imatinib experienced adverse events; however, most of the events were mild or mild to moderate. Patients treated with 800 mg/d imatinib had more grade 3 or above side effects compared to those treated with 400 mg/d in both phase III trials comparing 2 different doses. Most common grade 3-4 side effects in these 2 phase III trials were anemia, granulocytopenia, fatigue, fluid retention, muscle pain, and gastrointestinal symptoms^[29,42]. In the Euro-Australasian trial,

dose reductions and treatment interruptions, mostly because of toxic effects, were found to be more likely to occur in patients who received imatinib 400 mg twice daily. Additionally, side effects were mostly recorded during the first 8 wk of treatment^[43].

Fluid retention due to imatinib is generally mild, most frequently affects the periorbital region, and can be seen in 70%-80% of patients treated with imatinib. It responds well to intermittent low dose thiazide diuretics. On the other hand, muscle cramps can be seen in up to 40% of patients and can be very troublesome as a result of their long-term course. There are anecdotal reports of patients responding to magnesium/calcium supplements^[44], quinine^[45], and chlorthalidone^[46]. Hypophosphatemia can also be seen with imatinib use, and routine monitoring of phosphate levels has been suggested^[47]. In 2006, 10 individuals were reported to develop severe heart failure after receiving imatinib^[48]. However, whether imatinib causes cardiac toxicity in GIST patients is still unclear. Multiple randomized studies failed to show excess cardiotoxicity in patients treated with imatinib postoperatively^[37,49]. Tumor hemorrhage is a life-threatening toxicity for which patients with large, bulky tumors have a higher risk. Therefore, hemoglobin levels should be monitored upon starting GIST patients on imatinib.

Imatinib resistance

The advent of imatinib had completely changed the management and survival of patients with GISTs. However, imatinib resistance, both primary and secondary, is still a

Table 3 Prevalence of *c-kit* and *PDGFR-A* mutations and clinical responses in advanced GISTs to imatinib and sunitinib correlated with mutational status

Genotype	Prevalence ^[59-61]	Clinical benefit from imatinib ^[15,58,59]	Clinical benefit from sunitinib ^{[58]1}
KIT exon 9 mutation (%)	5-14	74-81	60
KIT exon 11 mutation (%)	57-69	83-93	35
KIT exon 13 mutation (%)	< 5	60-100 (few cases)	65
KIT exon 17 mutation (%)	< 5	75-80 (few cases)	
PDGFR-A mutations (%)	3-8	40-66	0 (few cases)
Both wild-type (%)	5-10	33-73	55

Clinical benefit is defined as complete, partial response or stable disease. ¹In imatinib-resistant GIST.

significant problem. In the Euro-Australasian phase III trial, 12% of patients exhibited initial resistance to imatinib^[29]. Furthermore, more than 40% of patients who were initially responsive to imatinib developed late resistance after a median follow-up of 25 mo.

Several different mechanisms of imatinib resistance were identified in the literature: (1) KIT or PDGFRA mutations resulting in intrinsic target resistance; (2) genomic amplification leading to target overexpression; and (3) alternate oncoprotein activation supplanting target expression^[24,50,51]. During imatinib treatment, resistance often develops as a result of secondary mutations, primarily in the kinase domains of KIT or PDGFR-A^[52-54]. One of the frequent secondary kinase domain mutations, V654A mutation, is intrinsically imatinib-resistant^[55].

Depending on the mechanism of the resistance, a higher dose of imatinib may be considered in patients with GISTs resistant to 400 mg/d imatinib. The effect of increasing the dose of imatinib to 800 mg daily in advanced GIST patients was assessed in 2 phase III trials. In the Euro-Australasian trial of 946 patients, Verweij *et al*^[29] reported that although there was no difference in response rates between daily and twice daily 400 mg dosing, progression-free survival was significantly better in patients who received the higher dose. On the other hand, the results of the phase III trial in North America did not reveal any difference between daily and twice daily 400 mg dosing in response rates, progression-free and overall survival. However, 31% of patients who progressed on 400 mg daily dosing and crossed over to 400 mg twice daily dosing had a partial response or stable disease suggesting that in a subset of patients increasing the imatinib dose may overcome the resistance to imatinib^[42].

In the North American phase III trial, patients with exon 11 mutations had a better response rate, time to progression and overall survival compared to those with exon 9 mutations and the wild-type *c-kit* gene. Although the survival rates of exon 9, exon 11 or wild-type GISTs were not affected by imatinib dose, the response rate for patients with an exon 9 mutation was significantly higher in those who were treated with a higher dose of imatinib (67% *vs* 17%, $P = 0.02$)^[56]. Furthermore, in a meta-analysis of 2 phase III trials, 800 mg/d imatinib was found to be associated with a significantly better progression-free survival in patients with the exon 9 mutation (median progression-free survival: 1.6 year *vs* 0.5 year; HR, 0.58,

$P = 0.017$)^[57]. Table 3 demonstrates the prevalence and response rates of mutations in different exons of *KIT* and *PDGFR-A* genes derived from 3 different clinical trials^[15,58,59], population^[60] and institution based studies^[61]. La-sota *et al*^[62] had published a detailed review on the role of *c-kit* and *PDGFR-A* gene mutations on GIST therapy^[62].

SUNITINIB

Sunitinib malate, an oral multitargeted inhibitor of KIT, PDGFRs, vascular endothelial growth factor receptors (VEGFR), FLT3 and RET receptor TKs, is FDA approved for treatment of imatinib-resistant GISTs and of advanced renal cell carcinoma. In addition to its antitumor effect through inhibition of KIT and PDGFR, sunitinib may have an antiangiogenic effect on GISTs through inhibition of VEGFR^[63].

In a phase I / II trial, sunitinib at different dosages and schedules was administered to 97 GIST patients with imatinib resistance (96%) or intolerance (4%). In phase I, sunitinib 50 mg/d for 4 wk followed by 2 wk off treatment was chosen for further development. Overall, 73% of patients experienced stable disease which lasted > 6 mo and 7% achieved a partial response. The median overall survival was 19 mo^[64].

The pivotal, randomized, placebo-controlled, multinational phase III trial of sunitinib in patients with unresectable, imatinib-resistant GISTs had to be unblinded early when interim data analysis showed a significantly longer median time to progression in patients who received sunitinib compared to those who received placebo (27.3 wk *vs* 6.4 wk; HR, 0.33, $P < 0.001$). Response rate was better in patients treated with sunitinib compared to those treated with placebo (6.8% *vs* 0%, $P = 0.006$). Despite the crossover after unblinding, overall survival was superior in the sunitinib arm (HR, 0.49; $P < 0.007$). This study established the role of sunitinib as a second-line therapy in patients with advanced imatinib-refractory and imatinib-intolerant GISTs^[65].

Although a clinical benefit of sunitinib treatment was observed in all major mutant types, the primary response rate was significantly higher for KIT exon 9 mutants. The inhibitory effect of sunitinib was not substantially affected by KIT mutations in TK1 whereas GISTs with KIT-TK2 mutations were resistant to sunitinib treatment. Specifically, the imatinib-resistant KIT mutation V654A was highly sensitive to sunitinib^[66,67].

Table 4 Novel agents being developed for GIST therapy

Agent	Molecular target	Clinical benefit in pilot studies
Kinase inhibitors		
Nilotinib	KIT, PDGFRs, bcr-abl	46%-77% ^[69,70]
Sorafenib	Raf, KIT, PDGFR-B, VEGFR, FLT3, RET	71% ^[71]
Dasatinib	Src, ABL, KIT, PDGFRs	Phase II ongoing in advanced sarcomas and accepting patients
AZD2171	VEGFR, KIT, PDGFRs	Phase II ongoing, not recruiting
OSI-930	VEGFR, KIT	Phase I ongoing, not recruiting
PTK787	VEGFR, KIT, PDGFRs	67% ^[74]
XL820	KIT, PDGFR-B, VEGFR	Phase II ongoing, not recruiting
AMG706	VEGFR, KIT, PDGFRs, RET	24%-27% ^[75,76]
mTOR and AKT inhibitors		
Perifosine	AKT	Phase II ongoing in combination with imatinib
Everolimus	mTOR	26% ^[73]
Temsirolimus	mTOR	Phase II ongoing, closed recruitment
Others		
IPI-504	Hsp90	78% ^[72] , phase III ended due to safety concerns
Flavopiridol	Transcription inhibitor	Phase I ongoing in combination with doxorubicin

Clinical benefit is defined as complete or partial response or stable disease.

Sunitinib was generally well tolerated in patients with GISTs, with adverse events mild to moderate in severity. In the phase III trial, the most frequent treatment-related adverse events were fatigue, diarrhea, hand-foot syndrome, rash, nausea, anorexia, and skin discoloration. Hypertension occurred in about 20% of treated patients, 5% being grade 3/4 in severity. Treatment discontinuation because of adverse events occurred in 9% and 8% of sunitinib and placebo recipients, respectively^[65]. Hypothyroidism, possibly secondary to inhibition of the protein product of the RET proto-oncogene found in normal thyroid has been described in as high as 36% of sunitinib-treated GIST patients^[68].

FUTURE DIRECTIONS

Currently, there is no established third-line treatment in patients with GISTs who failed to respond to both imatinib and sunitinib. Table 4 demonstrates the drugs in the pipeline for GIST therapy^[69-76]. Nilotinib, a second-generation TK inhibitor engineered to inhibit KIT, PDGFR-A, and bcr-abl, is approved for the treatment of imatinib-resistant CML. In a phase I study of nilotinib in imatinib-resistant GISTs, 13 (72%) of the 18 patients who received nilotinib experienced stable disease lasting more than 6 wk. One patient achieved a partial response. Median progression-free survival was 25 wk. Nilotinib 400 mg twice daily was the dose recommended for future studies^[69]. Recently, Montemurro *et al.*^[70] reported a retrospective analysis of 52 patients treated with nilotinib 400 mg twice daily as compassionate use in 12 European centers. All patients had failed both imatinib and sunitinib either due to resistance (96%) or intolerance (4%). One and 4 patients achieved complete and partial responses, respectively; whereas 19 had disease stabilization. The median progression-free survival was 12 wk (range 2 d-104 wk) and the median overall survival was 34 wk (range 2-135 wk). Most patients tolerated nilotinib well except 6

(12%) patients discontinued treatment due to grade 2-3 adverse effects^[70].

The efficacy of sorafenib, a multi-kinase inhibitor of raf kinase, VEGFR, PDGFR, and KIT, in imatinib- and sunitinib-resistant GISTs was studied in a phase II trial of 26 patients. Three (13%) patients achieved a partial response and 14 patients (58%) experienced stable disease. Median progression-free survival was 5.3 mo and median overall survival was 13.0 mo. The most common side effects were hand-foot syndrome (28%), hypertension (24%), and rash (20%)^[71].

Heat shock proteins (Hsp) control the proper folding, function, and stabilization of various proteins. Laboratory studies have demonstrated that inhibition of Hsp90 results in selective destruction of the mutated KIT in human GIST cell lines. In a phase I trial of IPI-504, an intravenous Hsp90 inhibitor, 78% of evaluable patients with imatinib-resistant (100%) and sunitinib-resistant (95%) GISTs experienced stable disease^[72]. However, a phase III trial of IPI-504 ended early because of safety concerns. Other strategies to inhibit growth of GISTs is to abrogate KIT mRNA expression with flavopiridol, a transcriptional inhibitor^[77]; and inhibition of the AKT-mTOR pathway by mTOR inhibitors such as everolimus^[73].

CONCLUSION

The discovery of near universal KIT protein expression, and activating KIT and PDGFR-A mutations advanced our understanding of GISTs significantly. Later, the finding of the high efficacy of imatinib in the treatment of GISTs changed the management and prognosis of this once very deadly malignancy. Imatinib is currently approved for the treatment of unresectable or metastatic GISTs, however, many centers around the world, including our own, use imatinib also in adjuvant and neoadjuvant settings. PET scans may be especially helpful in identifying imatinib-resistant tumors during neoadjuvant treatment. Further

studies are needed to tailor the imatinib treatment in these settings. On the other hand, imatinib should be continued until disease progression or intolerance in patients with metastatic or unresectable GISTs.

The standard therapy for metastatic GISTs resistant to imatinib is sunitinib. The efficacy of sunitinib in the first-line treatment of advanced GISTs and as adjuvant/neoadjuvant treatment should be studied in randomized trials. Resistance to imatinib and sunitinib continues to be a challenging issue. Upcoming treatment options are second-generation kinase inhibitors and inhibition of the growth of GISTs via pathways other than KIT and PDGFR- α . We believe the combination of Hsp90 inhibitors and kinase inhibitors could yield superior results.

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REFERENCES

- Joensuu H, Fletcher C, Dimitrijevic S, Silberman S, Roberts P, Demetri G. Management of malignant gastrointestinal stromal tumours. *Lancet Oncol* 2002; **3**: 655-664
- DeMatteo RP, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg* 2000; **231**: 51-58
- Nilsson B, Bümming P, Meis-Kindblom JM, Odén A, Drott A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era—a population-based study in western Sweden. *Cancer* 2005; **103**: 821-829
- Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465
- Tran T, Davila JA, El-Serag HB. The epidemiology of malignant gastrointestinal stromal tumors: an analysis of 1,458 cases from 1992 to 2000. *Am J Gastroenterol* 2005; **100**: 162-168
- Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. *Lancet* 2007; **369**: 1731-1741
- Mazur MT, Clark HB. Gastric stromal tumors. Reappraisal of histogenesis. *Am J Surg Pathol* 1983; **7**: 507-519
- Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; **279**: 577-580
- Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J* 1987; **6**: 3341-3351
- Rosnet O, Marchetto S, deLapeyriere O, Birnbaum D. Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. *Oncogene* 1991; **6**: 1641-1650
- Fletcher JA. Role of KIT and platelet-derived growth factor receptors as oncoproteins. *Semin Oncol* 2004; **31**: 4-11
- Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70-83
- Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol* 2005; **29**: 52-68
- Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Demetri GD, Fletcher CD, Fletcher JA. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001; **61**: 8118-8121
- Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003; **21**: 4342-4349
- Longley BJ, Reguera MJ, Ma Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* 2001; **25**: 571-576
- Chan PM, Ilangumaran S, La Rose J, Chakrabartty A, Rottapel R. Autoinhibition of the kit receptor tyrosine kinase by the cytosolic juxtamembrane region. *Mol Cell Biol* 2003; **23**: 3067-3078
- Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299**: 708-710
- Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* 2003; **125**: 660-667
- Lyseng-Williamson K, Jarvis B. Imatinib. *Drugs* 2001; **61**: 1765-1774; discussion 1775-1776
- Novartis Pharmaceuticals Corporation. Product information (US): Gleevec® (imatinib mesylate) tablets 100 and 400mg [online]. Accessed 2009 May 22. Available from: URL: <http://www.fda.gov/cder>
- Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. *Clin Pharmacokinet* 2005; **44**: 879-894
- Demetri GD, Wang Y, Wehrle E, Racine A, Nikolova Z, Blanke CD, Joensuu H, von Mehren M. Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors. *J Clin Oncol* 2009; **27**: 3141-3147
- Siddiqui MA, Scott LJ. Imatinib: a review of its use in the management of gastrointestinal stromal tumours. *Drugs* 2007; **67**: 805-820
- Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M, Druker B, Goldman J, O'Brien SG, Russell N, Fischer T, Ottmann O, Cony-Makhoul P, Facon T, Stone R, Miller C, Tallman M, Brown R, Schuster M, Loughran T, Gratwohl A, Mandelli F, Saglio G, Lazzarino M, Russo D, Baccarani M, Morra E. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002; **346**: 645-652
- Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 2001; **344**: 1052-1056
- Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002; **347**: 472-480
- Blanke CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg BL, Fletcher J, Corless CL, Wehrle E, Sandau KB, Joensuu H. Long-term follow-up of a phase II randomized trial in advanced gastrointestinal stromal tumor (GIST) pa-

- tients (pts) treated with imatinib mesylate. *J Clin Oncol* 2006; **24** (18S): 9528
- 29 **Verweij J**, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, Issels R, van Oosterom A, Hogendoorn PC, Van Glabbeke M, Bertulli R, Judson I. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* 2004; **364**: 1127-1134
 - 30 **Blay JY**, Le Cesne A, Ray-Coquard I, Bui B, Duffaud F, Delbaldo C, Adenis A, Viens P, Rios M, Bompas E, Cupissol D, Guillemet C, Kerbrat P, Fayette J, Chabaud S, Berthaud P, Perol D. Prospective multicentric randomized phase III study of imatinib in patients with advanced gastrointestinal stromal tumors comparing interruption versus continuation of treatment beyond 1 year: the French Sarcoma Group. *J Clin Oncol* 2007; **25**: 1107-1113
 - 31 **Huang HY**, Li CF, Huang WW, Hu TH, Lin CN, Uen YH, Hsiung CY, Lu D. A modification of NIH consensus criteria to better distinguish the highly lethal subset of primary localized gastrointestinal stromal tumors: a subdivision of the original high-risk group on the basis of outcome. *Surgery* 2007; **141**: 748-756
 - 32 **Miettinen M**, Makhlof H, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. *Am J Surg Pathol* 2006; **30**: 477-489
 - 33 **Hornick JL**, Fletcher CD. The role of KIT in the management of patients with gastrointestinal stromal tumors. *Hum Pathol* 2007; **38**: 679-687
 - 34 **DeMatteo RP**, Owzar K, Antonescu CR, Maki R, Demetri GD, McCarter M, von Mehren M, Pisters P, Brennan MF, Ballman KV. Efficacy of adjuvant imatinib mesylate following complete resection of localized, primary gastrointestinal stromal tumor (GIST) at high risk of recurrence: the U.S. Intergroup phase II trial ACOSOG Z9000. American Society of Clinical Oncology 2008 Gastrointestinal Cancers Symposium, 25-27 January 2008, Orlando, FL
 - 35 **DeMatteo RP**, Antonescu CR, Chadaram V, You YN, McCall L, Maki R, Murgo A, Demetri G, Pisters P, Brennan MF; American College of Surgeons Oncology Group (ACOSOG). Adjuvant imatinib mesylate in patients with primary high risk gastrointestinal stromal tumor (GIST) following complete resection: safety results from the U.S. intergroup phase II trial ACOSOG Z9000. *J Clin Oncol* 2005; **23** (16S): 818
 - 36 **DeMatteo RP**, Owzar K, Maki R, Pisters P, Blackstein M, Antonescu C, Blanke C, Demetri G, von Mehren M, Ballman K. American College of Surgeons Oncology Group (ACOSOG) Intergroup Adjuvant GIST Study Team. Adjuvant imatinib mesylate increases recurrence free survival (RFS) in patients with completely resected localized primary gastrointestinal stromal tumor (GIST): North American Intergroup Phase III trial ACOSOG Z9001. 2007 ASCO Annual Meeting Abstract, A10079
 - 37 **DeMatteo RP**, Ballman KV, Antonescu CR, Maki RG, Pisters PW, Demetri GD, Blackstein ME, Blanke CD, von Mehren M, Brennan MF, Patel S, McCarter MD, Polikoff JA, Tan BR, Owzar K. Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial. *Lancet* 2009; **373**: 1097-1104
 - 38 **Kang Y**, Kang B, Ryu M, Im S, Park S, Kang W, Kim T, Oh D, Jung K, Lee J. A phase II study of imatinib mesylate as adjuvant treatment for curatively resected high-risk localized gastrointestinal stromal tumors with c-kit exon 11 mutation. American Society of Clinical Oncology 2009 Gastrointestinal Cancers Symposium, Abstract 95
 - 39 **Li J**, Gong FJ, Li J, Wu WA, Shen L. Adjuvant therapy with imatinib in gastrointestinal stromal tumor (GIST) patients with intermediate or high risk: Analysis from a single-center contrast study. *J Clin Oncol* 2009; **27** (15S): A10556
 - 40 **Eisenberg BL**, Harris J, Blanke CD, Demetri GD, Heinrich MC, Watson JC, Hoffman JP, Okuno S, Kane JM, von Mehren M. Phase II trial of neoadjuvant/adjuvant imatinib mesylate (IM) for advanced primary and metastatic/recurrent operable gastrointestinal stromal tumor (GIST): early results of RTOG 0132/ACRIN 6665. *J Surg Oncol* 2009; **99**: 42-47
 - 41 **Fiore M**, Palassini E, Fumagalli E, Pilotti S, Tamborini E, Stacchiotti S, Pennacchioli E, Casali PG, Gronchi A. Preoperative imatinib mesylate for unresectable or locally advanced primary gastrointestinal stromal tumors (GIST). *Eur J Surg Oncol* 2009; **35**: 739-745
 - 42 **Blanke CD**, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, Raymond AK, Bramwell VH, Baker LH, Maki RG, Tanaka M, Hecht JR, Heinrich MC, Fletcher CD, Crowley JJ, Borden EC. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J Clin Oncol* 2008; **26**: 626-632
 - 43 **Verweij J**, van Oosterom A, Blay JY, Judson I, Rodenhuis S, van der Graaf W, Radford J, Le Cesne A, Hogendoorn PC, di Paola ED, Brown M, Nielsen OS. Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. *Eur J Cancer* 2003; **39**: 2006-2011
 - 44 **Deininger MW**, O'Brien SG, Ford JM, Druker BJ. Practical management of patients with chronic myeloid leukemia receiving imatinib. *J Clin Oncol* 2003; **21**: 1637-1647
 - 45 **Zekri JM**, Robinson MH, Woll PJ. Relative hypocalcaemia and muscle cramps in patients receiving imatinib for gastrointestinal stromal tumour. *Sarcoma* 2006; **2006**: 48948
 - 46 **Medeiros BC**, Lipton JH. Chlordiazepoxide for imatinib-induced muscular cramps. *Eur J Haematol* 2006; **77**: 538
 - 47 **Berman E**, Nicolaides M, Maki RG, Fleisher M, Chanel S, Scheu K, Wilson BA, Heller G, Sauter NP. Altered bone and mineral metabolism in patients receiving imatinib mesylate. *N Engl J Med* 2006; **354**: 2006-2013
 - 48 **Kerkela R**, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med* 2006; **12**: 908-916
 - 49 **Verweij J**, Casali PG, Kotasek D, Le Cesne A, Reichardt P, Judson IR, Issels R, van Oosterom AT, Van Glabbeke M, Blay JY. Imatinib does not induce cardiac left ventricular failure in gastrointestinal stromal tumours patients: analysis of EORTC-ISC-AGITG study 62005. *Eur J Cancer* 2007; **43**: 974-978
 - 50 **Fletcher JA**, Corless CL, Dimitrijevic S. Mechanisms of resistance to imatinib mesylate in advanced gastrointestinal stromal tumor [Abstract no. 3275]. *Proc Am Soc Clin Oncol* 2003; **22**: 815
 - 51 **Debiec-Rychter M**, Cools J, Dumez H, Sciot R, Stul M, Mentens N, Vranckx H, Wasag B, Prenen H, Roels J, Hagemeyer A, Van Oosterom A, Marynen P. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology* 2005; **128**: 270-279
 - 52 **Chen LL**, Trent JC, Wu EF, Fuller GN, Ramdas L, Zhang W, Raymond AK, Prieto VG, Oyedede CO, Hunt KK, Pollock RE, Feig BW, Hayes KJ, Choi H, Macapinlac HA, Hittelman W, Velasco MA, Patel S, Burgess MA, Benjamin RS, Frazier ML. A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. *Cancer Res* 2004; **64**: 5913-5919
 - 53 **McLean SR**, Gana-Weisz M, Hartzoulakis B, Frow R, Whelan J, Selwood D, Boshoff C. Imatinib binding and cKIT inhibition is abrogated by the cKIT kinase domain I missense mutation Val654Ala. *Mol Cancer Ther* 2005; **4**: 2008-2015
 - 54 **Tamborini E**, Bonadiman L, Greco A, Albertini V, Negri T, Gronchi A, Bertulli R, Colechia M, Casali PG, Pierotti MA,

- Pilotti S. A new mutation in the KIT ATP pocket causes acquired resistance to imatinib in a gastrointestinal stromal tumor patient. *Gastroenterology* 2004; **127**: 294-299
- 55 **Heinrich MC**, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K, McDougall K, Ou WB, Chen CJ, Fletcher JA. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006; **24**: 4764-4774
- 56 **Heinrich MC**, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD, Ryan CW, von Mehren M, Blanke CD, Rankin C, Benjamin RS, Bramwell VH, Demetri GD, Bertagnolli MM, Fletcher JA. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol* 2008; **26**: 5360-5367
- 57 **van Glabbeke MM**, Owzar K, Rankin C, Simes J, Crowley J. GIST Meta-analysis Group (MetaGIST). Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors (GIST): A meta-analysis based on 1,640 patients (pts). *J Clin Oncol* 2007; **25** (18S): A10004
- 58 **Heinrich MC**, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, Town A, McKinley A, Ou WB, Fletcher JA, Fletcher CD, Huang X, Cohen DP, Baum CM, Demetri GD. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; **26**: 5352-5359
- 59 **Debiec-Rychter M**, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, Blay JY, Leyvraz S, Stul M, Casali PG, Zalcberg J, Verweij J, Van Glabbeke M, Hagemeijer A, Judson I. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2006; **42**: 1093-1103
- 60 **Steigen SE**, Eide TJ, Wasag B, Lasota J, Miettinen M. Mutations in gastrointestinal stromal tumors--a population-based study from Northern Norway. *APMIS* 2007; **115**: 289-298
- 61 **Penzel R**, Aulmann S, Moock M, Schwarzbach M, Rieker RJ, Mechttersheimer G. The location of KIT and PDGFRA gene mutations in gastrointestinal stromal tumours is site and phenotype associated. *J Clin Pathol* 2005; **58**: 634-639
- 62 **Lasota J**, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. *Histopathology* 2008; **53**: 245-266
- 63 **Faivre S**, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**: 25-35
- 64 **Morgan JA**, Demetri GD, Fletcher JA. Patients with imatinib mesylate-resistant GIST exhibit durable responses to sunitinib malate (SU11248). 13th European Cancer Conference; 2005 Oct 30-Nov 3, Paris, A1456
- 65 **Demetri GD**, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368**: 1329-1338
- 66 **Heinrich MC**, Corless CL, Liegl B, Fletcher CD, Raut CP, Donsky R, Bertagnolli MM, Harlow A, Demetri GD, Fletcher JA. Mechanisms of sunitinib malate (SU) resistance in gastrointestinal stromal tumors (GISTs). *J Clin Oncol* 2007; **25** (18S): 10006
- 67 **Heinrich MC**, Maki RG, Corless CL, Antonescu CR, Fletcher JA, Fletcher CD, Huang X, Baum CM, Demetri GD. Sunitinib (SU) response in imatinib-resistant (IM-R) GIST correlates with KIT and PDGFRA mutation status. ASCO Annual Meeting Proceedings Part I. *J Clin Oncol* 2006; **24** (18S): 9502
- 68 **Desai J**, Yassa L, Marqusee E, George S, Frates MC, Chen MH, Morgan JA, Dychter SS, Larsen PR, Demetri GD, Alexander EK. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. *Ann Intern Med* 2006; **145**: 660-664
- 69 **von Mehren M**, Reichardt P, Casali PG, Blay J, Debiec-Rychter M, Dumez H, Cheung W, Feifel B, Veronese M, Demetri GD. A phase I study of nilotinib alone and in combination with imatinib (IM) in patients (pts) with imatinib-resistant gastrointestinal stromal tumors (GIST) - Study update. *J Clin Oncol* 2007; **25** (18S): 10023
- 70 **Montemurro M**, Schöffski P, Reichardt P, Gelderblom H, Schütte J, Hartmann JT, von Moos R, Seddon B, Joensuu H, Wendtner CM, Weber E, Grünwald V, Roth A, Leyvraz S. Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib. *Eur J Cancer* 2009; **45**: 2293-2297
- 71 **Wiebe L**, Kasza KE, Maki RG, D'Adamo DR, Chow WA, Wade JL, Agamah E, Stadler WM, Vokes EE, Kindler HL. Activity of sorafenib (SOR) in patients (pts) with imatinib (IM) and sunitinib (SU)-resistant (RES) gastrointestinal stromal tumors (GIST): A phase II trial of the University of Chicago Phase II Consortium. *J Clin Oncol* 2008; **26** (18S): 10502
- 72 **Wagner AJ**, Morgan JA, Chugh R, Rosen LS, George S, Gordon MS, Devine CM, van den Abbeele AD, Grayzel D, Demetri GD. Inhibition of heat shock protein 90 (Hsp90) with the novel agent IPI-504 in metastatic GIST following failure of tyrosine kinase inhibitors (TKIs) or other sarcomas: Clinical results from phase I trial. *J Clin Oncol* 2008; **26** (18S): 10503
- 73 **von Mehren M**. Beyond imatinib: second generation c-KIT inhibitors for the management of gastrointestinal stromal tumors. *Clin Colorectal Cancer* 2006; **6** Suppl 1: S30-S34
- 74 **Joensuu H**, De Braud F, Coco P, De Pas T, Putzu C, Spreafico C, Bono P, Bosselli S, Jalava T, Laurent D, Casali PG. Phase II, open-label study of PTK787/ZK222584 for the treatment of metastatic gastrointestinal stromal tumors resistant to imatinib mesylate. *Ann Oncol* 2008; **19**: 173-177
- 75 **Benjamin RS**, Schöffski P, Hartmann JT. Initial results of a multicenter single arm phase 2 study of AMG 706, an oral multikinase inhibitor, for the treatment of advanced imatinib-resistant gastrointestinal stromal tumors (GIST) [abstract 641]. 12th Annual Meeting of the Connective Tissue Oncology Society; November 2-4, 2006; Venice, Italy
- 76 **Yamada Y**, Sawaki A, Nishida T, Komatsu Y, Kanda T, Doi T, Koseki M, Baba H, Asami Y, Ohtsu T. Phase II study of motesanib diphosphonate (AMG 706) in Japanese patients with advanced gastrointestinal stromal tumors (GIST) who developed progressive disease or relapsed while on imatinib mesylate. 2008 ASCO Gastrointestinal Cancers Symposium; January 25-27, 2008; Orlando, FL, A107
- 77 **Sambol EB**, Ambrosini G, Geha RC, Kennealey PT, Decarolis P, O'Connor R, Wu YV, Motwani M, Chen JH, Schwartz GK, Singer S. Flavopiridol targets c-KIT transcription and induces apoptosis in gastrointestinal stromal tumor cells. *Cancer Res* 2006; **66**: 5858-5866

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Magnetic resonance imaging for local complications of acute pancreatitis: A pictorial review

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Abstract

Acute pancreatitis is a common disease characterized by sudden upper abdominal pain and vomiting. Alcoholism and choledocholithiasis are the most common factors for this disease. The choice of treatment for acute pancreatitis might be affected by local complications, such as local hemorrhage in or around the pancreas, and peripancreatic infection or pseudoaneurysm. Diagnostic imaging modalities for acute pancreatitis have a significant role in confirming the diagnosis of the disease, helping detect the extent of pancreatic necrosis, and for diagnosing local complications. Magnetic resonance imaging (MRI) might be indicated in acute pancreatitis for detecting and characterizing local complications of acute pancreatitis that involve necrotic, hemorrhagic, infectious, vascular, and pseudocyst disorders. The general MRI sequences for pancreatitis require the combined use of T1-weighted, T2-weighted sequences, and magnetic resonance chol-

angiopancreatography. For imaging of pancreatic necrosis, the combination of T1-weighted and T2-weighted findings with dynamic contrast-enhanced imaging gives a comprehensive evaluation of the extent of necrosis and full range of inflammatory extension. For imaging of infectious complications, dynamic contrast-enhanced examinations might help differentiate pancreatic cellulitis or abscesses, from pancreatic fluid collection or simple pseudocysts. For vascular abnormalities, the combination of cross-sectional pancreatic parenchyma imaging with MRA represents a single diagnostic modality for the full evaluation of peripancreatic artery and vein involvement, such as arterial pseudoaneurysms and venous thromboses. The purpose of this pictorial review is to examine the MRI appearances of various local complications of acute pancreatitis and to discuss the practical setup of MRI in local complications of acute pancreatitis.

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Key words: Pancreas; Pancreatitis; Complication; Magnetic resonance imaging; Pictorial review

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INTRODUCTION

Acute pancreatitis is a common and protean disease characterized by sudden upper abdominal pain and vomiting,

and is triggered by the leakage of activated pancreatic digestive enzymes. Alcoholism and choledocholithiasis are the most common physiological factors for acute pancreatitis^[1,2]. Traditional management of acute pancreatitis tends to promote the use of conservative management. However, the choice of treatment can be influenced by local complications, such as local hemorrhage in or around the pancreas, and peripancreatic infection or pseudoaneurysm^[2,3].

Increased levels of serum and/or urinary pancreatic amylase and lipase have been detected in most individuals with acute pancreatitis after the onset of symptoms. Therefore, acute pancreatitis is often diagnosed by clinical manifestations and laboratory examinations. However, diagnostic imaging modalities for acute pancreatitis have a significant role in confirming the diagnosis of the disease, helping detect the extent of pancreatic necrosis, and for diagnosing local complications^[2,3].

Among a variety of imaging tools, computed tomography (CT) is considered the standard of reference for patients with acute pancreatitis; however, it has an increased radiation burden that can result from follow-up examinations^[4] and displays different results for the potential aggravation of acute pancreatitis resulting from the use of iodinated contrast media^[5-7]. The specific reasons for using magnetic resonance imaging (MRI) are as follows: (1) MRI is a diagnostic imaging method with no radiation hazard, which might be suitable for patients with multiple follow-up reviews; (2) MRI is a reliable method of staging the severity of acute pancreatitis, which has predictive value for the prognosis of the disease. It also has fewer contraindications than CT^[8]; (3) MR cholangiopancreatography (MRCP) has the unique capability of providing noninvasive images of the pancreatic ducts and can demonstrate possible communication of a pancreatic pseudocyst with pancreatic ducts^[9]; and (4) MRI in combination with MR angiography (MRA), a single diagnostic modality, is useful for assessing the signal consistency of fluid exudation or pseudocysts to identify local hemorrhage in or around the pancreas or pseudoaneurysm, which might help plan the surgery.

However, the possible drawbacks of MRI with respect to CT are cost and time consumption. Moreover, several MRI sequences require patients to hold their breath, which might be difficult for patients with severe acute pancreatitis.

MRI of pancreatitis and its complications has been reported^[10]. However, to our knowledge, there is no report thoroughly assessing MRI for local complications of acute pancreatitis, including necrotic, hemorrhagic, infectious, vascular, and pseudocyst disorders.

In this pictorial essay, after briefly discussing the MRI technique, we review the MRI appearances of various local complications of acute pancreatitis, ranging from relatively common necrotic and hemorrhagic conditions to rare conditions, such as peripancreatic abscess and pseudoaneurysm. Knowledge of the classic MRI findings of these complications allows prompt recognition of the related pathologic condition, and might influence the choice of treatment.

MRI TECHNIQUE

For the appropriate evaluation of local complications of acute pancreatitis, it is necessary to assess all parts of the pancreas, including the peripancreatic parenchyma and the vasculature^[1]. The general MRI sequences for pancreatitis require the combined use of T1-weighted sequences [e.g. fast spin-echo (FSE) imaging with multiple breath-hold acquisitions or single-breath-hold gradient echo (GRE) imaging], T2-weighted sequences [e.g. fast recovery fast spin-echo (FRFSE) or single-shot fast spin-echo (SSFSE) imaging], and MRCP (e.g. a thick-slab, SSFSE MRCP). The advantages or limitations of these sequences are as follows: (1) T1-weighted scan with fat suppression improves the delineation of pancreatic borders and the pancreas itself, and it has important value in MRI acquisition for evaluation of hemorrhagic complications of acute pancreatitis^[3]. However, T1-weighted sequences with breath holding require patient cooperation, otherwise, respiratory artifacts will occur, which might affect the visualization of the whole pancreas and its adjacent structure^[1-3]; (2) T2-weighted sequences have significant advantages in demonstrating fluid-filled lesions in or around the pancreas and the pancreatic duct, and SSFSE T2-weighted sequence can be used to guide acquisition of an MRCP series^[2,3]. Using respiratory triggered or navigator-echo-based techniques allows the investigating of less cooperative patients; and (3) MRCP is obtained before gadolinium administration, allows noninvasive evaluation of pancreatic ducts and the whole extrahepatic biliary tract, and provides few respiratory artifacts or susceptibility effects^[1-3]. However, the main weakness of MRCP for ductal visibility is the potential overlap of other fluid-containing organs (e.g. the stomach and duodenum)^[3].

The methodology of contrast-enhanced MRI for patients with acute pancreatitis in our institute is detailed as follows. A 19-gauge needle was placed in the right antecubital vein. A dose of 0.2 mL/kg Gd-DTPA (469.01 mg/mL Bayer Schering Pharma AG; Berlin, Germany) was then administered. A bolus of 20 mL of physiological saline was given immediately following gadolinium administration. The contrast agent and physiological saline were injected at an infusion rate of 3 mL/s with a power injector (Spectris MR Injector System; Medrad, USA). After gadolinium chelate injection, the optimal dynamic contrast-enhanced method [e.g. T1-weighted acquisition performed with liver acquisition with volume acceleration (LAVA)] was used to assess the extent of pancreatic necrosis for the initial staging of acute pancreatitis, which has been shown to correlate with the course of the disease^[1-3]. MRI LAVA scanning starts at 14-16 s after the intravenous administration of gadolinium. It costs a total of 65 s for three-phase dynamic contrast-enhanced scans (15 s for each phase and 10 s of the interval of phases for breathing). A total of (80-112) × 3 contrast-enhanced frames were obtained. Moreover, MRA, the post-processing technique after MRI LAVA, can be performed to supplement the information for visualization of pancreatic vascular network

Table 1 MRI parameters

Sequence	TR (ms)	TE (ms)	Slice thickness (mm)	Slice space (mm)	Matrix	Field of view (mm)	Number of excitation	Flip angle
FSE T1W	300	10	5	0.5-1	256 × 192	380 × 340	2	90°
GRE T1W	195	1.5	5-7	0.5-1	256 × 192	360 × 340	1	70°
FRFSE T2W	8000-13 500	90-120	5	0.5-1	256 × 224	400 × 340	1	90°
SSFSE T2W	Not applicable	80-197	5	0.5	256 × 192	390 × 330	1	90°
SSFSE MRCP	Not applicable	830-1100	40-50	0	256 × 192	320 × 240	2	90°
LAVA T1W	3.8-6.1	1.8-2.1	5 ¹	0	256 × 224	380 × 340	1	15°-20°

¹A section thickness of 5 mm for LAVA is the section thickness of automatic reconstruction images from this sequence. MRI: Magnetic resonance imaging; FSE: Fast spin-echo; GRE: Gradient echo; FRFSE: Fast recovery fast spin-echo; SSFSE: Single-shot fast spin-echo; LAVA: Liver acquisition with volume acceleration; T1W: T1-weighted; T2W: T2-weighted.

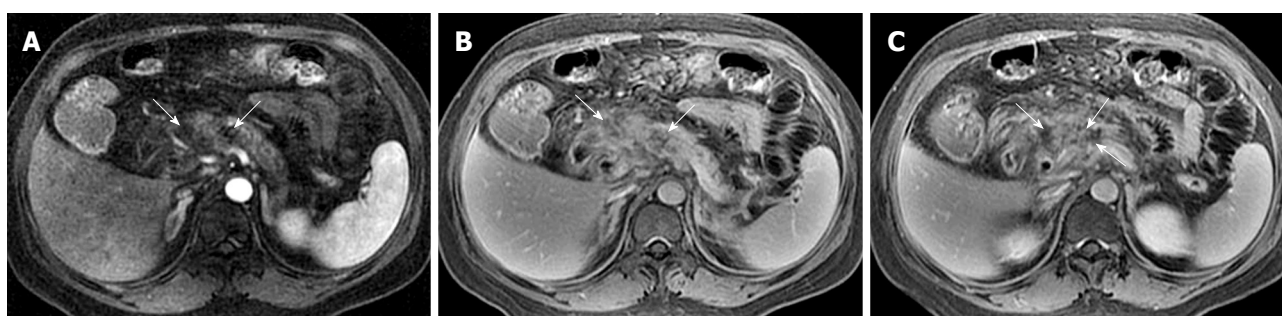


Figure 1 43-year-old woman with acute pancreatitis. Contrast-enhanced axial T1-weighted images during arterial phase (A) and late venous phase (B, C) show spotted, patchy non-enhanced areas in the pancreatic head and body (arrows).

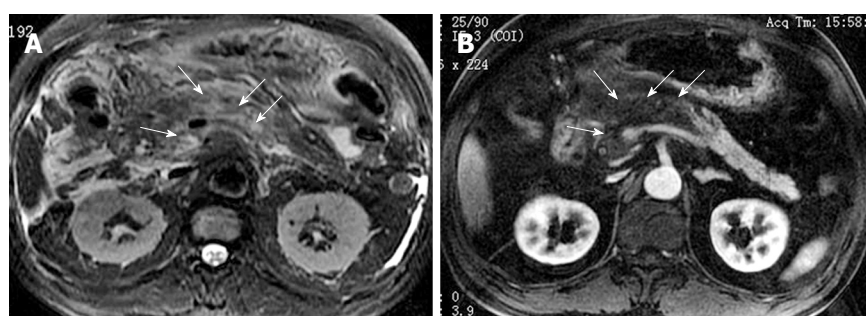


Figure 2 65-year-old man with acute pancreatitis. Axial T2-weighted image (A) shows the high-signal intensity in the pancreatic head and body (arrows). The arterial phase MR image (B) shows a large necrotic area ("black pancreas") in the pancreatic head and body (arrows).

and vascular complications of acute pancreatitis. The detailed protocols used with our 1.5-T MR imager for these sequences are listed in Table 1.

MRI FINDINGS

Necrosis

Pancreatic necrosis refers to a pathological collection of devitalized tissue in the pancreas, which is a relatively common local complication of acute pancreatitis^[10].

Necrosis is diagnosed when focal or diffuse, conspicuous hypointense areas on T1WI corresponding to hyperintense areas on T2WI and well-margined areas of non-enhancing pancreatic parenchyma in comparison to the signal intensity of the normal pancreatic parenchyma are present^[1-3]. Dynamic contrast-enhanced MRI examination can identify surviving pancreatic tissue^[10]. The focal pancreatic necrosis is characterized by multiple spotted, patchy non-enhancing areas (like "pepper") on

contrast-enhanced MR images (Figure 1). The large diffuse non-enhanced area of the pancreas (the so-called "black pancreas") in acute pancreatitis on enhanced MR images represents diffuse pancreatic necrosis (Figure 2).

Necrosis of peripancreatic fatty tissue, which is a form of inflammatory extension, involves the spread of peripancreatic intra-abdominal fatty tissue and adipose tissue in the retroperitoneal space^[2]. The covering of the pancreas is surrounded by the formation of the capsule, which is only a series of loose connective tissue. Therefore, the pancreatic capsule forms almost no block on the role of inflammatory extension. MRI might show the intra-abdominal inflammatory involvement located in omental or mesenteric fatty contents regions (Figure 3). The affected retroperitoneal space manifests the inflammation extension from the retroperitoneal space to anterior pararenal space of the left kidney, which is complicated by retroperitoneal steatonecrosis (Figure 4)^[2,3]. It is important and helpful in evaluating the prognosis of acute pancre-

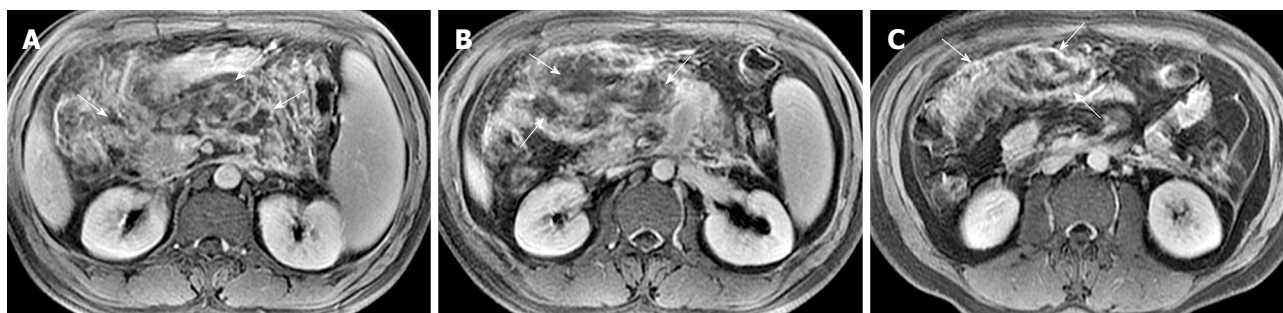


Figure 3 34-year-old man with acute pancreatitis. Contrast-enhanced axial T1-weighted images show peripancreatic inflammation extension (arrows) along the mesenteric adipose tissue region (A, B). There is addition irregular thickening and enhancement of the adjacent intestinal wall (arrows, C).



Figure 4 31-year-old man with acute pancreatitis. Contrast-enhanced axial T1-weighted images with fat suppression reveal thickening and enhancement of dorsal peritoneum (arrow, A). The peripancreatic inflammation extended from the retroperitoneal space to the anterior pararenal space of the left kidney (arrows, B and C).



Figure 5 30-year-old man with acute pancreatitis. Unenhanced axial T1-weighted image with fat suppression shows patchy hemorrhagic foci (arrows) in the pancreas and in the fatty tissue behind the pancreas.

atitis to differentiate between necrosis of peripancreatic tissue and central pancreas necrosis. Neoptolemos *et al*^[11] reported that the evaluation of the integrity of the main pancreatic duct might be helpful in making a distinction between mild pancreatitis and severe pancreatitis, and for determining the necessity for surgery for local pancreatic complications in severe pancreatitis.

Hemorrhage

Hemorrhage of the pancreas is seen in 2%-5% of patients with acute pancreatitis and might occur in the setting of severe necrotizing pancreatitis^[10]. With conversion from hemoglobin to methemoglobin in the hemorrhage regions, MRI findings of hemorrhagic lesions show spotted or patchy high signal intensity (like “salt”) in T1-weighted images with fat suppression (Figure 5).

Peripancreatic hemorrhage might be secondary to the necrosis of peripancreatic tissue, which is visualized as patchy or large diffuse areas with hyperintensity on T1-weighted images with fat suppression^[2] (Figure 6). Martin *et al*^[12] reported that elevated peripancreatic signal on fat-suppressed T1-weighted spoiled gradient echo images is associated with poor outcome in acute pancreatitis. This might represent a simplified imaging method in regards to its relationship to clinical prognosis. Other local complications of acute pancreatitis, such as pancreatic pseudocysts and peripancreatic vascular invasion, can also show hemorrhage (mentioned below).

Infection

Pancreatic cellulitis is an infectious complication in patients with acute pancreatitis, whose incidence varies from 8.3% to 10.6%, and can be difficult to differentiate clinically from pancreatic abscess^[13,14]. It is a clinical or radiological entity in the pancreas and peripancreatic region as a result of pathological pancreatic swelling, inflammatory cell infiltration, and tissue necrosis^[15]. Contrast-enhanced MRI examination can show the range of the involvement of pancreatic cellulitis. MRI findings of pancreatic cellulitis include the formation of an ill-defined mass with ring-like and separated enhancement (like “Hornet’s nest”), and non-enhanced regions within the central part of the mass (Figure 7). Pancreatic cellulitis can be absorbed completely and might also progress to a pancreatic abscess.

Pancreatic abscess is a typical infectious manifestation of acute necrotizing pancreatitis, whose incidence varies from 1% to 9%, and can result in high mortality in acute

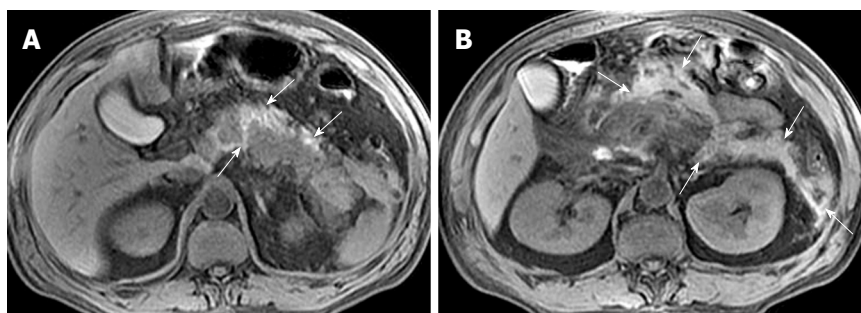


Figure 6 50-year-old man with acute pancreatitis. Unenhanced axial T1-weighted images with fat suppression reveal pancreatic and peripancreatic hemorrhage (arrows, A and B); Peripancreatic hemorrhage involvement include the retroperitoneal space and anterior pararenal space of the left kidney (arrows, B).

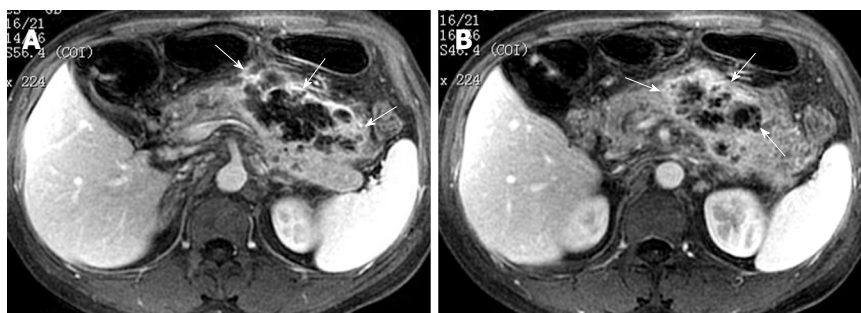


Figure 7 39-year-old man with acute necrotizing pancreatitis and peripancreatic cellulitis. Contrast-enhanced T1-weighted images show a ring-like and separated enhancing mass in pancreatic adjacent retroperitoneal space (arrows, A and B).

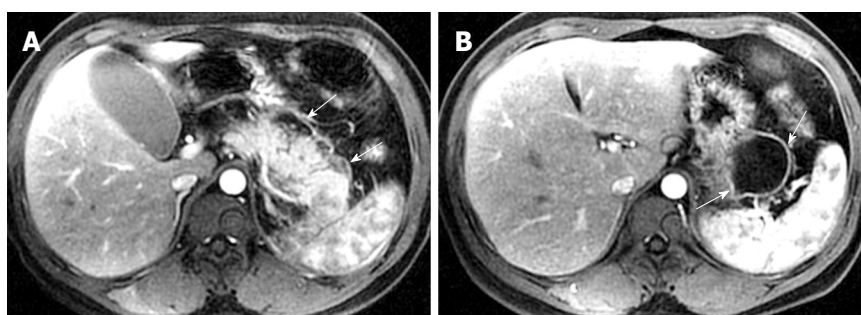


Figure 8 33-year-old man with acute necrotizing pancreatitis and peripancreatic abscess. Contrast-enhanced axial T1-weighted images reveal abnormal peripancreatic enhancement (arrows, A) and a liquid-like mass with a well-defined, thickened, and enhancing wall (arrows, B).

pancreatitis patients^[16]. If pancreatic cellulitis was not well controlled, abscesses might occur 3-5 wk after the onset of acute pancreatitis and might be similar to pseudocysts^[10]. Pancreatic abscesses with acute pancreatitis induce a systemic infection poisoning reaction resulting in symptoms of fever and local peritonitis. The performance of a pancreatic abscess on MRI might present as an encapsulated liquid-form mass with a significantly enhancing wall (Figure 8).

Pseudocyst

Pancreatic pseudocysts are local complications of acute or chronic pancreatitis, and they can be classified as acute pseudocysts in patients suffering from acute post-necrotic pancreatitis, and chronic pseudocysts that develop in patients already suffering from chronic pancreatitis^[17]. The pathogenesis of pancreatic pseudocysts might be associated with an actively secreting pancreas in acute or chronic pancreatitis; an encapsulated cyst communicating with pancreatic ducts and/or partial pancreatic ductal obstruction. Patients with pancreatic pseudocysts are treated conservatively or percutaneously with aspiration under ultrasound guidance or surgically with external catheter drainage^[17].

Pancreatic pseudocysts might be located within the pancreatic tissue or surrounding the pancreas. They often occur approximately four weeks after a first episode of

acute pancreatitis^[18-20]. MRI findings of a simple pseudocyst include a round or oval fluid collection surrounded by a thin or thick wall, with liquid signal performance of hypointensity on T1-weighted images and hyperintensity on T2-weighted images (Figure 9). Pancreatic pseudocysts can also present as complex pseudocysts associated with mucus, protein, and hemorrhage^[2]. This often manifests as a heterogeneous lesion dominated by hyperintensity on T1-weighted images with fat suppression (Figure 10). Therefore, compared with CT, MRI might be more favorable when the a suspected pseudocyst with bleeding occurs.

Vascular disorders

Vascular complications of acute pancreatitis represent a spectrum of vascular abnormalities, such as peripancreatic artery involvement including pseudoaneurysm, and vein invasion including phlebotrombosis^[1-4]. Dörffel *et al*^[21] assessed vascular complications in acute pancreatitis by color duplex ultrasonography: the incidence of arterial pseudoaneurysms was 7% in acute pancreatitis, and the incidence of venous thromboses was 30% in acute non-necrotizing pancreatitis and 57% in necrotizing pancreatitis. MRI findings of artery invasion consist of the loss of normal vascular flowing void effect, the blurred edge of the local involving arterial wall and the

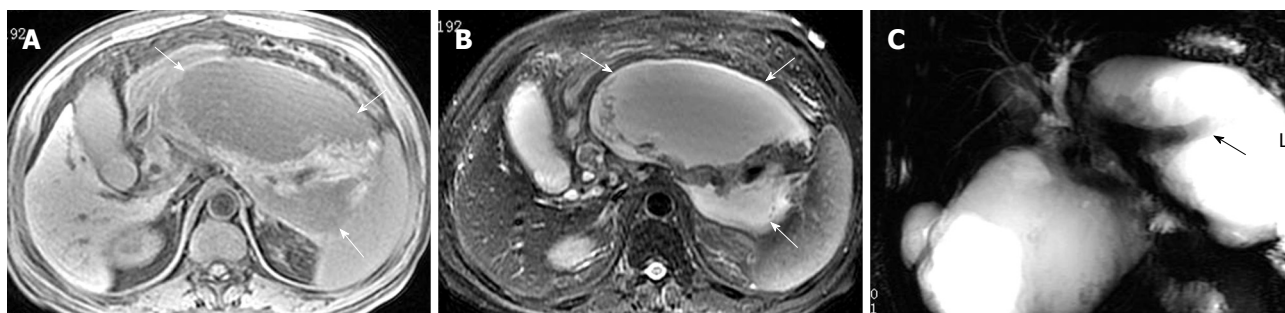


Figure 9 41-year-old man with a large peripancreatic pseudocyst who presented as abdominal distension for follow-up after acute pancreatitis. Unenhanced axial T1-weighted (A) and axial T2-weighted (B) images show a large lesion with typical liquid signal intensity around the pancreas (arrows). MRCP image (C) reveals the pancreas wrapped inside the large pseudocyst, just like the swimming fish (arrow).



Figure 10 35-year-old man with multiple pseudocysts complicated hemorrhage. Patient had previously suffered from acute pancreatitis. Unenhanced axial T1-weighted images (A and B) with fat suppression and axial T2-weighted image (C) with fat suppression reveal hemorrhagic pseudocysts with a mixed signal intensity dominated by hyperintensity (arrows).

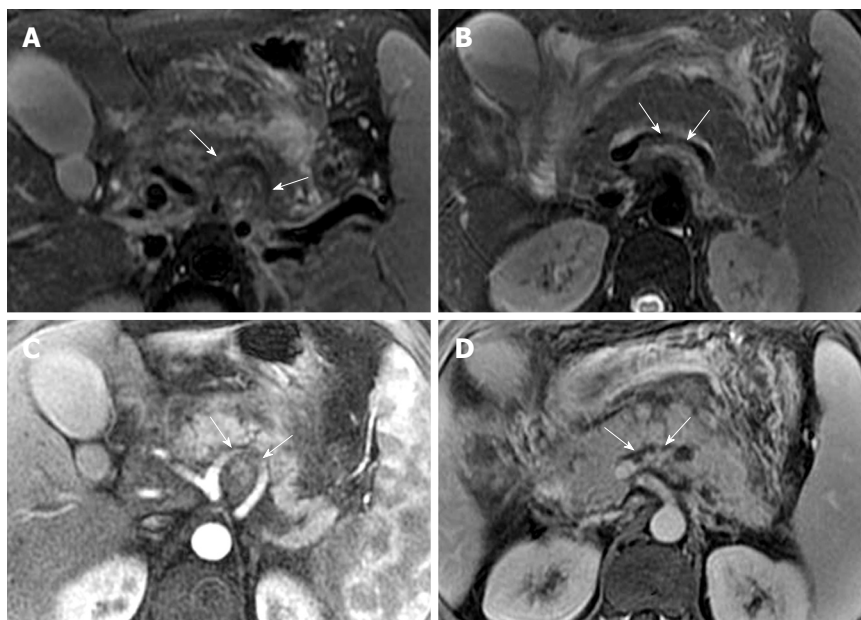


Figure 11 30-year-old man with acute pancreatitis and splenomegaly. Axial T2-weighted images (A and B) show the involved segments (arrows) of splenic artery (A) and splenic vein (B). Enhanced arterial phase (C) and venous phase (D) images reveal clearly the involved segments (arrows) of the splenic artery (C) and vein (D).

poor enhancement at the involving segment on contrast-enhanced arterial phase images (Figure 11). The splenic artery, gastroduodenal artery, and pancreaticoduodenal artery are more frequently involved^[10,22].

Pseudoaneurysm is an uncommon, usually delayed, complication of acute pancreatitis and might constitute a life-threatening emergency if rupture occurs^[10]. MRI can directly show the pseudoaneurysm cavity that communi-

cates with the adjoining arteries and the mural thrombosis in the pseudoaneurysm cavity. On enhanced MR images, the enhancement of pseudoaneurysm cavity corresponds with the arteries, whereas the non-enhanced region is illustrated as the mural thrombus (resembling a Chinese “Yin-Yang” diagram)^[23] (Figure 12).

The splenic vein is the most common vein invaded by pancreatic inflammatory extensions due to its prox-

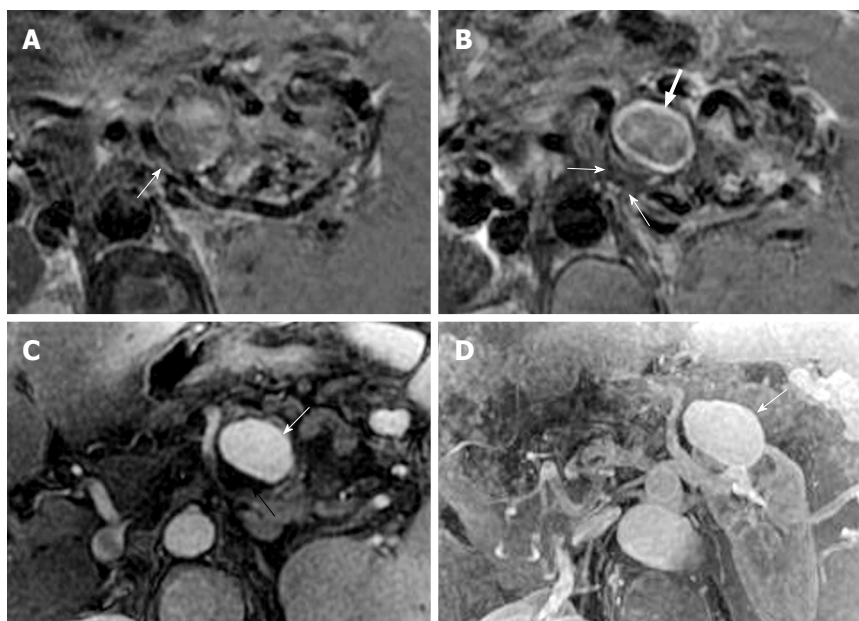


Figure 12 36-year-old man with a history of acute pancreatitis and a splenic artery pseudoaneurysm. The involved segment of the splenic artery (arrow, A) and an aneurysmal dilatation structure (large arrow, B) are seen on axial T2-weighted images. On enhanced arterial phase image (C), the enhancement of the pseudoaneurysm cavity (white arrow) and the crescent-form filling defect (black arrow) are similar to the Chinese “Yin-Yang” diagram. This pseudoaneurysm is also illustrated on contrast-enhanced MR angiography (arrow, D).

imity to the pancreas^[10,22]. The involving vein segment might be complicated by local venous thrombosis. MRI shows the loss of normal vascular flowing void effect at this involving vein segment. After administration of a contrast agent, an intravenous filling defect can be seen on enhanced venous phase images (Figure 11).

Practical setup of MRI in local complications of acute pancreatitis

Cross-sectional T2-weighted imaging and contrast-enhanced fat-suppressed T1-weighted imaging combined with MRCP can be used to assess the severity of acute pancreatitis before therapeutic planning^[1-3]. However, dynamic contrast-enhanced MRI in acute pancreatitis is not routinely performed, because, for the majority of patients, pancreatic change is easy to establish with various T1-weighted and T2-weighted sequences in transverse and coronal sections. Therefore, in our current pancreatic imaging practice, the decision process as to whether patients receive dynamic studies is as follows: (1) For imaging of pancreatic necrosis, the combination of T1-weighted and T2-weighted findings with dynamic contrast-enhanced appearances represents a comprehensive evaluation of the extent of necrosis and full range of inflammatory extension. We recommend that enhanced-MRI is essential; (2) For imaging of infectious complications, dynamic contrast-enhanced examinations might help differentiate pancreatic cellulitis or abscesses from pancreatic fluid collection or simple pseudocysts; and (3) For imaging of vascular abnormalities, the combination of cross-sectional pancreatic parenchyma imaging with MRA offers the possibility of a single diagnostic modality for the full evaluation of peripancreatic artery and vein involvement, such as arterial pseudoaneurysms and venous thromboses.

CONCLUSION

In summary, MRI might be indicated in acute pancreati-

tis for detecting and characterizing local complications of acute pancreatitis. Enhanced-MRI is essential for a comprehensive evaluation of the extent of pancreatic necrosis.

REFERENCES

- 1 Balthazar EJ, Freeny PC, vanSonnenberg E. Imaging and intervention in acute pancreatitis. *Radiology* 1994; **193**: 297-306
- 2 Lenhart DK, Balthazar EJ. MDCT of acute mild (nongangrenous) pancreatitis: abdominal complications and fate of fluid collections. *AJR Am J Roentgenol* 2008; **190**: 643-649
- 3 Matos C, Cappeliez O, Winant C, Coppens E, Devière J, Metens T. MR imaging of the pancreas: a pictorial tour. *Radiographics* 2002; **22**: e2
- 4 Blackstone MO. Contrast medium worsens pancreatitis? *Gastroenterology* 1994; **107**: 321-322
- 5 McMenamin DA, Gates LK Jr. A retrospective analysis of the effect of contrast-enhanced CT on the outcome of acute pancreatitis. *Am J Gastroenterol* 1996; **91**: 1384-1387
- 6 Uhl W, Roggo A, Kirschstein T, Anghelacopoulos SE, Gloor B, Müller CA, Malfertheiner P, Büchler MW. Influence of contrast-enhanced computed tomography on course and outcome in patients with acute pancreatitis. *Pancreas* 2002; **24**: 191-197
- 7 Hwang TL, Chang KY, Ho YP. Contrast-enhanced dynamic computed tomography does not aggravate the clinical severity of patients with severe acute pancreatitis: reevaluation of the effect of intravenous contrast medium on the severity of acute pancreatitis. *Arch Surg* 2000; **135**: 287-290
- 8 Arvanitakis M, Delhaye M, De Maertelaere V, Bali M, Winant C, Coppens E, Jeanmart J, Zalcmann M, Van Gansbeke D, Devière J, Matos C. Computed tomography and magnetic resonance imaging in the assessment of acute pancreatitis. *Gastroenterology* 2004; **126**: 715-723
- 9 Gillams AR, Kurzawinski T, Lees WR. Diagnosis of duct disruption and assessment of pancreatic leak with dynamic secretin-stimulated MR cholangiopancreatography. *AJR Am J Roentgenol* 2006; **186**: 499-506
- 10 Miller FH, Keppke AL, Dalal K, Ly JN, Kamler VA, Sica GT. MRI of pancreatitis and its complications: part 1, acute pancreatitis. *AJR Am J Roentgenol* 2004; **183**: 1637-1644
- 11 Neoptolemos JP, London NJ, Carr-Locke DL. Assessment of main pancreatic duct integrity by endoscopic retrograde

- pancreatography in patients with acute pancreatitis. *Br J Surg* 1993; **80**: 94-99
- 12 **Martin DR**, Karabulut N, Yang M, McFadden DW. High signal peripancreatic fat on fat-suppressed spoiled gradient echo imaging in acute pancreatitis: preliminary evaluation of the prognostic significance. *J Magn Reson Imaging* 2003; **18**: 49-58
- 13 **Fan ST**, Choi TK, Chan FL, Lai EC, Wong J. Pancreatic phlegmon: what is it? *Am J Surg* 1989; **157**: 544-547
- 14 **Maroun Marun C**, Uscanga L, Lara F, Passareli L, Quiroz-Ferrari F, Robles-Díaz G, Campuzano-Fernández M. [Pancreatic phlegmon: a potentially fatal form of acute pancreatitis] *Rev Invest Clin* 1992; **44**: 507-512
- 15 **Kune GA**, King R. The late complications of acute pancreatitis: pancreatic swelling, cyst and abscess. *Med J Aust* 1973; **1**: 1241-1246
- 16 **Hill MC**, Dach JL, Barkin J, Isikoff MB, Morse B. The role of percutaneous aspiration in the diagnosis of pancreatic abscess. *AJR Am J Roentgenol* 1983; **141**: 1035-1038
- 17 **Andrén-Sandberg A**, Dervenis C. Pancreatic pseudocysts in the 21st century. Part I: classification, pathophysiology, anatomic considerations and treatment. *JOP* 2004; **5**: 8-24
- 18 **Merkle EM**, Görich J. Imaging of acute pancreatitis. *Eur Radiol* 2002; **12**: 1979-1992
- 19 **Robinson PJ**, Sheridan MB. Pancreatitis: computed tomography and magnetic resonance imaging. *Eur Radiol* 2000; **10**: 401-408
- 20 **Kim YH**, Saini S, Sahani D, Hahn PF, Mueller PR, Auh YH. Imaging diagnosis of cystic pancreatic lesions: pseudocyst versus nonpseudocyst. *Radiographics* 2005; **25**: 671-685
- 21 **Dörffel T**, Wruck T, Rückert RI, Romaniuk P, Dörffel Q, Wermke W. Vascular complications in acute pancreatitis assessed by color duplex ultrasonography. *Pancreas* 2000; **21**: 126-133
- 22 **Mortelé KJ**, Mergo PJ, Taylor HM, Wiesner W, Cantisani V, Ernst MD, Kalantari BN, Ros PR. Peripancreatic vascular abnormalities complicating acute pancreatitis: contrast-enhanced helical CT findings. *Eur J Radiol* 2004; **52**: 67-72
- 23 **Lupattelli T**. The yin-yang sign. *Radiology* 2006; **238**: 1070-1071

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Unlocking the ultrastructure of colorectal cancer cells *in vitro* using selective staining

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Abstract

AIM: To characterise differences between three widely used colorectal cancer cell lines using ultrastructural selective staining for glycogen to determine variation in metastatic properties.

METHODS: Transmission electron microscopy was used in this investigation to help identify intracellular structures and morphological features which are precursors of tumor invasion. In addition to morphological markers, we used selective staining of glycogen as a marker for neoplastic cellular proliferation and determined whether levels of glycogen change between the three different cell lines.

RESULTS: Ultrastructural analysis revealed morphological differences between the cell lines, as well as differentiation into two sub-populations within each

cell line. Caco-2 cells contained large glycogen deposits as well as showing the most obvious morphological changes between the two sub-populations. SW480 cells also contained large glycogen stores as well as deep cellular protrusions when grown on porous filter membranes. HT-29 cells had trace amounts of glycogen stores with few cellular projections into the filter pores and no tight junction formation.

CONCLUSION: Morphology indicative of metastatic properties coincided with larger glycogen deposits, providing strong evidence for the use of selective staining to determine the neoplastic properties of cells.

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Key words: Cancer; Colorectal; Electron microscopy; Glycogen; Potassium ferrocyanide

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INTRODUCTION

Cultured human colon carcinoma Caco-2, HT-29, and SW480 cell lines have been used as model cell lines by many researchers to investigate the cell biology and metastatic properties of colorectal cancer. The main tissue of origin of these cell lines, the colon, is a structure comprising a dynamic epithelium where there is continuous

renewal of normal colonic epithelial cells that originate in the crypts (secretory cells) and migrate to the tips of the villous folds (absorptive cells)^[1]. This, therefore, results in cellular heterogeneity of all epithelial cells that are derived from any human adenocarcinoma cell line. The present study is designed to examine these heterogeneous features ultrastructurally within the three chosen cell lines to determine whether varying malignant characteristics are expressed.

All epithelia form a barrier made of polarized cells in which the apical and basolateral domains of the cells can be separated into functional domains^[2]. Cell adhesion is critical in the organisation of normal epithelia, in particular the establishment of the adherens junction, mediated by the presence of E-cadherin, which initiates a cascade of events leading to the formation of the tight junction, gap junction, and desmosomes, all key regulators of the junctional complex^[3-5] and mediators of cellular polarisation. The loss of these structures results in the breakdown of the epithelium, leading to increased permeability and pathogenesis of disease^[4]. An ultrastructural investigation into the various features of the junctional complex will provide evidence of cellular communication and adhesion to determine whether cellular polarisation is present.

Additionally, the invasive properties of cells are also reflected by cell shape, whereby epithelioid cells appear to be less invasive than spindle-shaped cells^[6] due to cellular polarisation and establishment of a tight junction. Additional ultrastructural assays that might help determine the differences in the invasive properties of the aforementioned cell lines is their ability to leave the compartment to which they are normally restricted and to determine whether cells have the potential to carry out invasive growth. By culturing these cells on porous membrane filters, cellular anchorage and cellular sensing movement can be investigated by analysing the degree of cellular migration into the filter pores. By examining these characteristics at an ultrastructural level, comparative invasive properties can be evaluated in each of the cell lines.

In addition to using cellular morphology as a marker for invasive properties, storage of glycogen will also be analysed. Functionally, glycogen storage in the epithelium provides an energy store for cellular proliferation^[7-9]. Large quantities of glycogen stores have also been reported in a variety of cultured cells, in particular cells undergoing neoplastic transformations^[9-11]. Glycogen metabolism has been widely investigated in malignant cells originating from tissues which normally store glycogen, i.e. hepatomas^[11,12] and cancers of the cervix^[13]. Surprisingly, elevated stores of glycogen have been reported in all malignant cell lines (in a 58 cell line culture study) irrespective of tissue of origin^[9], and in particular colon adenocarcinomas, originating from human colonocytes where glycogen is normally absent^[9,10]. Furthermore, the variation in the amount of glycogen storage has been utilized as a marker for malignant processes in cells, whereby increasing levels of glycogen are indicative of more malignant cells due to abnormal cellular proliferation^[9]. Glycogen storage has been reported in Caco-2, HT-29, and SW480 cells *via* a glycogen quantitative assay. The highest glycogen

levels were found in Caco-2 cells, intermediate levels in HT-29 cells, and the lowest levels in SW480^[10], suggesting that Caco-2 cells undergo extensive abnormal cellular proliferation compared to HT-29 and SW480 cells. These assays have not, however, yielded any information on the ultrastructural position of glycogen in the cells, as accumulation of glycogen particles are first observed in the apical cytoplasm followed by a basal distribution when glycogen particles continue to accumulate^[7]. By incorporating a selective staining method to detect the presence of glycogen in the cell, this investigation will contribute to the ultrastructural localization of glycogen particles in these colorectal cancer cell lines for the evaluation of malignant properties.

Osmium tetroxide has been widely used by electron microscopists to routinely fix and stain intercellular membrane systems^[14,15]; however, primary fixation in an osmium tetroxide-potassium ferrocyanide [K₄Fe(CN)₆] mixture combines selective fixation and staining of various cellular components, in particular glycogen^[16,17] and intracellular membranes^[14,18]. Osmium-ferrocyanide has been shown to bind selectively through the C₂ and C₃ dihydroxyl groups in glycogen. Chelation of the osmium-ferrocyanide complexes by donor atoms in the tissue helps reduce the osmium to a more stable oxidation state, allowing greater osmium deposition than that observed through post-fixation with osmium tetroxide alone. Therefore the osmium-ferrocyanide complex provides excellent preservation and contrast of glycogen complexes, which in many cases might otherwise remain unstained^[17]. The osmium-ferrocyanide method will be incorporated into the present study to determine whether there is an ultrastructural variation of glycogen distribution between different colorectal cancer cell lines.

This study will use three cell lines: Caco-2, HT-29 and SW480 cells. The human colon adenocarcinoma cell line, Caco-2, undergoes spontaneous differentiation in cell culture resulting in structural and functional characteristics which resembles intestinal epithelium^[19-23]. The Caco-2 cell line, although derived from a tumor of the human colon epithelium (colonocytes), surprisingly exhibits characteristics of foetal ileal epithelial cells from the small intestine (enterocytes)^[23]. Many reports have identified the formation of a heterogeneous population of Caco-2 cells in culture^[7,20,24-26]. One population consists of dome-forming simple columnar polarized epithelium with a homogenous distribution of microvilli (brush border)^[27] and established junctional complexes, in particular the tight junction^[4,5]. The variant population comprises multilayered cuboidal cells^[28], exhibiting large intercellular spaces (cysts) characteristic of motile cells^[24,29]. Due to this functional differentiation, Caco-2 cells provide a model to investigate the intestinal barrier as well as drug transport across the intestinal mucosa^[19,22] and provide our study with a model cell line that exhibits a heterogeneous cell population.

HT-29 cells, although not homogenous in nature, when grown in control Dulbecco's modified Eagle's medium (DMEM) remain morphologically undifferentiated and comprise of a multilayer of unpolarized cells

that contain features of mucous secreting cells^[30-32]. The junctional complex and, in particular, the tight junction is absent^[33], resulting in disorderly development of cells. A unique feature of HT-29 cells is their ability to undergo polarisation and reversible differentiation into enterocytes after administration of galactose into the culture medium^[31]. The undifferentiated form of HT-29 cells will be analysed in this investigation to avoid confounding effects attributable to cell culture medium to allow for a comparative framework between cells lines to investigate junctional properties, glycogen content, and other microanatomy of the HT-29 cell line.

The SW480 cell line also consists of a heterogenous population of cells, but these cells do not differentiate^[34]. The predominant cell type in the SW480 cell line consist of flat polygonal cells which form typical epithelial colonies (E-type); the variant R-type cells consist of round, refractile cells with a disoriented morphology. The R-type cells display anchorage-independent growth, form multilayers and are regarded as more malignant^[35]. Cellular morphology, glycogen content, and desmosome formation to determine cellular contact and organisation will be used to identify whether the predominant population of cells in the investigation consist of the E- or R-type.

The comparative ultrastructural and glycogen storage data will be examined in the Caco-2, HT-29 and SW480 cell lines at day 1 and day 12 post-confluency to help promote cellular differentiation. The data collected from this investigation will contribute to the utilisation of microanatomical cellular features to determine neoplastic transformations in human adenocarcinoma cell lines and to help identify sub-populations in the chosen cell lines.

MATERIALS AND METHODS

Cell culture

Caco-2, HT-29, and SW480 cell lines were obtained from the American Type Culture Collection. Caco-2 and HT-29 cells were cultured in advanced Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum, 0.5 mL Glutamax, and 0.5 mL antibiotics per 40 mL of medium. SW480 cells were cultured in L15 medium supplemented with 10% fetal calf serum, 0.5 mL Glutamax, and 0.5 mL antibiotics per 40 mL of medium. The cells were incubated at 37°C in a humidified incubator with 5% CO₂ in air and medium was changed every day after the cells reached confluency.

Transmission electron microscopy

Cells were cultured on 0.4 µm pore size cell culture inserts (BD Falcon, NSW Australia) and collected on day 1 and day 12 post-confluency. Four culture inserts per treatment day were fixed in 2.5% glutaraldehyde (ProSci Tech, Queensland, Australia) in 0.1 mol/L sodium cacodylate buffer with 0.1 mol/L sucrose pH 7.4 for 1 h. Cells were next postfixed for 1 h in a 1% osmium tetroxide solution containing 0.8% potassium ferrocyanide in 0.1 sodium cacodylate buffer (to enhance plasma membranes and

glycogen particles), dehydrated in graded alcohols, and infiltrated with 50% Spurr's resin/absolute ethanol for 1 h. The cells were then re-embedded in fresh Spurr's resin (Agar Scientific, Essex, UK) overnight under gentle agitation, re-embedded in fresh Spurr's resin, and left to polymerized at 60°C for 24 h. Two areas of each cell culture insert ($n = 8$) were cut using a Leica ultracut UCT ultramicrotome (Leica, Heerbrugg, Switzerland) and silver-gold sections (80-90 nm) were mounted onto 200-mesh copper grids. Sections were stained with a 2% solution of aqueous uranyl acetate for 10 min, followed by Reynold's lead citrate stain for 10 min. Stained sections were then viewed using a JEOL 1400 (Tokyo, Japan) transmission electron microscope operating at 120 kV.

RESULTS

Overall, the application of the potassium-ferrocyanide methods results in selective staining of glycogen particles (approximate 20 nm in diameter), electron dense demarcation of plasma membranes, and demarcation of intranuclear indentations (intranuclear rods).

Ultrastructure of Caco-2

When grown on membrane filters, Caco-2 cells differentiated into two morphologically different populations, which will be described separately (Table 1).

Sub-population 1

On day 1 post-confluency, cells were simple squamous with irregular sparse microvilli (Figure 1A). Cells contained round euchromatic nuclei and showed evidence of all other cellular organelles, including mitochondria, rER, and Golgi (Figure 1B). Intercellular spaces between cells were evident, as well as tight junctions and desmosomes. When grown on porous membrane filters (Figure 1C), cellular processes marginally invaded into filter pores (Figure 1D). On day 12 post-confluency, cellular morphology and organisation changed to a stratified cuboidal formation and intercellular spaces increased, resulting in the loss of the tight junction, yet desmosomes were still evident (Figure 1E and F). Glycogen granules were distributed within the cytoplasm with a supranuclear predisposition (Figure 1E and G). Extensive intranuclear rods (indentations of the nuclear membrane) were evident (Figure 1F) and no invasive processes were observed projecting into the filter pores (Figure 1H).

Sub-population 2

On day 1 post-confluency, cells were simple cuboidal and contained regular microvilli (Figure 2A). No intercellular spaces were evident and a tight junction was present along the apical region of the lateral plasma membrane (Figure 2B). Small amounts of glycogen particles were evident and regularly distributed (Figure 2C). Several cellular processes projected into the filter pores (Figure 2D). On day 12 post-confluency, cells became simple columnar with a homogenous population of microvilli (Figure 1E). Glycogen accumulation increased in the cytoplasm of

Table 1 Ultrastructural and morphological features of Caco-2 (sub-population 1 and 2, HT-29 and SW480 cell lines)

Cell line	Days post-confluency	Cell type	Nucleus	Intercellular space	Microvilli	Invasive processes	Mitochondria	Golgi	sER	rER	Junctions	Glycogen	Vacuoles
Caco-2 sub-pop 1	1	Simple squamous	Indented (I/N rods)	++	+/-	+/-	+	+	+	+	+Des -TJ	-	-
Caco-2 sub-pop 1	12	Stratified cuboidal	I/N rods	+++	+	+/-	+	+	+	+	+Des -TJ	+++	-
Caco-2 sub-pop 2	1	Simple squamous	Round	+	++	+/-	+	++	+	+	++Des +TJ	-	+
Caco-2 sub-pop 2	12	Simple columnar	Round	+	+++	-	++	++	+	++	+++Des +TJ	+++	+
HT29	1	Stratified cuboidal	Round	+++	+++	-	+	+	+	+	++Des -TJ	-	-
HT29	12	Stratified cuboidal	I/N rods	+++	+++	-	+	+	+	+++	++Des -TJ	+/-	+++
SW480	1	Simple squamous	Round	+	+++	+++ 4 µm	+++	++	+	+++	+/-Des -TJ	-	-
SW480	12	Simple squamous (spindle shape)	Round	+	+++	+++ 8 µm	+++	++	+	+++	+/-Des -TJ	+	-

-. Absent; +/-: Present; +: Small; ++: Intermediate; +++: Large; I/N: Intracellular; Des: Desmosomes; TJ: Tight junction.

these cells, exhibiting a supra- and sub-nuclear distribution (Figure 2F). Mucous storing vacuoles also occupied a large volume of these cells and were located in close proximity of the glycogen particles. Intercellular spaces were evident in the basal region of the lateral plasma membrane and the tight junction and plasma membrane interdigitation was also present (Figure 2F). No invasive projections into the filter pores were evident (Figure 2H).

Ultrastructure of HT-29 cells

No morphological heterogeneity was observed in the HT-29 cell line.

On day 1 post-confluency, the cells were stratified cuboidal with microvilli projecting into large intercellular spaces between cells (Figure 3A and B). Desmosomes were evident at meeting points along the lateral plasma membrane (Figure 3C and D). The nuclei were round and euchromatic. Mitochondria, rER and Golgi bodies were all present. The apical-most cells of the multilayer contained regular microvilli; no invasive processes were present and there were no obvious glycogen stores. On day 12 post-confluency, cells remained stratified cuboidal, expressing large intercellular spaces with microvilli projecting into them (Figure 3E). Golgi bodies were more evident, as well as large accumulations of mucous secreting vacuoles (Figure 3F and G). Desmosomes were present along contact sites of the lateral plasma membrane (Figure 3G) and no significant invasive processes or glycogen stores were evident (Figure 3H).

Ultrastructure of SW480 cells

On day 1 post-confluency, cells were simple squamous with irregular microvilli (Figure 4A). Mitochondria were densely distributed through the cell cytoplasm (Figure 4B) and tight junction formation was evident when upper leaflets of the plasma membrane were in close contact (Figure 4C). Long invasive cellular projections were present in the filter pores (Figure 4D). On day 12 post-confluency, cells were

simple cuboidal with irregular sparse microvilli (Figure 4E). Large numbers of mitochondria with filamentous branching were evident, as well as the presence of glycogen accumulation (Figure 4F and G). Small intercellular spaces were present, but no desmosomes were evident (Figure 4F). Extremely long invasive processes projected into the filter pores and these projections contained cellular organelles, such as mitochondria (Figure 4H).

DISCUSSION

Utilising selective staining to examine differences in metastatic properties of Caco-2, HT-29, and SW480 cells advances our understanding of the commonly used colorectal cancer cell lines. Morphologically, after reaching confluency, many differences were apparent between the cell lines in this investigation. When grown in standard culture conditions, spontaneous differentiation predominantly occurred in the Caco-2 cell line; however, ultrastructural differences suggesting heterogenous populations also became evident in the HT-29 and SW480 cell lines when cells were examined 12 d post-confluency.

Evidence of a heterogenous population already existed in the Caco-2 cells one day after reaching confluency. Although both Caco-2 cell populations were simple squamous, one population exhibited regular microvilli and tight junction formation (sub-population 2), whereas the other population was void of these features (sub-population 1). By day 12 post-confluency, this heterogeneity was even more apparent, as population 2 cells exhibited a tight junction and became a simple layer of columnar polarized cells with regular microvilli indicative of regular epithelial cell assembly. No intercellular spaces were evident between adjoining cells and there was no evidence of invasive projections into membrane pores; glycogen accumulation, however, was abundant. These features were indicative of intestinal epithelial cells^[19,20] and suggested that this sub-population of cells originated from the absorptive

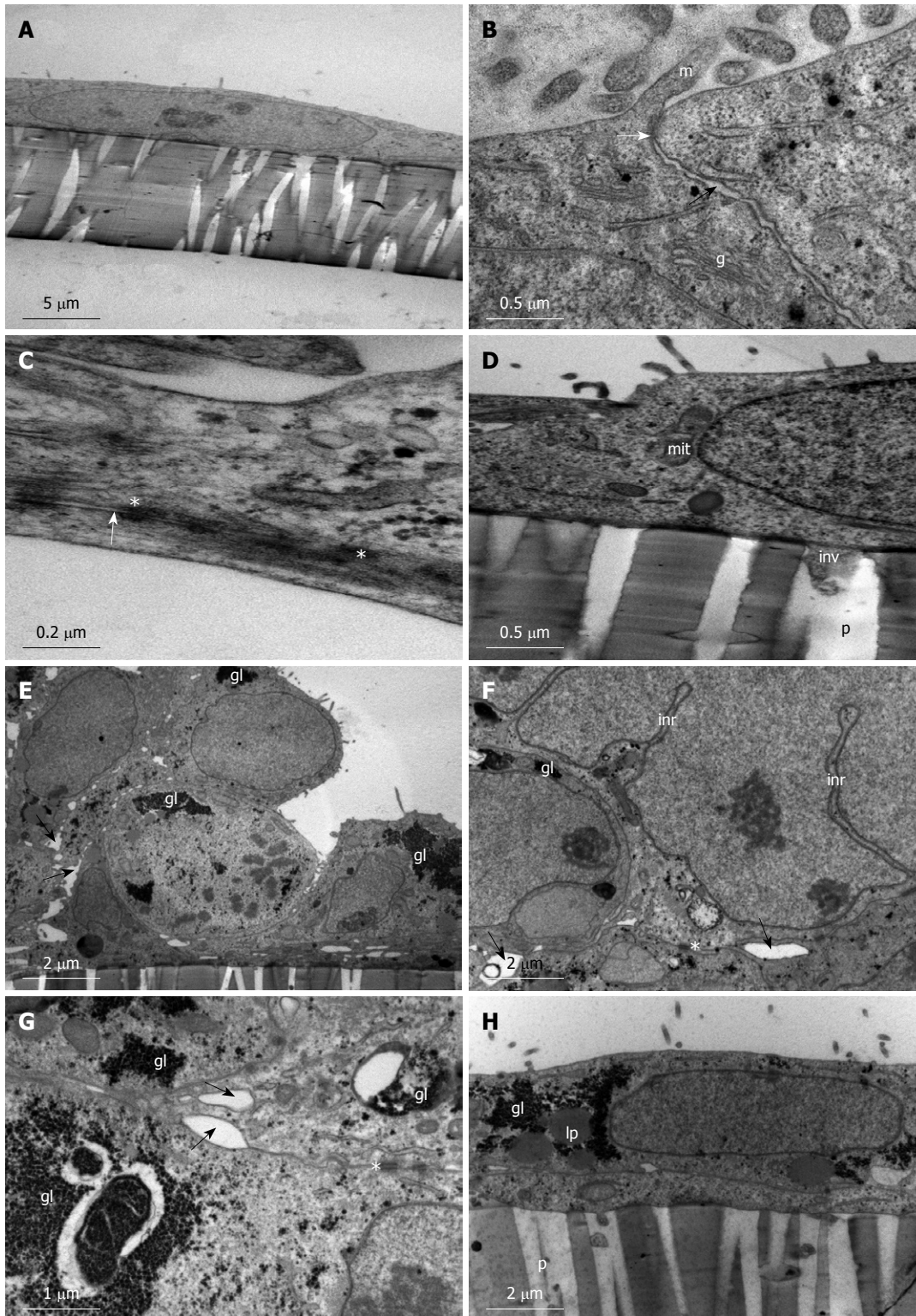


Figure 1 Spontaneous differentiation of Caco-2 into sub-population 1 of stratified cuboidal cells. A-D: Caco-2 cells grown on membrane filters examined on day 1 one post-confluency. A: Simple squamous cells with irregular sparse microvilli; B: Junction between two cells (black arrow) and establishment of a tight junction (white arrow) and the presence of microvilli (m) and golgi bodies (g); C: Desmosome (*) formation along the lateral plasma membrane between two cells (white arrow); D: Basolateral plasma membrane depicting attachment to membrane filter with evidence of small cell protrusions (inv) into the membrane pore (p). Mitochondria (mit); E-H: Caco-2 cells grown on membrane filters examined 12 d post-confluency. E: Cells appear stratified and cuboidal with large intercellular spaces (black arrows); large glycogen stores are evident (gl); F: Intercellular spaces (black arrows) and desmosomes (*) are evident between cells with glycogen deposits (gl) and indentations of the nucleus form large intranuclear rods (inr); G: High power image of intercellular spaces (black arrows) and desmosomes (*) present along the lateral plasma membrane with large glycogen deposits (gl) found in the cytoplasm; H: Basolateral plasma membrane showing attachment of the cell to the membrane filter. No invasive processes are evident projecting into the filter pores (p). Glycogen (gl) and some lipid (lp) droplets are present in the cell cytoplasm.

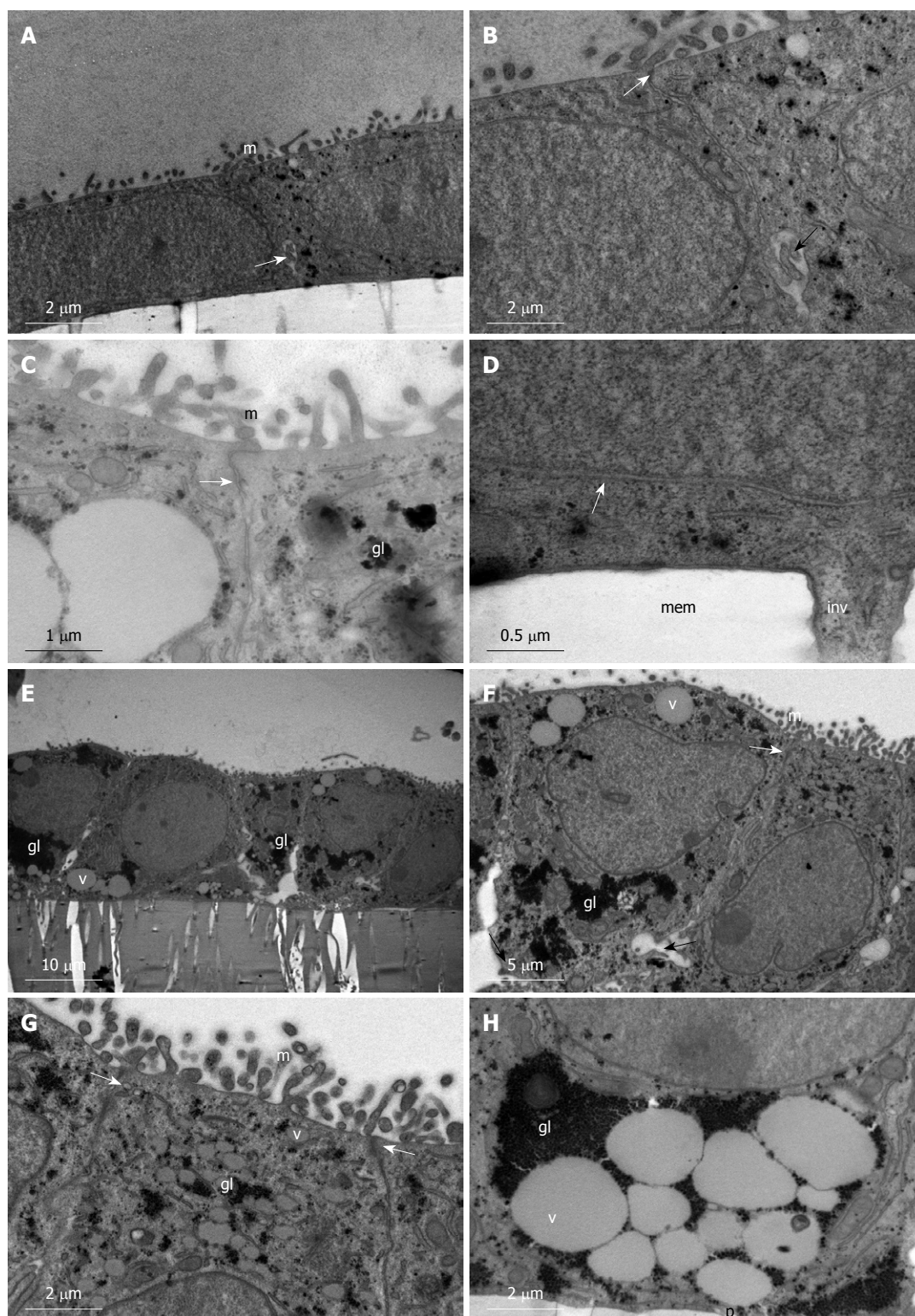


Figure 2 Spontaneous differentiation of Caco-2 into sub-population 2 of simple columnar monolayer of polarized epithelial cells. A-D: Caco-2 cells grown on membrane filters examined on day 1 post-confluency. A: Simple cuboidal epithelium with regular microvilli (m) and intercellular junctions (white arrow); B: Intercellular junction with a tight junction (white arrow) evident along the apical part of the lateral plasma membrane. A small intercellular space (black arrow) is evident near the basolateral region of the plasma membrane; C: High power image of a tight junction (white arrow) between cells showing glycogen particles (gl) and microvilli (m); D: Basolateral plasma membrane showing intercellular junction (white arrow) and small invasive projection (inv) into the filter pore. Membrane (mem); E-H: Caco-2 cells grown on membrane filters examined on day 12 post-confluency. E: Simple columnar polarized epithelium is present with large glycogen stores (gl), mucin filled vacuoles (v), and intercellular spaces along the basal part of the lateral plasma membrane; F: Junction between two columnar cells (white arrow); regular microvilli (m) are present as well as large glycogen stores (gl) and mucin filled vacuoles (v). Intercellular space (black arrow); G: High power micrograph showing the apical domain of cells with regular microvilli (m), glycogen stores (gl), mucin vacuoles (v), and tight junction formation (white arrows); H: High power image of the basal domain of cells with large mucin filled vacuoles (v) enclosed by large glycogen stores (gl). No invasive processes are evident projecting into filter pores (p).

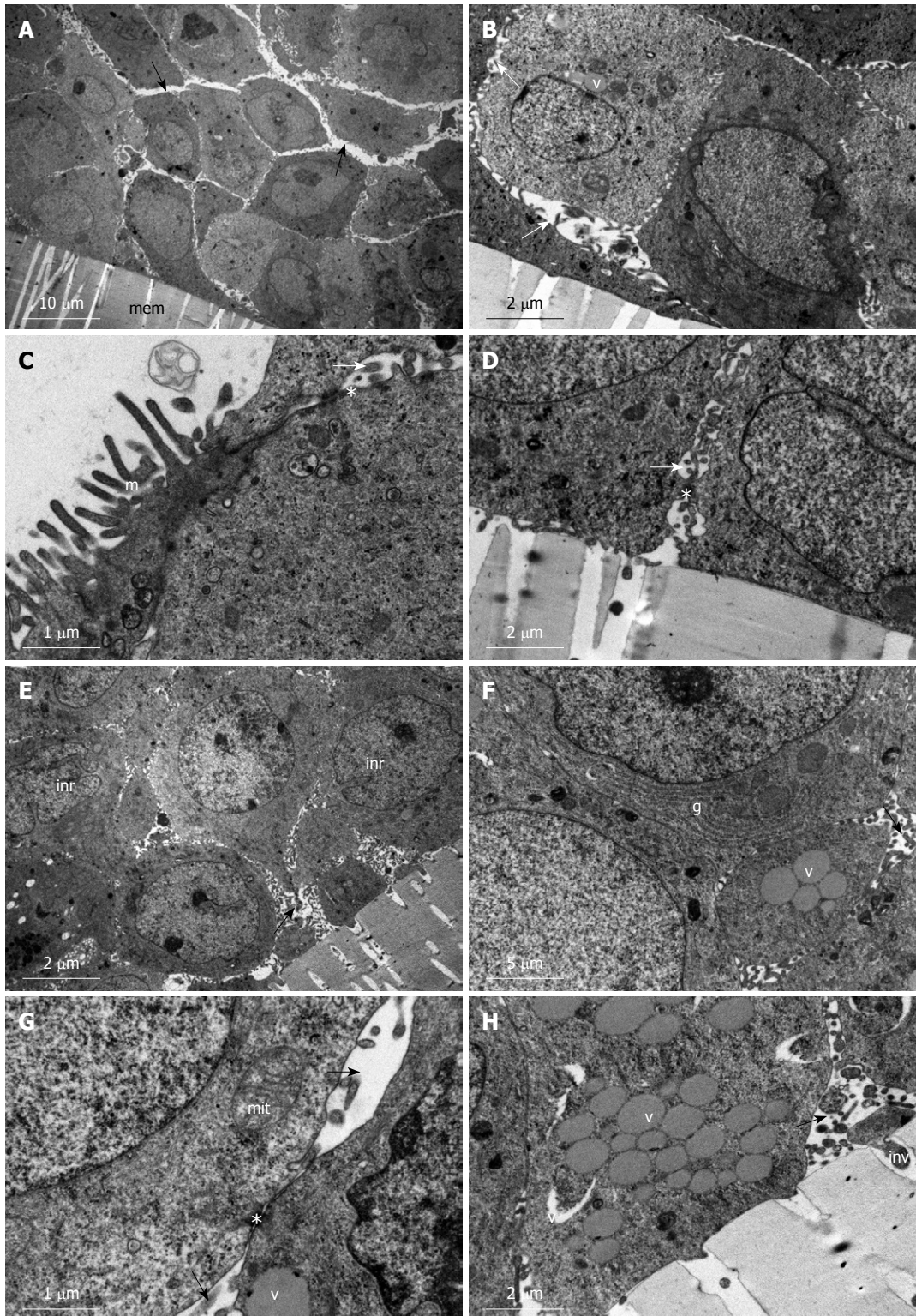


Figure 3 Undifferentiated heterogenous population of HT-29 cells. A-D: HT-29 cells grown on membrane filters (mem) and examined day 1 post-confluency. A: Stratified cuboidal cells arranged in clumps. Large intercellular spaces (black arrows) are evident with microvilli projecting into them; B: High power micrograph showing large intercellular spaces (approximate 2 μ m) (white arrows) with microvillar projections and formation of small vacuoles (v); C: Microvillous (m) apical plasma membrane is present and desmosomes (*) are evident along contact sites of the lateral plasma membrane of adjoining cells and presence of intercellular spaces (white arrow); D: No invasive projections are evident along the basolateral plasma membrane. Desmosomes (*) are present at contact sites of the plasma membrane, which otherwise shows intercellular spaces (white arrow); E-H: HT-29 cells grown on membrane filters (mem) and examined day 12 post-confluency. E: Stratified cuboidal cells with microvillar projections entering the large intercellular space (black arrow). Intranuclear rods are evident (inr); F: High power micrograph of junction between two cells. Golgi bodies (g) as well as mucin filled vacuoles (v) are distributed throughout the cell cytoplasm and intercellular spaces are evident with microvilli projecting into their lumen; G: High power micrograph of a contact site between two opposing plasma membranes showing a desmosome (*). Large intercellular spaces (black arrows) are evident above and below the desmosomes contact site. Mitochondria (mit) and mucin filled vacuoles (v) are also evident; H: Basolateral attachment site showing minor invasive projections (inv) into the filter pore. Intercellular spaces (black arrow) and vacuoles (v) are evident.

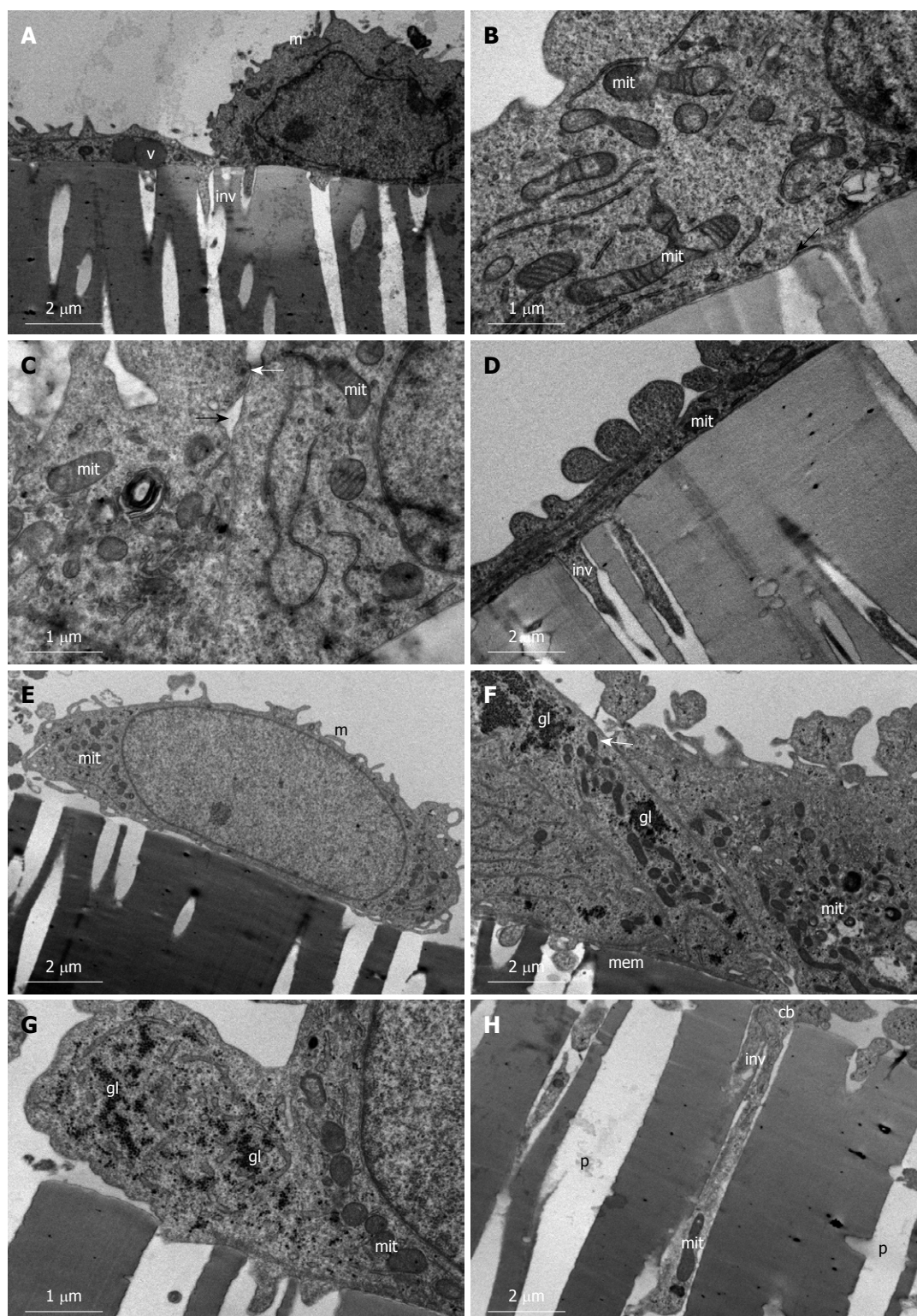


Figure 4 Undifferentiated heterogenous population of SW480 cells. A-D: SW480 cells grown on membrane filters and examined day 1 post-confluency. A: Simple squamous (left) and simple cuboidal (right) distinctions are already evident in cells on day 1 post-confluency. Cells exhibit vacuoles (v), microvilli (m), and invasive processes (inv) are evident projecting into filter pores; B: Large population of mitochondria (mit) are evident which exhibit branching characteristics; C: Intercellular junctions (black arrow) exhibit early formation of the tight junction (white arrow). Mitochondria (mit) with numerous cristae are present; D: Simple squamous cells exhibiting long invasive processes (inv) into the filter pores. Mitochondria (mit); E-H: SW480 cells grown on membrane filters and examined day 12 post-confluency. E: Cells remain a monolayer with microvillar (m) projections and abundant mitochondria (mit); F: Junction between two adjoining cells growing on membrane (mem) showing no intercellular spaces, tight junction formation (white arrow), abundant mitochondria (mit), and glycogen (gl) stores; G: High power micrograph depicting the glycogen (gl) granules in the cytoplasm amongst mitochondria (mit); H: Basolateral region of cell body (cb) showing deep invasive processes (inv) projecting into filter pores (p). These processes extend to depths of approximately 10 μ m and cellular organelles such as mitochondria (mit) are present in these processes, showing deep anchorage of cell.

enterocyte population when the original adenocarcinoma was excised. The polarisation of the Caco-2 epithelial layer indicated that epithelial organisation had been achieved^[3] and the cells were contained in the epithelial monolayer without morphological indications of metastatic properties. The presence of glycogen however, suggested that neoplastic proliferation was still taking place in this population. This finding might be significant for tumor excision and treatment, as glycogen stores suggest a neoplastic cell; however, the morphological architecture indicates a contained tumor with possible clear margins and a better probability of complete surgical excision.

Sub-population 1 of Caco-2 cells did not form regular microvilli, and tight junctions did not polarise 12 d post-confluency. This cell population continued to grow in clumps of stratified cuboidal epithelium with large intercellular spaces between individual cells, suggesting high cellular motility (particularly in the apical population of cells)^[24]. In the basal distribution of the cells, desmosomes were evident in areas of established cellular contact. Glycogen particles were abundant, with both an apical and basal distribution. Another additional feature that became apparent using a combined osmium tetroxide and potassium ferrocyanide selective staining, which would otherwise not have been adequately sampled, was the presence of intranuclear rods. These structures are indentations of the cytoplasm into the nucleus, whereby they increase the nucleocytoplasmic interactions^[36] and carry out nucleocytoplasmic exchange, which is the most important transport phenomena in cells^[37]. In the case of malignant colorectal cancer cells, intranuclear rods are suggested to carry out nucleocytoplasmic exchange of glycogen^[37]. In a previous report in the Ehrlich-Létré mouse ascites tumor, glycogen production that originated in the nucleus shifted from the nucleus to the cytoplasm *via* a transfer mechanism that allows the intranuclear glycogen to pour out into the cytoplasm *via* the intranuclear indentations (rods)^[37]. Intranuclear rods are prevalent in the Caco-2 cell line (in particular sub-population 1); however, glycogen is predominantly expressed in the cytoplasm, which suggested that the nucleocytoplasmic exchange has occurred. Due to their disorganized arrangement, large intercellular spaces and lack of polarisation and tight junctional formation, sub-population 1 of Caco-2 cells did not form an impermeable monolayer or “classical epithelium”. A lack of tight junction formation allows for dissociation of individual cells from the primary tumor due to a decrease in cell-cell adhesion, which consequently allows for the cancerous cells to invade the surrounding tissue, leading to metastasis^[38]. Furthermore, for successive metastasis to occur, the tight junctions present in the surrounding tissue must be compromised to enable the tumor cells to penetrate^[38]. In a previous investigation of colorectal cancer cells, using the tight junctional protein claudin-4 as a marker, a disruption of claudin-4 mediated tight junction formation enhanced cancer cell invasion and metastasis in colorectal cancer^[3]. Finally, the morphology of the cells in sub-population 1 of the Caco-2 cell line did not provide strong evidence indicating the formation of enterocytes, and the high glycogen content signifies metastatic pro-

liferation. Thus, data associated with future functional and transport studies carried out on sub-population 1 of Caco-2 cells might be restricted.

When grown under standard conditions, the HT-29 cells, although not homogenous, did not differentiate into a monolayer of polarized cells, as there is no evidence of any tight junction formation, which again suggests that cells can break away from the primary tumor and begin a metastatic process in surrounding tissue. These results are consistent with previous reports of a heterogeneous HT-29 cell population^[31,33,39]. At day 1 post-confluency, the cells were arranged into a large clump of microvillous stratified cuboidal epithelium up to seven layers thick. By day 12 post-confluency, large intercellular spaces were observed suggesting that cells remain relatively motile^[24], even though some desmosomes were evident at points of plasma membrane contact of cells in the basal population. No invasive cellular projections were evident in the membrane filters, and trace amounts of glycogen particles were present in HT-29 cells when compared to the abundant glycogen stores found in both populations of Caco-2 cells. Reduced glycogen storage suggested that these cells were not undergoing comparable metastatic proliferation as the two sub-populations of the Caco-2 cell line. Previous glycogen assay studies have provided comparative data, where less glycogen content was present in HT-29 cells during both the exponential and stationary growth phase than in Caco-2 cells^[10], indicating that the selective staining produced by the potassium-ferrocyanide fixation as a glycogen marker yields comparative results. An additional feature of the HT-29 cell line was the presence of mucin filled vacuoles, which provides evidence that these cells potentially originated from a mucous secreting population of the intestinal epithelium. Previous investigations of the HT-29 cell line have also isolated mucous-secreting sub-clones in the population, but this was achieved by inducing cellular differentiation by the administration of galactose to the culture medium^[31]. This induced differentiation also resulted in polarisation and monolayer formation of the HT-29 cells^[31]. The present study did not include differentiation of HT-29 cells by adding galactose; therefore, the presence of the mucin filled vacuoles might be indicative of a population of precursor cells that later form secretory intestinal epithelial cells.

When the SW480 cells were cultured under standard conditions, a non-homogenous population of undifferentiated cells was observed. One cell type was simple squamous with irregular microvilli, and the other population of cells were simple spindle-shaped with irregular microvilli. These results were similar to those reported by previous investigations, which also described a heterogeneous undifferentiated population of SW480 cells with comparable morphological features^[35]. By day one post-confluency, cell-to-cell contacts have been established, with no evidence of intercellular spaces; however, no desmosomes or tight junctions were evident. The glycogen content was equivalent in both populations of SW480 cells, indicating that comparable metastatic properties were present in both sub-populations of this cell line. By day one post-confluency, abundant mitochondria were

present throughout the cell cytoplasm when compared to the cytoplasm of the other two cell lines, and some mitochondria appeared to be branching. Large numbers of mitochondria indicated that these cells were highly active.

At 12 d post-confluency, glycogen particles were also evident, representing intermediate glycogen expression when compared to the large amounts found in Caco-2 cells and trace amounts found in the HT-29 cells. The glycogen stores were distributed amongst mitochondrial populations; however, no intranuclear rods were evident projecting into the nucleus. Another striking feature of the SW480 cell line, irrespective of cell type, was their ability to send deep invasive processes into the membrane filter pores (approximate 8 μ m deep). These processes often contained cell organelles, such as mitochondria, which demonstrated deep cell anchorage. Previous reports have also reported that spindle-shaped SW480 cells exhibited vast migration through uncoated membrane filters, whereas cells with a more epithelioid shape (Caco-2 and HT-29) scarcely invaded membranes, even those coated with Matrigel^[6]. These potentially invasive characteristics were not observed in Caco-2 or HT-29 cells in this investigation. Invasive processes have, however, been reported in Caco-2 cells in other literature when invasion was induced by adding a collagen matrix to the membrane filters^[19]. The addition of the collagen promoted cell migration through the filter pores, which eventually led to the depolarisation of the model cells^[19]. No invasion inducing agents were administered in this investigation and there is clear evidence that the SW480 cells presented the most invasive capabilities of the three cell lines. These results were comparable with a previous investigation where metastatic capacities were examined in the SW480 cell line using orthotopic tumor models^[40]. When SW480 cancer cells were injected or implanted into the caecum of mice, SW480 cells had a 100% take rate, when compared to a take rate of 69% in HT-29 and 40% in Caco-2 cells. The SW480 cells also had the highest growth index, which was represented by the time it took between implantation and the appearance of disease symptoms^[40]. The ultrastructural features observed in this study of the SW480 cancer cell line supported other literature that considered this cell line to be highly malignant^[35].

In conclusion, this was the first study to incorporate selective staining for transmission electron microscopy to help identify glycogen storage and nucleocytoplasmic exchange of glycogen by identifying intranuclear rods. Ultrastructurally, the loss of the tight junction in sub-population 1 of Caco-2 cells, and in HT-29 and SW480 cells is indicative of highly disorganized epithelia, whereby individual tumor cells have the potential to dissociate from the primary tumor and begin a metastatic process in other tissue. Conversely, sub-population 2 of Caco-2 cells remained as a polarized monolayer of epithelia with extensive tight junction formation, suggesting organised epithelia with strong cell-cell adhesion with lower disassociation potential. Their glycogen storage, however, suggested that Caco-2 and SW480 cells were undergoing the largest amount of neoplastic transformation, and the

abundant mitochondria present in both populations of SW480 cells was also indicative of that feature. The ultrastructural features examined in this study have contributed to our already vast knowledge of colorectal cancer cells. We propose the structural parameters detailed in this systemic study can be used as a quality control in the study of the ultrastructure of Caco-2, HT-29, and SW480 cells *in vitro*. The table herein presented might serve as a morphometric guide for others who wish to utilise these cell lines. Future 3D tomographical work will further capture the nucleocytoplasmic exchange and assist in identifying other key ultrastructural regulators of the invasive process of malignant cells. The identification of glycogen stores in these cells might result in drug therapy trials targeted against these abundant intracellular features.

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COMMENTS

Background

The extensive variability that exists in the morphology, growth rate, and cell viability between different colorectal cancer cells in culture results in wide-ranging observations and the inability to standardise data. This investigation is designed to compile relevant ultrastructural data on three commonly used cancer cell lines to determine whether ultrastructural features and the presence of large glycogen stores can be used as indicators for the metastatic properties of cells.

Research frontiers

Large quantities of glycogen stores have been reported in a variety of cultured cells, particularly cells undergoing neoplastic transformations, even when the cells from the tissue of origin lack glycogen. By incorporating selective staining using an osmium tetroxide and a potassium ferrocyanide solution in routine transmission electron microscopy, these glycogen stores can be ultrastructurally identified and used as diagnostic markers for the metastatic properties of cells.

Innovations and breakthroughs

This is the first study to combined ultrastructural and functional features of colorectal cancer cells with selective staining for other neoplastic markers, such as glycogen.

Applications

The compilation of ultrastructural features and glycogen storage data from three commonly used colorectal cancer cell lines will assist future investigators in selecting appropriate cell models depending on their area of interest, particularly for drug therapy trials that might be directed against excessive intracellular glycogen stores.

Peer review

This investigation emphasizes the variability in morphology, behaviour, and metastatic properties of colorectal cancer cells and provides readers with a coherent guide on three commonly used colorectal cancer cell lines for use as a morphometric tool for future investigations.

REFERENCES

- 1 Welsh MJ, Smith PL, Fromm M, Frizzell RA. Crypts are the site of intestinal fluid and electrolyte secretion. *Science* 1982; **218**: 1219-1221
- 2 Simons K, Fuller SD. Cell surface polarity in epithelia. *Annu Rev Cell Biol* 1985; **1**: 243-288
- 3 Gout S, Marie C, Lainé M, Tavernier G, Block MR, Jacquier-Sarlin M. Early enterocytic differentiation of HT-29 cells:

- biochemical changes and strength increases of adherens junctions. *Exp Cell Res* 2004; **299**: 498-510
- 4 **Li N**, Lewis P, Samuelson D, Liboni K, Neu J. Glutamine regulates Caco-2 cell tight junction proteins. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G726-G733
 - 5 **Schreider C**, Peignon G, Thenet S, Chambaz J, Pinçon-Raymond M. Integrin-mediated functional polarization of Caco-2 cells through E-cadherin-actin complexes. *J Cell Sci* 2002; **115**: 543-552
 - 6 **de Both NJ**, Vermey M, Dinjens WN, Bosman FT. A comparative evaluation of various invasion assays testing colon carcinoma cell lines. *Br J Cancer* 1999; **81**: 934-941
 - 7 **Mestres P**, Diener M, Rummel W. Storage of glycogen in rat colonic epithelium during induction of secretion and absorption in vitro. *Cell Tissue Res* 1990; **261**: 195-203
 - 8 **Prothmann C**, Wellard J, Berger J, Hamprecht B, Verleysdonk S. Primary cultures as a model for studying ependymal functions: glycogen metabolism in ependymal cells. *Brain Res* 2001; **920**: 74-83
 - 9 **Rousset M**, Zweibaum A, Fogh J. Presence of glycogen and growth-related variations in 58 cultured human tumor cell lines of various tissue origins. *Cancer Res* 1981; **41**: 1165-1170
 - 10 **Rousset M**, Chevalier G, Rousset JP, Dussaulx E, Zweibaum A. Presence and cell growth-related variations of glycogen in human colorectal adenocarcinoma cell lines in culture. *Cancer Res* 1979; **39**: 531-534
 - 11 **Staedel C**, Beck JP. Resurgence of glycogen synthesis and storage capacity in cultured hepatoma cells. *Cell Differ* 1978; **7**: 61-71
 - 12 **Callaghan RC**, Gil-Benso R, Pellin A, Llombart-Bosch A. Cytophotometric analysis of glycogen, protein and DNA of a glycogen-storing rat hepatoma (N13) cell line. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1991; **60**: 271-278
 - 13 **Alpers JB**, Wu R, Racker E. Regulatory mechanisms in carbohydrate metabolism. VI. Glycogen metabolism in HeLa cells. *J Biol Chem* 1963; **238**: 2274-2280
 - 14 **Jinguji Y**, Ishikawa H. An osmium-ferricyanide staining method for the intercellular space in the small intestinal epithelium. *J Electron Microsc* (Tokyo) 1990; **39**: 59-62
 - 15 **Schnepf E**, Hausmann K, Herth W. The osmium tetroxide-potassium ferrocyanide (OsFeCN) staining technique for electron microscopy: a critical evaluation using ciliates, algae, mosses, and higher plants. *Histochemistry* 1982; **76**: 261-271
 - 16 **Goldfischer S**, Kress Y, Coltoff-Schiller B, Berman J. Primary fixation in osmium-potassium ferrocyanide: the staining of glycogen, glycoproteins, elastin, an intranuclear reticular structure, and intercostal trabeculae. *J Histochem Cytochem* 1981; **29**: 1105-1111
 - 17 **Pelliniemi LJ**, Kellokumpu-Lehtinen P, Hoffer AP. Glycogen accumulations in differentiating mesonephric ducts and tubuli in male human embryos. *Anat Embryol (Berl)* 1983; **168**: 445-453
 - 18 **Forbes MS**, Plantholt BA, Sperelakis N. Cytochemical staining procedures selective for sarcotubular systems of muscle: modifications and applications. *J Ultrastruct Res* 1977; **60**: 306-327
 - 19 **Hilgers AR**, Conradi RA, Burton PS. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm Res* 1990; **7**: 902-910
 - 20 **Peterson MD**, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the C2BBE clones of the human intestinal cell line, Caco-2. *J Cell Sci* 1992; **102** (Pt 3): 581-600
 - 21 **Pinto M**, Robine-Leon S, Appay MD, Keding M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol Cell* 1983; **47**: 323-330
 - 22 **Sambuy Y**, De Angelis I, Ranaldi G, Scarino ML, Stamatii A, Zucco F. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol Toxicol* 2005; **21**: 1-26
 - 23 **Engle MJ**, Goetz GS, Alpers DH. Caco-2 cells express a combination of colonocyte and enterocyte phenotypes. *J Cell Physiol* 1998; **174**: 362-369
 - 24 **Briske-Anderson MJ**, Finley JW, Newman SM. The influence of culture time and passage number on the morphological and physiological development of Caco-2 cells. *Proc Soc Exp Biol Med* 1997; **214**: 248-257
 - 25 **Ferruzza S**, Scarino ML, Rotilio G, Ciriolo MR, Santaroni P, Muda AO, Sambuy Y. Copper treatment alters the permeability of tight junctions in cultured human intestinal Caco-2 cells. *Am J Physiol* 1999; **277**: G1138-G1148
 - 26 **Giannasca KT**, Giannasca PJ, Neutra MR. Adherence of Salmonella typhimurium to Caco-2 cells: identification of a glycoconjugate receptor. *Infect Immun* 1996; **64**: 135-145
 - 27 **Menconi MJ**, Salzman AL, Unno N, Ezzell RM, Casey DM, Brown DA, Tsuji Y, Fink MP. Acidosis induces hyperpermeability in Caco-2BBE cultured intestinal epithelial monolayers. *Am J Physiol* 1997; **272**: G1007-G1021
 - 28 **Herold G**, Rogler G, Rogler D, Stange EF. Morphology of CaCo-2 cells varies in different cell batches. *In Vitro Cell Dev Biol Anim* 1994; **30A**: 289-291
 - 29 **Chantret I**, Barbat A, Dussaulx E, Brattain MG, Zweibaum A. Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: a survey of twenty cell lines. *Cancer Res* 1988; **48**: 1936-1942
 - 30 **Hekmati M**, Polak-Charcon S, Ben-Shaul Y. A morphological study of a human adenocarcinoma cell line (HT29) differentiating in culture. Similarities to intestinal embryonic development. *Cell Differ Dev* 1990; **31**: 207-218
 - 31 **Huet C**, Sahuquillo-Merino C, Coudrier E, Louvard D. Absorptive and mucus-secreting subclones isolated from a multipotent intestinal cell line (HT-29) provide new models for cell polarity and terminal differentiation. *J Cell Biol* 1987; **105**: 345-357
 - 32 **Lesuffleur T**, Barbat A, Dussaulx E, Zweibaum A. Growth adaptation to methotrexate of HT-29 human colon carcinoma cells is associated with their ability to differentiate into columnar absorptive and mucus-secreting cells. *Cancer Res* 1990; **50**: 6334-6343
 - 33 **Cohen E**, Ophir I, Henis YI, Bacher A, Ben Shaul Y. Effect of temperature on the assembly of tight junctions and on the mobility of lipids in membranes of HT29 cells. *J Cell Sci* 1990; **97** (Pt 1): 119-125
 - 34 **Duranton B**, Holl V, Schneider Y, Carnesecchi S, Gossé F, Raul F, Seiler N. Polyamine metabolism in primary human colon adenocarcinoma cells (SW480) and their lymph node metastatic derivatives (SW620). *Amino Acids* 2003; **24**: 63-72
 - 35 **Tomita N**, Jiang W, Hibshoosh H, Warburton D, Kahn SM, Weinstein IB. Isolation and characterization of a highly malignant variant of the SW480 human colon cancer cell line. *Cancer Res* 1992; **52**: 6840-6847
 - 36 **Colonnier M**. On the nature of intranuclear rods. *J Cell Biol* 1965; **25**: 646-653
 - 37 **Paweletz N**, Granzow C. Elimination of intranuclear glycogen and its transport to the cytoplasm in Ehrlich-Létré mouse-ascites-tumour. *Z Zellforsch Mikrosk Anat* 1972; **135**: 71-86
 - 38 **Martin TA**, Jiang WG. Loss of tight junction barrier function and its role in cancer metastasis. *Biochim Biophys Acta* 2009; **1788**: 872-891
 - 39 **Cohen E**, Ophir I, Shaul YB. Induced differentiation in HT29, a human colon adenocarcinoma cell line. *J Cell Sci* 1999; **112** (Pt 16): 2657-2666
 - 40 **Flatmark K**, Maeldandsmo GM, Martinsen M, Rasmussen H, Fodstad Ø. Twelve colorectal cancer cell lines exhibit highly variable growth and metastatic capacities in an orthotopic model in nude mice. *Eur J Cancer* 2004; **40**: 1593-1598

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Hydrophobic protein in colorectal cancer in relation to tumor stages and grades

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CONCLUSION: Stomatin-like protein 2 was found to be a promising biomarker for CRC, especially in female patients. The differentially expressed proteins identified were associated with CRC and may act as drug target candidates.

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Key words: Biomarker; Colorectal cancer; Liquid chromatography tandem mass spectrometry; Proteomic approach; 2D-polyacrylamide gel electrophoresis

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Abstract

AIM: To identify differentially expressed hydrophobic proteins in colorectal cancer.

METHODS: Eighteen pairs of colorectal cancerous tissues in addition to tissues from normal mucosa were analysed. Hydrophobic proteins were extracted from the tissues, separated using 2-D gel electrophoresis and analysed using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). Statistical analysis of the proteins was carried out in order to determine the significance of each protein to colorectal cancer (CRC) and also their relation to CRC stages, grades and patients' gender.

RESULTS: Thirteen differentially expressed proteins which were expressed abundantly in either cancerous or normal tissues were identified. A number of these proteins were found to relate strongly with a particular stage or grade of CRC. In addition, the association of these proteins with patient gender also appeared to be significant.

INTRODUCTION

Cancer of the colon and rectum is termed colorectal cancer (CRC). CRC is ranked the third most common cancer worldwide. Statistical data from the World Cancer Report revealed that there are more than 940 000 cases of CRC each year with an annual mortality of around 500 000^[1]. In Malaysia, CRC is the most common type of cancer amongst men and the 3rd most common cancer amongst women after breast and cervical cancer^[2].

Proteomics in cancer is used to study the protein expression pattern of normal and cancerous tissues in order to elucidate the molecular mechanism associated with disease development and progression^[3]. Membrane proteins which make up approximately 30% of the human proteome are important components of cells^[4]. Membrane-associated proteins are involved in various

fundamental biological processes in cells including signal transduction, immune regulation and transportation^[5]. Nevertheless, the hydrophobic nature of membrane proteins make the extraction of these proteins challenging as they are difficult to extract and analyse^[6]. Solubilisation of membrane proteins requires the use of stringent reagents and the addition of urea and thiourea^[7,8]. Moreover, these proteins are prone to precipitation at their isoelectric points which may lead to protein loss during 2D-Polyacrylamide Gel electrophoresis (2D-PAGE) separation^[9,10]. Thus, the proteome of membrane proteins is normally under-represented. Many investigations have been carried out to improve extraction, enrichment and separation of membrane and membrane-associated proteins^[11,12].

In this study, a mixture of reagents was added to the extraction buffer in order to increase the solubility of membrane proteins. Although it is impossible to extract all the membrane proteins from tissues, we obtained a consistent pattern of protein maps for cancerous and normal tissues, respectively. This enabled us to identify unique or differentially expressed membrane proteins in CRC tissues which may be useful for the diagnosis or treatment of CRC. The significance of these proteins in relation to cancer stages, grades and patient gender was also evaluated.

MATERIALS AND METHODS

Tissue samples

Surgically removed colorectal cancerous tissues and their respective normal mucosa were obtained from 18 patients who received treatment at Penang General Hospital, Malaysia. Written informed consent was obtained from all patients before surgery. The tissue specimens were kept in -80°C until analysis. The tissues were grouped according to cancer stage, grade and patient gender (Table 1). The tumor type obtained was adenocarcinoma and normal mucosa was obtained from a site at least 10 cm away from the tumor. None of the patients received preoperative neo-adjuvant chemotherapy or radiotherapy. All the patients were > 40 years old at the time of surgery. The tissues were pathologically confirmed by the hospital's pathologist. Frozen sections of tissue were taken from cancerous tissues in the anterior and deep region to ensure adequacy of tumor and only cancerous tissue that contained > 90% malignant cells was used in this study.

Protein extraction

The membrane or membrane-associated proteins were extracted from the homogenised tissues using thiourea buffer (7 mol/L urea, 2 mol/L thiourea, 4% CHAPS and 0.2% carrier ampholytes). Briefly, 200 mg of tissue was homogenised in 1 mL of Tris buffer (40 mmol/L Tris) followed by sonication for 30 s and chilled on ice for 2 min. The lysate was then vortexed for 5 min before being centrifuged (12 000 r/min, 15 min, 20°C). The su-

Table 1 Clinical information of the patients studied

No.	Age (yr)	Sex	Duke's system	Localization	Degree of differentiation
1	57	F	C	Right colon	Well-differentiated
2	64	M	C	Caecum	Moderately-differentiated
3	45	M	B	Sigmoid colon	Well-differentiated
4	91	F	C	Sigmoid colon	Well-differentiated
7	75	F	B	Rectosigmoid	Well-differentiated
8	62	M	C	Rectum	Well-differentiated
9	71	F	C	Sigmoid colon	Moderately-differentiated
10	76	M	B	Rectosigmoid	Well-differentiated
12	70	M	C	Sigmoid colon	Moderately-differentiated
13	63	M	B	Rectum	Moderately-differentiated
14	62	F	B	Rectosigmoid	Well-differentiated
15	54	F	B	Descending colon	Well-differentiated
17	74	M	B	Rectosigmoid	Moderately-differentiated
19	67	M	B	Rectum	Moderately-differentiated
20	53	F	C	Rectosigmoid	Moderately-differentiated
21	79	F	C	Rectosigmoid	Moderately-differentiated
23	67	M	C	Sigmoid colon	Moderately-differentiated
24	42	M	B	Descending colon	Well-differentiated

pernatant was kept for a separate experiment while the pellet was rinsed twice with Tris buffer. The pellet was then suspended in 150 µL of thiourea buffer, sonicated, vortexed and centrifuged (12 000 r/min, 15 min, 20°C) and the supernatant was collected for analysis.

2-D gel electrophoresis

The protein concentration of the thiourea buffer extracts was determined using the RCDC protein assay kit (Bio-Rad, USA), and 500 µg of the extract in 185 µL of rehydration buffer (same composition as thiourea buffer) was used to rehydrate IPG strips (4–7 pH, 11 cm) for 15 h and focused using IEF Cell (Bio-Rad, USA) starting from 0 to 250 V within 15 min, followed by 250 to 8000 V within 2.5 h and maintained at 8000 V until 35 000 V-h was achieved. Subsequently, the IPG strips were equilibrated for 15 min with gentle shaking in Sodium Dodecyl Sulfate-Polyacrylamide Gel electrophoresis (SDS-PAGE) Equilibration Buffer I (6 mol/L urea, 0.375 mol/L Tris pH 8.8, 2% SDS, 20% glycerol, 2% DTT and a trace amount of bromophenol blue), followed by another 15 min of gentle shaking in SDS-PAGE Equilibration Buffer II (same composition as SDS-PAGE Equilibration Buffer I, however, 2.5% iodoacetamide was used instead of 2% DTT). Second dimension separation was carried out under constant voltage of 200 V for approximately 3 h in 10% SDS-PAGE. The gel was stained overnight using Coomassie Blue (0.2% Coomassie Brilliant Blue R250, 50% MeOH and 2% acetic acid) and destained for 2 h.

Gel imaging and statistical analysis

The 2D-PAGE images were acquired by using the Versadoc system (Bio-Rad, USA). The gel images were processed and analyzed using PDQuest version 7.3 (Bio-Rad, USA). The software was used to create a matchset to compare the images of cancerous and normal colorectal tissues. The matchset was used to analyze quantitative

and qualitative differences in protein spots between the images. The intensity of the protein spot was measured after normalization of the protein spot as a percentage of the total density of all proteins spots on each gel in order to minimise the variation which may be caused by the amount of sample loaded. β -actin protein spot was used as the landmark for gel image analysis. A protein was up-regulated if its expression level in cancerous tissues was 1.5-fold or more compared to normal colorectal tissue, and was down-regulated when the opposite occurred. A protein was uniquely expressed if it was found exclusively in either normal or cancerous colorectal tissue only. In addition, the statistical significance of the protein's change in expression was determined using the Wilcoxon signed-rank test (PDQuest version 7.3) at 95% level of confidence.

In-gel digestion

In-gel digestion was carried out according to Gam *et al.*^[13]. In brief, the protein spots of interest were excised from the gel. The Coomassie blue stain on the protein spot was removed by dehydrating the gel pieces in acetonitrile and rehydrating in 100 mmol/L NH_4HCO_3 . This step was repeated three times. The gel pieces were then incubated in a volume of trypsin buffer (50 mmol/L NH_4HCO_3 and 5 mmol/L CaCl_2) containing 12.5 ng/ μL trypsin and chilled at 4°C for 45 min. The trypsin buffer with trypsin was then replaced by trypsin buffer without trypsin at a volume sufficient to wet the gel pieces and incubated overnight at 37°C. The peptides were then eluted from the gel pieces and dried using a sample dryer (Technique, UK) under a continuous flow of nitrogen gas and stored at -20°C prior to analysis.

Liquid chromatography tandem mass spectrometry analysis

The dry peptides were suspended in 15 μL of ultrapure ddH₂O and were subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis using an electrospray ionisation-ion trap mass spectrometer (Agilent). A 5 μL volume of the reconstituted sample was injected into a RPC-column (C18, 300 Å, 5 μm , 1 mm \times 150 mm) connected to a HPLC (1100 Series, Agilent). A capillary pump was used to pump the mobile phase (A and B) at a flow rate of 15 $\mu\text{L}/\text{min}$. The linear gradient used was 5% B to 95% B in 65 min. Mobile phase A was 0.05% formic acid in deionized water and B was 0.05% formic acid in acetonitrile. The HPLC was interfaced to the mass spectrometer detector. An experimental method comprising 2 scan events was used for analysis. The first scan event was a full scan MS whilst the second scan was the data dependent MS/MS scan which is dependent on the results of the first scan event. Two of the most intense ions in the MS scan which surpassed the threshold set were automatically isolated and excited to the MS/MS scan. The MS parameters used were; dry gas flow rate of 15 $\mu\text{L}/\text{min}$, nebulizer pressure of 30.0 psi and dry gas temperature of 300°C. The parameters for the MS/MS

scan were; default collision energy (voltage) of 1.15 V, charge state of 2, minimum threshold of 1000 counts, and isolation width of 2 m/z. The MS/MS data from the analysis were used to search for their corresponding protein identity in Swiss-Prot using MASCOT Search engine version 2.2 from Matrix Science (www.matrixscience.com). The search parameters used were *Homo sapiens* for taxonomy, trypsin for enzyme, carboxymethyl for fixed modifications, peptide tolerance of ± 2 Da, MS/MS tolerance of ± 0.8 Da and average experimental mass value. Further analysis of proteins was carried out using the ProtParam programme available at the EXPASY website (<http://www.expasy.org/tools/protparam.html>) for calculation of the proteins grand average of hydrophobicity (GRAVY). The Tmpred programme (http://www.ch.embnet.org/software/TMPRED_form.html) was used to determine the transmembrane domain of the proteins.

Western blotting

Western blotting was carried out using a semi-dry blotting method^[14]. Protein extracts were separated by SDS-PAGE according to Laemmli^[15]. A similar quantity of protein was loaded on to SDS-PAGE, after electrophoresis separation, the proteins in the gel were transferred using a TE 70 Semiphor semi-dry transfer unit (Hoefer Scientific, Germany) at 134 mA for 1.5 h to a nitrocellulose membrane. The membrane was incubated in 20 mL of mouse anti-SLP-2 antibody (Abnova, Taiwan) at 1:250 followed by incubation with 50 mL of HRP conjugated anti-mouse secondary antibody (Bio-Rad, USA). The reaction of HRP and its substrate 4-Chloro Naphthol (4CN) indicated the presence of stomatin-like protein 2 (SLP-2).

RESULTS

Two-dimensional gel electrophoresis for protein separation was carried out on a linear pH range of 4 to 7. A total of 13 differentially expressed proteins which were expressed abundantly in either cancerous or normal tissues were identified (Figure 1). Identification of differential protein expression in individual patients was accomplished by conducting a pair-wise comparison between the cancerous and normal tissues for each patient and is displayed in Figure 2. An average of 177.35 ± 26.60 protein spots was detected on 2D gel, with a coefficient variation of 15%. Eight proteins, namely tubulin α -1 chain (S4), tubulin β -2 chain (S5), chaperonin GroEL (S6), heat shock 70 kDa protein (S7), SLP-2 (S8), annexin A3 (S9), annexin A4 (S10) and ATP synthase D chain (S13) were up-regulated although only the up-regulation of tubulin β -2 chain, SLP-2, annexin A3 and annexin A4 were significant ($P < 0.05$) in CRC. Figure 3 shows the comparative analysis of spot intensity between normal and cancerous tissues for SLP-2, annexin A3 and annexin A4.

The identity of the proteins was determined by amino acid sequencing *via* tandem mass spectrometric analysis followed by protein database search. The representative

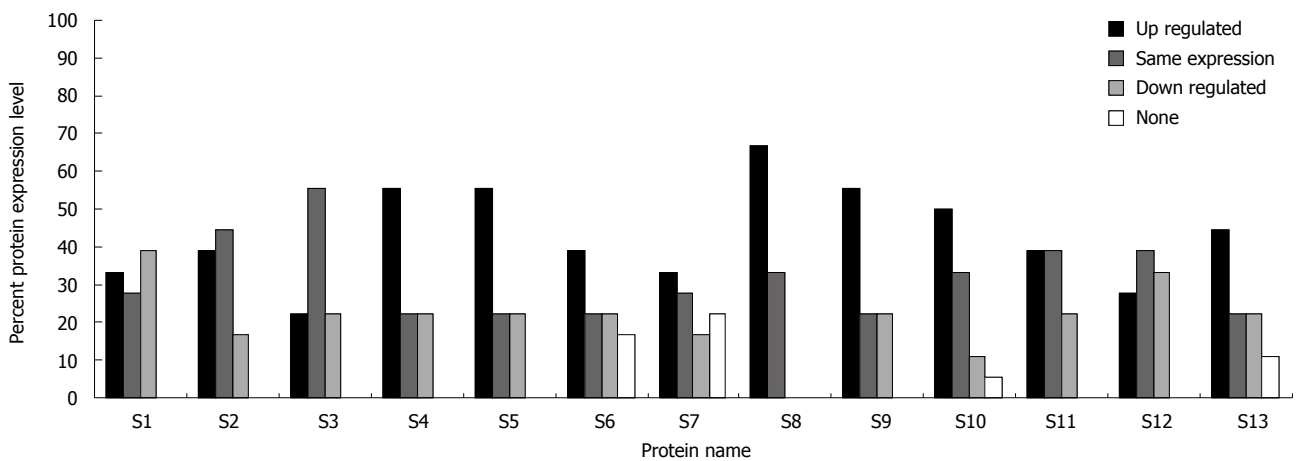
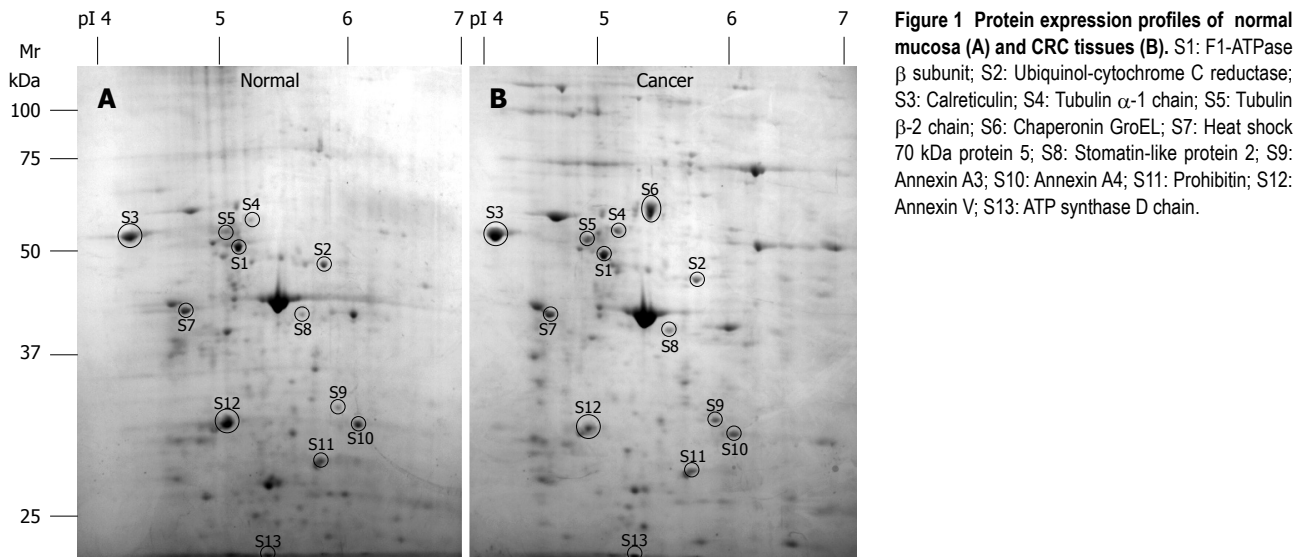


Figure 2 Distribution of proteins in cancerous tissues vs normal tissues in all 18 patients. An up-regulated protein is a protein with a higher expression level in cancerous tissues than in normal tissues, and *vice versa* for down-regulated proteins. Same expression is when the expression levels are the same between cancerous and normal tissues. None expression is when the protein was not found in either cancerous or normal tissues.

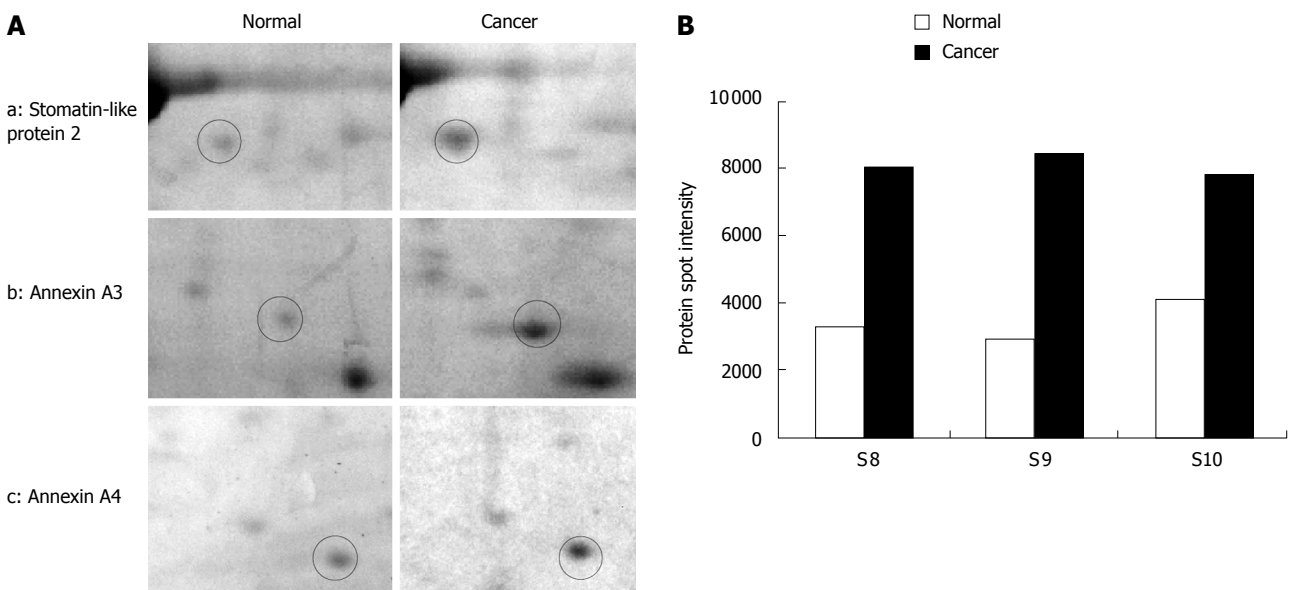


Figure 3 Protein spot analysis (A) and quantification of spot intensity (B) of (a) stomatin-like protein 2 (S8), (b) annexin A3 (S9) and (c) annexin A4 (S10) from normal and colorectal cancerous tissues in the same patient.

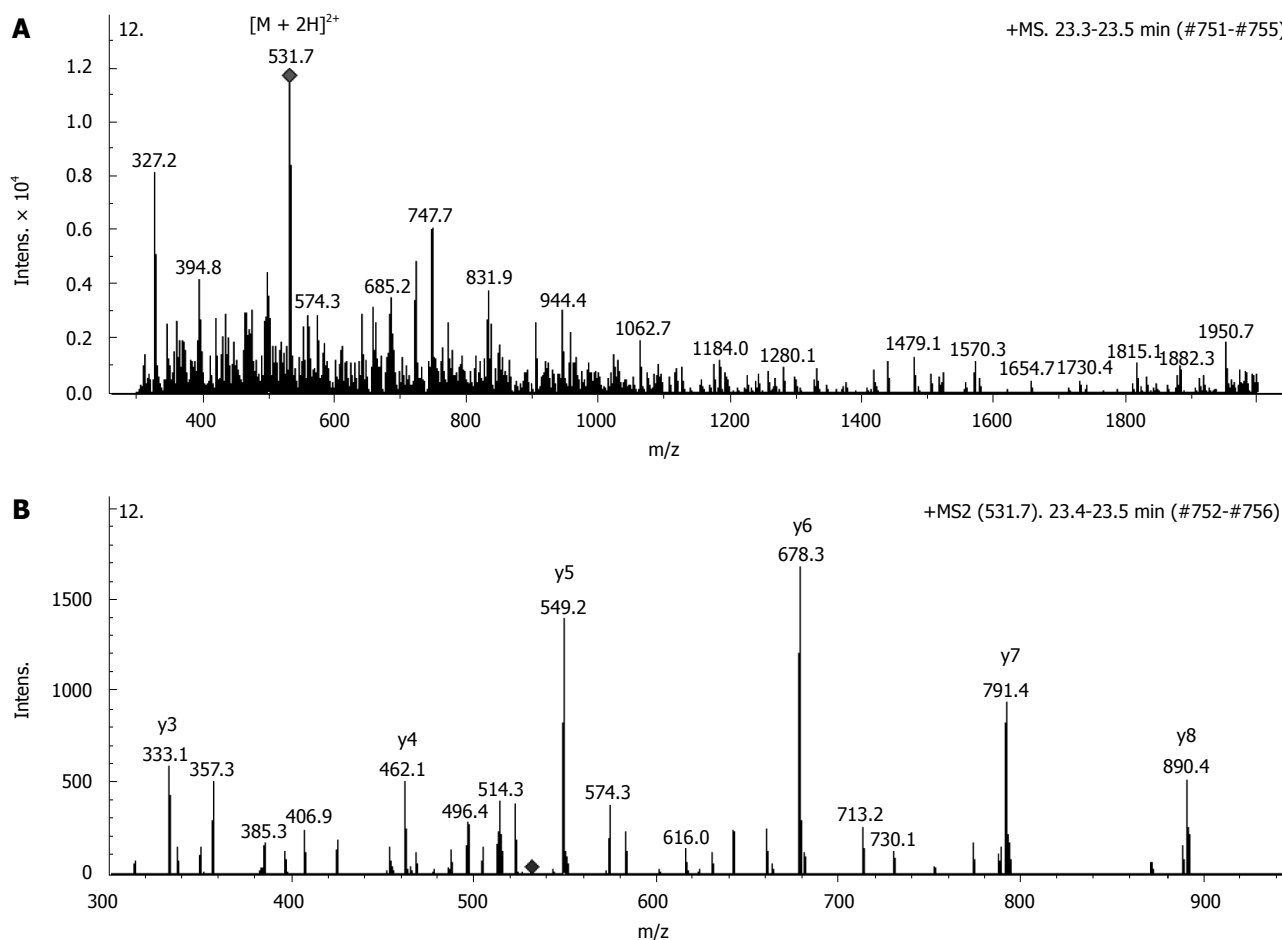


Figure 4 LC/MS/MS analysis of protein. A: Full scan MS spectra of 531.7 m/z peptide; B: MS/MS spectra of 531.7 m/z peptide ions of stomatin-like protein 2.



Figure 5 Western blotting for stomatin-like protein 2. Lane 1 and 2 are proteins extracted from normal and cancerous tissues, respectively, from the same patient.

MS and MS/MS spectra of SLP-2 are shown in Figure 4. Western blotting was used to confirm the results obtained from mass spectrometric analysis. Figure 5 shows a Western blot image of SLP-2 extracted from normal and cancerous tissue in the same patients, SLP-2 was only detected in cancerous tissue. Table 2 shows the 13 differentially expressed proteins identified in this study. The GRAVY score indicated the hydrophobic property of each protein, and the change in the protein expression levels is indicated as fold change (calculated as the ratio of total spot intensity of the protein in normal and cancerous tissues in all 18 patients). A positive value indicated that the protein expression level was higher in cancer compared to normal tissue or that it was up-regulated, while a negative value showed that the protein was down-regulated. Chaperon

nin GroEL was shown to have the greatest fold change (+265.0) although its up-regulation in all 18 patients was not statistically significant ($P < 0.05$).

Analysis of protein expression was carried out by comparing protein expression profiles of cancerous and normal tissues within and between cancer stage, pathological status (grade) and gender of the patients. The tissue specimens collected comprised 9 each of Duke's B and Duke's C, respectively. Figure 6A shows the level of up-regulation of the 13 proteins in Duke's B and C. The proteins which were up-regulated in $> 50\%$ of Duke's B cancer were SLP-2 (S8) and annexin A4 (S10), while F1-ATPase β subunit (S1), ubiquinol-cytochrome c reductase (S2), tubulin α -1 chain (S4), tubulin β -2 chain (S5), SLP-2 (S8), annexin A3 (S9) and ATP synthase D chain (S13) were up-regulated in $> 50\%$ of Duke's C cancer. The up-regulation of both SLP-2 and annexin A4 was significant ($P < 0.05$) in Duke's B cancer, whilst only tubulin β -2 chain and SLP-2 were significantly ($P < 0.05$) up regulated in Duke's C cancer.

As for cancer grade, the tissues collected comprised 9 each of well-differentiated adenocarcinoma (WDC) and moderately-differentiated adenocarcinoma (MDC), respectively. Figure 6B shows the level of up-regulation of the 13 proteins in WDC and MDC. SLP-2 was expressed at $> 50\%$ in WDC and its up-regulation in WDC was

Table 2 Differentially expressed proteins in CRC

Protein spot	Protein name	SwissProt accession No.	Score	Theoretical molecular weight (Da)	Theoretical pI	Sequence coverage (%)	GRAVY	Fold change ¹	TMR
S1	F1-ATPase β subunit	P06576	364	58 013	5.80	19	-0.030	+58.5	1
S2	Ubiquinol-cytochrome c reductase	P31930	171	53 308	5.94	7	-0.141	+32.8	1
S3	Calreticulin	P27797	73	47 092	4.30	11	-1.191	-31.9	1
S4 ^{1,f}	Tubulin α -1 chain	Q71U36	61	50 800	4.94	6	-0.229	+10.8	1
S5 ^{1,c,f}	Tubulin β -2 chain	P68371	299	48 142	4.70	25	-0.347	+20.8	1
S6	Chaperonin GroEL	P10809	185	61 348	5.70	8	-0.076	+265.0	3
S7	Heat shock 70 kDa protein	P11021	775	72 488	5.07	42	-0.487	+5.1	1
S8 ^{a,b,c,d,e,f,g}	Stomatin-like protein 2	Q9UJZ1	151	38 644	6.88	28	-0.161	+27.9	1
S9 ^{a,e}	Annexin A3	P12429	140	36 396	5.63	22	-0.430	+34.9	0
S10 ^{a,b,c,e,f}	Annexin A4	P09525	165	35 983	5.85	33	-0.447	+29.6	0
S11	Prohibitin	P35232	421	29 890	5.57	41	+0.024	+11.8	1
S12	Annexin A5	P08758	195	35 994	4.94	39	-0.330	-5.2	0
S13	ATP Synthase D chain	O75947	117	18 406	5.22	32	-0.569	+8.3	0

¹Fold change is given as the ratio of the spot intensity in normal mucosa over tumour tissue (negative variation or decrease) or tumor tissue over normal tissue (positive variation or increase). ^aDifferentially expressed proteins that are statistically significant ($P < 0.05$) among all 18 patients; ^bDifferentially expressed proteins that are statistically significant ($P < 0.05$) in Duke's B stage; ^cDifferentially expressed proteins that are statistically significant ($P < 0.05$) in Duke's C stage; ^dDifferentially expressed proteins that are statistically significant ($P < 0.05$) in WDC; ^eDifferentially expressed proteins that are statistically significant ($P < 0.05$) in MDC; ^fDifferentially expressed proteins that are statistically significant ($P < 0.05$) in female patients; ^gDifferentially expressed proteins that are statistically significant ($P < 0.05$) in male patients. pI: Isoelectric point; GRAVY: Grand average of hydrophobicity; TMR: Transmembrane region.

significant ($P < 0.05$). Tubulin α -1 chain (S4), tubulin β -2 chain (S5), SLP-2 (S8), annexin A3 (S9) and annexin A4 (S10) were up-regulated in $> 50\%$ MDC, however, only the expression levels of SLP-2, annexin A3 and annexin A4 were significant ($P < 0.05$).

There were 10 male patients and 8 female patients included in this study. All 13 proteins identified in the study were equal to or $> 50\%$ up-regulated in female patients except for calreticulin (S3) and heat shock 70 kDa protein (S7) (Figure 6C). In contrast, all 13 proteins were up-regulated in $< 50\%$ of male patients except SLP-2 (S8) and annexin A3 (S9) which were up-regulated in 50% of male patients (Figure 6C). Only SLP-2 was significantly up-regulated ($P < 0.05$) in CRC male patients, while tubulin α -1 chain, tubulin β -2 chain, SLP-2 and annexin A4 were significantly up-regulated in CRC female patients.

DISCUSSION

The potential use of membrane proteins in drug targeted therapy and diagnosis of diseases is enormous, where many of the biomarkers for indication of diseases are of membrane origin^[16]. The membrane or membrane-associated proteins contain potential antibody recognition sites that may provide identification of cancer development^[6]. Although the extraction of membrane proteins is difficult, a combination of reagents can be used to enhance the solubility of membrane proteins. In this study, tissues were homogenised and treated with Tris buffer prior to thiourea buffer extraction. This pre-treatment removed aqueous soluble proteins and minimised proteolysis. Thus, the remaining tissue pellet contained mainly hydrophobic proteins which were then extracted using thiourea buffer. Thiourea buffer is usually used as soluble buffer for hy-

drophobic proteins^[17]. Dowling *et al.*^[18] and Alvarez-Chaver *et al.*^[9] reported proteomic data of membrane proteins isolated from colorectal cancer by using Triton X-114. Although some common differentially expressed proteins were identified in our study and in the study by Alvarez-Chaver *et al.*^[9], there were also different types of proteins reported by both studies which indicated the crucial roles of reagents in determining the type of protein extracted.

Separation of proteins was carried out using 2-D gel electrophoresis. A similar quantity of proteins from cancerous and normal tissues was used in the analysis to ensure that the change in protein intensity was a factor of tissue type and not tissue weight, this is because the density of cancerous tissues is generally higher than that of normal tissues. A total of 500 μ g of protein was shown to be an ideal load for 2-D gel separation as it allows the visualisation of minute proteins with good spot quality, while abundant proteins were kept under the saturation level when stained with Coomassie Blue.

In this study, only those proteins that were consistently expressed in all the tissues were targeted, we believe that this approach will minimise the identification of false-positive proteins that result from tissue heterogeneity and sample handling. In addition, the normalisation of protein spots during gel image analysis also serves as a control for the determination of differential protein expression levels. We observed a consistent pattern of proteomes for normal and cancerous tissues, respectively (Figure 1). Nevertheless, the expression levels of the proteins varied between patients, where no single protein was solely up-regulated or down-regulated in either cancerous or normal tissues in all patients; i.e. an un-regulated protein in one patient can be expressed as a down-regulated protein in another patient. This observation may support the phe-

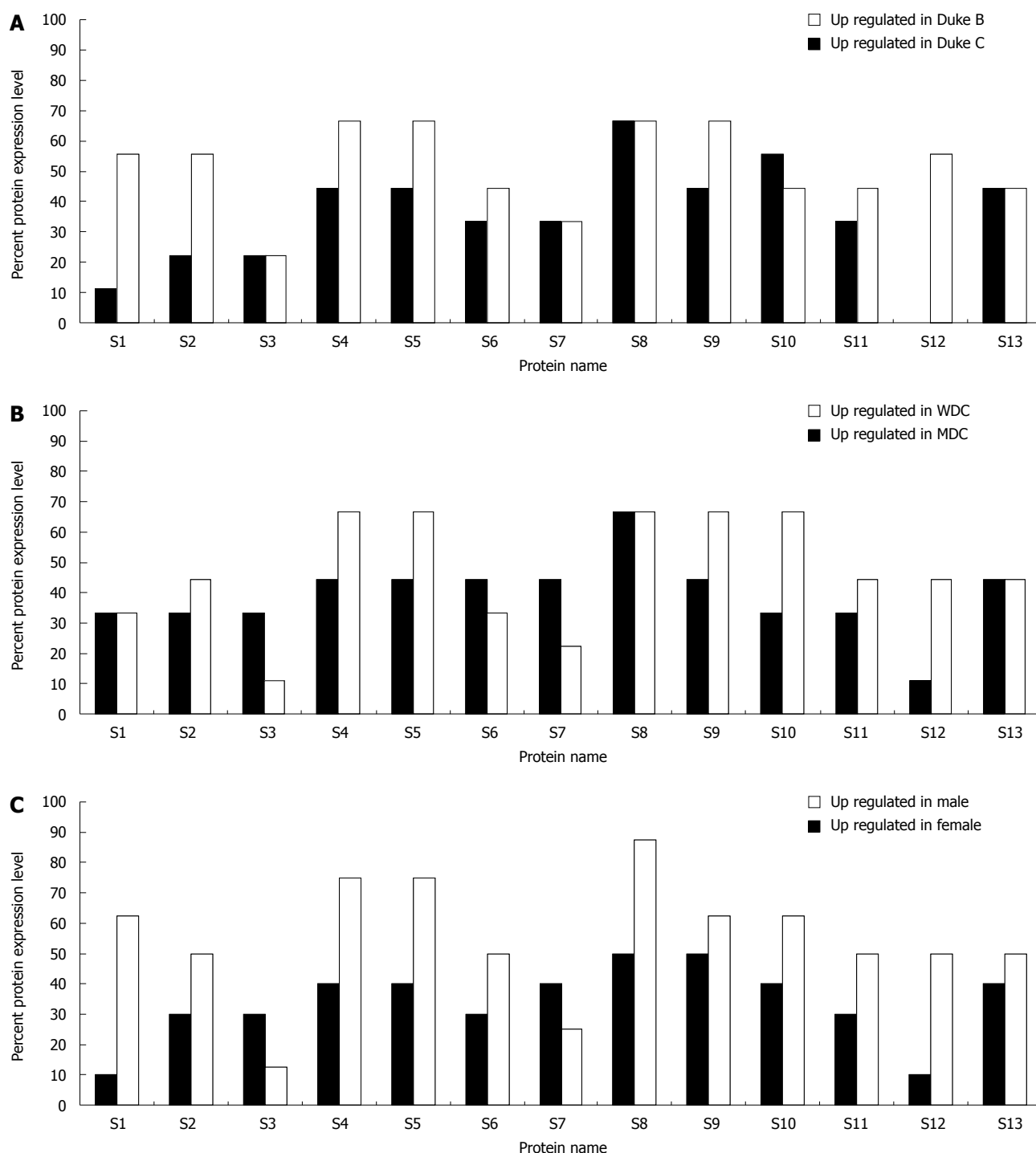


Figure 6 Distribution of proteins in cancerous tissues vs normal tissues in all patients in relation to cancer stage (A), grade (B) and gender (C). An up-regulated protein is a protein with a higher expression level in cancerous tissues than in normal tissues, and *vice versa* for down-regulated proteins.

nomenon that one drug does not fit all and therefore, customized drugs have become the current trend for the treatment of cancer. Nevertheless, protein expression level was found to be more consistent when the tissues were analysed according to cancer stage, grade and gender of the patients. The association of protein expression with stage and grade of CRC and gender of the patients showed the potential of developing stage, grade or gender specific treatment for CRC patients.

Duke's classification of tumor invasion has been proved to correlate with patient survival^[19]. A greater number of proteins were found to be consistently up-regulated in Duke's C tissues compared to Duke's B tissues. In Duke's B cancer, the tumor has not yet metastasized and therefore may still undergo the process of differentiation, nevertheless, when it advances to Duke's C stage, where cancer has metastasized to the lymph nodes, the tumor may also reach a certain level of maturity and reveal

a greater consistency in the protein expression profile. In contrast, Kwong *et al.*^[20] reported that changes in the number of highly expressed proteins do not correlate with the progression of colorectal cancer from Duke's stage B to D.

Well-differentiated adenocarcinoma is less aggressive than moderately-differentiated adenocarcinoma, where the former has a greater resemblance to normal cells. We found that the pattern of protein expression in well-differentiated adenocarcinoma was closer to normal tissues, where only 1 protein was up-regulated in > 50% of well-differentiated tissues compared to 5 proteins in moderately-differentiated tissues.

With regard to the gender of patients, female patients showed a greater consistency in protein expression compared with male patients. In general, all 13 proteins identified had a much greater level of expression in female patients than in male patients except for heat shock 70 kDa protein and calreticulin. Calreticulin was identified as a down-regulated protein in this study. It has been reported that men have higher probabilities of developing polyps and tumors than women, although women are more likely to develop right-sided polyps and right-sided tumors than men^[21], which may explain the different protein expression profiles in the two genders.

All 13 proteins identified in this study were among the highly expressed proteins in either cancerous or normal colorectal tissues. Of these proteins, SLP-2 has been shown to be a promising biomarker for CRC, particularly for female patients, where it was up-regulated in 87.5% of female patients, whereas in male patients, it was found to be up-regulated in 50% of patients. Moreover, in Duke's B, Duke's C, WDC and MDC, its up-regulation levels were 66.7%. Up-regulation of SLP-2 was statistically significant ($P < 0.05$) in both male and female patients and in the stages and grades of CRC tested. Its detection in CRC has not been reported, although it was reported to be up-regulated in other types of cancer. Over expression of SLP-2 was identified in human esophageal squamous cell carcinoma, lung cancer, laryngeal cancer, and endometrial adenocarcinoma which indicated that SLP-2 over expression is very common in cancer development. SLP-2 was associated with different stages of tumor progression from normal tissue to premalignant and malignant lesions of the esophagus^[22]. High expression of SLP-2 was also attributed to advanced stages of breast cancer^[23]. SLP-2 was reported to be involved in regulating cell growth and cell adhesion in human oesophageal squamous cell carcinoma^[22]. SLP-2 plays an important role in sustaining T cell activation through the antigen receptor that is required for T cell differentiation during immunomodulation. It may also be involved in regulating ion channel conductance and/or the organisation of sphingolipid and cholesterol-rich lipid rafts^[24].

The GRAVY score analysis of the thirteen proteins identified in this study showed that only prohibitin had a positive GRAVY score (0.024) indicating its hydrophobic nature. Nevertheless, Blonder *et al.*^[25] suggested that the GRAVY calculation does not reliably predict the hydro-

phobic nature of a protein. Therefore, the presence of the transmembrane domain of a protein is collectively used to predict the nature of a protein. Tmpred analysis of human SLP-2 (356 amino acids) has shown that it contains a single transmembrane domain, which putatively consists of 19 hydrophobic amino acids (amino acid 5-24). SLP-2 is a novel and unusual member of the stomatin gene superfamily. It is a peripheral membrane protein^[24].

Besides SLP-2, other up-regulated proteins with significant expression were annexin A3, annexin A4 and tubulin β -2 chain. Annexin A3 and annexin A4 are members of the annexin family, a family of calcium-regulated phospholipid-binding proteins. Annexin A3 induces the migration and tube formation of vascular endothelial cells by inducing hypoxia-inducible factor-1 (HIF-1), which in turn causes the secretion of vascular endothelial growth factor (VEGF), an important factor in angiogenesis^[26]. Annexin A4 forms complexes with protein kinase C, which has roles in cancer progression and was shown to be up-regulated in colorectal cancer^[27]. Annexins have been reported to be involved in disease processes such as neoplasia. Furthermore, changes in the expression of annexins were linked with tumorigenesis^[28]. Over expression of annexins in primary CRC increase significantly with advancing tumor stage, which suggests that annexins play a role in the progression and development of CRC^[29]. Over expression of annexin A4 with advancing tumor stage was correlated with its role in promoting tumor cell migration. The distinct localization of annexin A4 in tumor cells was implicated to the loss of cell-to-cell adhesion and therefore increased tumor cell spread^[30]. Our data showed that up-regulation of annexin A3 increased when the cancer stage advanced from Duke's B to Duke's C, while the opposite was observed for annexin A4. There have been no previous reports of annexin A3 participating in tumorigenesis or its association with different stages of CRC. Annexin A3 was reported to be up-regulated in colorectal tumor tissues and its cellular location was predominantly membrane-associated as revealed by immunohistochemistry assay^[31]. Immunohistochemistry staining of annexin A3 in prostate cancer has shown its possible relationship with cancer grade^[32]. In this study, we also observed that up-regulation of annexin A3 and annexin A4 in CRC increased when the cancer progressed from well-differentiated adenocarcinoma to moderately-differentiated adenocarcinoma.

The tubulin β -2 chain is one of the components of the cytoskeleton which plays a complex role in cells^[33]. Significant differences in β -tubulin expression in polyps and invasive colon cancers indicates its possible roles in invasive cancer development^[34]. A significant relationship between the expression of tubulins and stages of rectal cancer was suggested to be useful in identifying Dukes' B and Duke's C rectal cancer^[34]. In this study, we found that the expression of tubulin β -2 chain increased significantly when the cancer advanced from Duke's B to Duke's C.

In conclusion, we have identified four hydrophobic proteins, namely SLP-2, tubulin β -2 chain, annexin A3

and annexin A4 which were abundantly expressed in cancerous tissues compared with normal tissues of the colon. Although a limited number of tissues were tested, the expression of these proteins in colorectal cancer was found to be significant suggesting that the possible use of these protein biomarkers in drug targeted therapy and in the diagnosis of colorectal cancer are worth further investigation.

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COMMENTS

Background

Colorectal cancer (CRC) is the third most common cancer worldwide. In Malaysia, the incidence of CRC is increasing. An evaluation of the protein profile of CRC tissues may lead to an understanding of the changes in protein expression when cancer progresses. Proteomics is an emerging tool to study proteins and therefore can be used for the identification of potential biomarkers in the detection and treatment of CRC.

Research frontiers

Membrane proteins comprise approximately 30% of the total human proteome. They are important component of cells and perform vital cellular functions. Due to their hydrophobic nature, many membrane proteins are difficult to extract and remain under-represented in protein profiles.

Innovations and breakthroughs

The authors have successfully extracted hydrophobic proteins from CRC patients. This group of proteins are differentially expressed and significantly correlated with stage and grade of cancer and gender of the patients. In their study, stomatin-like protein 2 (SLP-2) was significantly up-regulated in cancerous tissues. SLP-2 has not been previously reported in CRC.

Applications

Hydrophobic proteins are mainly located on the cell membrane. Therefore, they have the potential to be cell surface markers that can be used in drug targeted therapies for CRC.

Terminology

A hydrophobic protein is a protein that contains a stretch of amino acids that have hydrophobic side chains. These amino acids are arranged so that the hydrophobic side chains are placed outside the protein in the three dimensional structure of the protein. This part of the protein will tend to embed itself in lipid structures such as cell membranes.

Peer review

This manuscript describes the identification of a number of protein biomarkers that are up regulated in colorectal cancer. Membrane proteins from both cancer and normal mucosa were isolated using 2-D gel electrophoresis and subjected to LC/MS/MS analysis.

REFERENCES

- 1 **World Health Organization.** Cancer: Fact sheet. 2009 [cited 31th August 2009]; Available from: URL: <http://www.who.int/mediacentre/factsheets/fs297/en/index.html>
- 2 **Lim GCC, Yahaya H, Lim TO.** The first report of the national cancer registry, cancer incidence in malaysia. Kuala Lumpur: Ministry of Health, 2003: 57-58
- 3 **Alessandro R, Belluco C, Kohn EC.** Proteomic approaches in colon cancer: promising tools for new cancer markers and drug target discovery. *Clin Colorectal Cancer* 2005; **4**: 396-402
- 4 **Wallin E, von Heijne G.** Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. *Protein Sci* 1998; **7**: 1029-1038
- 5 **Tan S, Tan HT, Chung MC.** Membrane proteins and membrane proteomics. *Proteomics* 2008; **8**: 3924-3932
- 6 **Canelle L, Bousquet J, Pionneau C, Hardouin J, Choquet-Kastylevsky G, Joubert-Caron R, Caron M.** A proteomic approach to investigate potential biomarkers directed against membrane-associated breast cancer proteins. *Electrophoresis* 2006; **27**: 1609-1616
- 7 **Rabilloud T.** Use of thiourea to increase the solubility of membrane proteins in two-dimensional electrophoresis. *Electrophoresis* 1998; **19**: 758-760
- 8 **Rabilloud T.** Solubilization of proteins in 2-D electrophoresis. An outline. *Methods Mol Biol* 1999; **112**: 9-19
- 9 **Alvarez-Chaver P, Rodríguez-Piñero AM, Rodríguez-Berocal FJ, Martínez-Zorzano VS, Páez de la Cadena M.** Identification of hydrophobic proteins as biomarker candidates for colorectal cancer. *Int J Biochem Cell Biol* 2007; **39**: 529-540
- 10 **Molloy MP.** Two-dimensional electrophoresis of membrane proteins using immobilized pH gradients. *Anal Biochem* 2000; **280**: 1-10
- 11 **Dreger M.** Subcellular proteomics. *Mass Spectrom Rev* 2003; **22**: 27-56
- 12 **Görg A, Weiss W, Dunn MJ.** Current two-dimensional electrophoresis technology for proteomics. *Proteomics* 2004; **4**: 3665-3685
- 13 **Gam LH, Leow CH, Man CN, Gooi BH, Singh M.** Analysis of differentially expressed proteins in cancerous and normal colonic tissues. *World J Gastroenterol* 2006; **12**: 4973-4980
- 14 **Laurière M.** A semidry electroblotting system efficiently transfers both high- and low-molecular-weight proteins separated by SDS-PAGE. *Anal Biochem* 1993; **212**: 206-211
- 15 **Laemmli UK.** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685
- 16 **Lund R, Leth-Larsen R, Jensen ON, Ditzel HJ.** Efficient isolation and quantitative proteomic analysis of cancer cell plasma membrane proteins for identification of metastasis-associated cell surface markers. *J Proteome Res* 2009; **8**: 3078-3090
- 17 **Görg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W.** The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 2000; **21**: 1037-1053
- 18 **Dowling P, Meleady P, Dowd A, Henry M, Glynn S, Clynes M.** Proteomic analysis of isolated membrane fractions from superinvasive cancer cells. *Biochim Biophys Acta* 2007; **1774**: 93-101
- 19 **Deans GT, Parks TG, Rowlands BJ, Spence RA.** Prognostic factors in colorectal cancer. *Br J Surg* 1992; **79**: 608-613
- 20 **Kwong KY, Bloom GC, Yang I, Boulware D, Coppola D, Haseman J, Chen E, McGrath A, Makusky AJ, Taylor J, Steiner S, Zhou J, Yeatman TJ, Quackenbush J.** Synchronous global assessment of gene and protein expression in colorectal cancer progression. *Genomics* 2005; **86**: 142-158
- 21 **McCashland TM, Brand R, Lyden E, de Garmo P.** Gender differences in colorectal polyps and tumors. *Am J Gastroenterol* 2001; **96**: 882-886
- 22 **Zhang L, Ding F, Cao W, Liu Z, Liu W, Yu Z, Wu Y, Li W, Li Y, Liu Z.** Stomatin-like protein 2 is overexpressed in cancer and involved in regulating cell growth and cell adhesion in human esophageal squamous cell carcinoma. *Clin Cancer Res* 2006; **12**: 1639-1646
- 23 **Cao W, Zhang B, Liu Y, Li H, Zhang S, Fu L, Niu Y, Ning L, Cao X, Liu Z, Sun B.** High-level SLP-2 expression and HER-2/neu protein expression are associated with decreased breast cancer patient survival. *Am J Clin Pathol* 2007; **128**: 430-436
- 24 **Wang Y, Morrow JS.** Identification and characterization of human SLP-2, a novel homologue of stomatin (band 7.2b)

- present in erythrocytes and other tissues. *J Biol Chem* 2000; **275**: 8062-8071
- 25 **Blonder J**, Goshe MB, Moore RJ, Pasa-Tolic L, Masselon CD, Lipton MS, Smith RD. Enrichment of integral membrane proteins for proteomic analysis using liquid chromatography-tandem mass spectrometry. *J Proteome Res* 2002; **1**: 351-360
 - 26 **Park JE**, Lee DH, Lee JA, Park SG, Kim NS, Park BC, Cho S. Annexin A3 is a potential angiogenic mediator. *Biochem Biophys Res Commun* 2005; **337**: 1283-1287
 - 27 **Gökmen-Polar Y**, Murray NR, Velasco MA, Gatalica Z, Fields AP. Elevated protein kinase C betaII is an early promotive event in colon carcinogenesis. *Cancer Res* 2001; **61**: 1375-1381
 - 28 **Rand JH**. The annexinopathies: a new category of diseases. *Biochim Biophys Acta* 2000; **1498**: 169-173
 - 29 **Duncan R**, Carpenter B, Main LC, Telfer C, Murray GI. Characterisation and protein expression profiling of annexins in colorectal cancer. *Br J Cancer* 2008; **98**: 426-433
 - 30 **Zimmermann U**, Balabanov S, Giebel J, Teller S, Junker H, Schmoll D, Protzel C, Scharf C, Kleist B, Walther R. Increased expression and altered location of annexin IV in renal clear cell carcinoma: a possible role in tumour dissemination. *Cancer Lett* 2004; **209**: 111-118
 - 31 **Madoz-Gúrpide J**, López-Serra P, Martínez-Torrecuadrada JL, Sánchez L, Lombardía L, Casal JJ. Proteomics-based validation of genomic data: applications in colorectal cancer diagnosis. *Mol Cell Proteomics* 2006; **5**: 1471-1483
 - 32 **Wozny W**, Schroer K, Schwall GP, Poznanović S, Stegmann W, Dietz K, Rogatsch H, Schaefer G, Huebl H, Klocker H, Schrattenholz A, Cahill MA. Differential radioactive quantification of protein abundance ratios between benign and malignant prostate tissues: cancer association of annexin A3. *Proteomics* 2007; **7**: 313-322
 - 33 **Sullivan KF**. Structure and utilization of tubulin isotypes. *Annu Rev Cell Biol* 1988; **4**: 687-716
 - 34 **Giarnieri E**, De Francesco GP, Carico E, Midiri G, Amanti C, Giacomelli L, Tucci G, Gidaro S, Stroppa I, Gidaro G, Giovagnoli MR. Alpha- and beta-tubulin expression in rectal cancer development. *Anticancer Res* 2005; **25**: 3237-3241

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Relationship between clinicopathological features and mucin phenotypes of advanced gastric adenocarcinoma

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Abstract

AIM: To investigate a relationship between the clinicopathological features and mucin phenotypes in advanced gastric adenocarcinoma (AGA).

METHODS: Immunohistochemical staining was performed to determine the mucin phenotypes in 38 patients with differentiated adenocarcinomas (DACs), 9 with signet-ring cell carcinomas (SIGs), and 48 with other diffuse-type adenocarcinomas (non-SIGs) of AGA. The mucin phenotypes were classified into 4 types: gastric (G), gastrointestinal (GI), intestinal, and unclassified.

RESULTS: The G-related mucin phenotypes were highly expressed in all the histological subtypes of AGA. The expression of the GI phenotype in SIG patients was lower than that in DAC patients ($P = 0.02$), and this phenotype was observed in 56% of the non-SIG patients in the intramucosal layer. Among non-SIG cases, the expression of the GI phenotype was significantly higher

in patients with extended adenocarcinomas and those with positive rates of lymph node metastasis. There was no difference between the expressions of the G and other GI phenotype factors. Among DAC and non-SIG patients, there were no differences between the survival rates of the corresponding patient groups.

CONCLUSION: The GI phenotype might possess more invasive characteristics than the G phenotype in non-SIG. Neither of the phenotypes indicated a poor prognosis of DAC and non-SIG.

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Key words: Mucins; Phenotype; Diffuse type; Undifferentiated type; Gastric neoplasms; Adenocarcinoma; Prognosis

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INTRODUCTION

Recently, an immunohistochemical method for mucin staining has been developed, and the expression of the mucin phenotype in gastric adenocarcinoma has been reported^[1-4]. Lauren^[5] and Nakamura *et al*^[6] histologically classified gastric adenocarcinomas into 2 main types: in-

testinal type of Lauren's histology or differentiated type, and diffuse type or poorly differentiated type. The diffuse type adenocarcinomas, as classified using Lauren's method^[7] (non-solid type of poorly differentiated adenocarcinoma and the signet-ring cell carcinoma according to a Japanese classification^[8]: PDC), which do not show clustering or glandular formation, can be further divided into 2 histological subtypes. In one of these subtypes, the adenocarcinomas are predominantly (> 50%) composed of isolated malignant cells containing intracellular mucin and the nucleus is located at the periphery of the cytoplasm (signet-ring cell carcinoma: SIG according to the Japanese classification). In the other subtype, the adenocarcinoma contains few (< 50%) signet-ring cancer cells (non-SIGs in PDC).

On the basis of the differences in immunohistochemical staining, the mucin phenotypes in gastric adenocarcinoma can generally be divided into 4 types: gastric (G), mixed or gastrointestinal (GI), intestinal (I), and unclassified (UC). Some studies on the intestinal or differentiated types of adenocarcinoma have described the relationship between the expression of mucin phenotypes and the occurrence, progression, and clinicopathological features of adenocarcinomas^[9-14]. The expression of the mucin phenotype in diffuse or poorly differentiated adenocarcinomas has also been studied^[15-22]. However, some of these studies have been restricted to SIG^[15-22], and there have been few studies on advanced gastric adenocarcinoma (AGA)^[23-27]. Therefore, there is little information on the effects of the mucin phenotypes on the clinicopathological and histological subtype-based features of AGA, particularly in the diffuse type as defined by Lauren's method^[7]. To clarify the role of the mucin phenotype in the clinicopathological features and prognosis of AGA, further studies should take into account the histological subtypes of AGA.

To this end, we examined the expression of mucin phenotypes in the above-mentioned histological subtypes of AGA. The mucin phenotype-based analysis was performed according to the classification proposed by Watanabe *et al.*^[28,29]. We also determined the relationship between the expression of the mucin phenotypes and the clinicopathological features, including the prognosis of patients, who were grouped according to the different histological subtypes of gastric adenocarcinoma.

MATERIALS AND METHODS

We investigated 95 subjects with AGA who had undergone gastric resection at the Surgery I Department of Gunma University Hospital between 1994 and 2000. Among the 95 subjects, 38 had differentiated adenocarcinoma (DAC), 9 had SIG, and 48 had non-SIG of PDC. We defined AGA as an adenocarcinoma that invades deep into the muscularis propria. The definition of DAC includes papillary and tubular adenocarcinomas classified using the Japanese classification^[8]. The definitions of SIG and PDC are described above. The definition of non-SIG is the adenocarcinoma of NSC excluding SIG.

We used a staining method which had been previously reported^[25,26]. In brief, the samples obtained after gastric resection were fixed in 10% buffered neutral formalin, macroscopically examined, and photographed. Thereafter, the resected tumor, which included the tumor center, was cut into 3-4 mm wide slices. These slices were then embedded in paraffin and stained with hematoxylin-eosin (HE). The slices were then examined and color images were used for the histochemical mapping of the tissues and for measuring tumor size. For immunohistochemistry, we selected 1 or 2 HE-stained sections obtained from the tumor areas with the largest diameters and the deepest mucosal invasion. Paraffin blocks containing the selected HE-stained sections were cut into consecutive 3 μ m sections for immunohistochemical staining.

The following protocol was employed for staining. Deparaffinized sections were treated with citrate buffer (pH 6.0), heated in a microwave oven for 20 min, and allowed to be cooled to room temperature. Endogenous peroxidase activity was blocked by incubating the sections for 20 min with 0.3% hydrogen peroxidase in absolute methanol, and the sections were then washed in tap water. Non-specific binding was blocked by using normal serum (Nichirei, Japan). The sections were incubated with a primary antibody overnight at 4°C and then incubated with a biotinylated secondary antibody for 30 min at room temperature (Nichirei, Japan). Immunohistochemical staining was performed using a streptavidin-biotin-peroxidase kit (Nichirei, Japan) according to the manufacturer's instructions, with slight modifications. We used MUC5AC (diluted 1:100; antibody CLH2, Novocastra, UK) and human gastric mucin (HGM, diluted 1:50; antibody 45M1, Novocastra) antibodies as markers for the gastric foveolar phenotype; MUC6 (diluted 1:100; antibody CLH5, Novocastra) and M-GGMC-1 (diluted 1:50; antibody HIK1083, Kantou Chemicals, Japan) antibodies as markers for the pyloric gland phenotype; MUC2 (diluted 1:500; antibody Ccp58, Novocastra) as a marker for intestinal goblet cell mucin; and CD10 (diluted 1:200; antibody 56C6, Novocastra) as a marker for small intestinal enterocytes.

The markers HGM, M-GGMC-1, and CD10 exhibited both cytoplasmic and luminal membranous reactivity, whereas MUC5AC, MUC6, and MUC2 exhibited only cytoplasmic reactivity. The reactivity was estimated on the basis of the percentage of stained cells among the total number of tumor cells in each stained section. On the basis of the frequency of positive staining for the relevant marker, the adenocarcinoma phenotypes were classified into 4 groups: G, GI, I, and UC. The following criteria were used for the classification of the mucin phenotypes: (1) if more than 5% of the cells were positive for HGM, MUC5AC, MUC6, or M-GGMC-1, the phenotype was classified as G; (2) if more than 5% of the cells were positive for MUC2, the phenotype was classified as I; and (3) if even a single cell was positive for CD10, the phenotype was classified as I. If none of the above criteria were met, the phenotype was classified as UC. The mucin phenotype was determined by examining the intramucosal layer.

Table 1 Clinical profiles and tumor characteristics according to the histological subtypes

Histological subtype	Gender		Age at operation (yr)	Tumor site			Tumor size (cm)	
	Male	Female		U	M	L	< 5	> 5
DAC (<i>n</i> = 38)	28	10	58	9	12	17	17	21
SIG (<i>n</i> = 9)	3 ^a	6 ^a	62	2	1	6	3	6
Non-SIG (<i>n</i> = 48)	24	24	65	7	27	14	14	34

DAC: Differentiated-type adenocarcinoma; SIG: Signet-ring cell carcinoma; Non-SIG: Other diffuse-type adenocarcinomas; U: Upper segment of the stomach; M: Middle segment of the stomach; L: Lower segment of the stomach. ^a*P* = 0.05.

Table 2 Expression rates of the various mucin phenotypes at the intramucosal layer among patients classified according to the histological subtype

Antigen/histological subtype	HGM (%)	MUC5AC (%)	MUC6 (%)	M-GGMC-1 (%)	MUC2 (%)	CD10 (%)
DAC (<i>n</i> = 38)	97	95	84	90 ^{a,b}	76 ^c	0
SIG (<i>n</i> = 9)	100	100 ^d	56	44 ^{a,d}	33 ^c	0
Non-SIG (<i>n</i> = 48)	96	98 ^e	65	52 ^{b,e}	56	0

HGM: Human gastric mucin. ^a*P* < 0.01, ^b*P* < 0.01, ^c*P* = 0.02, ^d*P* = 0.02, ^e*P* < 0.01.

In addition to determining the mucin phenotype, we reviewed the patients' profiles (age at operation and gender), the tumor site (upper segment of the stomach, U; middle segment of the stomach, M; and lower segment of the stomach, L), the tumor size, the histological findings (lymphatic and vessel invasion, metastasis to the lymph nodes and other sites), and patient survival. The relationships among these factors, particularly the comparison between the G and GI phenotypes, were determined.

The survival rates of the patients with G and GI phenotypes grouped according to the histological subtypes were examined by Kaplan-Meier analysis.

Statistical analysis was performed using the Pearson χ^2 test [Fisher's exact test (extended)] and the Student *t*-test. Two-tailed *P*-values < 0.05 were considered significant.

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and all the participants gave their written informed consent.

RESULTS

Clinical profiles and tumor characteristics of the patients with DAC, SIG, and non-SIG adenocarcinomas

The clinical profiles and tumor characteristics of the patients with DAC, SIG, and non-SIG are summarized in Table 1. Among women, the number with SIG was significantly higher than those with DAC (*P* = 0.05). There were no intergroup differences in the age at operation or size of tumor. For the site of tumor, the number in the lower segment in DAC was higher than that in SIG or DAC.

Typical photographs of a patient with SIG expressing the G phenotype and of a patient with non-SIG expressing the GI phenotype

Typical photographs of a patient with SIG expressing

the G phenotype are shown in Figure 1. Immunohistochemical staining revealed a large number of HGM- or M-GGMC-1-positive cells in the mucosal portion of the tumor. No MUC2-positive cells were detected in the tumor area. The cancer cells, most of which were signet-ring cells, were scattered without clustering or glandular formation.

Figure 2 shows typical photographs of a patient with non-SIG expressing the GI phenotype. Immunohistochemical staining revealed a number of M-GGMC-1-positive cells in the mucosal portion of the tumor. We also observed sporadically distributed MUC2-positive cells. No HGM-positive cells were detected in the tumor area of the examined tissues. We observed scattered non-SIG cells similar to the SIG shown in Figure 1. No cluster and glandular formation of cancer cells was detected in any region of tumor.

Expression rates of the various mucin phenotypes at the mucosal layer among patients classified according to the histological subtypes

The expression rates of various mucin phenotypes at the mucosal layer among the patients classified according to the histological subtype are shown in Table 2. The expression rates of HGM and MUC5AC were higher than those of MUC6 and M-GGMC-1 in all the histological subtypes, particularly in the cases of SIG (*P* = 0.02) and non-SIG (*P* < 0.01). The expression rate of M-GGMC-1 in the cases of DAC was higher than that in the cases of SIG (*P* < 0.01) and non-SIG (*P* < 0.01).

The MUC2-expression rates in the cases of DAC, SIG, and non-SIG were 76%, 33%, and 56%, respectively. The MUC2-expression rate was lowest in the cases of SIG, and the expression rate in the cases of DAC was higher than that in the cases of SIG (*P* = 0.02) and non-SIG (*P* = 0.06). No CD10 expression was detected in any of the cases.

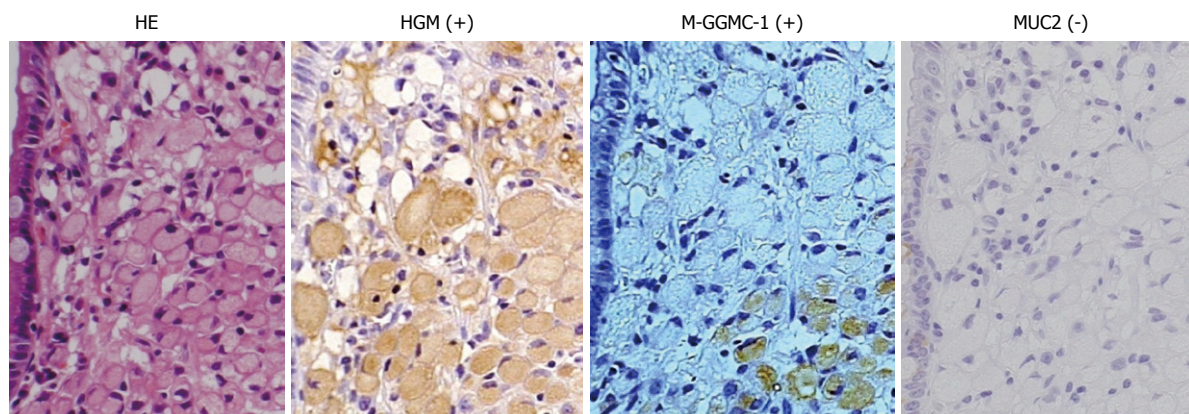


Figure 1 Typical photographs of a patient with signet-ring cell carcinoma (SIG) expressing the gastric (G) phenotype.

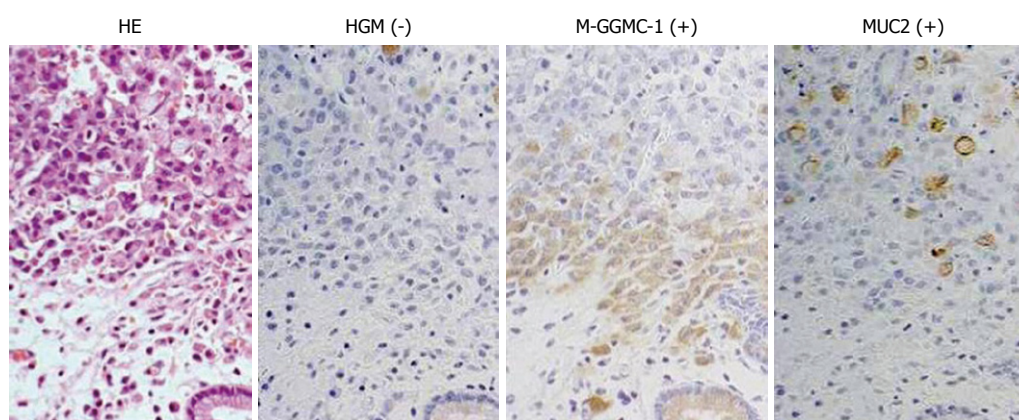


Figure 2 Typical photographs of a patient with non-SIG expressing the gastrointestinal (GI) phenotype.

Table 3 Expression rates of the various mucin phenotypes at the intramucosal layer of gastric adenocarcinomas according to the histological subtypes *n* (%)

Mucin phenotype/histological subtype	G	GI	I	UC
DAC (<i>n</i> = 38)	9/38 (24)	29/38 (76) ^{a,b}	0/38 (0)	0/38 (0)
SIG (<i>n</i> = 9)	6/9 (67)	3/9 (33) ^a	0/9 (0)	0/9 (0)
Non-SIG (<i>n</i> = 48)	19/48 (40)	27/48 (56) ^b	0/48 (0)	2/48 (4)

G: Gastric phenotype; GI: Gastrointestinal phenotype; I: Intestinal phenotype; UC: Unclassified phenotype. ^a*P* = 0.02; ^b*P* = 0.06.

Expressions of G, GI, I, and UC phenotypes at the mucosal layer among patients classified according to the histological subtypes

The expressions of the G, GI, I, and UC phenotypes at the mucosal layer among the patients classified according to the different histological subtypes are shown in Table 3. The expressions of the G, GI, I, and UC phenotypes in patients with DAC were 24%, 76%, 0%, and 0%, respectively; the expressions in patients with SIG were 67%, 33%, 0%, and 0%, respectively; and the expressions in patients with non-SIG were 40%, 56%, 0%, and 4%, respectively. For all the histological subtypes, the expressions of the I and UC phenotypes were extremely low

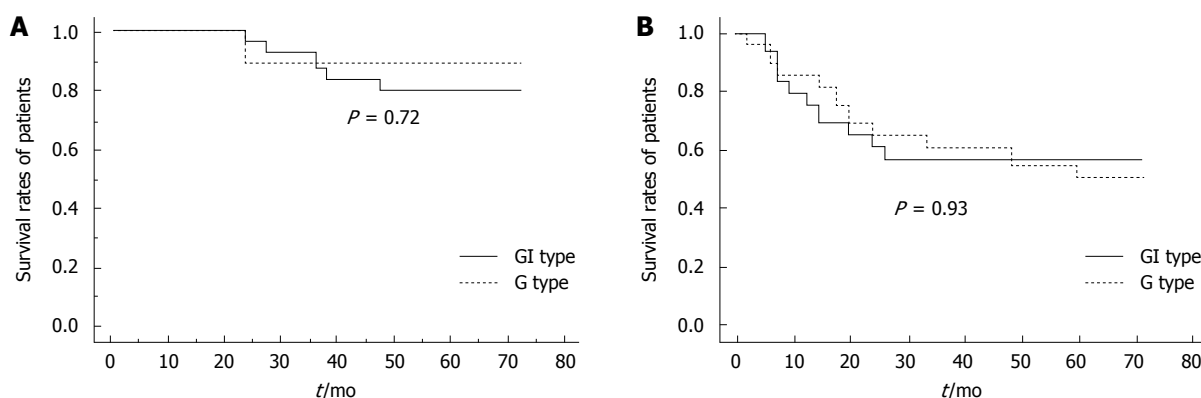
and those of the G and GI phenotypes were extremely high. The expression of the GI phenotype in the cases of DAC was higher than that in the cases of SIG (*P* = 0.02) and non-SIG (*P* = 0.06).

Comparison between the expressions of the G and GI phenotypes with respect to clinical and histological findings in patients classified according to the histological subtypes

Table 4 presents a comparison between the expressions of the G and GI phenotypes with respect to the clinical profiles, tumor site, tumor size, metastasis rate, and histological findings among the patients classified according to the histological subtypes. In SIG, all 6 female patients expressed the G phenotype and all 3 male patients expressed the GI phenotype (*P* = 0.01). Among the subjects with non-SIG, the number of GI phenotype cases with a tumor diameter greater than 5 cm was significantly higher than the corresponding number of G phenotype cases (*P* = 0.01). The positive rate of lymph node metastasis in the GI phenotype cases was also significantly higher than the corresponding value in the G phenotype cases (*P* = 0.01). There were no significant differences in other factors between the patients expressing the G and GI phenotype.

Table 4 Comparisons between the expression of G and GI phenotypes in clinical profiles, tumor site and tumor size, metastasis rate, and histological findings, according to the histological subtype

Histological subtype	DAC		SIG		Non-SIG	
	G (n = 9)	GI (n = 29)	G (n = 6)	GI (n = 3)	G (n = 19)	GI (n = 27)
Male:female	6:3	12:17	0:6 ^a	3:0 ^a	9:10	10:17
Mean age (yr)	57.2	60.3	63.2	55.3	68.2	60.9
Tumor site						
U	2	7	2	0	2	5
M	2	10	0	1	9	18
L	5	12	4	2	8	4
Tumor size (cm)						
< 5	3	17	3	0	9 ^b	3 ^b
> 5	6	12	3	3	10 ^b	24 ^b
Metastasis rate (expression rate, %)						
Lymph node	67	45	83	100	42 ^c	81 ^c
Other site	22	3	50	33	11	19
Histological findings (expression rate, %)						
Vessel invasion	67	69	67	100	84	93
Lymph invasion	100	86	83	100	95	100

^aP = 0.01, ^bP = 0.01, ^cP = 0.01.**Figure 3** Survival rates of patients with the G and GI phenotypes in differentiated adenocarcinoma (DAC) (A) and non-SIG (B). The Kaplan-Meier method was used for the analysis.

Survival rates of the patients expressing the G and GI phenotype classified according to the histological subtypes

The survival rates of the G and GI phenotype cases classified according to the histological subtypes are shown in Figure 3. Among the patients with DAC, there was no significant difference between the survival rates of patients expressing the G phenotype and those expressing the GI phenotype (Figure 3A). The survival rates among the non-SIG patients expressing the G phenotype and those expressing the GI phenotype (Figure 3B) were also not significantly different ($P = 0.93$).

DISCUSSION

In the present study, HGM and MUC5AC were highly expressed in all histological subtypes. Among the SIG and non-SIG cases examined, the expression rates of MUC6 and M-GGMC-1 were lower than those of HGM and MUC5AC. The expression rates of MUC6 and M-GGMC-1 in the DAC were higher than those in the SIG and non-SIG cases. Pinto-de-Sousa *et al.*^[18] showed

that the mucin phenotype is associated with the tumor site. In the SIG and non-SIG cases in the present study, the upper or middle segments of the stomach were the most common tumor sites. The differences between the rates of expression of the G-related phenotypes were believed to have been influenced by the tumor location. In addition, most cases of AGA showed the presence of gastric foveola phenotype. Further, we observed that the proportion of women in the SIG group was high and that none of the women with SIG expressed the GI phenotype. With regard to the clinical features of the mucin phenotype, the SIGs in women had specific clinical features.

On the basis of the examination of various mucin phenotype-expressing antigens, we classified almost all the AGAs (over 95%) as having either a G or GI phenotype. Furthermore, we failed to detect the expression of the pure I phenotype in any of the cases. The expression rate of the UC phenotype was also extremely low. Pinto-de-Sousa *et al.* studied the mucin phenotypes of 23 diffuse-type adenocarcinomas classified according to Lauren's method^[7] and showed that the MUC5AC-ex-

pression rate in these adenocarcinomas was significantly higher than that in the unclassified and expansive adenocarcinomas^[18]. Further, Reis *et al*^[4] studied the expression of MUC5AC in early gastric adenocarcinomas and suggested that all gastric adenocarcinomas retain at least some G phenotype cells in the initial stages of neoplasm development. Therefore, we postulate that almost all the cases of advanced DAC, SIG, and non-SIG exhibit or retain the features of G-related phenotypes in the intramucosal layer.

In the present study, the I phenotype (GI phenotype) was expressed in 56% of the non-SIG cases. Barresi *et al*^[21] reported that 8 out of every 10 cases of diffuse-type adenocarcinomas were MUC2-positive. Tajima *et al*^[23] studied the expression rates of the GI and I phenotypes in the cases of undifferentiated type AGA and reported that the I phenotype was expressed in half of the cases. Yamachika *et al*^[15] and Bamba *et al*^[16] reported that the progression of SIG was associated with a phenotypic shift from the G-type to the I-type expression. Other studies have described the invasive features of the I phenotype in gastric adenocarcinoma^[19,22,26]. The results of our study and the abovementioned studies suggest that more than 50% of the cases with non-SIG AGA exhibit the features of the I phenotype and that this phenotypic feature is acquired or transformed during the initial progression stage of these adenocarcinomas. In addition, our results indicate that the GI phenotype is associated with invasive features.

The expression rate of the I phenotype (GI phenotype) among the SIG cases was 33% and the number of SIG cases was low (9 cases). We assume that SIG cells with the G phenotype cannot progress to the deep layer. It is also suggested that the morphological features of the SIG cells change and are subsequently classified as non-SIG during tumor progression.

In the present study, we also examined the relationships between the expression of mucin phenotypes and the clinicopathological features and prognosis of patients with DAC, SIG, and non-SIG. Among the non-SIG cases, the expression rate of the GI phenotype in both the patients with extended tumors and those with lymph node metastasis was significantly high. The survival rates of the DAC and non-SIG patients who expressed the G or GI phenotypes were not significantly different. These results indicate that the acquisition of the I phenotype in patients with non-SIG AGA is related to tumor extension and lymph node metastasis and that the existence or acquisition of this phenotype does not affect the survival rate of these patients. Our results indicate that the GI phenotype is associated with invasive features as described above. In SIG, the relationship between the survival rates of G and GI phenotype patients is unknown due to the small number of patients with these phenotypes.

The high rate of lymph node metastasis and the invasive tendency of the non-SIG cells with the GI phenotype may influence the 5-year survival rate, and the patients with the GI phenotype may thus show poor

prognosis. However, in the present study, there was no difference between the survival rates of G and GI phenotype patients showing these features. With regard to the features of the mucin phenotype in differentiated-type gastric adenocarcinoma, the G phenotype has been reported to have malignant features^[12,13]. In contrast to our study, Tajima *et al*^[23] studied the expression of the mucin phenotype in patients with AGA and reported that patients with the G phenotype adenocarcinomas had a poorer outcome than those with the I phenotype adenocarcinomas. Mizoshita *et al*^[24] reported that AGA patients expressing the GI phenotype had a relatively good prognosis. Although the reason for the difference is unclear, it could be attributed to the difference in the histological subtypes of the examined adenocarcinomas; this is because almost all the studies reporting the malignant potential of the G phenotype have considered differentiated adenocarcinomas^[12,13]. Further, we mainly studied the diffuse type of AGA, which was classified into the SIG and non-SIG types, namely the restricted diffuse type of Lauren's classification. In addition, we assume that the prognosis of the patients with advanced non-SIG will also depend on postoperative treatments such as postoperative chemotherapy.

Therefore, we assume that the expression of the G or GI phenotypes in cases of the advanced non-SIG type of pure diffuse-type gastric adenocarcinoma does not indicate a poor prognosis during the 5-year postoperative period.

In conclusion, the GI phenotype showed a high expression rate (56%) in patients with advanced non-SIG, thereby indicating the acquisition of I phenotypic features during the progression of adenocarcinomas. In patients with advanced non-SIG, although the GI phenotype may be associated with greater invasiveness than the G phenotype, the survival rates of patients expressing either phenotype are similar, suggesting that neither the G nor the I phenotype indicates a poor prognosis in this type of adenocarcinoma. However, the presence of the I phenotype in patients with advanced SIG is unknown.

COMMENTS

Background

The relationship between the clinicopathological features and mucin phenotypes in advanced gastric adenocarcinoma (AGA) classified according to the histological subtype [differentiated adenocarcinoma (DAC), signet-ring cell carcinoma (SIG), and diffuse-type adenocarcinoma (non-SIG)] is unclear.

Research frontiers

Recently, the expression of the mucin phenotype in gastric adenocarcinoma was reported. Lauren and Nakamura histologically classified gastric adenocarcinomas into 2 main types: intestinal type of Lauren's histology or differentiated type, and diffuse type or poorly differentiated type. There is little information on the effects of the mucin phenotypes on the clinicopathological and histological subtype-based features of AGA, particularly in the diffuse type as defined by Lauren's method.

Innovations and breakthroughs

This study is the first report that investigated the mucin phenotype in advanced differentiated, SIG and non-SIG gastric adenocarcinomas. The GI phenotype might possess more invasive characteristics than the G phenotype in non-SIG patients. However, neither of the phenotypes indicates a poor prognosis of DAC and non-SIG.

Applications

Mucin phenotypes of gastric adenocarcinoma are related to the biological features in these cancers. The study of mucin phenotype is useful to clarify the biological features in gastric adenocarcinoma.

Terminology

Mucins of stomach are heavily glycosylated glycoproteins that are the major components of the mucous viscous gel covering gastric surface mucous cells, pyloric gland cells, intestinal goblet cells of the mature gastrointestinal tract, and the brush border of intestinal epithelial cells, etc.

Peer review

The data presented are interesting and helpful for further understanding the possible clinical value of mucin phenotypes of gastric carcinomas. The conclusions extracted are reasonable, although most of them are disproving.

REFERENCES

- 1 **Tatematsu M**, Furihata C, Katsuyama T, Miki K, Honda H, Konishi Y, Ito N. Gastric and intestinal phenotypic expressions of human signet ring cell carcinomas revealed by their biochemistry, mucin histochemistry, and ultrastructure. *Cancer Res* 1986; **46**: 4866-4872
- 2 **Saito K**, Shimoda T. The histogenesis and early invasion of gastric cancer. *Acta Pathol Jpn* 1986; **36**: 1307-1318
- 3 **Tahara E**. Genetic alterations in human gastrointestinal cancers. The application to molecular diagnosis. *Cancer* 1995; **75**: 1410-1417
- 4 **Reis CA**, David L, Nielsen PA, Clausen H, Mirgorodskaya K, Roepstorff P, Sobrinho-Simões M. Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. *Int J Cancer* 1997; **74**: 112-121
- 5 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49
- 6 **Nakamura K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 1968; **59**: 251-258
- 7 Members of Working Group in World Health Organization Classification of Tumors. Tumor of the stomach. In: Hamilton SR, Aaltonen LA, editors. *Pathology and Genetics: Tumors of the Digestive System*. Lyon, France: IARC Press, 2000: 37-52
- 8 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma. 13th ed. Tokyo: Kanahara Inc., 1996: 26
- 9 **Endo Y**, Tamura G, Mtoyama T, Ajioka Y, Watanabe H. Well-differentiated adenocarcinoma mimicking complete-type intestinal metaplasia in the stomach. *Hum Pathol* 1999; **30**: 826-832
- 10 **Yoshikawa A**, Inada Ki K, Yamachika T, Shimizu N, Kaminiishi M, Tatematsu M. Phenotypic shift in human differentiated gastric cancers from gastric to intestinal epithelial cell type during disease progression. *Gastric Cancer* 1998; **1**: 134-141
- 11 **Nakamura T**, Yao T, Kabashima A, Nishiyama K, Maehara Y, Tsuneyoshi M. Loss of phenotypic expression is related to tumour progression in early gastric differentiated adenocarcinoma. *Histopathology* 2005; **47**: 357-367
- 12 **Shibata N**, Watari J, Fujiya M, Tanno S, Saitoh Y, Kohgo Y. Cell kinetics and genetic instabilities in differentiated type early gastric cancers with different mucin phenotype. *Hum Pathol* 2003; **34**: 32-40
- 13 **Koseki K**, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K. Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. *Cancer* 2000; **89**: 724-732
- 14 **Yamazaki K**, Tajima Y, Makino R, Nishino N, Aoki S, Kato M, Sakamoto M, Morohara K, Kaetsu T, Kusano M. Tumor differentiation phenotype in gastric differentiated-type tumors

and its relation to tumor invasion and genetic alterations. *World J Gastroenterol* 2006; **12**: 3803-3809

- 15 **Yamachika T**, Inada K, Fujimitsu Y, Nakamura S, Yamamura Y, Kitou T, Itzkowitz SH, Werther JL, Miki K, Tatematsu M. Intestinalization of gastric signet ring cell carcinomas with progression. *Virchows Arch* 1997; **431**: 103-110
- 16 **Bamba M**, Sugihara H, Kushima R, Okada K, Tsukashita S, Horinouchi M, Hattori T. Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the human stomach. *Virchows Arch* 2001; **438**: 49-56
- 17 **Saito A**, Shimoda T, Nakanishi Y, Ochiai A, Toda G. Histologic heterogeneity and mucin phenotypic expression in early gastric cancer. *Pathol Int* 2001; **51**: 165-171
- 18 **Pinto-de-Sousa J**, David L, Reis CA, Gomes R, Silva L, Pimenta A. Mucins MUC1, MUC2, MUC5AC and MUC6 expression in the evaluation of differentiation and clinicobiological behaviour of gastric carcinoma. *Virchows Arch* 2002; **440**: 304-310
- 19 **Aihara R**, Mochiki E, Nakabayashi T, Akazawa K, Asao T, Kuwano H. Clinical significance of mucin phenotype, beta-catenin and matrix metalloproteinase 7 in early undifferentiated gastric carcinoma. *Br J Surg* 2005; **92**: 454-462
- 20 **Kabashima A**, Yao T, Maehara Y, Tsuneyoshi M. Relationship between biological behavior and phenotypic expression in undifferentiated-type gastric carcinomas. *Gastric Cancer* 2005; **8**: 220-227
- 21 **Barresi V**, Vitarelli E, Grosso M, Tuccari G, Barresi G. Relationship between immunoexpression of mucin peptide cores MUC1 and MUC2 and Lauren's histologic subtypes of gastric carcinomas. *Eur J Histochem* 2006; **50**: 301-309
- 22 **Tian MM**, Zhao AL, Li ZW, Li JY. Phenotypic classification of gastric signet ring cell carcinoma and its relationship with clinicopathologic parameters and prognosis. *World J Gastroenterol* 2007; **13**: 3189-3198
- 23 **Tajima Y**, Shimoda T, Nakanishi Y, Yokoyama N, Tanaka T, Shimizu K, Saito T, Kawamura M, Kusano M, Kumagai K. Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. *Oncology* 2001; **61**: 212-220
- 24 **Mizoshita T**, Tsukamoto T, Nakanishi H, Inada K, Ogasawara N, Joh T, Itoh M, Yamamura Y, Tatematsu M. Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. *J Cancer Res Clin Oncol* 2003; **129**: 727-734
- 25 **Tajima Y**, Yamazaki K, Nishino N, Morohara K, Yamazaki T, Kaetsu T, Suzuki S, Kawamura M, Kumagai K, Kusano M. Gastric and intestinal phenotypic marker expression in gastric carcinomas and recurrence pattern after surgery-immunohistochemical analysis of 213 lesions. *Br J Cancer* 2004; **91**: 1342-1348
- 26 **Yamagishi M**, Noda M, Tatsumi Y, Mukaisho K, Mitsufuji S, Sugihara H, Okanoue T, Hattori T. Correlation between cyclooxygenase-2, proliferative activity, and mucin phenotype in human advanced gastric cancer. *J Gastroenterol* 2004; **39**: 1143-1149
- 27 **Kim GH**, Song GA, Park DY, Lee SH, Lee DH, Kim TO, Jo HJ, Heo J, Kang DH, Cho M. CDX2 expression is increased in gastric cancers with less invasiveness and intestinal mucin phenotype. *Scan J Gastroenterol* 2006; **41**: 880-886
- 28 **Shiroshita H**, Watanabe H, Ajioka Y, Watanabe G, Nishikura K, Kitano S. Re-evaluation of mucin phenotypes of gastric minute well-differentiated-type adenocarcinomas using a series of HGM, MUC5AC, MUC6, M-GGMC, MUC2 and CD10 stains. *Pathol Int* 2004; **54**: 311-321
- 29 **Watanabe G**, Watanabe H, Ajioka Y, Shiroshita H, Nishikura K. Well-differentiated type adenocarcinomas of gastric mucin phenotype transform into intestinal type carcinomas. *Stomach and Intestine* 2003; **38**: 693-700

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Protective effect of recombinant human IL-1Ra on CCl₄-induced acute liver injury in mice

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Abstract

AIM: To evaluate the effects of positive regulation of recombinant human interleukin 1 receptor antagonist (rhIL-1Ra) on hepatic tissue recovery in acute liver injury in mice induced by carbon tetrachloride (CCl₄).

METHODS: Acute liver damage was induced by injecting 8-wk-old mice with CCl₄ 1 mL/kg (1:3 dilution in corn oil) intraperitoneally (ip). Survival after liver failure was assessed by injecting 8-wk-old mice with a lethal dose of CCl₄ 2.6 mL/kg (1:1 dilution in corn oil) ip. Mice

were subcutaneously injected with 1 mg/kg recombinant human IL-1Ra twice a day after CCl₄ treatment for 5 d. Serum alanine amino transferase (ALT) and aspartate aminotransferase (AST) levels were determined with a commercial assay kit. Serum IL-1 β , IL-1Ra levels were measured by enzyme-linked immunosorbent assay kit. Quantitative real-time polymerase chain reaction was used to determine liver IL-1 β , IL-1Ra and IL-6 expression during CCl₄-induced acute liver injury. Liver sections were stained with hematoxylin-eosin. A histology-injury grading system was used to evaluate the degree of necrosis after acute liver injury. Proliferating cell nuclear antigen (PCNA) staining was used to evaluate the role of rhIL-1Ra in promoting hepatocyte proliferation.

RESULTS: Quantitative analysis showed a higher level of IL-6 mRNA expression and reduced serum AST and ALT levels in the livers of the rhIL-1Ra-treated group at the early phase of CCl₄-induced acute liver injury. Histological examination indicated a decrease in centrilobular necrotic areas in mice treated with rhIL-1Ra, and a novel role of rhIL-1Ra in promoting hepatocyte proliferation was also supported by an increase of PCNA staining. All these results, accompanied by a strong survival benefit in rhIL-1Ra-treated *vs* PBS-treated groups, demonstrated that rhIL-1Ra administration ameliorated the histological damage and accelerated the regeneration and recovery process of the liver.

CONCLUSION: rhIL-1Ra could be further developed as a novel therapeutic agent for the treatment of acute liver injury because of its ability to reduce hepatocellular damage and facilitate liver regeneration.

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Key words: Recombinant human interleukin 1 receptor antagonist; Carbon tetrachloride; Liver injury; Hepatocyte proliferation

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INTRODUCTION

The interleukin (IL)-1 family includes the structurally related proteins IL-1 α , IL-1 β , and interleukin 1 receptor antagonist (IL-1Ra) that bind to the same cell surface receptor. However, IL-1Ra functions as a competitive inhibitor of IL-1 α and IL-1 β ^[1]. IL-1 α and IL-1 β play a key role in inflammation^[2]; in response to stimuli such as inflammatory agents, infections, or microbial endotoxins, a dramatic increase of IL-1 mediated by macrophages and various other cell types is seen^[3,4]. IL-1Ra is a naturally occurring anti-inflammatory protein; it competitively blocks the binding of IL-1 α and IL-1 β to type I IL-1 receptor, but exerts no agonist activity^[5,6]. IL-1Ra has been shown to inhibit the effects of IL-1 both *in vitro* and *in vivo*, and as an acute-phase protein, it can reduce the severity of several animal models of inflammatory disease^[7]. Serum levels of IL-1Ra can rise dramatically during different inflammatory and noninflammatory conditions^[8]. Human IL-1Ra is synthesized as a 177 amino acid precursor that contains a 25 amino acid signal sequence plus a 152 amino acid mature region^[9,10].

Acute administration of carbon tetrachloride (CCl₄) is used to establish an experimental model of severe hepatocellular damage involving generation of oxidative stress and recruitment of inflammatory cells^[11-13], which is reported to induce liver architectural and functional damage^[14-16]. Liver regeneration involves a complex regulated response to CCl₄-induced acute liver injury^[17,18].

In this study, we aimed to examine the effects of IL-1Ra as an acute phase protein in reducing hepatic injury and accelerating hepatocyte proliferation following CCl₄ administration. Although the anti-inflammatory effect of IL-1Ra has been described, the contribution of this cytokine to protect from the liver injury remains unclear. The present findings indicate that IL-1Ra is a critical factor that shows a potent antihepatotoxic activity in recovery of hepatocellular necrosis and in acceleration of liver regeneration during injury. IL-1Ra could provide a novel therapeutic approach by stimulating liver regeneration.

MATERIALS AND METHODS

Animals

Procedures were performed in male C57BL/6 mice (purchased from SLC Shanghai, China) 8 wk after birth, main-

tained in a conventional clean facility and in accordance with the National Animal Care and Use Committee.

Cytokine and reagents

Recombinant human IL-1Ra (rhIL-1Ra) was obtained from Dr Wei Han's Laboratory at the School of Pharmacy, Shanghai Jiao Tong University. Endotoxin level of the rhIL-1Ra was under 0.1 EU/ μ g. CCl₄ was purchased from Sigma, USA.

Acute liver injury and lethal dose performance

Acute liver injury was induced by injecting 8-wk-old mice with CCl₄ 1 mL/kg (1:3 dilution in corn oil) intraperitoneally (ip). A lethal dose was administered by injecting 8-wk-old mice with CCl₄ 2.6 mL/kg (1:1 dilution in corn oil) ip.

rhIL-1Ra and PBS injection

Mice were subcutaneously injected with 1 mg/kg rhIL-1Ra (diluted to 0.5 mg/mL with PBS) twice a day after CCl₄ administration for 5 d because human and murine IL-1Ra show an overall homology of 77% with no apparent species specificity^[19]. The first rhIL-1Ra injection was performed at 1 h after CCl₄ treatment. The control group of mice was subcutaneously injected with the same volume of PBS.

Serum aspartate aminotransferase and alanine amino transferase

Serum aspartate aminotransferase (AST) and alanine amino transferase (ALT) levels were determined with a commercial assay kit (Nanjing Jiancheng Biological Technology, Inc., China). Enzyme activities were expressed as an international unit per liter (IU/L).

Enzyme-linked immunosorbent assay

Serum IL-1 β and IL-1Ra level were measured by enzyme-linked immunosorbent assay (ELISA) kit (R&D system, Minneapolis, MN, USA) according to the manufacturer's instructions.

Histology-injury grading

Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin-eosin for the histological investigations. To evaluate the degree of necrosis after acute liver injury we created an injury grading score (Grade I - IV) based on severity of necrotic lesions in the liver parenchyma (Table 1).

Proliferating cell nuclear antigen staining

For proliferating cell nuclear antigen (PCNA) immunohistochemical staining, de-paraffinized sections of liver blocks were used. Liver tissues were fixed for 24 h in neutral buffered formalin, processed routinely and embedded in wax. Immunohistochemical staining was performed as previously described^[16]. The sectioned liver tissues were stained using a mouse monoclonal antibody against PCNA and the SABC Staining Kit (Wuhan Boster Biological Technology, Wuhan, China) according to manufac-

Table 1 Injury grade

No. of mice	Day + 2 ¹	Day + 3 ¹	Day + 5 ¹	Day + 7 ¹
Group A (rhIL-1Ra)				
1	III	II	I	0
2	III-IV	I	I	0
3	III	I	0	0
4	III	II	I	0
5	III-IV	I	0	1
6	IV	I	0	0
Group B (PBS)				
1	III-IV	II-III	I-II	0
2	IV	III	II	1
3	III-IV	III	II	0
4	IV	II-III	II	0
5	IV	III	II	1
6	III-IV	III	I	1

¹Day of sacrifice after CCl₄ 1 mL/kg (1:3 dilution in corn oil) ip treatment. Injury grading with respect to severity of necrosis in liver parenchyma: Grade 0: Normal histology; Grade I: Presence of degenerated hepatocytes with only rare foci of necrosis; Grade II: Mild centrilobular necrosis around the central vein, occupying only a part of Rappaport's zone III; Grade III: Established necrosis limited to zone III; Grade IV: Extensive, confluent centrilobular necrosis involving Rappaport's zone III and II. rhIL-1Ra: Recombinant human interleukin 1 receptor antagonist.

turer's protocol, then subjected to photomicroscopic observation (NIS-Elements Basic Research, Nikon Eclipse 50i, Kanagawa, Japan).

Quantitative real-time polymerase chain reaction

Total RNA was obtained from the liver of mice and was prepared using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). The quantification and qualification of RNA were determined by UV absorbance and electrophoresis in 1.2% agarose. RNA quality was satisfied when the 28s rRNA banding was twice the intensity of the 18s rRNA without significant smearing of the rRNA bands. Quantitative real-time polymerase chain reaction (RT-PCR) reactions were performed with the MJ chromo 4 RT-PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Specific primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and their sequences are listed as follows: IL-1 β (sense) 5'TGAGCACCTTCCTTTTCCTTC3', IL-1 β (anti-sense) 5'GTTTCATCTCGGAGCCTGTAG3'; IL-1Ra (sense) 5'AGACCTTGTGTCTCTGTTTAGC3', IL-1Ra (anti-sense) 5'GGTCAATAGGCACCATGTCT3'; IL-6 (sense) 5'CCACTCCCAACAGACCTGTCTATAC3', IL-6 (anti-sense) 5'CACAACCTCTTTTCTCATTTCCACGA3'; β -actin (sense) 5'AGCCTTCCTTCTTGGGTATG3', β -actin (anti-sense) 5'GTGTTGGCATAGAGGTCTTTAC3'. For the RT-PCR reaction, the following procedure was followed. Total RNA (5 μ g) was used as a template for synthesizing the first-strand of cDNA with M-MuLV reverse transcriptase (MBI Fermentas, Vilnius, Lithuania) in a 20 mL reaction volume. PCR reactions were carried out by adding 100 \times diluted cDNAs, 100 nmol/L of each primer, and SYB Premix Ex Taq (TaKaRa, Dalian, China) in 20 μ L reactions. PCR conditions were optimized using

Opticon monitor 3 software (Bio-Rad Laboratories) and involved the following steps: 95°C for 5 min, 1 cycle; 95°C for 5 s and 60°C for 30 s, 40 cycles. Final data were analyzed with Opticon monitor 3 software (Bio-Rad Laboratories), presented as ratios to β -actin for each time point.

Statistical analysis

Results are expressed as mean \pm SD. Statistically significant differences over time in the same treatment group, or among different treatment groups at a single time point, were determined by Student's *t* test. *P* < 0.05 was considered to be statistically significant. Results from survival experiments were analyzed using the log-rank test and expressed as Kaplan-Meier survival curves.

RESULTS

IL-1 β , IL-1Ra and IL-6 expression during CCl₄-induced acute liver injury

Expression of IL-1 β mRNA decreased in the first 12 h, and reached its lowest point at day 1.5 (Figure 1A). In contrast, expression of IL-1Ra mRNA was rapidly induced and reached a peak within 12 h following 1 mL/kg CCl₄ administration (Figure 1B). Serum level of IL-1 β did not increase so rapidly (Figure 1C). We found that serum IL-1Ra enhanced markedly after CCl₄ administration (Figure 1D), induced by generation of oxidative stress and recruitment of inflammatory cells. We confirmed that an adequate ratio of serum IL-1Ra to IL-1 was crucial to the recovery of liver injury (Figure 1E), and we found that the ratio reached a peak at day 1.5 after CCl₄ administration. Furthermore, the expression of IL-6 mRNA was also stimulated by excessive treatment with rhIL-1Ra (Figure 1F).

rhIL-1Ra protects mice from acute hepatocellular damage

CCl₄-induced acute liver injury that results in a quantifiable liver damage recovers naturally within 7 d, as mice sacrificed 7 d after CCl₄ injection appear with normal liver histology. To confirm the role of rhIL-1Ra in protecting from hepatic damage, we investigated the effect of rhIL-1Ra on CCl₄-induced acute liver injury. Mice were subcutaneous injected with rhIL-1Ra and PBS after CCl₄ administration. Animals were sacrificed 1, 3, 5 and 7 d after CCl₄ administration for AST and ALT determination. The serum level of ALT or AST rapidly elevated to reach a peak at day 1 then decreased thereafter in PBS-treated control mice, while rhIL-1Ra treatment significantly inhibited the elevation of ALT and AST from day 1 to day 5 (Figure 2A and B). The reduction of serum AST and ALT indicated that rhIL-1Ra has a direct protective effect on hepatocytes. To evaluate the effect of rhIL-1Ra on hepatocellular necrosis and inflammation, histological changes in the liver after CCl₄ administration with or without rhIL-1Ra treatment were examined by histology-injury grading (Table 1). Liver sections from PBS-treated animals showed hepatocellular necrosis and inflammation at day 3 after CCl₄ administration; in contrast, liver sections from the rhIL-1Ra-treated group demonstrated only mild hepatocellular necrosis and in-

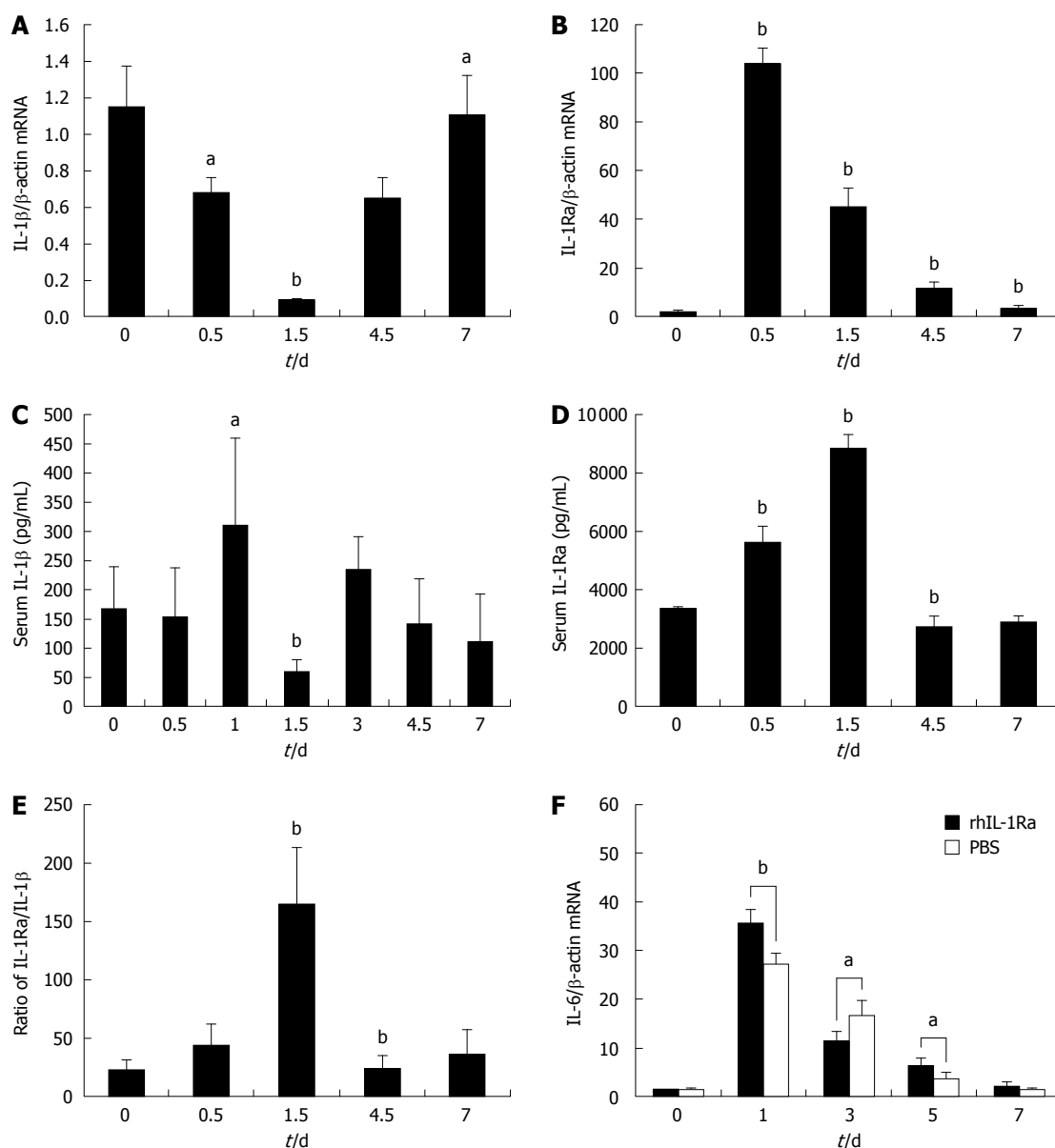


Figure 1 Interleukin (IL)-1 β , interleukin 1 receptor antagonist (IL-1Ra) and IL-6 expression after carbon tetrachloride (CCl₄) (1 mL/kg) administration. A: Quantification of IL-1 β mRNA levels; B: IL-1Ra mRNA levels; C: Serum IL-1 β ; D: Serum IL-1Ra; E: Ratio of serum level of IL-1Ra to IL-1 β ; F: Quantification of IL-6 mRNA levels of the livers treated with recombinant human IL-1Ra (rhIL-1Ra) or PBS. ^a $P < 0.05$, ^b $P < 0.01$.

flammation was dramatically decreased. We found the necrotic areas were significantly diminished around the central vein (Figure 2D and E) and centrilobular regions (Figure 2C) in rhIL-1Ra-treated mice at day 3. However, rhIL-1Ra did not cause any liver injury to healthy mice (Figure 2F). These findings indicate that rhIL-1Ra has a potent anti-hepatotoxic activity in reducing hepatocellular necrosis around the central vein.

rhIL-1Ra promotes hepatocyte proliferation from an early phase

We also investigated the proliferation of hepatocytes by immunostaining of PCNA in sections of liver tissue at days 2 and 3. Our PCNA staining confirmed that the number of positive cells increased sharply at day 2 (Figure 3C). Great numbers of hepatocytes (Figure 3E) could be de-

tected in the liver sections of rhIL-1Ra-treated mice at day 3, which demonstrated that rhIL-1Ra significantly increased the number of PCNA⁺ cells. In contrast, PBS-treated mice showed a much fewer number of PCNA⁺ cells (Figure 3D and F). In our study, we also confirmed rhIL-1Ra was unable to induce hepatocyte proliferation (Figure 3A and B) in normal mice. Numbers of PCNA⁺ cells (Figure 3G) in at least 12 mm² tissue sections were counted for each mouse, which showed that mice receiving rhIL-1Ra after CCl₄ injection gained the potent advantage of accelerating hepatocyte proliferation from an early phase.

rhIL-1Ra increases probability of survival after a lethal dose performance

In dose-response experiments, we found that 2.6 mL/kg

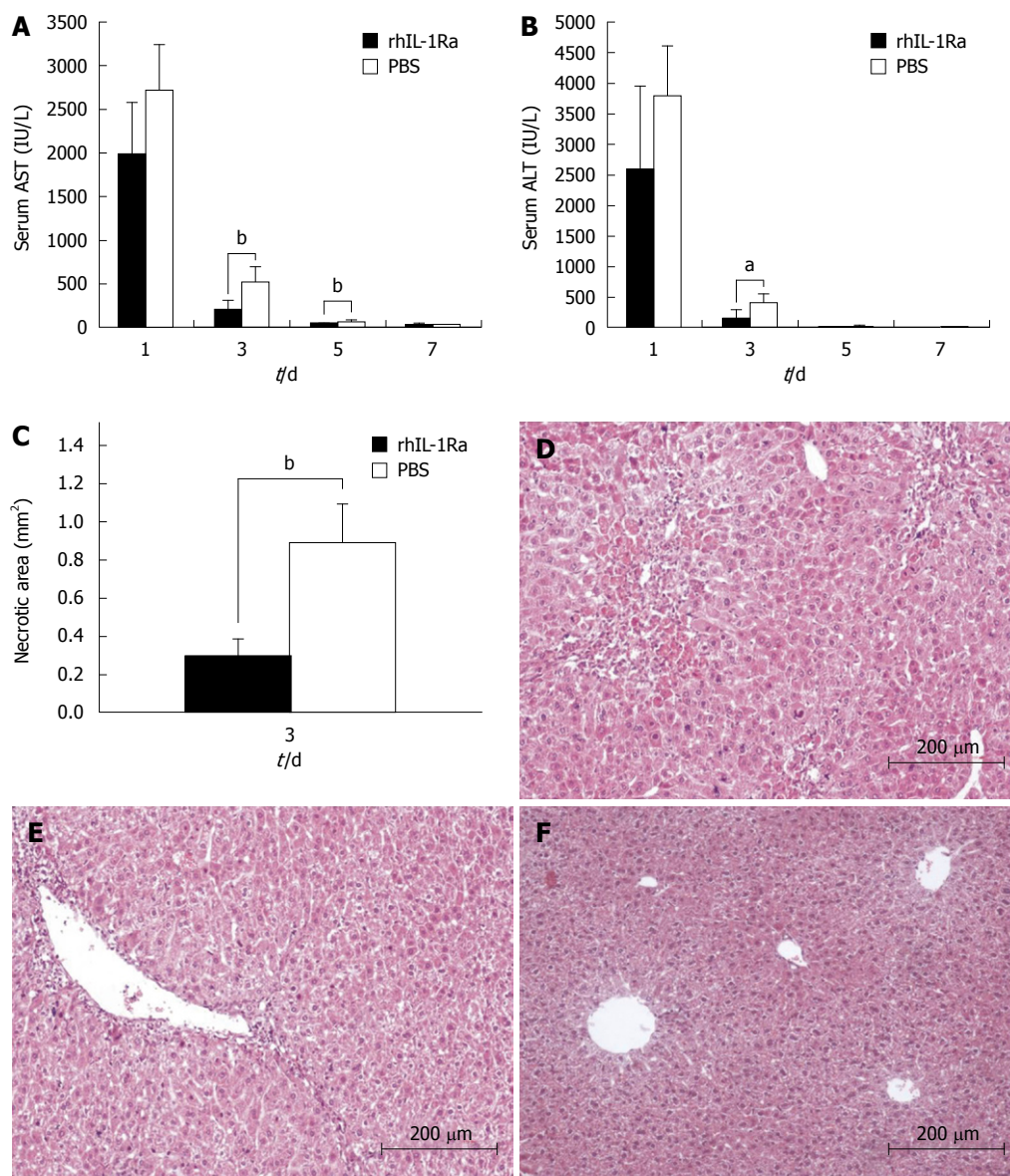


Figure 2 Acute liver injury (1 mL/kg CCl₄ administration) ± rhIL-1Ra. A: Serum aspartate aminotransferase (AST); B: Serum alanine amino transferase (ALT), with rhIL-1Ra or PBS; C: Necrotic areas. Representative findings from at least 12 mm² tissue sections were counted for each mouse; D-F: Hematoxylin and eosin (HE) stained liver sections; D: Group received PBS at day 3 after CCl₄ administration, shows necrosis with clusters of inflammatory cells around central vein (original magnification, × 100); E: Group received rhIL-1Ra at day 3 after CCl₄ administration, demonstrates mostly histological recovery with only inconspicuous necrosis still remaining around central vein and very few inflammatory cells are present (original magnification, × 100); F: Normal liver histology with rhIL-1Ra treatment, which shows no difference from normal liver tissue histology. ^a*P* < 0.05, ^b*P* < 0.01.

CCl₄ is a median lethal dose (mortality 50%, data not shown) within 24 h. rhIL-1Ra treatment in the CCl₄-induced acute liver failure model offers a survival benefit in treated mice, increasing the probability of survival significantly from 10.0% to 55.0% at day 3 after CCl₄ injection (*P* = 0.006, Figure 4).

DISCUSSION

The model of acute intoxication with CCl₄ has been used for decades to investigate the response of acute and chronic liver injury, because the elementary lesions caused by this hepatotoxin replicate those seen in most cases of human liver diseases. Pro-inflammatory

cytokines such as IL-6 and IL-1β are believed to play a key role in the pathogenesis of CCl₄-induced liver injury^[8,18,20,21], which make it a good model for us to study signal transduction and cell cycle events in a synchronized manner *in vivo*. The CCl₄-induced acute liver injury model is generated with 1 mL/kg dose for a typical hepatic injury, which would function as a strong regenerative stimulus. Regarding liver damage, IL-1Ra plays a critical role in the prevention of fatty liver and hypercholesterolemia under inflammatory conditions^[22-24]. In this study, we investigated the severity of CCl₄-induced acute liver injury in mice after rhIL-1Ra treatment; the results demonstrate that rhIL-1Ra hypodermal injection affords protection from liver injury.

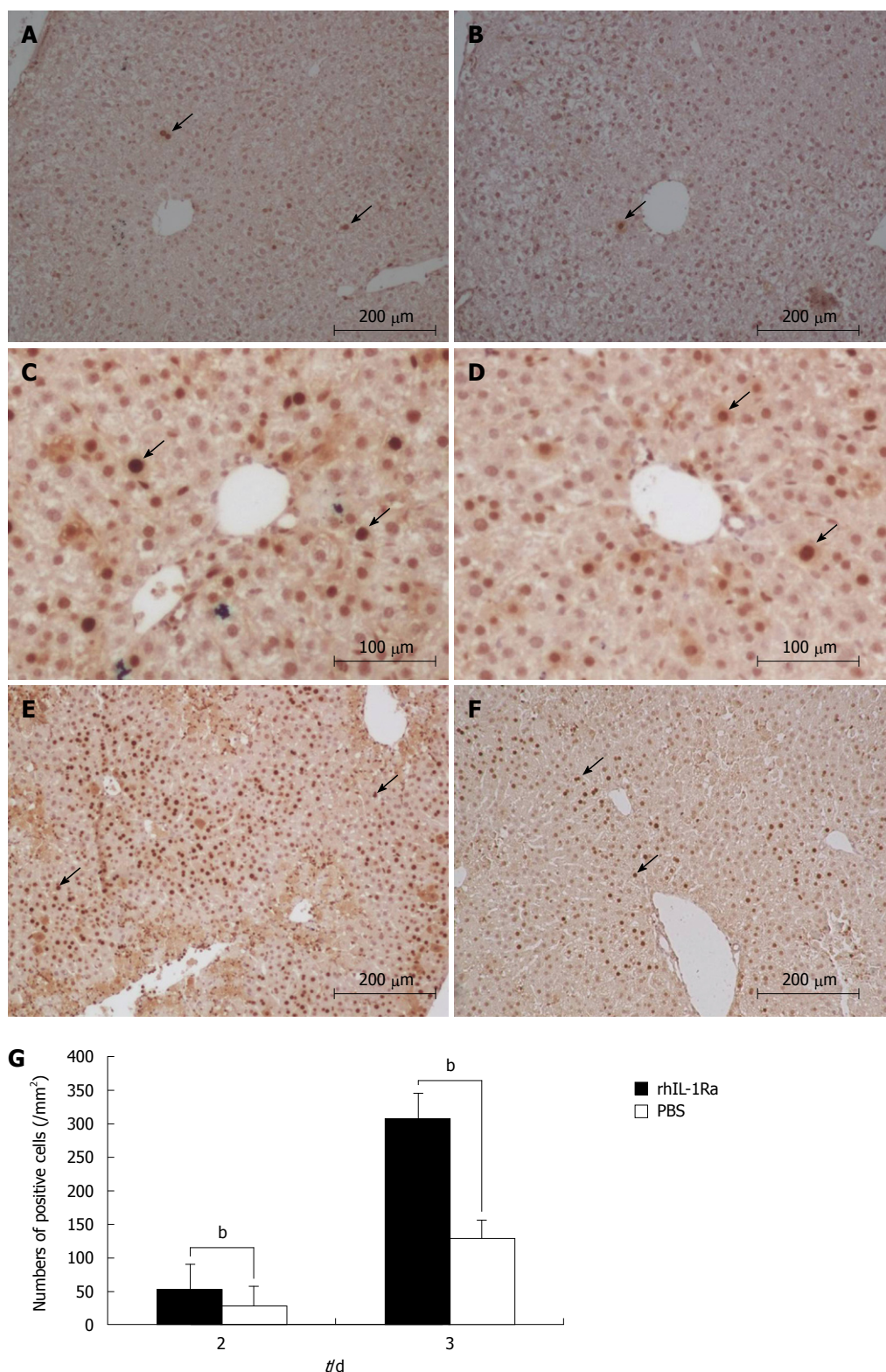


Figure 3 Immunostaining of proliferating cell nuclear antigen (PCNA) shows rhIL-1Ra promotes hepatocyte proliferation. A: Normal liver (original magnification, $\times 100$); B: Normal liver in rhIL-1Ra treated mice (original magnification, $\times 100$); C: Group received rhIL-1Ra at day 2 after CCl₄ administration, numerous PCNA+ hepatocytes in centrilobular areas and scattered PCNA+ hepatocytes at the edge of hepatocellular necrosis (original magnification, $\times 200$); D: Group received PBS at day 2 after CCl₄ administration, few PCNA+ hepatocytes in centrilobular areas at day 2 (original magnification, $\times 200$); E: Group received rhIL-1Ra at day 3 after CCl₄ administration, numerous positive cells in centrilobular areas around central vein (original magnification, $\times 100$); F: Group received PBS at day 3 after CCl₄ administration shows fewer numbers of positive cells (original magnification, $\times 100$); G: Numbers of PCNA+ cells after CCl₄ administration with rhIL-1Ra or PBS, at least 12 mm^2 tissue sections were counted for each mouse. Arrows point to PCNA+ hepatocytes. ^b $P < 0.01$.

The balance between IL-1 β and IL-1Ra has been extensively studied in a variety of experimental animal

models of disease; either local overproduction of IL-1 β or underproduction of IL-1Ra predisposes to the devel-

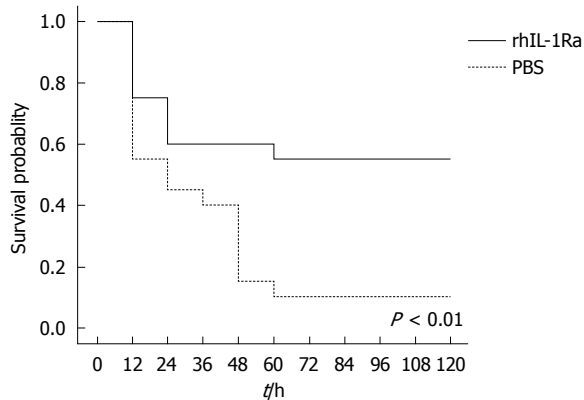


Figure 4 rhIL-1Ra treatment increased probability of survival after a lethal dose administration of CCl₄ (2.6 mL/kg). Mice were administered with either PBS as a control ($n = 20$) or rhIL-1Ra ($n = 20$) twice a day for 5 d. Survivals were scored twice a day for 5 d, and the results were analyzed using the log-rank test and expressed as the Kaplan-Meier survival curves. $P = 0.006$.

opment of disease and the therapeutic administration of IL-1Ra is efficacious in preventing tissue damage^[25]. Serum IL-1 β and IL-1Ra are mediators of inflammation. Down-regulation in the release of the pro-inflammatory cytokine IL-1 β and the up-regulation of its antagonist (IL-1Ra) may be a part of the inflammatory response to infection^[8]. It is necessary to functionally inhibit the biologic effects of IL-1 β on hepatocytes. We confirmed that liver IL-1 β mRNA originally decreased in the first 12 h after CCl₄ treatment, and reached its lowest level at day 1.5. On the other hand, liver IL-1Ra mRNA increased to its highest level at day 1.5. These results show that the liver has a strong power to trigger compensatory growth mechanisms, which suppress the liver IL-1 β expression. Several observations have emphasized that an adequate ratio of IL-1Ra to IL-1 β is protective in inflammatory or immune liver disease^[26-28]. Both serum IL- β and serum IL-1Ra were elevated after CCl₄ treatment, but serum IL-1Ra increased 100-fold greater than IL-1 β at day 1.5. Serum IL-1Ra to IL-1 β ratio was much higher in contrast to normal mice, which suggests a beneficial effect of IL-1Ra on CCl₄-induced acute liver injury. By analyzing the ELISA data of IL-1Ra and IL-1 β , we found that serum IL-1 β increased after CCl₄ treatment, which simultaneously stimulated the production of IL-1Ra as a compensatory reflection. The effects of IL-1Ra on blocking receptor binding of IL-1 β during the acute-phase response may serve to suppress the inflammatory consequences of early IL-1 β release after CCl₄-induced acute liver injury; with subsequent recovery, serum IL-1Ra decreased.

The level of serum aminotransferases is a very important marker to judge the severity of acute hepatic injury. After CCl₄ treatment the level of serum AST and ALT was significantly elevated, and attenuated by rhIL-1Ra in our experiment. The histological studies also showed that rhIL-1Ra inhibited inflammation and necrosis, which are the most common characteristics of CCl₄-induced liver damage. These findings suggest that rhIL-

1Ra protects hepatocytes from the oxidative damage caused by CCl₄, which is likely due to inhibition of the pro-inflammatory mediators.

Serum IL-1Ra can rise dramatically during different inflammatory and non-inflammatory conditions such as sepsis^[29] and chronic rheumatic diseases^[30-32]. In addition, it plays a crucial role in regulating IL-1 signaling in various inflammatory states. IL-1Ra deficiency has been associated with major metabolic dysfunctions^[33,34]. Serum levels of IL-1Ra were found to correlate with serum IL-6 concentrations^[35], and administration of either IL-1 or IL-6 to patients increased the circulating levels of IL-1Ra^[36,37]. Liver is a recognized target organ for pro-inflammatory cytokines such as TNF α , IL-1 and IL-6^[38,39]. Regarding liver regeneration, IL-6 is thought to result in enhanced transcription, triggering hepatocytes to leave their quiescent state (G0) and enter a prereplicative phase (G1). Expression of IL-6 appears to be essential for the priming of hepatocytes^[40,41]. Previous studies showed that IL-1Ra production was enhanced by IL-1 β , and increasing IL-1 β and IL-6 exhibits a strong stimulatory effect on the acceleration of IL-1Ra expression^[8]. IL-6 is a marked signal to trigger liver regeneration, as demonstrated in a previous study^[42], and in this current study, we found that the production of IL-6 could also be enhanced by excessive rhIL-1Ra treatment. *In vitro*, IL-1 β inhibits hepatocyte proliferation^[43]. Isoda *et al*^[33] found that mRNA levels of IL-1 β were significantly elevated in livers of IL-1Ra $^{-/-}$ mice, and liver growth is also inhibited by hepatocyte proliferation inhibitor and IL-1 β ^[44,45]. It is of particular interest that excessive rhIL-1Ra inhibits the activity of IL-1 β , which rapidly and significantly increased the number of PCNA $^{+}$ hepatocytes.

IL-1Ra is well tolerated clinically and has a short half-life, making it an ideal protective agent for acute hepatocellular damage and for accelerating liver regeneration. Healthy humans are the most sensitive indicators of IL-1 agonist activity: 1 ng/kg of intravenous IL-1 β produces symptoms^[46]. In contrast, the intravenous infusion of 10 mg/kg of IL-1Ra in healthy humans, a 10 million-fold molar excess, gives no effect^[47]. Histopathological studies showed that rhIL-1Ra-treated healthy mice show no changes in contrast with normal liver, and excessive rhIL-1Ra treatment could not induce hepatocellular proliferation in healthy mice. We used very classical methods to judge whether rhIL-1Ra has an effect on repairing the damage of mice CCl₄-induced acute liver injury and accelerating liver regeneration. The work indicates that rhIL-1Ra administration accelerates recovery from acute CCl₄-induced liver injury. To confirm that rhIL-1Ra dramatically prevented CCl₄-induced liver injury, a lethal dose of CCl₄ was used, and rhIL-1Ra treatment resulted in survival benefits in mice with acute hepatic failure. This work indicates that rhIL-1Ra accelerates recovery from acute CCl₄-induced liver injury and offers a strong survival advantage in injured mice.

Although additional studies are necessary to confirm this effect in humans, our findings provide a rationale

to develop new pharmacological strategies in the clinical management of patients with acute liver injury. This approach might also provide a novel therapeutic tool for regenerative liver cell therapy.

COMMENTS

Background

Interleukin 1 receptor antagonist (IL-1Ra) is a member of the interleukin 1 cytokine family and is a major anti-inflammatory cytokine. It acts as a natural inhibitor of the pro-inflammatory cytokines IL-1 α and IL-1 β through its action of blocking the binding of interleukin-1 to cell-surface receptors, and modulates a variety of IL-1-related immune and inflammatory responses. Although the anti-inflammatory effect of IL-1Ra has been described, the contribution of this cytokine in protecting from liver injury remains unclear.

Research frontiers

IL-1Ra is well tolerated clinically and has a short half-life, making it an ideal protective agent for acute hepatocellular damage and for accelerating liver regeneration. The authors used very classical methods to judge whether recombinant human IL-1Ra has effects on repairing the damage of carbon tetrachloride (CCl₄)-induced acute liver injury in mice and on accelerating liver regeneration.

Innovations and breakthroughs

The authors aimed to examine the effects of IL-1Ra as an acute phase protein in accelerating hepatocyte proliferation following CCl₄-induced liver injury. The results indicate that IL-1Ra is a critical factor that shows a potent antihepatotoxic activity in recovery of hepatocellular necrosis and accelerates liver regeneration during injury. IL-1Ra could provide a novel therapeutic approach by stimulating liver regeneration.

Applications

Although additional studies are necessary to confirm this effect in humans, the authors' findings provide a rationale to develop new pharmacological strategies in the clinical management of patients with acute liver injury. This approach might also provide a novel therapeutic tool for regenerative liver cell therapy.

Peer review

It is interesting and well-written. I suggest the authors perform electron microscopic examination in order to investigate the ultrastructural features of the hepatocytes of normal compared to recombinant human IL-1Ra-treated mice, and CCl₄-compared to recombinant human interleukin 1 receptor antagonist-treated mice.

REFERENCES

- Arend WP. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. *J Clin Invest* 1991; **88**: 1445-1451
- Dinarelli CA. Biologic basis for interleukin-1 in disease. *Blood* 1996; **87**: 2095-2147
- Dinarelli CA. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991; **77**: 1627-1652
- Dinarelli CA. The interleukin-1 family: 10 years of discovery. *FASEB J* 1994; **8**: 1314-1325
- Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 1991; **266**: 10331-10336
- Granowitz EV, Clark BD, Mancilla J, Dinarelli CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. *J Biol Chem* 1991; **266**: 14147-14150
- Cominelli F, Nast CC, Clark BD, Schindler R, Lierena R, Eysselein VE, Thompson RC, Dinarelli CA. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J Clin Invest* 1990; **86**: 972-980
- Gabay C, Smith MF, Eidlen D, Arend WP. Interleukin 1 receptor antagonist (IL-1Ra) is an acute-phase protein. *J Clin Invest* 1997; **99**: 2930-2940
- Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol* 1998; **16**: 27-55
- Gabay C, Porter B, Fantuzzi G, Arend WP. Mouse IL-1 receptor antagonist isoforms: complementary DNA cloning and protein expression of intracellular isoform and tissue distribution of secreted and intracellular IL-1 receptor antagonist in vivo. *J Immunol* 1997; **159**: 5905-5913
- Slater TF. Free-radical mechanisms in tissue injury. *Biochem J* 1984; **222**: 1-15
- Poli G. Liver damage due to free radicals. *Br Med Bull* 1993; **49**: 604-620
- Johnson SJ, Hines JE, Burt AD. Macrophage and perisinusoidal cell kinetics in acute liver injury. *J Pathol* 1992; **166**: 351-358
- Sasaki S, Yoneyama H, Suzuki K, Suriki H, Aiba T, Watanabe S, Kawauchi Y, Kawachi H, Shimizu F, Matsushima K, Asakura H, Narumi S. Blockade of CXCL10 protects mice from acute colitis and enhances crypt cell survival. *Eur J Immunol* 2002; **32**: 3197-3205
- Morimoto J, Yoneyama H, Shimada A, Shigihara T, Yamada S, Oikawa Y, Matsushima K, Saruta T, Narumi S. CXC chemokine ligand 10 neutralization suppresses the occurrence of diabetes in nonobese diabetic mice through enhanced beta cell proliferation without affecting insulinitis. *J Immunol* 2004; **173**: 7017-7024
- Kalinichenko VV, Bhattacharyya D, Zhou Y, Gusarova GA, Kim W, Shin B, Costa RH. Foxf1 +/- mice exhibit defective stellate cell activation and abnormal liver regeneration following CCl₄ injury. *Hepatology* 2003; **37**: 107-117
- Steinman L, Martin R, Bernard C, Conlon P, Oksenberg JR. Multiple sclerosis: deeper understanding of its pathogenesis reveals new targets for therapy. *Annu Rev Neurosci* 2002; **25**: 491-505
- Morio LA, Chiu H, Sprowles KA, Zhou P, Heck DE, Gordon MK, Laskin DL. Distinct roles of tumor necrosis factor-alpha and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. *Toxicol Appl Pharmacol* 2001; **172**: 44-51
- Dana MR, Yamada J, Streilein JW. Topical interleukin 1 receptor antagonist promotes corneal transplant survival. *Transplantation* 1997; **63**: 1501-1507
- DeCicco LA, Rikans LE, Tutor CG, Hornbrook KR. Serum and liver concentrations of tumor necrosis factor alpha and interleukin-1beta following administration of carbon tetrachloride to male rats. *Toxicol Lett* 1998; **98**: 115-121
- Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009; **71**: 171-186
- Hardardóttir I, Grünfeld C, Feingold KR. Effects of endotoxin and cytokines on lipid metabolism. *Curr Opin Lipidol* 1994; **5**: 207-215
- Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* 2004; **45**: 1169-1196
- Feige U, Hu YL, Gasser J, Campagnuolo G, Munyakazi L, Bolon B. Anti-interleukin-1 and anti-tumor necrosis factor-alpha synergistically inhibit adjuvant arthritis in Lewis rats. *Cell Mol Life Sci* 2000; **57**: 1457-1470
- Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; **13**: 323-340
- Sekiya KD, Yoshida M, Thomson AW. Circulating pro-inflammatory cytokines (IL-1 beta, TNF-alpha, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* 1994; **98**: 71-77
- Gramantieri L, Casali A, Trerè D, Gaiani S, Piscaglia F, Chicco P, Cola B, Bolondi L. Imbalance of IL-1 beta and IL-1 receptor antagonist mRNA in liver tissue from hepatitis C virus (HCV)-related chronic hepatitis. *Clin Exp Immunol*

- 1999; **115**: 515-520
- 28 **Conti F**, Breton S, Batteux F, Furlan V, Houssin D, Weill B, Calmus Y. Defective interleukin-1 receptor antagonist production is associated with resistance of acute liver graft rejection to steroid therapy. *Am J Pathol* 2000; **157**: 1685-1692
 - 29 **Granowitz EV**, Santos AA, Poutsika DD, Cannon JG, Wilmore DW, Wolff SM, Dinarello CA. Production of interleukin-1-receptor antagonist during experimental endotoxaemia. *Lancet* 1991; **338**: 1423-1424
 - 30 **Prieur AM**, Kaufmann MT, Griscelli C, Dayer JM. Specific interleukin-1 inhibitor in serum and urine of children with systemic juvenile chronic arthritis. *Lancet* 1987; **2**: 1240-1242
 - 31 **Gabay C**, Gay-Croisier F, Roux-Lombard P, Meyer O, Mainetti C, Guerne PA, Vischer T, Dayer JM. Elevated serum levels of interleukin-1 receptor antagonist in polymyositis/dermatomyositis. A biologic marker of disease activity with a possible role in the lack of acute-phase protein response. *Arthritis Rheum* 1994; **37**: 1744-1751
 - 32 **Suzuki H**, Takemura H, Kashiwagi H. Interleukin-1 receptor antagonist in patients with active systemic lupus erythematosus. Enhanced production by monocytes and correlation with disease activity. *Arthritis Rheum* 1995; **38**: 1055-1059
 - 33 **Isoda K**, Sawada S, Ayaori M, Matsuki T, Horai R, Kagata Y, Miyazaki K, Kusuhara M, Okazaki M, Matsubara O, Iwakura Y, Ohsuzu F. Deficiency of interleukin-1 receptor antagonist deteriorates fatty liver and cholesterol metabolism in hypercholesterolemic mice. *J Biol Chem* 2005; **280**: 7002-7009
 - 34 **Matsuki T**, Horai R, Sudo K, Iwakura Y. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. *J Exp Med* 2003; **198**: 877-888
 - 35 **De Benedetti F**, Pignatti P, Massa M, Sartirana P, Ravelli A, Martini A. Circulating levels of interleukin 1 beta and of interleukin 1 receptor antagonist in systemic juvenile chronic arthritis. *Clin Exp Rheumatol* 1995; **13**: 779-784
 - 36 **Bargetzi MJ**, Lantz M, Smith CG, Torti FM, Eisenberg SP, Starnes HF Jr. Interleukin-1 beta induces interleukin-1 receptor antagonist and tumor necrosis factor binding protein in humans. *Cancer Res* 1993; **53**: 4010-4013
 - 37 **Tilg H**, Trehu E, Atkins MB, Dinarello CA, Mier JW. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood* 1994; **83**: 113-118
 - 38 **Michalopoulos GK**, DeFrances MC. Liver regeneration. *Science* 1997; **276**: 60-66
 - 39 **Fausto N**, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006; **43**: S45-S53
 - 40 **Diehl AM**, Rai RM. Liver regeneration 3: Regulation of signal transduction during liver regeneration. *FASEB J* 1996; **10**: 215-227
 - 41 **Streetz KL**, Luedde T, Manns MP, Trautwein C. Interleukin 6 and liver regeneration. *Gut* 2000; **47**: 309-312
 - 42 **Gabay C**, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454
 - 43 **Boulton R**, Woodman A, Calnan D, Selden C, Tam F, Hodgson H. Nonparenchymal cells from regenerating rat liver generate interleukin-1alpha and -1beta: a mechanism of negative regulation of hepatocyte proliferation. *Hepatology* 1997; **26**: 49-58
 - 44 **LaBrecque D**. Liver regeneration: a picture emerges from the puzzle. *Am J Gastroenterol* 1994; **89**: S86-S96
 - 45 **Friedman JM**, Chung EY, Darnell JE Jr. Gene expression during liver regeneration. *J Mol Biol* 1984; **179**: 37-53
 - 46 **Tewari A**, Buhles WC Jr, Starnes HF Jr. Preliminary report: effects of interleukin-1 on platelet counts. *Lancet* 1990; **336**: 712-714
 - 47 **Granowitz EV**, Porat R, Mier JW, Pribble JP, Stiles DM, Bloedow DC, Catalano MA, Wolff SM, Dinarello CA. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. *Cytokine* 1992; **4**: 353-360

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Anxiety and depression in adult patients with celiac disease on a gluten-free diet

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METHODS: The levels of anxiety, depression and of a probable anxiety or depressive disorder were assessed by the Hospital Anxiety and Depression Scale in 441 adult patients with CD recruited by the German Celiac Society, in 235 age- and sex-matched patients with inflammatory bowel disease (IBD) in remission or with slight disease activity, and in 441 adult persons of a representative German general population sample (GP). Potential demographic (age, sex, social class, family status) and disease-related (latency to diagnosis, duration of GFD, compliance with GFD, thyroid disease) predictors of anxiety and depression in CD were tested for by regression analyses.

RESULTS: The level of anxiety in CD patients was predicted ($R^2 = 0.07$) by female gender ($P = 0.01$). Female sex (OR = 3.6, 95% CI: 1.3-9.4, $P = 0.01$) was associated with a probable anxiety disorder. Living alone (OR = 0.5, 95% CI: 0.2-0.9, $P = 0.05$) was associated with a reduced risk of an anxiety disorder. The level of depression and a probable depressive disorder were not predicted by any of the demographic and medical variables tested for. The levels of anxiety in patients with CD (6.6 ± 3.4) and with IBD (6.9 ± 3.7) were higher than those of persons in the GP (4.6 ± 3.3) (both $P < 0.001$). The levels of depression in persons with CD (4.2 ± 3.4), IBD (4.6 ± 3.4) and of the GP (4.2 ± 3.8) did not differ ($P = 0.3$). The prevalence of a probable anxiety disorder in persons with CD (16.8%) and IBD (14.0%) was higher than that of the GP (5.7%) ($P < 0.001$). The prevalence of a probable depressive disorder did not differ significantly between the three groups ($P = 0.1$).

CONCLUSION: Anxiety in adult German female celiacs on a GFD is higher than in persons of the GP. Female celiacs on a GFD should be screened for anxiety.

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Key words: Celiac disease; Anxiety; Depression; Gender

Abstract

AIM: To compare anxiety and depression levels in adult patients with celiac disease (CD) on a gluten-free diet (GFD) with controls.

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INTRODUCTION

Celiac disease (CD) is an autoimmune disorder which is precipitated, in genetically predisposed persons, by the ingestion of gluten, the major storage protein of wheat and similar grains. Originally considered a rare malabsorption syndrome of childhood, CD is now recognized as a common condition that may be diagnosed at any age and that affects many organ systems. In most affected people, CD remains undiagnosed, although the rate of diagnosis is increasing. CD occurs in adults and children at rates approaching 1% of the population. The disease is recognized not only throughout Europe and in countries populated by persons of European ancestry but also in the Middle East, Asia, South America and North Africa^[1]. The highest CD prevalence in the world is found in a North African population originally living in the Western Sahara with a figure of 5.6%^[2]. Among adults, two to three times as many women have the disease as men, for unknown reasons. The therapy for the disease is a gluten-free diet (GFD); however, the response to therapy is poor in up to 30% of patients mainly because of non-adherence to the GFD^[1].

Along with the many gastrointestinal, nutritional and metabolic consequences of CD, there have been significant concerns about increased rates of psychological symptoms and mental disorders in celiacs (CDs)^[3]. Most studies have been on depression^[4-7]. Case series in Gastroenterological departments^[4] and case control studies in primary care demonstrated that depression can be a symptom of undiagnosed CD^[5]. Whether the levels of depression in CDs under a GFD are comparable to those of controls [general population (GP), other chronic somatic diseases] is under debate. Some studies found more depression in CDs compared to controls of the GP^[6-11,13,15,17], others did not^[12,14,16]. A few studies also explored anxiety. Some studies reported more anxiety in CDs compared to controls^[8,13], some did not^[7,16]. The majority of studies were performed in Italy^[6-11,13,17] and Scandinavia^[12,15,16]. No study has been performed in Germany until now. Only two studies included more than 200 CDs^[14,15].

The data regarding potential disease-related determinants of anxiety and depression in CDs on a GFD are also contradictory: one Italian study found no correlation between duration of, and compliance with, GFD

and depressed mood^[10], whereas another reported increasingly depressed mood with the duration of GFD^[13]. An elevated risk of depression in CD patients with type 1 diabetes mellitus and autoimmune thyroiditis has been reported by one study for each of these diseases^[14,9]. The influence of demographic factors which are associated with depression in the GP, such as marital status or social class index^[18], were only controlled for in a minority of studies^[7,8,13]. Moreover, gender aspects in anxiety/depression in CD have been considered only by a few studies, although Scandinavian studies found that female CDs reported more anxiety and depression than male patients^[16]. These open questions on anxiety and depression in CDs on a GFD provided us with the aims of our study which were: (1) to explore the potential impact of disease-related, demographic and gender variables on anxiety/depression in CDs; and (2) to compare the levels of anxiety/depression and the frequency of anxiety and depressive disorder in CDs with a sample of the GP and with patients having another chronic somatic disease, with adequate large sample sizes, in a central European country (Germany).

MATERIALS AND METHODS

Ethics

The surveys were approved by the ethics committees of the University of Tübingen and Leipzig and of the board of physicians of the Saarland.

Patients

CD: Within the German Celiac Health Survey of the German Celiac Society DZG, a set of questionnaires was sent out in May 2005 by the DZG to 1000/18355 of their members ≥ 18 years old, self-reporting a diagnosis of CD according to the membership directory of the DZG, following consecutive postal codes 0-9. To ensure a geographically representative sample every 18th member of the list was contacted by a letter signed by the board of directors of the DZG in which the aims of the study were explained. The letter included a set of questionnaires with a stamped self-addressed envelope enclosed. The patients were asked to give their written informed consent and to fill out and send back the questionnaires within four weeks. Patients < 18 years were not included in the study. There were no other primary exclusion criteria. All patients gave their voluntary informed consent after full written explanation of the aims of the study, including considerations regarding ethics and data protection and the anonymous deposition of the questionnaires^[19].

Inflammatory bowel diseases: We selected inflammatory bowel diseases (IBDs) (patients with IBD) for comparison because both diseases have similar gastrointestinal (abdominal pain, diarrhea) and general (fatigue) symptoms and disease-associated disorders (e.g. bone, skin, liver).

Consecutive adult patients (≥ 18 years) with diagnosis of Crohn's disease or ulcerative colitis confirmed

by endoscopy and histology, attending three tertiary care centers for evaluation and/or therapy and members of the German's Crohn's Disease/Ulcerative Colitis Foundation (DCCV), were invited to participate in a study on health-related quality of life (HRQOL)^[20]. Patients unable to speak German or with suspicious diagnosis and indeterminate colitis were excluded. Based on the hospital registry and medical documentation, a total of 730 adults were identified who had been attending the specialized outpatient clinic of the Tübingen University Hospital at least once in the last three years with confirmed diagnosis of either Crohn's disease or ulcerative colitis. Every one of these patients received a set of questionnaires by mail and was asked to return it once completed. One reminder was sent out three weeks after initial postal contact. Forty-five consecutive IBD patients of the coloproctological outpatient department of the Department of General and Abdominal Surgery of the University of the Saarland and 23 consecutive IBD inpatients of the Department of Internal Medicine I of the Klinikum Saarbrücken were asked to participate in the study during regular outpatient visits or during a hospital stay after admission for any acute complications of their disease. Physicians were trained to offer instruction and help if persons did not understand the meaning of questions when needed, to collect the questionnaires and to record clinical data using standardized forms. The study took place between January and June, 2000. In edition 4 (2000) of the journal of the German Crohn's disease/Ulcerative Colitis Foundation (DCCV) "Bauchredner", the main topic was "Health-related quality of life and life satisfaction in IBD" and all readers suffering from IBD were invited to participate in our study. The DCCV is the largest patient association for IBD in Germany with around 15000 members (patients, relatives, physicians). Seventy-six patients indicated their interest to the DCCV and received the set of questionnaires by mail and were asked to return the completed set to the study center in Saarbrücken.

Disease activity was measured by the German Inflammatory Bowel Disease activity Index (GIBDI^{Crohn's disease} resp. GIBDI^{Ulcerative colitis}). The GIBDI had been validated for the assessment of disease activity in surveys within the German Competence Network Inflammatory Bowel Diseases^[21].

General German population: A representative sample of the German GP was selected with the assistance of a demographic consulting company (USUMA, Berlin, Germany). The random selection was based on multi-stage sampling with three stages (according to the typical random selection procedure in national surveys in Germany). First, 258 sample point regions were randomly drawn from the last political election register, covering rural and urban areas from all regions in Germany. The second stage was a random selection of households using the random route procedure (based on a starting address). The third stage was a random selection of household respondents with the Kish selection grid. The

sample aimed to be representative in terms of age, gender, and education for the general German population. The inclusion criteria for the study were age at or above 14 and the ability to read and understand the German language. All subjects were visited by a study assistant informed about the investigation. Self-rating questionnaires were presented. The assistant waited until participants answered all questionnaires, and offered help if persons did not understand the meaning of questions. Data collection took place in 1998^[22].

Questionnaires

Medical questionnaires: In both patient surveys a self-developed medical questionnaire assessing the history of the disease and therapy, current symptoms necessary to calculate activity scores, disease-related comorbidities and current medication was included. CDs were asked about comorbid dermatitis herpetiformis, diabetes mellitus type 1, autoimmune thyroiditis, autoimmune liver-biliary tract disease, recurrent aphthae, anemia, Addison's disease, osteoporosis and duodenal carcinoma or lymphoma. Latency of diagnosis was calculated by the reported difference of the time between first medical assessment because of CD-associated symptoms and final diagnosis.

The demographic questionnaire of the German Competence Network "Inflammatory Bowel Diseases"^[21] was included in both patient surveys. By questions on education, the occupational status and the available income were used to calculate a social index. Data of this social index can be compared to a representative sample of the general German population^[23].

The Hospital Anxiety and Depression Scale (HADS) was specifically designed for the assessment of anxiety and depression in patients with physical illness. With 7 items each on a 4-point Likert scale ranging from 0 to 3, subscale scores for anxiety and depression can be calculated. Scores ≥ 11 on the anxiety scale are indicative of a probable anxiety disorder (mainly general anxiety disorder). Scores ≥ 11 on the depression scale are indicative for a probable depressive disorder^[24,25]. The HADS is a reliable and valid psychological screening tool for anxiety and depressive disorder in physically ill persons^[25] with a validated German version available^[26].

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Release 17.0, Chicago 2008). One missing item of each subscale of the HADS was substituted by the individual median. If more items were missing the patient was excluded from analysis.

Data derived from descriptive statistical analysis are presented in the form of percentages for categorical variables and of the mean and the standard deviation (SD) for continuous data. Categorical data were compared using the χ^2 -test and continuous data by *t*-tests or analysis of variance with post-hoc Bonferroni adjusted pairwise comparisons. In addition, anxiety and depres-

sion scores were compared between CDs and IBDs using analysis of covariance adjusting social class index.

Because we were interested in assessing the possible impact of demographic and medical factors on anxiety and depression, the anxiety and depression scores of the HADS served as dependent variables for a multiple regression analysis. Present age, family status, gender, social class index, latency of diagnosis, duration of and compliance with GFD and CD-associated thyroid disease were included as independent variables in a direct multiple regression analysis.

In order to estimate the risk for a possible anxiety and depressive disorder (coded 1 = no, 2 = yes), a logistic regression (all independent variables were included within a single step; no automated stepwise selection procedures were used) was performed with the same variables. The variables were coded as follows for the regression analyses: (1) Demographic variables: sex (1 = male, 2 = female), present age (continuous), living in a family or partnership (1 = yes; 2 = no) and social class index (1 = low, 2 = middle, 3 = high); and (2) CD-associated variables: reported latency between first medical assessments because of CD-associated symptoms and final CD diagnosis (continuous), reported duration of GFD (continuous), reported adherence to GFD (1 = rarely; 2 = sometimes; 3 = most of the time; 4 = all of the time), thyroid disease (1 = no, 2 = yes), DM type 1 (1 = no, 2 = yes).

The internal validity of the model of the logistic regression analysis was tested by the omnibus test of the model coefficient and the Hosmer-Lemeshow-test.

A prevalence of mental disorders of 10% estimated by the HADS in the general German population^[23] given a sample size of 219 for each group was calculated to provide 80% power to assess a 10% difference between CD and controls.

Due to multiple comparisons the level of significance was set to $P = 0.01$ for group comparisons.

RESULTS

Return rates

CD: Almost half of the total questionnaires (52.2%, 522 of 1000) were returned. Since six questionnaires were excluded due to missing data, a total of 516 questionnaires were usable for further analyses. Of these, 213 (41.3%) of the respondents indicated that the CD diagnosis was made by duodenal biopsy, 37 (7.2%) by serological tests (CD-specific antibodies), 34 (6.6%) by stool tests (transglutaminase antibodies), and 232 (45.0%) by duodenal biopsy plus serological tests. The 445 patients reporting a biopsy-proven CD were included for further analysis. HADS data were available from 441 patients. Patients who took part in the survey did not differ from those who did not send back the questionnaires in terms of age, sex and geographical region of Germany.

IBD: The total response rate was 63.4% (550/868). Of

the 550 returned questionnaires, 128 were excluded from analysis due to colo- or ileostoma ($n = 59$), uncertain diagnosis ($n = 16$), impossibility of calculation of GIBDI because of missing data ($n = 51$), missing HADS data ($n = 6$) or missing declaration of consent ($n = 2$). Patients with moderate ($n = 40$) and severe ($n = 10$) disease activity were excluded from comparison. The IBD group thus consisted of 366 patients; 50% were females. To ensure a sex-matched comparison with CDs, 131 randomly selected male patients were excluded from analysis. Of the 235 included for comparison, 138 (56.3%) patients were in remission, 107 (43.7%) patients had a slight disease activity.

To check for a possible selection bias, the data of responders were compared to the known structural data on all outpatients of the Tübingen outpatient clinic (place of residence, diagnosis, gender, age, duration of disease, age at disease onset, number of consultations in 2000). No indication of systematic selection effect was found in respondents *vs* non-respondents. Likewise, there was no difference in terms of gender and age in patients from the departments of the Saarland University Clinics who did and did not participate. There were no significant differences in the demographic variables and HADS scores of the patients included in and excluded from comparison.

GP: The initial sample consisted of 2952 subjects of whom 2037 (69.0%, $n = 1142$ women) fully participated. Forty-five subjects < 18 years were excluded. Thus the GP sample consisted of 1992 adult persons. Of these, 441 GPs (persons of GP) (346 women, 95 men) were randomly selected for comparison. There were no significant differences in the demographic variables and HADS scores of the persons included in and excluded from comparison.

Medical and demographic data of the celiac sample

The sample consisted of 78.5% women. The most frequently self-reported CD-associated diseases were dermatitis herpetiformis Dühring ($n = 45$, 9.3%) and autoimmune thyroiditis ($n = 25$, 5.6%). Type 1 diabetes mellitus was reported by 2 patients (0.5%). There were no gender differences in the demographic and medical variables assessed. The level of anxiety ($P = 0.0002$) and the frequency of a probable anxiety disorder ($P = 0.01$) were higher in female than in male CDs (Table 1).

Predictors of anxiety and depression in CD

Anxiety was significantly predicted only by female gender ($R^2 = 0.07$, $P = 0.01$). Depression was not significantly predicted by any of the medical and sociodemographic variables tested.

Predictors of a probable anxiety and depressive disorder

Because only in 2/8 patients with CD and diabetes mellitus type 1 an anxiety or depressive disorder was diagnosed, this variable was not entered into the regression analysis.

Anxiety disorder: The model correctly classified 84.8%

Table 1 Demographic data of the celiac disease sample with gender comparison

Variable	Total sample	Females	Males	Test-value <i>P</i> -value
Sex (<i>n</i> , %)	441 (100)	346 (78.5)	95 (21.5)	
Age, mean (SD)	46.3 (15.1)	45.7 (15.0)	48.5 (15.0)	Not significant
Living in partnership (<i>n</i> , %)	334 (76.1)	253 (73.8)	80 (84.2)	Not significant
Social class index (<i>n</i> , %)				Not significant
Lower class	26 (6.3)	18 (5.6)	7 (7.5)	
Middle class	271 (65.3)	220 (68.8)	50 (53.8)	
Upper class	118 (28.4)	82 (25.6)	36 (38.7)	
Number of CD-associated diseases (<i>n</i> , %)				Not significant
None	240 (55.2)	175 (51.3)	65 (69.9)	
One	118 (27.1)	100 (29.3)	17 (18.3)	
Two	48 (11.0)	40 (11.7)	8 (8.6)	
> Two	29 (6.7)	26 (7.7)	3 (3.2)	
Latency to diagnosis, yr, mean (SD)	5.6 (10.0)	5.9 (9.8)	4.9 (10.7)	Not significant
Years since start of GFD, mean (SD)	8.5 (7.7)	8.5 (7.7)	8.7 (7.7)	Not significant
Compliance with GFD				Not significant
Rarely		20 (5.8)	2 (2.1)	
Sometimes		9 (2.9)	0	
Most of the time		86 (25.0)	30 (32.3)	
All of the time		229 (66.6)	61 (65.6)	
Anxiety, mean (SD)	6.7 (4.09)	7.1 (4.1)	5.3 (3.5)	$t = -3.7, P < 0.001$
Depression, mean (SD)	4.1 (3.6)	4.3 (3.8)	3.2 (3.9)	$t = -2.5, P = 0.01$
Probable anxiety disorder (<i>n</i> , %)	68 (15.4)	63 (18.2)	5 (5.2)	$\chi^2 = 9.6, P = 0.001$
Probable depressive disorder (<i>n</i> , %)	25 (5.7)	24 (6.9)	1 (1.0)	Not significant

Some discrepancies between total number of patients and absolute numbers of some variables are due to missing data. CD: Celiac disease; GFD: Gluten-free diet.

of the patients. Only female sex (OR = 3.6, 95% CI: 1.3-9.4, $P = 0.001$) was associated with a probable anxiety disorder. Living alone (OR = 0.45, 95% CI: 0.20-0.99, $P = 0.05$) was associated with a reduced risk of a probable anxiety disorder. The omnibus test of the model coefficient was significant ($\chi^2 = 20.2, P = 0.009$). The level of significance in the Hosmer Lemeshow test was $P = 0.5$ ($\chi^2 = 6.9$) above the predefined P -value of 0.05, thus confirming the adequacy of the model.

Depressive disorder: None of the variables tested was significantly associated with a probable depressive disorder. The omnibus test of the model coefficient was not significant ($\chi^2 = 13.5, P = 0.10$).

Comparison with GP and patients with chronic IBD

In the CD sample, there were more patients of a higher social class than in the IBD sample ($\chi^2 = 14.0, P = 0.006$). There were no differences between the samples in the other variables tested (Table 2).

The level of anxiety in CDs (6.7 ± 4.0) and IBDs (6.9 ± 3.7) was higher than that seen in GPs (4.6 ± 3.6) (all $P < 0.001$). After adjusting for social class there was no significant difference in the anxiety level between CDs and IBDs ($F = 1.3, P = 0.3$).

The level of depression in CDs (4.1 ± 3.6), IBDs (4.6 ± 3.4) and GPs (4.2 ± 3.8) did not differ ($P = 0.3$). After adjusting social class index, IBDs reported higher depression than CDs ($F = 3.9, P = 0.02$).

The frequency of a probable anxiety disorder was higher in CDs (15.4%) and IBDs (14.0%) than in GPs

(5.7%) (all $P < 0.001$). There was no difference in the frequency of a probable depressive disorder between the three groups ($P = 0.1$).

DISCUSSION

We assessed medical and demographic predictors of anxiety and depression in adult patients with CD and compared their levels of anxiety and depression and the frequency of an anxiety or depressive disorder with age- and sex-matched patients with IBD in remission or slight disease activity, and with a representative German population sample. Only female sex, but not medical variables such as duration of and compliance with GFD, predicted anxiety. Depression was not predicted by any of the medical and demographic variables. Anxiety, but not depression, was higher in CDs and IBDs than in GPs.

Comparisons of the results with other studies

Factors associated with anxiety and depression in CD: Two Italian studies found no gender differences in anxiety and depression^[10,27] whereas two Scandinavian studies reported lower levels of psychological well-being in female CD patients^[16,28]. The higher levels of anxiety in German and Scandinavian women might be explained by general gender differences and/or CD-specific factors. Higher levels of anxiety and frequency of anxiety disorder in women have also been found in the general German population^[22]. A higher psychosocial burden on female CDs because of buying and preparing food for the family can be presumed^[28,29].

Table 2 Comparisons of patients with celiac disease with patients with inflammatory bowel disease in remission or having slight disease activity and with subjects of the general population

Variable	Celiac disease <i>n</i> = 441 (1)	Inflammatory bowel disease <i>n</i> = 235 (2)	General population <i>n</i> = 441 (3)	Overall comparison	Comparison subgroups
Sex (female) (<i>n</i> , %)	346 (78.5)	183 (77.9)	346 (78.5)	Not significant	
Age (mean, SD)	46.3 (15.1)	44.9 (12.4)	49.9 (16.8)	Not significant	
Social class index (<i>n</i> , %)			No comparable data available	Not possible	$\chi^2 = 14.5$ $P < 0.01$
Lower	26 (6.3)	28 (11.9)			
Middle	271 (65.3)	167 (71.1)			
Upper	118 (28.4)	40 (17.0)			
Living in partnership or with family (<i>n</i> , %)	334 (76.1)	187 (80.3)	No comparable data available	Not significant	
Anxiety				$F = 45.9$ $P < 0.001$	1, 2 > 3
Total, mean (SD)	6.7 (4.0)	6.9 (3.7)	4.6 (3.3)		
Depression				Not significant	
Total, mean (SD)	4.1 (3.6)	4.6 (3.4)	4.2 (3.8)		
Probable anxiety disorder				$\chi^2 = 23.2$ $P < 0.001$	1, 2 > 3
Total (<i>n</i> , %)	68 (15.4)	33 (14.0)	25 (5.7)		
Probable depressive disorder				Not significant	
Total (<i>n</i> , %)	25 (5.7)	12 (5.1)	38 (8.6)		

In contrast to findings in the GP^[18,30], low social class index was not associated with anxiety and depression in CDs. In contrast to the GP^[18,30], living in a family was associated in CDs with the risk of an anxiety disorder. We speculate that the problems of buying and preparing food in a family with CDs and non-CDs might lead to financial and interpersonal problems which can contribute to an anxiety disorder.

In line with one Italian longitudinal study^[7] and one Italian cross-sectional study^[10], we found no association between depressed mood and the duration of GFD. We also found no association between anxiety and the duration of GFD. This finding is not contradictory to the results of longitudinal studies which reported a decrease of anxiety (state) after starting a GFD^[7,12]: a GFD may reduce psychological symptoms due to malabsorption of tryptophan, but may also increase psychological distress associated with the financial and social restrictions of GFD and disease-related worries^[6,31].

We confirm the results of two Italian studies which found no association between non-compliance with GFD and anxiety/depression^[10,13].

The limited number of CDs with diabetes mellitus type 1 prohibited a comparison with an American study which reported a higher prevalence of depression in CD patients with type 1 diabetes mellitus (5.8% of the sample) compared to celiacs without type 1 diabetes mellitus^[14]. We could not confirm a higher prevalence of depressive disorders in CDs with autoimmune thyroiditis^[9].

Anxiety/depression in CDs compared to controls:

The different instruments used to assess anxiety and depression do not allow a comparison of prevalence rates in studies on anxiety/depression in CD. Moreover, the studies differed in the ways of recruiting patients and in the type of controls used. Some studies used healthy subjects^[6-10,13,14,17], GP^[15,16] or patients with diabetes^[13], IBD^[6], irritable bowel syndrome^[14] and chronic hepatitis^[10] for

controls. Despite these limitations, we can state that our finding that CDs are not more depressed than controls is in line with the results of one American^[14] and two Scandinavian^[11,16] studies and in contrast to the findings of the Italian studies^[6-11,13,17]. Our finding of more anxiety in CDs compared to GP controls is in line with that of Italian studies^[7-9] and in contrast to Scandinavian studies^[11,16].

Limitations of the study

Some limitations of the study have to be considered. First, the study population consisted of adult CDs recruited among members of the German Celiac Society which may have induced a selection bias. A large hospital-based register of CD patients is not available in Germany. Furthermore, to our knowledge, most CDs attending tertiary care outpatient departments in Germany are associated with the German Celiac Society. Additionally, we cannot exclude a response bias of CDs sending back the questionnaires. Nevertheless, the CD sample of our study is the best available one in Germany. Second, we cannot exclude a response bias of CD and IBD patients sending back the questionnaires. Third, another potential bias of the study is the different approach to completing and returning the questionnaires. In the subsamples of the GP and the IBD patients of the departments of Homburg/Saar and Saarbrücken, help was offered if persons did not understand the meaning of questions and the questionnaires were re-collected by research assistants or physicians. The questionnaires of the celiac and IBD sample of the department of Tübingen were completed without potential external support and returned by mail. Fourth, all data on the history and type of diagnosis, CD-associated diseases and on adherence to regimen relied solely on the participants' self report. Due to data protection in this type of survey, information from clinical records, interviews or serological/radiological tests was not available. Fifth, due to the type of study (postal survey) we were unable to use standardized psychiatric interviews for the confirmation of a probable

mental disorder when the critical cut-off scores of the HADS were surpassed. Furthermore, the items on anxiety of the HADS cover general anxiety but not phobic symptoms. Therefore, our data cannot comment on the high frequency of social phobia seen in Italian patients^[8]. However, the HADS is one of the best screening tools available for anxiety and depressive disorders in patients with somatic diseases^[25]. Finally, because of the cross-sectional nature of the study the results show only associations between factors and do not allow for conclusions for causality.

In conclusion, German patients with CD on a GFD do not differ from the GP in levels of depression, but there is a subgroup of mainly female CDs with a probable anxiety disorder. Mental disorders increase the risk of a reduced health-related quality of life^[31] and of irritable bowel-like symptoms in CDs^[32]. A screening for comorbid anxiety disorders in female patients with CD on a GFD by general practitioners and gastroenterologists is therefore recommended, and screening is possible by using the ultra-brief screening scale for anxiety and depression, PHQ-4^[33]. The two questions on anxiety in the PHQ-4 can be directly asked: "Over the last two weeks, have you often been bothered by feeling nervous, anxious, or on edge? Over the last two weeks, have you often not been able to stop or control worrying?"

COMMENTS

Background

Previous studies have yielded conflicting results regarding the presence of an association of celiac disease (CD) in patients on a gluten-free diet (GFD) and anxiety/depression.

Research frontiers

Anxiety and depression are common symptoms in the general population (GP) as well as in other chronic somatic diseases and are associated with demographic factors such as age, gender and social class. Most studies on anxiety/depression in CD were not adequately powered to detect differences in anxiety and depression between CD and controls. In this study with adequately powered sample sizes, the authors demonstrate that anxiety in CD patients on a GFD is higher than in the GP and comparable to that of patients with inflammatory bowel disease who are in remission and have slight disease activity.

Innovation and breakthroughs

Recent reports have highlighted the importance of female gender and other autoimmune disorders associated with CD as predictors of depression in CD. This is the first study to report an association between anxiety disorder and female gender in CD. The study did not confirm findings that autoimmune thyroid disorder is a risk factor for depressive disorder in CD.

Applications

The study substantiated the necessity of exploring not only somatic but also anxiety symptoms in female CD patients on a GFD.

Peer review

This paper is worthy of publication as the first comprehensive study of anxiety and depression in German adult CD. It needs to be substantially revised, reduced in size, particularly in the Discussion and number of tables, and be less speculative.

REFERENCES

- Green PH, Cellier C. Celiac disease. *N Engl J Med* 2007; **357**: 1731-1743
- Catassi C, Rätsch IM, Gandolfi L, Pratesi R, Fabiani E, El Asmar R, Frijia M, Bearzi I, Vizzoni L. Why is coeliac disease endemic in the people of the Sahara? *Lancet* 1999; **354**: 647-648
- Addolorato G, Leggio L, D'Angelo C, Mirijello A, Ferrulli A, Cardone S, Vonghia L, Abenavoli L, Leso V, Nesci A, Piano S, Capristo E, Gasbarrini G. Affective and psychiatric disorders in celiac disease. *Dig Dis* 2008; **26**: 140-148
- Hallert C, Derefeldt T. Psychic disturbances in adult coeliac disease. I. Clinical observations. *Scand J Gastroenterol* 1982; **17**: 17-19
- Cannings-John R, Butler CC, Prout H, Owen D, Williams D, Hood K, Crimmins R, Swift G. A case-control study of presentations in general practice before diagnosis of coeliac disease. *Br J Gen Pract* 2007; **57**: 636-642
- Addolorato G, Stefanini GF, Capristo E, Caputo F, Gasbarrini A, Gasbarrini G. Anxiety and depression in adult untreated celiac subjects and in patients affected by inflammatory bowel disease: a personality "trait" or a reactive illness? *Hepatogastroenterology* 1996; **43**: 1513-1517
- Addolorato G, Capristo E, Ghittoni G, Valeri C, Mascianà R, Ancona C, Gasbarrini G. Anxiety but not depression decreases in coeliac patients after one-year gluten-free diet: a longitudinal study. *Scand J Gastroenterol* 2001; **36**: 502-506
- Addolorato G, Mirijello A, D'Angelo C, Leggio L, Ferrulli A, Vonghia L, Cardone S, Leso V, Miceli A, Gasbarrini G. Social phobia in coeliac disease. *Scand J Gastroenterol* 2008; **43**: 410-415
- Carta MG, Hardoy MC, Boi MF, Mariotti S, Carpinello B, Usai P. Association between panic disorder, major depressive disorder and celiac disease: a possible role of thyroid autoimmunity. *J Psychosom Res* 2002; **53**: 789-793
- Ciaci C, Iavarone A, Mazzacca G, De Rosa A. Depressive symptoms in adult coeliac disease. *Scand J Gastroenterol* 1998; **33**: 247-250
- Cicarelli G, Della Rocca G, Amboni M, Ciaci C, Mazzacca G, Filla A, Barone P. Clinical and neurological abnormalities in adult celiac disease. *Neurol Sci* 2003; **24**: 311-317
- Collin P, Kaukinen K, Mattila AK, Joukamaa M. Psychoneurotic symptoms and alexithymia in coeliac disease. *Scand J Gastroenterol* 2008; **43**: 1329-1333
- Fera T, Cascio B, Angelini G, Martini S, Guidetti CS. Affective disorders and quality of life in adult coeliac disease patients on a gluten-free diet. *Eur J Gastroenterol Hepatol* 2003; **15**: 1287-1292
- Garud S, Leffler D, Dennis M, Edwards-George J, Saryan D, Sheth S, Schuppan D, Jamma S, Kelly CP. Interaction between psychiatric and autoimmune disorders in coeliac disease patients in the Northeastern United States. *Aliment Pharmacol Ther* 2009; **29**: 898-905
- Ludvigsson JF, Reutfors J, Osby U, Ekblom A, Montgomery SM. Coeliac disease and risk of mood disorders--a general population-based cohort study. *J Affect Disord* 2007; **99**: 117-126
- Roos S, Kärner A, Hallert C. Psychological well-being of adult coeliac patients treated for 10 years. *Dig Liver Dis* 2006; **38**: 177-180
- Siniscalchi M, Iovino P, Tortora R, Forestiero S, Somma A, Capuano L, Franzese MD, Sabbatini F, Ciaci C. Fatigue in adult coeliac disease. *Aliment Pharmacol Ther* 2005; **22**: 489-494
- Akhtar-Danesh N, Landeen J. Relation between depression and sociodemographic factors. *Int J Ment Health Syst* 2007; **1**: 4
- Häuser W, Gold J, Stein J, Caspary WF, Stallmach A. Health-related quality of life in adult coeliac disease in Germany: results of a national survey. *Eur J Gastroenterol Hepatol* 2006; **18**: 747-754
- Janke KH, Klump B, Gregor M, Meisner C, Haeuser W. Determinants of life satisfaction in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 272-286
- Janke KH, Raible A, Bauer M, Clemens P, Meisner C, Häuser W, Steder-Neukamm U, Henrich G, Herschbach P, Gregor M, Klump B. Questions on life satisfaction (FLZM) in inflammatory bowel disease. *Int J Colorectal Dis* 2004; **19**: 343-353
- Hinz A, Schwarz R. [Anxiety and depression in the general

- population: normal values in the Hospital Anxiety and Depression Scale] *Psychother Psychosom Med Psychol* 2001; **51**: 193-200
- 23 **Deck R**, Röckelein E. Assessment of sociodemographic and socialmedical indicators within cooperatives of research in rehabilitation medicine. *DRV Schriften* 1999; **16**: 85-96
 - 24 **Zigmond AS**, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; **67**: 361-370
 - 25 **Bjelland I**, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J Psychosom Res* 2002; **52**: 69-77
 - 26 **Herrmann C**, Buss U, Snaith RP. HADS-D: Hospital Anxiety and Depression Scale - German Version. Bern: Hans Huber, 1995
 - 27 **Ciacchi C**, D'Agate C, De Rosa A, Franzese C, Errichiello S, Gasperi V, Pardi A, Quagliata D, Visentini S, Greco L. Self-rated quality of life in celiac disease. *Dig Dis Sci* 2003; **48**: 2216-2220
 - 28 **Hallert C**, Sandlund O, Broqvist M. Perceptions of health-related quality of life of men and women living with coeliac disease. *Scand J Caring Sci* 2003; **17**: 301-307
 - 29 **Sverker A**, Ostlund G, Hallert C, Hensing G. 'I lose all these hours...'--exploring gender and consequences of dilemmas experienced in everyday life with coeliac disease. *Scand J Caring Sci* 2009; **23**: 342-352
 - 30 **Jacobi F**, Wittchen HU, Holting C, Höfler M, Pfister H, Müller N, Lieb R. Prevalence, co-morbidity and correlates of mental disorders in the general population: results from the German Health Interview and Examination Survey (GHS). *Psychol Med* 2004; **34**: 597-611
 - 31 **Häuser W**, Stallmach A, Caspary WF, Stein J. Predictors of reduced health-related quality of life in adults with coeliac disease. *Aliment Pharmacol Ther* 2007; **25**: 569-578
 - 32 **Häuser W**, Musial F, Caspary WF, Stein J, Stallmach A. Predictors of irritable bowel-type symptoms and healthcare-seeking behavior among adults with celiac disease. *Psychosom Med* 2007; **69**: 370-376
 - 33 **Kroenke K**, Spitzer RL, Williams JB, Löwe B. An ultra-brief screening scale for anxiety and depression: the PHQ-4. *Psychosomatics* 2009; **50**: 613-621

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Segmental duodenectomy for gastrointestinal stromal tumor of the duodenum

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were 100% after SD and 0% after PD ($P < 0.05$). The median DFS was 13 mo in the PD group.

CONCLUSION: Whenever associated with clear surgical margins, SD is a reliable and curative option for most duodenal GISTs, and is compatible with long-term DFS.

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Key words: Gastrointestinal stromal tumor; Duodenal neoplasms; Segmental duodenectomy; Pancreaticoduodenectomy

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Abstract

AIM: To evaluate the results of segmental duodenectomy (SD) and pancreaticoduodenectomy (PD) for duodenal gastrointestinal stromal tumor (GIST) and help clinicians with surgical management.

METHODS: All patients who underwent surgery for non-metastatic GIST of the duodenum in a single institution since 2000 were prospectively followed up. Seven patients (median age 51 years, range: 41-73 years) were enrolled: five underwent SD and two underwent PD.

RESULTS: All the patients had a complete resection (R0), with no postoperative morbidity and mortality. Among the SD group, GIST was classified as low risk in two patients, intermediate risk in two, and high risk in one, according to the Fletcher scale, (vs two high risk patients in the PD group). With a median follow-up of 41 (18-85) mo, disease-free survival (DFS) rates

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract, with an estimated annual incidence between 10 and 20/10⁶ people^[1,2]. Although GISTs are encountered all along the digestive tract, the most frequent sites of occurrence are the stomach (50%) and small bowel (30%). Duodenal GISTs are less frequent and account for < 5% of cases, but still represent approximately 30% of primary duodenal tumors^[3].

Surgery is still the only curative approach for GIST^[4-6], but the optimal surgical procedure for duodenal GIST remains to be established. A number of authors have reported various procedures including pancreaticoduo-

denectomy (PD), pancreas-sparing duodenectomy, segmental duodenectomy (SD), or wedge local resection, but few have correlated the different options with oncological results^[7-16].

Two major tumor characteristics have to be considered for surgical resection of duodenal GIST, which differs from duodenal adenocarcinoma^[17]. First, GIST spreads specifically hematogenously and is rarely, if ever, associated with lymphatic invasion, as in other sarcomas^[18]. Secondly, GISTs are well encapsulated tumors that rarely have a tendency to local invasion^[4]. For these reasons, radical lymphadenectomy or extended resection of adjacent organs should not confer a survival advantage in non-metastatic duodenal GIST^[6,19,20].

Therefore, this study was undertaken to audit the oncological results of segmental duodenal resection in comparison with more extensive procedure such as PD for primary non-metastatic duodenal GIST.

MATERIALS AND METHODS

This was a prospective cohort study of all surgical patients treated in our department for primary non-metastatic duodenal GIST from 2000 to 2008. Inclusion criteria were patients presenting with suspicion of non-metastatic duodenal GIST in a single hospital (Figures 1 and 2). Exclusion criteria were a clearly metastatic disease, poor health condition that precluded laparotomy (severe pulmonary disease, non-treatable coagulation abnormality), and patient's refusal to participate in this study. All patients had complete surgical resection (R0). Seven cases were included. Median age was 51 years (range: 41-73 years).

Five patients had an SD and two a cephalic PD. PD was the operation chosen for relatively large and difficult-to-reach tumors (first and second part of the duodenum). Among the SD group, one patient had a duodenal patch resection of a small D1 GIST with direct closure (case 3, Table 1), the others had a complete SD with latero-lateral duodeno-jejunal reconstruction (Figure 3).

Pathological diagnosis of GIST was confirmed for all according to histological and immunohistochemical work-up. All tumors were c-kit positive. GIST was classified according to the Fletcher scale^[21] and our scale^[4].

The primary endpoint for this analysis was disease-free survival (DFS), which was defined as time from surgery to GIST recurrence. Follow-up was available for all patients at the date set for collecting data, November 2008. Follow-up was carried out through routine visits at our Outpatient Oncological Clinic. Clinical assessment was made every 3 mo during the first 2 years after surgery and every 6 mo thereafter, with detection of recurrence as soon as possible to allow adjuvant therapy with imatinib. Yearly chest X-rays and abdominal computed tomography (CT) were routinely performed in all patients and additional imaging was requested when clinical suspicion of GIST recurrence occurred. The median follow-up was 41 (18-85) mo.

Statistical analysis

Statistical analysis was performed using GraphPad InStat



Figure 1 Gastrointestinal stromal tumor (GIST) located in the third part of the duodenum with typical computed tomography (CT) appearance. Note the typical CT appearance of GIST with an area of necrosis (central cavitations with surrounding highly vascular tissue) (white arrow: pancreas; black arrow: GIST).



Figure 2 GIST located in the horizontal duodenum (patient 5, Table 1). Note the close relation between the GIST (short arrow) and the pancreas (long arrow), which was easily dissected during surgery.

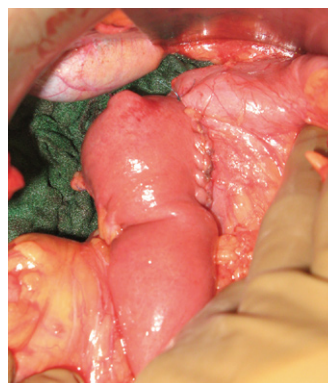


Figure 3 Duodeno-jejunal anastomosis. Latero-lateral duodeno-jejunal anastomosis between the second part of the duodenum and the first jejunal loop was easily performed after distal duodenectomy and Kocher maneuver.

(GraphPad Software, San Diego, CA, USA). When appropriate, data were analyzed using two-sided Fischer's test or two-sided *t* test. *P* < 0.05 was considered statistically significant.

RESULTS

Study population and pathological data

Seven patients were included in the analysis. Four patients presented with upper digestive tract hemorrhage,

Table 1 Patients characteristics and follow-up

Cases	Localization	Size (cm)	Mitosis/50 HPF	Fletcher grade	Surgery	Follow-up	DFS	Second-line therapy
1	D2	6.5	34	HR	PD	60	13	Imatinib and hepatectomy
2	D1	7	19	HR	PD	56	12	Imatinib
3	D1	2.5	3	LR	SD	85	85	-
4	D2	2	2	LR	Atypical duodenectomy	40	40	-
5	D2-3	10	10	HR	SD	37	37	-
6	D3	7	2	IR	SD	29	29	-
7	D4	5.5	5	IR	SD	18	18	-

D1: First part of the duodenum; D2: Second part of the duodenum; D3: Third part of the duodenum; D4: Fourth part of the duodenum; HR: High risk; IR: Intermediate risk; LR: Low risk GIST according to Fletcher scale; HPF: High-power field; DFS: Disease free survival.

the others with abdominal discomfort. In all patients, the diagnosis of duodenal GIST was made through CT after it was suspected by endoscopy in those with digestive bleeding. No preoperative biopsies were performed.

All GISTs were localized without metastases or peritoneal dissemination. According to the Fletcher classification, the GISTs were considered as high risk in the two patients in the PD group, and in the SD group as high risk in one, intermediate risk in two, and low risk in two patients ($P > 0.05$). According to our classification, the two GISTs in the PD were classified as malignant, as was one of the five GISTs in the SD group ($P < 0.05$).

Postoperative outcomes

No postoperative morbidity and mortality were recorded. All patients had complete surgical (R0) resection of their duodenal GIST. Five patients underwent SD and two PD (Table 1). GIST-free surgical margins along the duodenum ranged from 0.5 cm to 3 cm.

Follow-up results

The median follow-up was 41 mo (range: 18-85 mo). Median follow-up was 58 mo (range: 56-60 mo) and 37 mo (range: 18-85 mo) for the PD and SD group, respectively. Two patients in the PD group demonstrated recurrence with a median disease-free interval of 13 mo, whereas no recurrence was observed in the SD group ($P < 0.05$). All patients are alive and disease free in the SD group with a median DFS of 37 mo. The two patients with recurrence after PD presented with liver metastases, which were treated with imatinib mesylate, and one was also treated with partial hepatectomy. A statistically significant difference was detected between the PD and SD group for DFS ($P = 0.048$), however, this should be balanced by a higher rate of malignant GIST and longer median follow-up in the PD group.

DISCUSSION

The optimal surgical procedure for GISTs of the duodenum remains poorly defined in terms of oncological results. This study was undertaken to compare oncological results of SD and the more radical PD. According to our data, duodenal GIST prognosis is dependent on

tumor malignant potential when clear surgical margins can be achieved and not on size of surgical margins or lymphatic dissection. The data presented herein demonstrate that SD is associated with prolonged DFS.

Surgical resection is still the only curative therapy for GIST^[1,6,19]. GISTs are known to be resistant to chemotherapy and radiotherapy, and the recently developed molecular targeted therapies (imatinib mesylate and sunitinib), while being highly effective in disease control, are not curative^[1,5,6]. The optimal surgical procedure for duodenal GIST remains poorly defined in terms of oncologic results. The reports in the literature addressing surgical procedures for duodenal GIST demonstrate the feasibility of various surgical procedures: PD, pancreas-sparing duodenectomy, SD, or wedge local resection^[8,9,11-17,22-25]. These papers can help us a little to determine which surgical procedure is optimal in terms of short- and long-term oncological results. The largest series of duodenal GISTs ($n = 156$) evaluated prognosis according to tumor grade^[3]. In this pathological review, around 60% of patients underwent pancreas-preserving duodenectomy and 11% had PD, but due to the retrospective nature of this analysis, no correlation between type of operation and oncological results were reported. Very recently, Tien *et al.*^[26] have reported their experience, in which they compared nine PD with 16 limited operations (11 wedge resections, and five SDs) for duodenal GIST. They have shown that the type of operation is not correlated to operative risk or disease recurrence. They have concluded that limited procedures, like SD, should be attempted for duodenal GIST without involvement of the papilla of Vater. Others have reported similar results^[10].

The choice of surgical procedure for duodenal GIST can be guided by the size and exact location of the tumor^[6,11,17,25]. However, some principles of GIST surgical treatment have to be considered by a visceral surgeon when approaching duodenal GIST^[1,4,6,10,17]. First, GISTs are mesenchymal tumors that behave as other sarcomas and not like adenocarcinomas^[4,18]. GIST spreads specifically hematogenously and is rarely, if ever, associated with lymphatic invasion, as in other sarcomas^[4,6,18]. Therefore, lymphadenectomy is not recommended^[4,11,19]. This seems true for duodenal GIST as local lymph node invasion has never been described, even after PD^[3,11-13,17]. Secondly,

GISTs are well encapsulated tumors that rarely show a tendency to local invasion even for high risk tumors^[4,6,20]. They should be approached with the intention of performing complete *en bloc* removal (R0 resection) of the tumor and surrounding digestive tract tissue^[1,4,6,7,10,19]. The size of surgical margins along the segment of digestive tract involved are not formally defined, however there is little submucosal spread in GIST and clear margins of 1 or 2 cm are recommended^[4,6,19,20]. When extracapsular GIST mobilization is possible, there is no need for extensive surgical margins on adjacent organs and peri-tumoral resection with an intact capsule is sufficient^[4,6,19,20].

Segmental or atypical duodenectomy for duodenal GIST is in accordance with these principles and could be beneficial for patients because it does not involve the excessive resection and morbidity associated with PD. After complete surgical resection, duodenal GIST prognosis seems not to be influenced by the pancreatic margins, according to the present study and the sparse literature on the subject. Prognosis is mainly dependent on malignant status, which is determined by size and mitotic rate (Fletcher scale). This has been clearly shown for duodenal GIST in the study by Miettinen *et al.*^[3] and was true in our small series. However, some authors have advocated the need for PD as pancreatic invasion cannot be ruled out on preoperative studies^[17,27]. Although close contact between the GIST capsule and pancreas is usually the rule for large duodenal GISTs on CT (Figures 1 and 2), this is rarely correlated with pancreatic tumoral invasion, which allows treatment with pancreas-sparing duodenectomy^[12,28,29]. As a result of these considerations, we think that segmental or atypical duodenectomy is the optimal procedure for duodenal GIST, as previously proposed by others^[10-13,15,22-25,30]. The exact type of duodenectomy to perform might be influenced by GIST size and location, ranging from wedge resection with primary closure for small proximal duodenal GIST to SD with duodeno-jejunal anastomosis for large distal duodenal GIST^[11,15,30-32]. One exception to this might be periampullary or ampullary GIST, which can present with jaundice, for which pancreas-preserving duodenectomy can be challenging compared to cephalic PD, when the ampulla needs to be resected to obtain clear surgical margins^[8,27].

The present study has several limitations. First, it could be argued that a higher rate of malignant GIST is present in the PD group, because in part, PD was chosen for larger and more difficult-to-reach lesions. Furthermore, a longer median follow-up was available for the PD group. These two points could counter-balance the results. Finally, the sample was small, but duodenal GIST remains a rare tumor. Previous studies published in the literature have not reported large numbers of patients, and most of the time, only case reports^[8-17,19,22-27,30].

In conclusion, pancreas-preserving segmental or atypical duodenectomy seems to be a reliable and curative option in duodenal GIST. Despite being limited in their extent, these methods of resection, when performed with negative margins, are compatible with long-term DFS, and should be preferred, whenever possible, to PD. This

is related to the tumoral characteristics of GIST, which is generally well encapsulated, even when highly malignant and with extremely rare lymphatic spread. When clear surgical margins are achieved, prognosis depends on tumoral malignant potential and not on the extent of the surgical margins, especially the pancreatic margin, for duodenal GIST. However, PD remains a good alternative for tumors in the vicinity of the ampulla of Vater.

COMMENTS

Background

Duodenal gastrointestinal stromal tumors (GISTs) are rare primary duodenal tumors, and there are few guidelines to help the clinician in their surgical management. Surgery is still the only curative approach for GIST, but the optimal procedure remains to be established. Although, numerous authors have reported various surgical procedures, few have correlated their results with oncological outcomes.

Research frontiers

This study was designed to assess the optimal surgical procedure for duodenal GIST, and to compare segmental resection with more extensive pancreaticoduodenectomy (PD).

Innovations and breakthroughs

The authors reported good oncological outcomes with long-term disease-free survival (DFS) in the segmental duodenectomy (SD) group. Thus, whenever associated with clear surgical margins, SD is a reliable and curative option for most duodenal GISTs. However, PD remains a good alternative for tumors in the vicinity of the ampulla of Vater.

Applications

Segmental resection should be preferred, when possible, to more extensive procedures for duodenal GIST. However, for tumors located in the vicinity of the ampulla of Vater, PD remains a good option.

Peer review

The authors evaluated the results of SD (five cases) and PD (two cases) for duodenal GIST. The very low number of patients is certainly a weakness of this study; on the other hand, duodenal GIST is very rare. The authors obtained good DFS following limited resection (SD) with clear margins.

REFERENCES

- 1 Bucher P, Villiger P, Egger JF, Buhler LH, Morel P. Management of gastrointestinal stromal tumors: from diagnosis to treatment. *Swiss Med Wkly* 2004; **134**: 145-153
- 2 Miettinen M, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med* 2006; **130**: 1466-1478
- 3 Miettinen M, Kopczynski J, Makhlof HR, Sarlomo-Rikala M, Gyorkffy H, Burke A, Sobin LH, Lasota J. Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases. *Am J Surg Pathol* 2003; **27**: 625-641
- 4 Bucher P, Egger JF, Gervaz P, Ris F, Weintraub D, Villiger P, Buhler LH, Morel P. An audit of surgical management of gastrointestinal stromal tumours (GIST). *Eur J Surg Oncol* 2006; **32**: 310-314
- 5 Dematteo RP, Heinrich MC, El-Rifai WM, Demetri G. Clinical management of gastrointestinal stromal tumors: before and after STI-571. *Hum Pathol* 2002; **33**: 466-477
- 6 Gold JS, Dematteo RP. Combined surgical and molecular therapy: the gastrointestinal stromal tumor model. *Ann Surg* 2006; **244**: 176-184
- 7 Aparicio T, Boige V, Sabourin JC, Crenn P, Ducreux M, Le Cesne A, Bonvalot S. Prognostic factors after surgery of primary resectable gastrointestinal stromal tumours. *Eur J Surg*

- Oncol* 2004; **30**: 1098-1103
- 8 **Cavallini M**, Cecera A, Ciardi A, Caterino S, Ziparo V. Small periampullary duodenal gastrointestinal stromal tumor treated by local excision: report of a case. *Tumori* 2005; **91**: 264-266
- 9 **Chiarugi M**, Galatioto C, Lippolis P, Zocco G, Seccia M. Gastrointestinal stromal tumour of the duodenum in childhood: a rare case report. *BMC Cancer* 2007; **7**: 79
- 10 **Goh BK**, Chow PK, Kesavan S, Yap WM, Wong WK. Outcome after surgical treatment of suspected gastrointestinal stromal tumors involving the duodenum: is limited resection appropriate? *J Surg Oncol* 2008; **97**: 388-391
- 11 **Goh BK**, Chow PK, Ong HS, Wong WK. Gastrointestinal stromal tumor involving the second and third portion of the duodenum: treatment by partial duodenectomy and Roux-en-Y duodenojejunostomy. *J Surg Oncol* 2005; **91**: 273-275
- 12 **Kwon SH**, Cha HJ, Jung SW, Kim BC, Park JS, Jeong ID, Lee JH, Nah YW, Bang SJ, Shin JW, Park NH, Kim DH. A gastrointestinal stromal tumor of the duodenum masquerading as a pancreatic head tumor. *World J Gastroenterol* 2007; **13**: 3396-3399
- 13 **Lanuke K**, Bathe OF, Mack LA. Local excision of duodenal gastrointestinal stromal tumor. *J Surg Oncol* 2007; **95**: 267-269
- 14 **Stratopoulos C**, Soonawalla Z, Piris J, Friend PJ. Hepatopancreatoduodenectomy for metastatic duodenal gastrointestinal stromal tumor. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 147-150
- 15 **Takeda A**, Watanabe Y, Uehara T, Maruyama T, Tanaka H, Matsuzaki H, Arima H, Natsune T, Kudo H, Sakama A, Tohnosu N, Shimada H, Sato H. Successful surgical resection of a huge gastrointestinal stromal tumor of the third portion of the duodenum. *J Gastroenterol Hepatol* 2007; **22**: 283-284
- 16 **Yildirgan MI**, Başoglu M, Atamanalp SS, Albayrak Y, Gürsan N, Onbaş O. Duodenal stromal tumor: report of a case. *Surg Today* 2007; **37**: 426-429
- 17 **Winfield RD**, Hochwald SN, Vogel SB, Hemming AW, Liu C, Cance WG, Grobmyer SR. Presentation and management of gastrointestinal stromal tumors of the duodenum. *Am Surg* 2006; **72**: 719-722; discussion 722-723
- 18 **Woodall CE**, Scoggins CR. Retroperitoneal and visceral sarcomas: issues for the general surgeon. *Am Surg* 2007; **73**: 631-635
- 19 **DeMatteo RP**, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg* 2000; **231**: 51-58
- 20 **Joensuu H**, Fletcher C, Dimitrijevic S, Silberman S, Roberts P, Demetri G. Management of malignant gastrointestinal stromal tumours. *Lancet Oncol* 2002; **3**: 655-664
- 21 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465
- 22 **De Marco G**, Roviello F, Marrelli D, De Stefano A, Neri A, Rossi S, Corso G, Rampone B, Natri G, Pinto E. A clinical case of duodenal gastrointestinal stromal tumor with a peculiarity in the surgical approach. *Tumori* 2005; **91**: 261-263
- 23 **De Nicola P**, Di Bartolomeo N, Francomano F, D'Aulerio A, Innocenti P. Segmental resection of the third and fourth portions of the duodenum after intestinal derotation for a GIST: a case report. *Suppl Tumori* 2005; **4**: S108-S110
- 24 **Kurihara N**, Kikuchi K, Tanabe M, Kumamoto Y, Tsuyuki A, Fujishiro Y, Otani Y, Kubota T, Kumai K, Kitajima M. Partial resection of the second portion of the duodenum for gastrointestinal stromal tumor after effective transarterial embolization. *Int J Clin Oncol* 2005; **10**: 433-437
- 25 **Sun YH**, Wang XF, Hou YY, Qin XY. [Clinical characteristics and surgical treatment of 18 cases of duodenal gastrointestinal stromal tumors] *Zhonghua Weichang Waikexue* 2007; **10**: 26-28
- 26 **Tien YW**, Lee CY, Huang CC, Hu RH, Lee PH. Surgery for gastrointestinal stromal tumors of the duodenum. *Ann Surg Oncol* 2010; **17**: 109-114
- 27 **Filippou DK**, Pashalidis N, Skandalakis P, Rizos S. Malignant gastrointestinal stromal tumor of the ampulla of Vater presenting with obstructive jaundice. *J Postgrad Med* 2006; **52**: 204-206
- 28 **Benjamin RS**, Choi H, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Charnsangavej C. We should desist using RECIST, at least in GIST. *J Clin Oncol* 2007; **25**: 1760-1764
- 29 **Hong X**, Choi H, Loyer EM, Benjamin RS, Trent JC, Charnsangavej C. Gastrointestinal stromal tumor: role of CT in diagnosis and in response evaluation and surveillance after treatment with imatinib. *Radiographics* 2006; **26**: 481-495
- 30 **Sakamoto Y**, Yamamoto J, Takahashi H, Kokudo N, Yamaguchi T, Muto T, Makuuchi M. Segmental resection of the third portion of the duodenum for a gastrointestinal stromal tumor: a case report. *Jpn J Clin Oncol* 2003; **33**: 364-366
- 31 **Nauta RJ**. Duodenojejunostomy as an alternative to anastomosis of the small intestine at the ligament of Treitz. *Surg Gynecol Obstet* 1990; **170**: 172-174
- 32 **Sarmiento JM**, Thompson GB, Nagorney DM, Donohue JH, Farnell MB. Pancreas-sparing duodenectomy for duodenal polyposis. *Arch Surg* 2002; **137**: 557-562; discussion 562-563

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Reduction of the closure time of postoperative enterocutaneous fistulas with fibrin sealant

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shortens the closure time of postoperative enterocutaneous fistulas (ECFs).

METHODS: The prospective case-control study included 70 patients with postoperative ECFs with an output of < 500 mL/d, a fistulous tract of > 2 cm and without any local complication. They were divided into study ($n = 23$) and control groups ($n = 47$). Esophageal, gastric and colcutaneous fistulas were monitored under endoscopic visualization, which also allowed fibrin glue application directly through the external hole. Outcome variables included closure time, time to resume oral feeding and morbidity related to nutritional support.

RESULTS: There were no differences in mean age, fistula output, and follow-up. Closure-time for all patients of the study group was 12.5 ± 14.2 d and 32.5 ± 17.9 d for the control group ($P < 0.001$), and morbidity related to nutritional support was 8.6% and 42.5%, respectively ($P < 0.01$). In patients with colonic fistulas, complete closure occurred 23.5 ± 19.5 d after the first application of fibrin glue, and spontaneous closure was observed after 36.2 ± 22.8 d in the control group ($P = 0.36$). Recurrences were observed in 2 patients because of residual disease. One patient of each group died during follow-up as a consequence of septic complications related to parenteral nutrition.

CONCLUSION: Closure time was significantly reduced with the use of fibrin sealant, and oral feeding was resumed faster. We suggest the use of fibrin sealant for the management of stable enterocutaneous fistulas.

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Key words: Enterocutaneous fistulas; Fibrin sealant; Spontaneous closure

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Abstract

AIM: To assess whether the use of fibrin sealant

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INTRODUCTION

The formation of enterocutaneous fistulas (ECFs) can be either spontaneous or as a consequence of intra-abdominal surgery. The incidence of spontaneous fistulas is around 15%-25%^[1,2]. These can be secondary to Crohn's disease, malignancy, infectious diseases such as tuberculosis and deep mycosis, diverticulitis, vascular failure, radiation exposure and ischemia of the mesentery^[3]. Postoperative fistulas account for 75%-85% out of all fistulas in the digestive tract and they arise from unintentional enterotomy, dehiscence of an anastomosis because of tension in the suture line, a foreign body located close to the anastomosis, inadequate suture techniques, distal obstructions, hematomas, abscess formation at the site of the anastomosis, or tumors^[3-5]. Morbidity and mortality associated with postoperative fistulas are substantial as they are highly associated with nutritional deficits, septic complications and concomitant diseases that may appear during prolonged hospital stays^[5-7]. The conventional treatment for postoperative fistulas includes intestinal rest, correction of electrolytic disturbances, parenteral nutrition, protection of the skin surrounding the fistula, and treatment and prophylaxis of any related local or systemic septic complications^[5,6]. Spontaneous closure of ECFs occurs after 6-8 wk in 60%-70% of the cases after specific medical management^[8]. Fistulas that do not close with conservative medical treatment require surgery^[2,7].

Biological fibrin glues have long been used extensively in many surgical and speciality fields^[9,10]. Fibrin glue contains high concentrations of human fibrinogen and thrombin and it has been widely applied clinically as a biological adhesive system for tissue adhesion or hemostasis^[11-13]. Additionally, in comparison with other adhesives, this compound has several advantages in terms of biocompatibility, biodegradation and hemostasis^[14-16]. Its use has also been extended for the management of untreatable fistulas that have not responded to conservative treatment^[11,12]. The efficacy of fibrin varies depending on a number of features, such as the output volume of the fistula, the location of the fistula, and the presence of a fistulous tract long enough (greater than 2 cm) to allow fixation of the patch^[17]. Following the placement of the fibrin patch, it is replaced by collagen after about 4 wk, leading to cessation of drainage and promotion of

closure of the fistula^[9,16] with avoidance of inflammatory processes, finally resulting in improved healing^[18].

The aim of this study was to determine the outcomes and advantages of treating low-output-volume ECFs with the addition of fibrin glue and compare them with conservative management without the use of adjuvant application of fibrin glue into the fistulous tract.

MATERIALS AND METHODS

Over an 8-year period, 70 male and female adults with stable, non-complicated, low-output ECFs were included in this non-randomized prospective case-control study. During the same period, a total of 218 patients were evaluated for postoperative enterocutaneous fistulas. One hundred and forty-eight patients were not considered for the study because of high output volume (> 500 mL/24 h), abdominal infection, fistulous tract < 2 cm, entero-atmospheric fistulas and residual intestinal or peritoneal disease. Patients were divided into 2 groups, a study group ($n = 23$) in which patients received variable doses of fibrin glue through the external opening of the ECFs, and a control group ($n = 47$) of patients treated without fibrin glue. Both groups were assessed to compare endpoint variables. Patients belonging to the control group were selected according to the origin of the fistula, using 1 case per 2 controls. Both groups were treated with the same conservative management, known to promote spontaneous closure. Patients with any concomitant condition that impeded spontaneous closure were excluded from the study.

Evaluation of the external fistula and medical treatment

Patients with a fistula output volume of less than 500 mL/24 h for at least 3 consecutive days were chosen to participate in the study. We evaluated the characteristics of the fistulas with contrast fistulography, computed tomography (CT) scans and proximal or distal endoscopy to confirm the absence of any condition that might impede spontaneous closure of the fistula, such as complex tracts, associated abscesses, intra-abdominal sepsis, residual disease, foreign bodies or distal obstruction, and to determine the length of the fistulous tract. Those cases with purulent collections were treated with percutaneous drainage before the application of the fibrin glue. Fistulous tracts measuring less than 2 cm were not included in the study because it was not possible to produce an adequate adhesion area for the patch into the fistulous tract. Pure pancreatic fistulas were not included.

Conservative treatment consisted of fasting, suppression of gastric secretion with oral or intravenous omeprazole or ranitidine, subcutaneous octreotide and nutritional support, either parenteral or enteral according to the anatomic location of the fistula. All patients received skin protection with barriers to prevent burns. The most important endpoint variables were the changes in the output volume, the time required to reduce the output of the fistula to zero for 2 consecutive days, the time re-

Table 1 General characteristics of the patients with postoperative enterocutaneous fistulas and final results (mean \pm SD)

Type of fistula	Sex (Male/female)	Age (yr)	Time of evolution (d)	Fistula output (mL/24 h)	Fistula closure time (d)	Restart oral intake (d)
Esophago-gastrocutaneous						
Study group (<i>n</i> = 3)	3/0	48.6 \pm 12.2	23.3 \pm 7	70 \pm 42.7	8 \pm 4	12 \pm 3.6
Control group (<i>n</i> = 7)	7/0	49.1 \pm 10.5	18.8 \pm 4.4	88.1 \pm 19	19.1 \pm 6	24 \pm 8.6
¹ <i>P</i> value		<i>P</i> = 0.85	<i>P</i> = 0.24	<i>P</i> = 0.15	<i>P</i> < 0.05	<i>P</i> < 0.05
Gastrocutaneous						
Study group (<i>n</i> = 5)	4/1	58.6 \pm 9.4	93.8 \pm 85.1	151.4 \pm 146.1	7 \pm 3.1	9.8 \pm 2.4
Control group (<i>n</i> = 10)	6/4	47.4 \pm 16.7	86.6 \pm 69.8	131.4 \pm 28.8	35.2 \pm 18.7	39.7 \pm 20.1
¹ <i>P</i> value		<i>P</i> = 0.19	<i>P</i> = 0.86	<i>P</i> = 0.67	<i>P</i> < 0.01	<i>P</i> < 0.01
Duodeno-jejunocutaneous						
Study group (<i>n</i> = 5)	2/3	38.2 \pm 10.4	25.6 \pm 4.9	111.6 \pm 75.9	7.6 \pm 2.6	11.6 \pm 4.3
Control group (<i>n</i> = 10)	6/4	44.1 \pm 11.2	22.8 \pm 4.2	123.6 \pm 19.9	30 \pm 17.1	34.9 \pm 17.2
¹ <i>P</i> value		<i>P</i> = 0.34	<i>P</i> = 0.27	<i>P</i> = 0.63	<i>P</i> < 0.01	<i>P</i> < 0.01
Ileocutaneous						
Study group (<i>n</i> = 6)	2/4	53.1 \pm 5	28.1 \pm 9.6	132.8 \pm 47.6	16.1 \pm 21.6	18.3 \pm 20.6
Control group (<i>n</i> = 12)	6/6	55.4 \pm 13.1	23 \pm 4.7	143 \pm 36	37.8 \pm 17.2	42.5 \pm 17.7
¹ <i>P</i> value		<i>P</i> = 0.69	<i>P</i> = 0.13	<i>P</i> = 0.61	<i>P</i> < 0.05	<i>P</i> < 0.05
Colocutaneous						
Study group (<i>n</i> = 4)	3/1	61.5 \pm 8.3	40.2 \pm 17	87.7 \pm 40.8	23.5 \pm 19.5	25 \pm 17.8
Control group (<i>n</i> = 8)	5/3	60.1 \pm 11.9	33.1 \pm 13.2	106.1 \pm 16.5	36.2 \pm 22.8	40 \pm 23.1
¹ <i>P</i> value		<i>P</i> = 0.84	<i>P</i> = 0.44	<i>P</i> = 0.28	<i>P</i> = 0.36	<i>P</i> = 0.25
All types of enterocutaneous fistulas						
Study group (<i>n</i> = 23)	14/9	51.9 \pm 11.6	43.3 \pm 46.3	116.2 \pm 81.5	12.5 \pm 14.2	15.3 \pm 13.3
Control group (<i>n</i> = 70)	30/17	51.1 \pm 13.7	37.5 \pm 40.9	121.9 \pm 31.5	32.5 \pm 17.9	37.2 \pm 18.4
² <i>P</i> value		<i>P</i> = 0.81	<i>P</i> = 0.59	<i>P</i> = 0.74	<i>P</i> = 0.000	<i>P</i> = 0.000

¹*P* value from the Mann Whitney *U* test; ²*P* value from the Student's *t* test.

quired to resume normal oral intake, and fistula reopening. In addition, we studied complications related to the use of nutritional support.

Fibrin glue application

To allow the adhesion of the fibrin glue patch, all fistulous tracts were debrided with an endoscopic brush to produce a smooth surface. The adhesive was composed of fibrinogen at a concentration of 80 mg/mL and 1000 IU/mL of thrombin (both of human extraction) combined with tranexamic acid and calcium chloride (Quixil®; Omrix Biopharmaceuticals Ltd., Tel-Hashomer, Israel). Prior to application, the adhesive was defrosted and components were placed separately in a double-syringe system with distal mixing device. The application of the glue through the external opening of the fistula was controlled endoscopically to assure total occlusion of the internal hole, in those patients with esophageal, gastric and colocutaneous fistulas. Patients with fistulas arising from the duodenum (after gastrectomy and gastrojejunostomy), jejunum and ileum, the control of the glue application was performed with fistulography to establish the distance between the internal and external holes, fistulous tract diameter and estimated volume of fibrin glue to administrate into the fistulous tract. We considered therapeutic failure when the fistula output persisted after the third application of the adhesive without a limit time.

Ethical considerations

This study was conducted according to the declaration of Helsinki of 1989 and Mexican Health Guidelines. The

protocol was approved by the Ethical Committee of the Western National Medical Center at the Mexican Institute of Social Security. Full written informed consent was obtained from all patients before their inclusion to this study.

Statistical analysis

Results are described as percentages and central tendency and dispersion measures. Qualitative variables were analyzed using χ^2 or Fisher's exact test, and for quantitative variables the Mann Whitney *U* test or Student's *t* test was used according to the resulting distribution. All *P* values less than 0.05 were considered statistically significant.

RESULTS

Between January of 2000 and December of 2007, 70 patients were included. The study group consisted of 23 patients (14 male, 9 female) with a mean age of 51.95 \pm 11.67 years, and a control group of 47 patients (30 male, 17 female) with a mean age of 51.17 \pm 13.78 years. In both groups, all external fistulas were classified according to the anatomical origin (Table 1).

Esophago-gastrocutaneous fistulas

We included 10 patients with esophago-gastrocutaneous fistulas secondary to esophageal resection of distal carcinoma, caustic burns or high-grade dysplasia associated with Barrett's metaplasia. In all cases, the esophagus was replaced by gastric transposition through the posterior mediastinum. There were 3 cases in the study group and 7 in the control group. There was no difference in mean

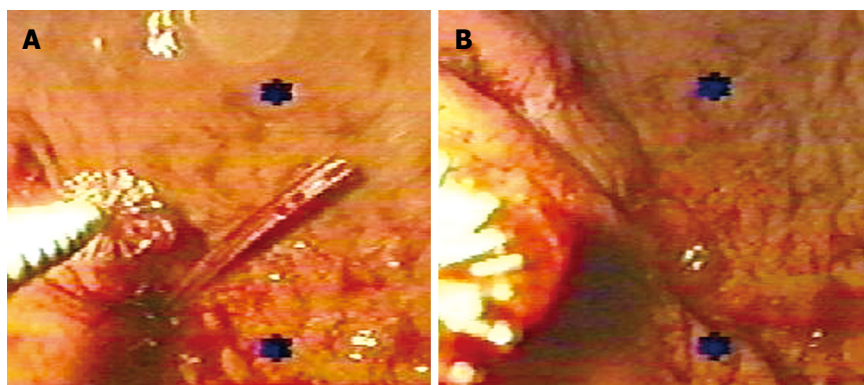


Figure 1 Endoscopic control of the application of fibrin glue in a patient with a gastrocutaneous fistula. A: The sealant was instilled externally; B: For better results, a complete occlusion of the internal hole of the fistulous tract is required.

age between groups ($P = 0.85$). The average output of the fistulas was 70 ± 42.7 mL and 88.1 ± 19 mL, respectively ($P = 0.15$). The fistulous tract measured 3.6 ± 0.36 cm and 3.65 ± 0.48 cm, respectively ($P = 0.86$). The mean quantity of fibrin glue applied into the external opening was 9 mL (range 7–11 mL). The time to obtain complete closure following the initial application was 8 ± 4 d in the study group. Spontaneous closure was observed after 19.1 ± 6 d of medical treatment in the control group ($P < 0.05$).

Gastrocutaneous fistulas

Five patients with gastrocutaneous fistulas were included in the study group and 10 in the control group. Gender distribution was similar in both groups and there were no differences in age ($P = 0.19$). Average fistula output was 151.4 ± 146.1 mL and 131.4 ± 28.8 mL ($P = 0.67$). The majority originated from endoscopic or surgical gastrotomies. One case in the control group had a fistula that originated from a partial dehiscence of a gastrojejunostomy. The fistulous tract measured 4.3 ± 0.77 cm and 4.1 ± 0.61 cm ($P = 0.76$) in study and control groups, respectively. The adhesive application was performed under endoscopic visual control to ensure complete occlusion of the internal hole (Figure 1). The mean quantity of fibrin glue was 18 mL (range 6–30 mL). In the study group, complete closure was observed 7.0 ± 3.1 d after the application. In the control group, spontaneous closure occurred after 35.2 ± 18.7 d ($P < 0.01$).

Duodenum and jejuno-cutaneous fistulas

Fifteen patients, 8 male and 7 female, were included in this group. One patient in the study group and 2 patients in the control group had pure duodenal fistulas after total or partial gastric resections. The remaining 12 cases had external jejuno-cutaneous fistulas, 4 in the study group and 8 in the control group, all of which resulted from partial dehiscence of jejunorrhaphy or anastomoses after resection. There were no differences in age between groups ($P = 0.34$). The mean fistula output was 111.6 ± 75.9 mL in the study group and 123.6 ± 19.9 mL in the control group ($P = 0.63$). The fistulous tract measured 6.5 ± 0.56 cm and 6.9 ± 0.68 cm ($P = 0.26$), respectively. The mean quantity of the fibrin glue applied into the external opening was 12.5 mL (range 10–15 mL). Complete closure was observed 7.6 ± 2.6 d after the application of the

adhesive in the study group and at 30.0 ± 17.1 d in the control group ($P < 0.01$).

Ileocutaneous fistulas

Eighteen patients, 8 male and 10 female were included in this group, 6 in the study group and 12 in the control group. All fistulas were secondary to partial dehiscence of end-to-end anastomoses or enterorrhaphy. There was no age difference between the groups ($P = 0.69$). The average fistula output was 132.8 ± 47.6 mL *vs* 143.0 ± 36.6 mL in study and control groups, respectively ($P = 0.61$). The fistulous tract measured 10.4 ± 1.8 cm and 10.6 ± 1.9 cm, respectively ($P = 0.85$). The mean quantity of fibrin glue applied into the external opening was 14.5 mL (range, 10–19 mL). For the study group, complete closure was observed 16.1 ± 21.6 d after the first application and 37.8 ± 17.2 d in the control group ($P < 0.05$). In one of the patients the fibrin glue was applied endoscopically because the fistula originated from dehiscence of an ileocolic anastomosis 70 cm from the anal margin.

Colocutaneous fistulas

This group had 12 patients, 8 male and 4 female, 4 in the study group and 8 in the control group. There was no age difference between groups ($P = 0.84$). The average of the fistula output was 87.7 ± 40.8 mL in the study group and 106.1 ± 16.5 mL in the control group ($P = 0.28$). The fistulous tract measured 15.4 ± 2.1 cm and 14.1 ± 1.5 cm ($P = 0.24$). All fistulas were secondary to left colon resection, with manually constructed end-to-end anastomosis or primary closure after trauma. Diagnoses made before the resection were diverticular disease, sigmoid or proximal rectal cancer, and trauma with incidental perforation. The mean quantity of fibrin glue applied into the external opening was 14.5 mL (range 12–26 mL). In the study group, complete closure occurred 23.5 ± 19.5 d after the first application, and spontaneous closure was observed after 36.2 ± 22.8 d in the control group ($P = 0.36$).

Fistula recurrence, complications related to nutritional support and mortality

During an 18-mo follow-up period, reopening of fistulous tracts was observed in 2 patients with colocutaneous fistulas. Both patients belonged to the study group. One

patient suffered incidental perforation of the left colon during a necrosectomy with primary closure. After the reopening of the fistulous tract, endoscopic evaluation showed sigmoid stenosis, and a CT scan demonstrated a retroperitoneal inflammatory process probably related to a previous episode of acute pancreatitis, and after 8 mo he experienced spontaneous closure. The second case was a patient with residual diverticular disease of the left colon that was not identified during the initial evaluation before fibrin glue application. This patient underwent a segmental colon resection with subsequent resolution of the complication.

Morbidity related to nutritional support was significantly different between groups. In the study group, there was one minor and one major complication (8.6%). One patient required a change of jejunostomy tube because of obstruction, and the other patient, who had short bowel syndrome and was receiving long-term total parenteral nutrition, developed septic shock secondary to *Candida albicans* infection 11 mo after the resolution of the fistulous complication. This patient eventually died of multiple organ failure. In the control group, morbidity was observed in 20 patients (42.5%). This difference was statistically significant ($P < 0.01$). Minor complications such as tube obstruction, enteral nutrition-associated diarrhea, and central venous catheter obstruction were observed in 8 cases (17%). The remaining 12 patients developed major complications, such as fever related to a central venous catheter in 11 cases: 4 with negative cultures which were resolved with a new access; 7 patients with positive cultures, 6 with Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus epidermidis*); and the remaining patient died of septic complications caused by a systemic *C. albicans* infection. There were 2 additional deaths during the follow-up from non-related causes, including one patient from each group (acute myocardial infarction and diffuse abdominal carcinomatosis). There were no complications related to the application of the fibrin glue. Finally, when we considered all patients stratified only by the interventional maneuver, we observed highly significant results. The time to achieve total fistula closure was 12.5 ± 14.2 d in the study group (fibrin glue) and 32.5 ± 17.9 d in the control group ($P < 0.001$).

DISCUSSION

Fibrin sealant, also referred to as fibrin glue or fibrin tissue adhesive, is a surgical hemostatic agent derived from plasma coagulation proteins^[19]. Fibrin sealants are widely used for many surgical procedures in all fields of surgery^[20]. They can be used for hemostasis, wound closure, and tissue sealing, and in contrast to synthetic adhesives, they have the advantage of being biocompatible and biodegradable, and they are not associated with inflammatory processes, foreign body reactions, tissue necrosis, or extensive fibrosis. With normal wound healing, fibrin absorption occurs within days to weeks of application, depending on the type of surgery, the proteolytic activity at the treated site, and the amount of sealant used^[21].

Commercial concentrates containing human fibrinogen, factor XIII and bovine thrombin became available in Europe in the late 1970s and have been extensively used since then for hemostasis and other indications^[22]. All these products contained antifibrinolytic agents. However, when first introduced, fibrin sealants were excluded from use in the United States based on the risk of transmission of infection^[21,22]. The risk of viral transmission was reduced through careful donor selection followed by heat treatment of the human fibrinogen component. Virally inactivated human thrombin has replaced bovine thrombin in most European products^[23]. A number of researchers have described the postoperative development of antibodies to bovine thrombin in some patients^[24].

The first new generation fibrin sealants used in the United States, with removed or inactivated viruses, was Tisseel (Baxter Healthcare), which was approved by the US Food and Drug Administration (FDA) in 1998^[25]. Tisseel was used in the majority of the studies performed to assess the use of this adhesive. In the present study, we used a fibrin sealant known as a second-generation sealant, which received FDA approval in March 2003 and is known as Crosseal (American Red Cross)^[26]. Both Tisseel and Quixil are composed of human fibrinogen and human thrombin, which are combined at the time of use. The respective formula differs from each other because Tisseel contains bovine aprotinin (BA), while Quixil contains tranexamic acid, both known as antifibrinolytic agents^[26].

Adverse events associated with the use of fibrin sealant over the last 25 years have been inflammatory processes, allergic reactions and viral infections secondary to the bovine component^[27].

Hino *et al*^[28] recently reported 3 cases of iatrogenic parvovirus B19 infection after the use of a fibrin sealant. This viral transmission was attributed to the use of dry-heat viral inactivation, which is not effective against non-enveloped viruses. Thus far, there are no reports of human immunodeficiency virus seroconversion or hepatitis B or C infection after the use of fibrin sealant^[29]. However, the risk of virus transmission by fibrin sealants is still a subject of debate^[19]. Three cases of anaphylaxis have been reported in Japanese patients, one of which was linked to the bovine aprotinin component of the sealant^[30].

Fibrin adhesives have been used for several decades to close or obliterate fistulous tracts^[31] and their effectiveness varies depending on the output volume of the fistula, its location, and the presence of a sufficiently long fistulous tract (greater than 2 cm) to allow fixation of the patch^[32]. Postoperative fistulas of the digestive tract are relatively frequent in surgical practice. Serious technical difficulties such as variability in the location of the fistulas, quantification of drainage, time of evolution of the fistula, patient status and underlying pathology, have hindered scientific research comparing various therapeutic options and treatments^[32]. This is one of the reasons why there are just a few randomized controlled clinical trials with the use of fibrin sealants^[19].

In 1996, Hwang *et al.*^[32], published a small randomized clinical trial in patients with very low-output enterocutaneous fistulas (less than 20 mL). They used fibrin glue extracted from a blood bank and activated with bovine thrombin. The anatomic origin of the fistulas was varied and included upper and lower digestive tract fistulas. In 6 patients treated with external application of the adhesive, the time needed to close the fistulous tract was reduced significantly (2 ± 0.4 d), as was the hospital stay. Control patients ($n = 7$) received conventional treatment with total parenteral nutrition. In this group, spontaneous closure was observed after 13 ± 2 d ($P < 0.01$). The authors concluded that the use of fibrin glue is safe and effective for patients with stable and low-output enterocutaneous fistulas.

Waag *et al.*^[33] performed the first endoscopic application of an adhesive in 1979, for the treatment of a tracheo-esophageal fistula. Subsequently, with the introduction of different types of biological tissue adhesives and the development of dual and triple lumen catheters in 1984, fibrin glues have been used in the endoscopic treatment of fistulas of the digestive tract^[34]. However, only proximal and distal digestive tract fistulas are accessible for endoscopic evaluation and management. In 1990, Nakagawa *et al.*^[35] reported the use of a small-diameter endoscope for the evaluation and treatment of fistulous tracts with fibrin glue. Fifteen patients were included in this study, and they were submitted to endoscopic examination, the clinical significance of which was assessed, and it was concluded that fistuloscopy is a safe and easy technique that results in less stress for the patients and is considered to be effective for the examination and treatment of fistulas^[35].

Lange *et al.*^[36] investigated whether the endoscopic procedure could be used as an adjuvant technique for the sealing of gastrointestinal fistulas in 17 enterocutaneous fistulas. The success rate was 64.5%, and they described complementary treatment for abscesses associated with the fistulous tract. Some patients developed complications because of the high pressure of the fibrin glue application, and one patient died of pulmonary air embolism^[36].

As reported by Shand *et al.*^[37] in 1997, some adjuvants have been purported to favor the early closure of fistulous tracts. They described a case of a patient with a non-healing low-output gastrocutaneous fistula closed by endoscopic means with fibrin glue and surgical packs. The fistula reopened 3 d after the procedure, and closure of the fistula was reattempted by direct endoscopic application of surgical packs and adhesive. Complete closure was obtained 11 d after direct endoscopic application of the fibrin glue^[37].

Recently, Lomis *et al.*^[38] performed a study in which they evaluated 7 patients with persistent fistula, treating them with collagen plugs. Under fluoroscopic guidance and using direct-catheter techniques, collagen plugs were applied into the fistulas. The success rate was 85.7%; 6 of the 7 patients had resolution of the fistula with no evidence of fistula recurrence 30-180 d after the closure.

In 1990, Eleftheriadis *et al.*^[39] reported 7 cases of proximal digestive fistulas with high-volume output (700 mL in 24 h minimum) that were closed with fibrin gel by endoscopic means, with a 100% success rate. In 2002, the same authors reported a study in 14 patients; 7 with low-volume-output fistulas (discharging 20-50 mL/d) and 7 with high-volume-output fistulas (200-1000 mL/d), using fistuloscopy to evaluate the fistulous tracts and to measure the length of the fistula, with removal of necrotic material and non-absorbable sutures. Fibrin sealant was applied under direct observation. The fistulas healed within 2-17 d (mean 9.2 ± 5.1 d) in all patients except one with peritoneal carcinomatosis. The authors concluded that fistuloscopy could be used as both a diagnostic and a therapeutic tool in low- and high-output postoperative fistulas resistant to conservative treatment^[40]. More recently, in 2004, the same author reported the endoscopic application of fibrin sealant for the treatment of gastrocutaneous fistula after bariatric surgery in three morbidly obese patients^[41].

Kurokawa *et al.*^[42], reported an 85% success rate after the selective occlusion of complex digestive fistulas with fibrin glue application under fistuloscopy in patients in whom fistula closure had not occurred after 3 wk of conservative postoperative treatment.

Ramón Rábago *et al.*^[43], recently reported the largest series of postoperative digestive fistulas treated with transendoscopic application of fibrin glue. They included 30 patients with proximal, distal and internal fistulas of low- and high-output. The medium time of conservative treatment was 95 ± 199 d for all types of digestive fistulas. The success rate was 80% and 25% respectively and 55% for internal fistulas. They recommended that conservative treatment should not be prolonged beyond 2-4 wk, since most postoperative fistulas required 14 d to stabilize, and that endoscopic treatment should be performed at that stage. After follow-up periods ranging from 6 mo to more than 6 years, only one of the sealed fistulas reopened requiring a new endoscopic resealing. Rabago and his colleagues concluded that endoscopic treatment achieves a very high success rate, without complications and at a lower cost^[43].

The first Mexican experience with fibrin sealants was published in 1997 by Justo-Janeiro *et al.*^[44]. They evaluated the application of concentrated human fibrinogen activated with bovine thrombin, fundamentally for hemostatic purposes, protection of high-risk anastomosis, seroma prevention and closure of enterocutaneous fistulas (4 patients). The success rate in the early closure of fistulous tracts was limited (50%), and failures were a consequence of distal obstruction to the fistula origin and intense inflammatory reactions^[44].

Since 1998 commercially-prepared fibrin glue became available in our country and in 1999 our group designed protocols to evaluate the efficacy of the fibrin sealant in different surgical indications, including general surgery. In this study, we used human fibrin glue exclusively in low-output digestive fistulas, and we made comparisons with patients who received the same medical treatment but without the use of the adhesive. Our results are relevant

as the closure times achieved were significantly reduced, and the time needed to resume oral intake was also decreased. Application through the external opening is very useful when the characteristics of the fistulous tract are well known. Recently, Murakami *et al.*^[45], demonstrated adequate closure of complex postoperative digestive fistulas identifying the characteristics of the fistulous tract with an injection of contrast medium through the drainage tube. Eighteen patients were included in the study, with an 88% success rate (16 patients) with the applications of 1 to 9 treatment sessions of diluted solution of thrombin and fibrinogen 80 mg/mL. They studied the effect of thrombin dilution in saline solution and found the optimal concentration at 8 mg/mL of thrombin with a coagulation time of 45 ± 5 s. The delayed coagulation time permitted injection of the glue through all the complex fistulous tracts reaching a high success rate despite 60% of patients having abscesses associated with the fistulous tracts. The treatment was offered between 31 and 168 (66 ± 40.4) d after the surgical procedures.

In the series presented here, endoscopic control of the applications was performed in 13 cases with proximal or distal fistulas. The persistence of inflammation and inadvertent diverticular disease induced the recurrence of 2 colonic fistulas which eventually resolved with medical and surgical treatment. We attribute the success rate, even without performing a fistuloscopy or transendoscopic application, to the meticulous instillation of the fibrin glue, the low-volume output of the enterocutaneous fistulas, and the absence of complex fistulous tracts or any other condition that impeded spontaneous closure of the fistulas, such as associated abscesses, intra-abdominal sepsis, residual disease and foreign bodies or distal obstruction. Morbidity related to nutritional support was higher in the control group due to the prolonged time to feeding, and mortality was secondary to catheter-related sepsis in chronic parenteral nutrition. Since accelerated closure was not the purpose of this study, we proposed to treat patients as soon as the fistulas became stable and low-output without any associated obstruction, infection or residual disease, as other authors suggested in treatment of this complication^[32,43,45].

In summary, we recommend the use of fibrin glue in those patients who do not experience spontaneous closure, to reduce the time needed to complete the resolution of the fistula and to minimize the rate of complications related to the secretions of the fistulas. The sealant should be applied as soon as the fistula becomes stable with the lowest output to assure closure after one or more applications of the glue.

Endoscopic control of the fibrin glue application should be performed whenever possible to ensure complete occlusion of the internal hole. The technique employed in this study has the advantage of allowing the removal of necrotic tissue and non-absorbable sutures from the fistulous tracts. Radiological visualization of the fistulous tracts, as studied here, requires less technological support, and we successfully treated digestive fistulas originating from the small bowel, including the duodenum,

with external application of the adhesive. Patients did not present any adverse reactions, and recurrences were attributed to residual or untreated disease, particularly in those patients with colonic fistulas.

COMMENTS

Background

The formation of enterocutaneous fistulas can be either spontaneous or as a consequence of intra-abdominal surgery. The incidence of spontaneous fistulas is around 15%-25%, and postoperative fistulas account for 75%-85% of all fistulas of the digestive tract. Morbidity and mortality associated with postoperative fistulas are substantial as they are highly associated with nutritional deficits, septic complications and concomitant diseases that may appear during prolonged hospital stays. The conventional treatment for postoperative fistulas includes intestinal rest, correction of electrolytic disturbances, parenteral nutrition, and protection of the skin surrounding the fistula, and treatment and prophylaxis of any related local or systemic septic complications. Spontaneous closure occurs after 6-8 wk in 60%-70% of cases after specific medical management.

Research frontiers

Biological fibrin glues have long been used extensively in many surgical and specialty fields. Its use has also been extended for the management of untreatable fistulas that have not responded to conservative therapy. The efficacy of fibrin varies depending on the features of the fistula, such as output volume, location, and the presence of a fistulous tract long enough (> 2 cm) to allow fixation of the patch. Following the placement of the fibrin patch, it is replaced by collagen, leading to cessation of drainage and closure of the fistula with avoidance of inflammatory processes, finally resulting in improved healing.

Innovations and breakthroughs

This study determined that the application of fibrin glue through the external opening of stable enterocutaneous fistula reduced the closure time and the morbidity associated with nutritional support.

Applications

Instillation of variable quantities of fibrin glue (6 to 30 mL) according to the fistulous tract in one to 3 treatment sessions. The application of the glue was controlled endoscopically to assure total occlusion of the internal hole in proximal and distal fistulas.

Peer review

This is a good paper which targets a difficult and important topic.

REFERENCES

- 1 **Berry SM**, Fischer JE. Classification and pathophysiology of enterocutaneous fistulas. *Surg Clin North Am* 1996; **76**: 1009-1018
- 2 **Arenas-Marquez H**, Anaya-Prado R, Hurtado H, Juarez F, Fernandez J, Galindo-Mendoza L, Terrazas-Espitia F, Aiello V, Mondragón R, Gudiño-Lever I, Gutierrez de la Rosa JL, Athié-Athié AJ, Perez-Huacuja R, Gonzalez-Ojeda A, Campos PS, Sitges-Serra A, Palma-Vargas JM. Mexican consensus on the integral management of digestive tract fistulas. Ixtapa-Zihuatanejo, Mexico, August 21-23, 1997. *Nutrition* 1999; **15**: 235-238
- 3 **Metcalfe C**. Enterocutaneous fistulae. *J Wound Care* 1999; **8**: 141-142
- 4 **Berry SM**, Fischer JE. Enterocutaneous fistulas. *Curr Probl Surg* 1994; **31**: 469-566
- 5 **Evenson AR**, Fischer JE. Current management of enterocutaneous fistula. *J Gastrointest Surg* 2006; **10**: 455-464
- 6 **McIntyre PB**, Ritchie JK, Hawley PR, Bartram CI, Lennard-Jones JE. Management of enterocutaneous fistulas: a review of 132 cases. *Br J Surg* 1984; **71**: 293-296
- 7 **Rubelowsky J**, Machiedo GW. Reoperative versus conservative management for gastrointestinal fistulas. *Surg Clin North Am* 1991; **71**: 147-157
- 8 **Dorta G**. Role of octreotide and somatostatin in the treatment of intestinal fistulae. *Digestion* 1999; **60** Suppl 2: 53-56

- 9 **Jung M**, Manegold BC, Brands W. Endoscopic therapy of gastrointestinal fistulae with fibrin tissue sealant. In: Wacławiczek HW, editor. Progress in fibrin sealing. Berlin: Springer Verlag, 1989: 43-52
- 10 **Redl H**, Schlag G. Properties of different tissue sealants with special emphasis on fibrinogen-based preparations. Fibrin sealant in operative medicine, otolaryngology. Vol 1. Berlin, Heidelberg: Springer-Verlag, 1986: 27-38
- 11 **Venkatesh KS**, Ramanujam P. Fibrin glue application in the treatment of recurrent anorectal fistulas. *Dis Colon Rectum* 1999; **42**: 1136-1139
- 12 **Willetts IE**, Dudley NE, Tam PK. Endoscopic treatment of recurrent tracheo-oesophageal fistulae: long-term results. *Pediatr Surg Int* 1998; **13**: 256-258
- 13 **Jessen C**, Sharma P. Use of fibrin glue in thoracic surgery. *Ann Thorac Surg* 1985; **39**: 521-524
- 14 **McCarthy PM**. Fibrin glue in cardiothoracic surgery. *Transfus Med Rev* 1993; **7**: 173-179
- 15 **Stricker RB**, Lane PK, Leffert JD, Rodgers GM, Shuman MA, Corash L. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. *Blood* 1988; **72**: 1375-1380
- 16 **Schlag G**, Redl H. Fibrin sealant: Efficacy, quality and safety. In Wacławiczek HW, editor. Progress in fibrin sealant. Heidelberg: Springer, 1989: 3-17
- 17 **Lange V**, Maiwald G, Souvatzki T, Meyer G. Endoscopic approaches for occlusion of fistulas. En Schlang G, Wayand W, editors. Fibrin sealing in surgical and nonsurgical fields: endoscopy. Berlin: Springer, 1995: 58-64
- 18 **Sheppard BB**, De Virgilio C, Bleiweis M, Milliken JC, Robertson JM. Inhibition of intra-abdominal adhesions: fibrin glue in a long term model. *Am Surg* 1993; **59**: 786-790
- 19 **Jackson MR**. Fibrin sealants in surgical practice: An overview. *Am J Surg* 2001; **182**: 1S-7S
- 20 **Spotnitz WD**. Commercial fibrin sealants in surgical care. *Am J Surg* 2001; **182**: 8S-14S
- 21 **Reece TB**, Maxey TS, Kron IL. A prospectus on tissue adhesives. *Am J Surg* 2001; **182**: 40S-44S
- 22 **Martinowitz U**, Saltz R. Fibrin sealant. *Curr Opin Hematol* 1996; **3**: 395-402
- 23 **Jackson MR**, MacPhee MJ, Drohan WN, Alving BM. Fibrin sealant: current and potential clinical applications. *Blood Coagul Fibrinolysis* 1996; **7**: 737-746
- 24 **Stricker RB**, Lane PK, Leffert JD, Rodgers GM, Shuman MA, Corash L. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. *Blood* 1988; **72**: 1375-1380
- 25 **Tisseel VH**. [package insert]. Glendale, Calif: Baxter Healthcare Corp, 2000
- 26 **Crosseal** [package insert]. Washington, DC: American Red Cross, 2003
- 27 **Albala DM**, Lawson JH. Recent clinical and investigational applications of fibrin sealant in selected surgical specialties. *J Am Coll Surg* 2006; **202**: 685-697
- 28 **Hino M**, Ishiko O, Honda KI, Yamane T, Ohta K, Takubo T, Tatsumi N. Transmission of symptomatic parvovirus B19 infection by fibrin sealant used during surgery. *Br J Haematol* 2000; **108**: 194-195
- 29 **Spotnitz WD**, Prabhu R. Fibrin sealant tissue adhesive--review and update. *J Long Term Eff Med Implants* 2005; **15**: 245-270
- 30 **Mitsuhata H**, Horiguchi Y, Saitoh J, Saitoh K, Fukuda H, Hirabayashi Y, Togashi H, Shimizu R. An anaphylactic reaction to topical fibrin glue. *Anesthesiology* 1994; **81**: 1074-1077
- 31 **Hedelin H**, Nilsson AE, Teger-Nilsson AC, Thorsen G. Fibrin occlusion of fistulas postoperatively. *Surg Gynecol Obstet* 1982; **154**: 366-368
- 32 **Hwang TL**, Chen MF. Randomized trial of fibrin tissue glue for low output enterocutaneous fistula. *Br J Surg* 1996; **83**: 112
- 33 **Waag KL**, Joppich I, Manegold BC. Endoscopic closure of tracheoesophageal fistula. *Z Kinderchir* 1979; **27** (supl): 93-95
- 34 **Groittl H**, Scheele J. Initial experience with the endoscopic application of fibrin tissue adhesive in the upper gastrointestinal tract. *Surg Endosc* 1987; **1**: 93-97
- 35 **Nakagawa K**, Momono S, Sasaki Y, Furusawa A, Ujiie K. Endoscopic examination for fistula. *Endoscopy* 1990; **22**: 208-210
- 36 **Lange V**, Meyer G, Wenk H, Schildberg FW. Fistuloscopy--an adjuvant technique for sealing gastrointestinal fistulae. *Surg Endosc* 1990; **4**: 212-216
- 37 **Shand A**, Pendlebury J, Reading S, Papachrysostomou M, Ghosh S. Endoscopic fibrin sealant injection: a novel method of closing a refractory gastrocutaneous fistula. *Gastrointest Endosc* 1997; **46**: 357-358
- 38 **Lomis NN**, Miller FJ, Loftus TJ, Whiting JH, Giuliano AW, Yoon HC. Refractory abdominal-cutaneous fistulas or leaks: percutaneous management with a collagen plug. *J Am Coll Surg* 2000; **190**: 588-592
- 39 **Eleftheriadis E**, Tzartinoglou E, Kotzampassi K, Aletras H. Early endoscopic fibrin sealing of high-output postoperative enterocutaneous fistulas. *Acta Chir Scand* 1990; **156**: 625-628
- 40 **Eleftheriadis E**, Kotzampassi K. Therapeutic fistuloscopy: an alternative approach in the management of postoperative fistulas. *Dig Surg* 2002; **19**: 230-235; discussion 236
- 41 **Papavramidis ST**, Eleftheriadis EE, Papavramidis TS, Kotzampassi KE, Gamvros OG. Endoscopic management of gastrocutaneous fistula after bariatric surgery by using a fibrin sealant. *Gastrointest Endosc* 2004; **59**: 296-300
- 42 **Kurokawa T**, Okushiba S, Kadoya M, Miyamoto D, Kurashima Y, Kitagami H, Ikeda J, Sunaga M, Shinzato Y, Ozawa T, Kondo S, Katoh H. Selective occlusion with fibrin glue under fistuloscopy: seven cases of postoperative management for intractable complex fistulas. *Endoscopy* 2002; **34**: 220-222
- 43 **Ramón Rábago L**, Moral I, Delgado M, Guerra I, Quintanilla E, Castro JL, Llorente R, Martínez Veiga JL, Gea F. [Endoscopic treatment of gastrointestinal fistulas with biological fibrin glue] *Gastroenterol Hepatol* 2006; **29**: 390-396
- 44 **Justo-Janeiro JM**, Pavon-Vargas A. First clinical experience in Mexico with the use of fibrine glue. *Cir Gen* 1997; **19**: 103-108
- 45 **Murakami M**, Tono T, Okada K, Yano H, Monden T. Fibrin glue injection method with diluted thrombin for refractory postoperative digestive fistula. *Am J Surg* 2009; **198**: 715-719

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Post-gastrectomy spleen enlargement and esophageal varices: Distal vs total gastrectomy

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CONCLUSION: Endoscopy should be performed to detect EVs when the platelet count-to-spleen diameter ratio is < 2600.

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Key words: Spleen enlargement; Esophageal varices; Platelet count; Distal gastrectomy; Total gastrectomy

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Abstract

AIM: To study the relationship between platelet count-to-spleen diameter ratio and post-gastrectomy esophageal varices (EVs) development in patients without liver cirrhosis or hepatitis.

METHODS: We retrospectively studied 92 patients who underwent gastrectomy. They were divided into 2 groups on the basis of the surgical treatment: the distal gastrectomy (DG) group and total gastrectomy (TG) group. The incidence of EVs was determined and postoperative platelet counts, spleen diameters, and platelet count-to-spleen diameter ratios were compared between the 2 groups.

RESULTS: EVs were not detected during the first 6 mo after surgery in either group; however, at 12 mo after surgery, EVs were detected in 2 patients (3%) in the DG group and in 1 patient (3.6%) in the TG group; their mean platelet count-to-spleen diameter ratio was 2628 ± 409 , and 2604 ± 360 , respectively.

INTRODUCTION

Esophageal varices (EVs) are one of the major life-threatening complications of liver cirrhosis^[1], and its prevalence is approximately 40% at the time of diagnosis, and 60% in those with decompensated disease^[2,3]. When EVs rupture, the mortality rate ranges from 17% to 57%^[4-7]. Therefore, screening of all patients diagnosed with liver cirrhosis for the presence of EVs is recommended^[8,9]. Giannini *et al*^[10] performed a study to identify parameters that could aid noninvasive prediction of EVs, and reported that the platelet count-to-spleen diameter ratio was the best noninvasive predictor of EVs. However, spleen enlargement is frequently observed during follow-up of noncirrhotic patients who have

undergone gastrectomy, and, in addition, some of these patients developed EVs. Thus, we studied the relationship between the platelet count-to-spleen diameter ratio and the development of EVs, and compared distal and total gastrectomy (TG) with regard to these variables in patients without liver cirrhosis or hepatitis.

MATERIALS AND METHODS

Patients

We retrospectively studied 92 patients (66 men and 26 women; age range, 30-80 years; mean age, 68 ± 10 years) who underwent gastrectomy between May 2002 and April 2006 at the Department of Surgery, Social Insurance Yokohama Central Hospital, Yokohama, Japan. The following patients were excluded from our study: those who had undergone abdominal operation in the past, those with hepatitis, and those in whom disease recurrence was observed. Preoperative upper gastrointestinal endoscopy was performed in all the patients, further, EVs were not confirmed in any of the patients. These patients were divided into the following 2 groups on the basis of the surgical treatment they received: patients who underwent distal gastrectomy (DG), and patients who underwent TG. The maximum bipolar diameter of the spleen was measured by ultrasound scanning. The platelet count, spleen diameter, and platelet count-to-spleen diameter ratio were measured after surgery, and their relationship with the occurrence of EVs was studied.

Endoscopic classification of esophageal varices

On the basis of the endoscopic findings, the EVs were classified into 3 grades: grade 1, varices could be compressed with the endoscope; grade 2, varices could not be compressed with the endoscope; and grade 3, varices were confluent around the esophagus^[11].

Statistical analysis

Univariate analysis was performed using the Student *t* test for continuous variables and Fisher's exact test and the χ^2 test for categorical variables. A *P*-value of less than 0.05 was considered to be significant.

RESULTS

Table 1 shows the patient characteristics and preoperative variables. No differences were observed between the 2 groups with respect to mean age, sex ratio, and preoperative clinical data. The gastric cancers were staged according to the Japanese Classification of Gastric Carcinoma^[12], and their stages were found to be similar.

Table 2 shows the variables 3 mo after the operation. The mean platelet count, mean spleen diameter, and mean platelet count-to-spleen diameter ratio were $25.8 \times 10^4 \pm 4.2 \times 10^4/\mu\text{L}$, 93.9 ± 7.6 mm, and 2769 ± 453 , respectively, in the DG group, and $25.1 \times 10^4 \pm 3.9 \times 10^4/\mu\text{L}$, 96.8 ± 9.5 mm, and 2593 ± 328 , respectively, in the TG group. No differences were observed between the 2 groups, and no EVs developed in either group.

Table 1 Characteristics of patients *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Age (yr)	68.3 \pm 10.1	69.3 \pm 6.7	0.6522
Sex ratio (male:female)	46:18	20:8	0.9651
Stage			
I A	15 (23.4)	5 (17.9)	0.5504
I B	13 (20.3)	7 (25)	0.6160
II	16 (25.0)	7 (25)	1.00
III A	17 (26.6)	7 (25)	0.8752
III B	3 (4.7)	2 (7.1)	0.6382
Initial disease			
Hypertension	10 (15.6)	3 (10.7)	0.7474
Diabetes mellitus	9 (14.1)	3 (10.7)	0.7502
Atrial fibrillation	2 (3.1)	1 (3.6)	1.00
Apoplexy	2 (3.1)	0 (0)	0.5184
Parkinson disease	1 (1.6)	0 (0)	0.3043
Old pulmonary tuberculosis	4 (6.3)	1 (3.6)	1.00
Preoperative platelet count ($\times 10^4/\mu\text{L}$)	24.8 \pm 4.1	24.5 \pm 3.4	0.7386
Preoperative spleen diameter (mm)	90.8 \pm 8.5	91.9 \pm 10.4	0.5908
Preoperative Platelet count/spleen diameter ratio	2744 \pm 473	2674 \pm 369	0.4921

DG: Distal gastrectomy; TG: Total gastrectomy.

Table 2 Clinical data 3 mo postoperatively *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Platelet count ($\times 10^4/\mu\text{L}$)	25.8 \pm 4.2	25.1 \pm 3.9	0.4580
Spleen diameter (mm)	93.9 \pm 7.6	96.8 \pm 9.5	0.1251
Platelet count/spleen diameter ratio	2769 \pm 453	2593 \pm 328	0.0674
Esophageal varices			
Grade 1	0 (0)	0 (0)	1.00
Grade 2	0 (0)	0 (0)	1.00
Grade 3	0 (0)	0 (0)	1.00
Total occurrence rate	0 (0)	0 (0)	1.00

Grade 1: The varices could be depressed by the endoscope; Grade 2: The varices could not be depressed by the endoscope; Grade 3: The varices were confluent around the esophagus.

Table 3 shows the variables 6 mo after the operation. The mean platelet count, mean spleen diameter, and mean platelet count-to-spleen diameter ratio were $26.7 \times 10^4 \pm 4.3 \times 10^4/\mu\text{L}$, 95.6 ± 7.6 mm, and 2853 ± 458 , respectively, in the DG group, and $26.5 \times 10^4 \pm 3.9 \times 10^4/\mu\text{L}$, 100.6 ± 9.4 mm, and 2632 ± 373 , respectively, in the TG group. There was no significant difference between the 2 groups with respect to the platelet count; however, the mean spleen diameter in the TG group was significantly greater than that in the DG group ($P < 0.0078$), and the mean platelet count-to-spleen diameter ratio in the TG group was significantly lower than that in the DG group ($P < 0.0274$). No EVs developed in the patients in either group.

Table 4 shows the variables 12 mo after the operation. The mean platelet count, mean spleen diameter, and mean platelet count-to-spleen diameter ratio were $26.8 \times 10^4 \pm 3.9 \times 10^4/\mu\text{L}$, 102.4 ± 8.7 mm, and 2628 ± 409 , respectively, in the DG group, and $27.5 \times 10^4 \pm 3.7 \times 10^4/\mu\text{L}$, 105.9 ± 9.0 mm, and 2604 ± 360 , respective-

Table 3 Clinical data 6 mo postoperatively *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Platelet count ($\times 10^3/\mu\text{L}$)	26.7 \pm 4.3	26.5 \pm 3.9	0.8016
Spleen diameter (mm)	95.6 \pm 7.6	100.6 \pm 9.4	0.0078
Platelet count/spleen diameter ratio	2853 \pm 458	2632 \pm 373	0.0274
Esophageal varices			
Grade 1	0 (0)	0 (0)	1.00
Grade 2	0 (0)	0 (0)	1.00
Grade 3	0 (0)	0 (0)	1.00
Total occurrence rate	0 (0)	0 (0)	1.00

Table 4 Clinical data 12 mo postoperatively *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Platelet count ($\times 10^3/\mu\text{L}$)	26.8 \pm 3.9	27.5 \pm 3.7	0.4358
Spleen diameter (mm)	102.4 \pm 8.7	105.9 \pm 9.0	0.0843
Platelet count/spleen diameter ratio	2628 \pm 409	2604 \pm 360	0.7887
Esophageal varices			
Grade 1	2 (3)	1 (3.6)	1.00
Grade 2	0 (0)	0 (0)	1.00
Grade 3	0 (0)	0 (0)	1.00
Total occurrence rate	2 (3)	1 (3.6)	1.00

Table 5 Clinical data 24 mo postoperatively *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Platelet count ($\times 10^3/\mu\text{L}$)	26.4 \pm 3.7	25.8 \pm 4.0	0.4533
Spleen diameter (mm)	104.4 \pm 9.8	109.9 \pm 10.1	0.0164
Platelet count/spleen diameter ratio	2546 \pm 380	2357 \pm 365	0.00287
Esophageal varices			
Grade 1	2 (3)	2 (7.1)	0.5825
Grade 2	0 (0)	1 (3.6)	0.3043
Grade 3	0 (0)	0 (0)	1.00
Total occurrence rate	2 (3)	3 (10.7)	0.1629

Table 6 Clinical data 36 mo postoperatively *n* (%), (mean \pm SD)

	DG group (<i>n</i> = 64)	TG group (<i>n</i> = 28)	<i>P</i> -value
Platelet count ($\times 10^3/\mu\text{L}$)	26.3 \pm 3.8	25.5 \pm 4.3	0.3904
Spleen diameter (mm)	105.6 \pm 9.8	110.6 \pm 9.6	0.0147
Platelet count/spleen diameter ratio	2515 \pm 386	2317 \pm 381	0.0256
Esophageal varices			
Grade 1	3 (4.7)	3 (10.7)	0.3638
Grade 2	1 (1.6)	1 (3.6)	0.5184
Grade 3	0 (0)	0 (0)	1.00
Total occurrence rate	4 (6.3)	4 (14.3)	0.2082

ly, in the TG group. No differences were observed between the 2 groups. However, 2 patients (3%) in the DG group and 1 patient (3.6%) in the TG group had grade 1 EVs. There was no significant difference between the 2 groups with respect to the occurrence of EVs.

Table 5 shows the variables 24 mo after the operation. The mean platelet count, mean spleen diameter, and mean platelet count-to-spleen diameter ratio were $26.4 \times 10^4 \pm 3.7 \times 10^4/\mu\text{L}$, 104.4 ± 9.8 mm, and 2546 ± 380 , respectively, in the DG group, and the corresponding values were $25.8 \times 10^4 \pm 4.0 \times 10^4/\mu\text{L}$, 109.9 ± 10.1 mm, and 2357 ± 365 , respectively, in the TG group. No differences were observed between the 2 groups with respect to the platelet count; however, the mean spleen diameter in the TG group was significantly greater than that in the DG group ($P < 0.0164$), and the mean platelet count-to-spleen diameter ratio in the TG group was significantly lower than that in the DG group ($P < 0.00287$). Two patients (3%) in the DG group and 2 patients (7.1%) in the TG group had grade-1 EVs, and 1 patient (3.6%) in the TG group had grade 2 EVs; however, there was no significant difference between the 2 groups with respect to the occurrence of EVs.

Table 6 shows the variables 36 mo after the operation. The mean platelet count, mean spleen diameter, and mean platelet count-to-spleen diameter ratio were $26.3 \times 10^4 \pm 3.8 \times 10^4/\mu\text{L}$, 105.6 ± 9.8 mm, and 2515 ± 386 , respectively, in the DG group, and $25.5 \times 10^4 \pm 4.3 \times 10^4/\mu\text{L}$, 110.6 ± 9.6 mm, and 2317 ± 381 , respectively, in the TG group. No differences were observed between the

2 groups with respect to the platelet count; however, the mean spleen diameter in the TG group was significantly greater than that in the DG group ($P < 0.0147$), and the mean platelet count-to-spleen diameter ratio in the TG group was significantly lower than that in the DG group ($P < 0.0256$). However, 3 patients (4.7%) in the DG group and 3 patients (10.7%) in the TG group had grade 1 EVs, and 1 patient (1.6%) in the DG group and 1 patient (3.6%) in the TG group had grade 2 EVs. There was no significant difference between the 2 groups with respect to the occurrence of EVs.

DISCUSSION

Bleeding from ruptured EVs is the leading cause of death among patients with liver cirrhosis^[1], and the mortality rate from this complication varies between 17% and 57%^[4-7]. Thus, cirrhotic patients should be screened for the presence of EVs. Some authors suggested that repeated endoscopy at intervals of 2-3 years should be performed in patients without varices, and that it should be performed at intervals of 2 years in patients with small varices in order to evaluate the development or progression of EVs^[13,14]. However, in order to develop less invasive and cost-effective screening procedures for EVs, several studies have attempted to validate parameters that could be used for noninvasive screening^[15-21], and have found that platelet count and spleen diameter showed a good correlation with the presence of EVs. In addition, Giannini *et al*^[10] and Baig *et al*^[22] reported that

the platelet count-to-spleen diameter ratio has the highest accuracy for noninvasive prediction of EVs in cirrhotic patients. However, spleen enlargement is frequently observed after patients have undergone gastrectomy. Thus, we studied the relationship between the platelet count-to-spleen diameter ratio and the presence of EVs after gastrectomy, and compared distal and TG with regard to these parameters in patients without liver cirrhosis or hepatitis. In addition, in order to avoid surgical influence, patients who had undergone abdominal operation in the past were excluded from this study. All our patients were healthy and did not exhibit any recurrence during their follow-up. In patients who did not have liver cirrhosis or hepatitis, the occurrence rate of EVs was thought to be low; however, splenic arterial flow was thought to increase after but not before the surgery. In general, blood flow in the celiac artery (CA) is diverted into 3 arteries: the common hepatic artery (CHA), left gastric artery (LGA), and splenic artery (SA). During gastrectomy (distal and total), the LGA and left gastric vein are ligated and cut, and blood flow from the CA is diverted into 2 arteries - the CHA and SA. Thus, blood flow in the CHA and SA increases after surgery. Moreover, during lymph node dissection around the celiac axis, collateral veins from the splenic vein are ligated and cut; thus, in our patients, blood outflow from the spleen was thought to decrease after the surgery as compared to that before the surgery. Also, in the TG group, short gastric veins were ligated and cut, and blood outflow from the spleen was thought to be lower than that in the GD group patients. This may be one of the main reasons why spleen enlargement was greater in the TG group than in the DG group.

Giannini *et al*^[10] reported that the prevalence-adjusted positive and negative predictive values for a platelet count-to-spleen diameter ratio of 909 were 96% and 100%, respectively, and Baig *et al*^[22] reported that the prevalence-adjusted positive and negative predictive values for a platelet count-to-spleen diameter ratio of 1014 were 9.4% and 95.1%, respectively. In our study, grade 1 EVs were detected in 2 patients (3%) in the DG group, and in 1 patient (3.6%) in the TG group at 12 mo after surgery, and the mean platelet count-to-spleen diameter ratio of the patients was found to be 2628 ± 409 in the DG group, and 2604 ± 360 in the TG group. At 24 mo after surgery, grade 2 EVs were detected in 1 patient (3.6%) in the TG group, and the mean platelet count-to-spleen diameter ratio of these patients was found to be 2357 ± 365 . In the DG group, grade 2 EVs were detected 36 mo after the surgery, and the mean platelet count-to-spleen diameter ratio of the patients was found to be 2515 ± 386 . In our study, EVs developed in 4 patients each in the DG group (6.3%) and the TG group (14.3%) after the surgery; however, no patient exhibited grade 3 EVs. In addition, none of our patients had liver cirrhosis or hepatitis; this contributed to the higher platelet count-to-spleen diameter ratio than that reported by Giannini *et al*^[10] and Baig *et al*^[22]. To the best of our knowledge, studies on the relationship between the platelet count-to-spleen diameter ratio and the presence of EVs have not

been conducted in patients who underwent gastrectomy. Thus, on the basis of the results of our study, we suggest that the occurrence rate of EVs may increase after 6 mo post DG and when the platelet count-to-spleen diameter ratio is less than 2600. Thus, in the case of patients who have undergone gastrectomy for gastric cancer, the development of EVs after surgery is a strong possibility.

In conclusion, spleen enlargement after gastrectomy is greater in the case of TG than DG, and the platelet count-to-spleen diameter ratio is a useful parameter for the non-invasive prediction of EVs after gastrectomy. In addition, we think that the occurrence rate of EVs increases after 6 mo post gastrectomy, and therefore, when the platelet count-to-spleen diameter ratio is less than 2600, endoscopy should be performed to determine the presence of EVs.

COMMENTS

Background

Esophageal varices (EVs) are one of the major life-threatening complications of liver cirrhosis, and its prevalence rate is approximately 40% at the time of diagnosis, and 60% in those with decompensated disease. Therefore, screening of all patients diagnosed with liver cirrhosis for the presence of EVs is recommended.

Research frontiers

Spleen enlargement is frequently observed during follow-up of noncirrhotic patients who have undergone gastrectomy. The authors studied the relationship between the platelet count-to-spleen diameter ratio and the development of EVs, and compared distal and total gastrectomy (TG) with regard to these variables in patients without liver cirrhosis or hepatitis.

Innovations and breakthroughs

The authors retrospectively studied 92 patients who underwent gastrectomy. They were divided into 2 groups on the basis of the surgical treatment: the distal gastrectomy (DG) group and TG group. The incidence of esophageal varices was determined and postoperative platelet counts, spleen diameters, and platelet count-to-spleen diameter ratios were compared between the 2 groups.

Applications

Spleen enlargement after gastrectomy is greater in the case of TG than DG, and the platelet count-to-spleen diameter ratio is a useful parameter for the noninvasive prediction of EVs after gastrectomy.

Peer review

It is an interesting manuscript, though the background did not identify the real reason why the authors were embarking on their endeavor. The results were well presented and the discussion would be improved. It is a nice experiment.

REFERENCES

- 1 de Franchis R, Primignani M. Natural history of portal hypertension in patients with cirrhosis. *Clin Liver Dis* 2001; 5: 645-663
- 2 Schepis F, Cammà C, Niceforo D, Magnano A, Pallio S, Cinquegrani M, D'amico G, Pasta L, Craxi A, Saitta A, Raimondo G. Which patients with cirrhosis should undergo endoscopic screening for esophageal varices detection? *Hepatology* 2001; 33: 333-338
- 3 D'Amico G, Luca A. Natural history. Clinical-haemodynamic correlations. Prediction of the risk of bleeding. *Baillieres Clin Gastroenterol* 1997; 11: 243-256
- 4 Garceau AJ, Chalmers TC. The natural history of cirrhosis. I. Survival with esophageal varices. *N Engl J Med* 1963; 268: 469-473
- 5 Graham DY, Smith JL. The course of patients after variceal hemorrhage. *Gastroenterology* 1981; 80: 800-809
- 6 Rigo GP, Merighi A, Chahin NJ, Mastronardi M, Codeluppi

- PL, Ferrari A, Armocida C, Zanasi G, Cristani A, Cioni G. A prospective study of the ability of three endoscopic classifications to predict hemorrhage from esophageal varices. *Gastrointest Endosc* 1992; **38**: 425-429
- 7 **Jensen DM**. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. *Gastroenterology* 2002; **122**: 1620-1630
 - 8 **Grace ND**, Groszmann RJ, Garcia-Tsao G, Burroughs AK, Pagliaro L, Makuch RW, Bosch J, Stiegmann GV, Henderson JM, de Franchis R, Wagner JL, Conn HO, Rodes J. Portal hypertension and variceal bleeding: an AASLD single topic symposium. *Hepatology* 1998; **28**: 868-880
 - 9 **D'Amico G**, Garcia-Tsao G, Calés P, Escorsell A, Nevens F, Cestari R, Caletti G. Diagnosis of portal hypertension: how and when. In: De Franchis R, editor. *Proceedings of the Third Baveno International Consensus Workshop on Definitions, Methodology and Therapeutic Strategies*. Oxford: Blackwell Science, 2001: 36-63
 - 10 **Giannini E**, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* 2003; **52**: 1200-1205
 - 11 Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. *N Engl J Med* 1988; **319**: 983-989
 - 12 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma - 2nd English Edition - *Gastric Cancer* 1998; **1**: 10-24
 - 13 **Calés P**, Desmorat H, Vinel JP, Caucanas JP, Ravaud A, Gerin P, Brouet P, Pascal JP. Incidence of large oesophageal varices in patients with cirrhosis: application to prophylaxis of first bleeding. *Gut* 1990; **31**: 1298-1302
 - 14 **D'Amico G**, Pagliaro L, Bosch J. The treatment of portal hypertension: a meta-analytic review. *Hepatology* 1995; **22**: 332-354
 - 15 **Thomopoulos KC**, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Iconomou G, Nikolopoulou VN. Non-invasive predictors of the presence of large oesophageal varices in patients with cirrhosis. *Dig Liver Dis* 2003; **35**: 473-478
 - 16 **Madhotra R**, Mulcahy HE, Willner I, Reuben A. Prediction of esophageal varices in patients with cirrhosis. *J Clin Gastroenterol* 2002; **34**: 81-85
 - 17 **Chalasani N**, Imperiale TF, Ismail A, Sood G, Carey M, Wilcox CM, Madichetty H, Kwo PY, Boyer TD. Predictors of large esophageal varices in patients with cirrhosis. *Am J Gastroenterol* 1999; **94**: 3285-3291
 - 18 **Zaman A**, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liver disease. *Am J Gastroenterol* 1999; **94**: 3292-3296
 - 19 **Freeman JG**, Darlow S, Cole AT. Platelet count as a predictor for the presence of oesophageal varices in alcoholic cirrhotic patients. *Gastroenterology* 1999; **116**: A1211 (Abst)
 - 20 **Barcia HX**, Robalino GA, Molina EG. Clinical predictors of large varices in cirrhotic patients. *Gastrointest Endosc* 1998; **47**: A78 (Abst)
 - 21 **Alacorn F**, Burke CA, Larive B. Esophageal varices in patients with cirrhosis: Is screening endoscopy necessary for everyone? *Am J Gastroenterol* 1998; **93**: 1673 (Abst)
 - 22 **Baig WW**, Nagaraja MV, Varma M, Prabhu R. Platelet count to spleen diameter ratio for the diagnosis of esophageal varices: Is it feasible? *Can J Gastroenterol* 2008; **22**: 825-828

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Recurrence after endoscopic piecemeal mucosal resection for large sessile colorectal polyps

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Abstract

AIM: To evaluate the safety and outcomes of endoscopic piecemeal mucosal resection (EPMR) for large sessile colorectal polyps.

METHODS: The patients enrolled in this study were 47 patients with 50 large sessile polyps (diameter, 2 cm or greater) who underwent EPMR using a submucosal saline injection technique between December 2002 and October 2005. All medical records, including characteristics of the patients and polyps, complications, and recurrences, were retrospectively reviewed. The first follow-up endoscopic examination was performed at 3-6 mo after initial endoscopic resection, and the second at 12 mo post-EPMR. Subsequent surveillance colonoscopic examinations were individualized, taking risk factors into account.

RESULTS: The patients were 23 men and 24 women,

with a mean age of 60 years. Mean polyp size was 30.1 mm. Of 50 polyps identified, 34 (68%) were benign and 16 (32%) were malignant. There were 6 (12%) cases with EPMR-related bleeding: 5 intra-procedural and 1 early post-procedural bleeding. All bleeding episodes were managed by endoscopic clipping or argon beam coagulation. There were no perforations. Recurrence was identified in 5 cases (12.2%): 4 local recurrences detected at 3 mo post-EPMR and 1 local recurrence detected at 14 mo post-EPMR. The recurrence rate after EPMR was 3.1% for benign polyps and 33.3% for malignant polyps ($P < 0.05$). Median follow-up time was 37 mo.

CONCLUSION: EPMR is safe, but should be applied carefully in malignant polyps. Close follow-up endoscopic examinations are necessary for early detection of recurrence.

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Key words: Colonoscopy; Colorectal neoplasm; Endoscopic piecemeal mucosal resection; Large sessile polyps

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INTRODUCTION

Endoscopic resection of large sessile colorectal polyps

remains challenging because of its technical difficulty and high complication rate^[1]; even so, the endoscopic removal of colonic adenomatous polyps is a commonly used technique that reduces the incidence of colorectal cancer. Some investigators have reported that endoscopic piecemeal mucosal resection (EPMR) is a safe and effective procedure for large sessile colorectal polyps^[2-4]; however, this approach remains controversial because of the high possibility of coexisting malignancy and a high recurrence rate associated with large sessile polyps. Endoscopic submucosal dissection (ESD) has recently been attempted by expert endoscopists for *en bloc* resection of large sessile polyps^[5,6]; however, this procedure has a long procedure time and high complication rate, and is not currently widely used due to its technical difficulty^[7].

Several studies have shown that the incomplete removal of large sessile colorectal polyps, particularly by piecemeal resection, can contribute to a higher subsequent incidence of colorectal cancers^[8,9]. Thus, recent guidelines recommend that when large sessile polyps are removed by piecemeal resection, a repeat examination should be performed at a short interval (2-6 mo) to verify complete removal^[10]. However, few studies report the recurrence rate after EPMR, and the long-term outcomes of EPMR have yet to be established. This study was designed to evaluate the safety, efficacy, and long-term outcomes of EPMR of large sessile colorectal polyps.

MATERIALS AND METHODS

We retrospectively reviewed and identified 77 patients with 80 large sessile colorectal polyps (2 cm or greater) detected by colonoscopy at the National Cancer Center, Korea, between December 2002 and October 2005. Among these patients, 30 were excluded for the following reasons: co-existence of synchronous advanced colorectal cancer ($n = 15$), non-lifting tumor ($n = 4$), encircling lesion $> 70\%$ ($n = 4$), transfer to other institution ($n = 4$), suspicion of muscle invasion by endoscopic ultrasound ($n = 1$), and recurrent tumor ($n = 2$). A final total of 47 patients with 50 large sessile polyps who underwent EPMR using submucosal saline injection technique were enrolled in this study. We reviewed medical records, including patient demographics, endoscopic findings, histopathological reports, and follow-up data. The study was performed in accordance with the Declaration of Helsinki and informed consent was obtained from all patients.

Colonoscopy

Patients were prepared with mechanical bowel preparation. The patients received either two 45 mL doses of sodium phosphate (Fleet[®]; C.B. Fleet Co. Inc., Lynchburg, VA, USA) or 4 L of polyethylene glycol solution (Colyte-F[®]; Taejoon Pharm, Seoul, Korea) and underwent colonoscopy under conscious sedation with midazolam. Colonoscopy was performed to the cecum or terminal ileum with white light colonoscopic examination. Polyps suspicious of invasive cancer (the presence of ulceration, induration,

friable mucosa, or non-lifting sign^[11]) were referred for surgical resection. Polyp size (measured in comparison with open biopsy forceps) and morphology were generally estimated and recorded by the endoscopist.

EPMR technique

Endoscopic resection was performed using a snare piecemeal method with submucosal saline injection technique (Figure 1) according to the strip biopsy method described by Karita *et al.*^[12] Colonoscopy was performed with a standard video colonoscope (CF Q260L; Olympus Optical Co., Ltd., Tokyo, Japan). Briefly, the injection catheter was passed through the channel, and saline solution mixed with diluted epinephrine (1:100 000) was injected into the submucosal layer near the sessile polyp until the entire polyp was elevated. If the polyp was not elevated after one injection, additional injections were made around the polyp. The injection catheter was removed and a snare device was inserted through the channel. The surrounding normal mucosa, along with the lesion, was encircled by the snare. After the snare device was positioned, resection was performed using electrosurgical coagulation current, a combination of coagulation and blended currents in sequence. If necessary, argon plasma coagulation (APC) was used to treat the polyp base in an attempt to destroy any residual polyp.

Complications

EPMR-induced bleeding was defined as intraprocedural (occurring during EMR), early (within 24 h after EPMR), or delayed (≥ 24 h after EPMR), as described previously^[13]. The diagnosis of early or delayed bleeding was based on the passage of blood per rectum. Bleeding was controlled by endoscopic clip (HX-600-135, Olympus) placement and/or APC. Perforation was defined as the presence of free air on plain abdominal film or computed tomography (CT).

Histopathology

All resected material was retrieved for histopathologic evaluation. Specimens were collected using a basket or by aspiration into the suction channel. For entire retrieval of sessile polyps located in the right colon, the colonoscope was withdrawn and reinserted as many times as necessary. A single pathologist assessed all histopathologic specimens and was not blinded to the endoscopic findings. Malignant polyps with unfavorable histology, such as poor differentiation, angiolymphatic invasion, and deep submucosal invasion ($\geq 1000 \mu\text{m}$), or having a positive deep resection margin, were referred for surgical treatment after polypectomy.

Follow-up

Patients who had undergone endoscopic treatment alone were followed up with colonoscopy to evaluate whether endoscopic resection of the sessile polyps had been complete. Endoscopic examinations were scheduled as follows: the first follow-up endoscopic examination was

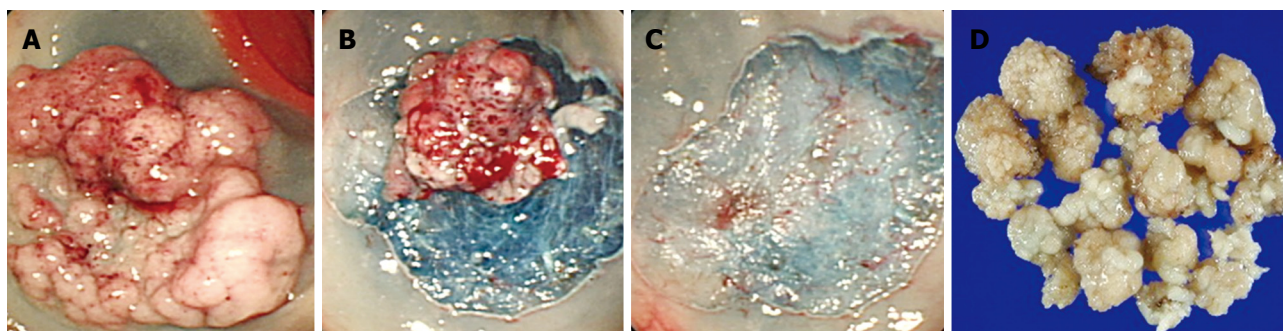


Figure 1 Endoscopic piecemeal mucosal resection of a large sessile polyp. A: Endoscopic view of the rectum showing a large sessile polyp (40 mm in diameter) after submucosal injection; B, C: Piecemeal polypectomy was performed; D: Retrieved specimens after resection of small pieces.

performed at 3-6 mo after the initial endoscopic resection, and the second was performed at 12 mo post-EPMR. Subsequent surveillance colonoscopic examinations were individualized with consideration of risk factors. If a polyp was detected on follow-up examinations, it was resected if possible. Residual polypoid tissue with the appearance of granulation tissue was biopsied, but not counted as residual adenoma. Recurrence or residual polyp was defined as the presence of any amount of adenomatous or carcinomatous tissue on follow-up, even as small as 1 mm, confirmed by histology at the site of prior piecemeal polypectomy^[14]. All recurrences were demonstrated by pathology to contain dysplastic (adenoma) or carcinomatous tissue. The difference in recurrence between EPMR for benign polyps and EPMR for malignant polyps was determined by the log-rank test using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). *P* values of < 0.05 were considered statistically significant.

RESULTS

The clinicopathologic characteristics of the 47 patients with 50 polyps are listed in Table 1. Among the patients, 5 had a history of non-steroidal anti-inflammatory drugs (NSAIDs) administration that was stopped at least 7 d before endoscopy. The mean size of resected polyps was 30.1 ± 10.9 mm (range, 20-60 mm). Locations of the large sessile polyps were as follows: cecum in 2 polyps, ascending in 7, hepatic flexure in 6, transverse in 4, splenic flexure in 1, descending in 5, sigmoid in 6, and rectum in 19. Of the 50 polyps, 34 (68%) were benign and 16 (32%) were malignant. Histological examination revealed tubular adenoma in 20 polyps, tubulovillous adenoma in 7, villous adenoma in 1, serrated adenoma in 4, hyperplastic polyp in 2, and carcinoma in 16 (Tis, 11; T1, 4; T2, 1).

The outcomes of EPMR for large sessile polyps are listed in Table 2. After EPMR, there were 6 (12%) cases of procedural bleeding: 5 intraprocedural and 1 early after initial endoscopic resection, which were managed by endoscopic means with the application of hemoclips alone in 4 cases, APC alone in 1 case, and hemoclips with APC plus fibrin glue in 1 case. There was no significant bleeding requiring blood transfusion or surgical intervention, and no delayed bleeding following polypectomy. No

Table 1 Clinicopathologic characteristics of 47 patients with 50 large sessile polyps treated by endoscopic piecemeal mucosal resection

Variable	<i>n</i>
Male/female	23/24
Mean age (range), yr	60 (27-78)
Mean polyp size (range), mm	30.1 (20-60)
Location	
Cecum	2
Ascending	7
Hepatic flexure	6
Transverse	4
Splenic flexure	1
Descending	5
Sigmoid	6
Rectum	19
Histology	
Benign (<i>n</i> = 34)	
Tubular adenoma	20
Tubulovillous adenoma	7
Villous adenoma	1
Serrated adenoma	4
Hyperplastic	2
Malignant (<i>n</i> = 16)	
Tis	11
T1	4
T2	1

patient suffered colonic perforation as a result of endoscopic resection. Bleeding prophylaxis was performed in 6 cases (12%) by applying a hemoclip. The median hospital stay (from procedure to discharge) was 3 d (range, 1-5 d), with the exception of 4 patients: 3 who were not admitted and 1 who underwent consecutive colonic resection.

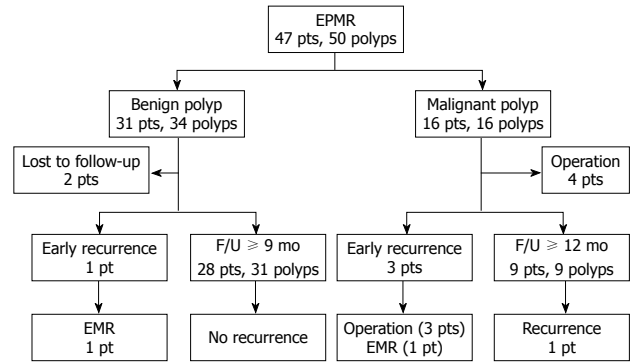
Of the 47 patients, follow-up data were available for 45 patients (95.7%); the exceptions were 2 without follow-up endoscopy. The median follow-up time was 37 mo (range, 3-72 mo). Recurrence was identified in 5 patients (12.2%): including 4 cases of local recurrence detected at 3 mo post-EPMR and 1 local recurrence detected at 14 mo post-EPMR (Table 3).

Of the 32 patients with benign polyps, there was 1 (3.1%) case of recurrence: a patient with benign recurrent adenoma who was successfully treated by endoscopic resection. Other follow-up endoscopy showed no recurrence. Of the 16 patients with malignant polyps, 4 polyps

Table 2 Clinical outcome of endoscopic piecemeal mucosal resection for large sessile polyps

Variable	n
Median follow-up ¹ (range), mo	37 (3-72)
Median hospital stay ² (range), d	3 (1-5)
Complications	
Bleeding (%)	6 (12)
Intraprocedural	5
Early	1
Delayed	0
Perforation	0
Recurrence (%)	5 (12.2)

¹2 patients lost to follow-up were excluded; ²Excluding 4 patients: 3 were not admitted and 1 underwent consecutive colonic resection.

**Figure 2** Long-term follow-up results for large sessile polyps. EPMR: Endoscopic piecemeal mucosal resection; F/U: Follow-up time; EMR: Endoscopic mucosal resection.**Table 3** Characteristics of 5 patients with residual/recurrent lesions after initial endoscopic piecemeal resection (EPMR)

No.	Age/sex	Location	Size (mm)	Primary histology	Time to recurrence (mo)	Recurrent histology	Method of treatment	Follow-up (mo)
1	72/M	Cecum	40	Adenoma	3	Adenoma	EMR	25
2	65/M	Rectum	40	Carcinoma (Tis)	3	Carcinoma (T2)	LAR	16
3	78/F	Rectum	40	Carcinoma (T1)	3	Adenoma	EMR	51
4	64/F	Rectum	60	Carcinoma (T1)	14	Carcinoma (Tis)	LAR	42
5	49/M	Sigmoid	40	Carcinoma (T1)	3	HGD adenoma	AR	40

HGD: High grade dysplasia; EMR: Endoscopic mucosal resection; LAR: Low anterior resection; AR: Anterior resection.

with unfavorable histology underwent surgery. Among the remaining 12 patients with malignant polyps, 4 (33.3%) had recurrence: 1 patient with benign recurrent adenoma was treated by endoscopic resection and 3 patients underwent surgery because their recurrent lesions could not be removed by endoscopic resection (Table 3).

The difference in recurrence between EPMR for benign polyps and that for malignant polyps was statistically significant (3.1% *vs* 33.3%, $P < 0.05$).

The follow-up results of the 50 large sessile polyps are shown in Figure 2.

DISCUSSION

Endoscopic resection of large sessile colorectal polyps (2 cm or greater) is increasingly used as an alternative to surgery, but remains challenging because of its technical difficulty, the high risk of complications such as bleeding or perforation, and the possibility of coexisting malignancy. Recent advances in endoscopic technique and equipment have enabled the development of techniques such as EPMR and ESD to remove large sessile colorectal polyps^[1-6]. Comparing these two techniques, the mean procedure times for EPMR^[4,6] and ESD^[6,15] ranged from 29 to 55 min and from 70.5 to 108 min, respectively. Regarding complications, the perforation rates for EPMR^[6,8,14] and ESD^[4,6,15] ranged from 0.8% to 1.3% and 6.2% to 10.0%, respectively. Because EPMR has a shorter procedure time and lower perforation rate than ESD, EPMR appears to be an easy and safe procedure; however, Saito *et al*^[6] dem-

onstrated that EPMR resulted in a higher recurrence rate compared with ESD (14% *vs* 2%). The rate of recurrence at the polypectomy site after EPMR was reported to be 20%-55% in several other studies^[8,14,16]. The recurrence rate after EPMR of 12.2% in the present study (11.36% in 44 polyps) is relatively low for the 44 large sessile polyps that had follow-up endoscopy. Several investigators have reported that additional techniques (e.g. APC) may further improve the success of polypectomy, and hence lower the recurrence rate^[3,14,17]. In the present study, 91% of cases were treated with APC. Our low recurrence rate was probably influenced by the high application rate of APC to the tumor bed following EPMR. With regard to histopathology of the polyps, the recurrence rate was 3.1% for benign polyps and 33.3% for malignant polyps; in other words, EPMR of malignant polyps resulted in a higher recurrence rate than that of benign polyps. In contrast, Conio *et al*^[13] reported similar recurrence rates for benign and malignant polyps. The recurrence rate of malignant colorectal polyps after EPMR varies among studies; however, it is difficult to compare the results of different series because there are wide variations in polyp size and the length of follow-up^[13]. In addition, it is difficult to explain the reason why the incidence of recurrence after EPMR in malignant lesions is higher than that in benign lesions. However, we should try to remove all cancer cells completely because microscopic residual cancer cells after EPMR can cause recurrences. Further studies are needed to confirm the usefulness of EPMR for malignant colorectal polyps.

The most common complication after polypectomy is bleeding; the risk of post-polypectomy bleeding ranges from 0.3% to 6.1%^[18,19]. The risk factors for bleeding include large polyp size and location in the proximal colon^[19,20]. In large sessile polyps (2 cm or greater), the incidence of bleeding during and after polypectomy has been reported to be as high as 13.5%^[2,3,8,14,16]. In the present study, bleeding occurred in 5 cases (12.2%), and all patients with bleeding were treated by endoscopic management, without surgery or blood transfusion. Thus, we can consider these cases as having “minor complications,” as described in a previous report^[3]. In the present study, none of the patients with bleeding were taking NSAIDs (aspirin) or had known coagulopathy at the time of the EPMR procedure. In our endoscopic database, any procedural bleeding requiring additional endoscopic treatment was described, and we classified EPMR-induced bleeding as intraprocedural, early, or delayed. Doniec *et al*^[21] suggested that it is doubtful whether hemorrhage should be classified as a complication during endoscopic treatment when it can be managed endoscopically; no surgeon would regard bleeding as a complication during an operation such as mucosectomy. We used submucosal injection of epinephrine-saline mixture 1:100 000 in all EPMR cases, not only for submucosal elevation but also to prevent procedure-related bleeding. Iishi *et al*^[2] injected only 0.9% saline solution alone to elevate sessile polyps, and reported bleeding in 7% of cases; however, no study has definitely proved the superiority of submucosal solution. In the present study, there was no delayed bleeding or perforations. Taking our experience into consideration, it is clear that the risk of perforation increases with increased width of the polypectomized colonic wall during snaring, rather than with increasing polyp size.

Rectal polyps are considered easy to remove due to the relatively low rate of perforation. In our study, 19 (40.4%) out of 47 polyps were located in the rectum. However, the recurrence rate after EPMR was not different between rectal polyps and colonic polyps (17.6% *vs* 7.4%, *P* = 0.359).

Previous studies have reported the risk of malignancy in large sessile colorectal polyps (2 cm or larger) as being up to 29%^[22,23]. In the present study, 16 polyps (32%) were found to be adenomas containing an area of carcinoma; of these, 5 (10%) were invasive cancer. We evaluated the lifting sign of the tumor using saline injection to the submucosal layer before EPMR in all cases. This technique may have caused the relatively low incidence of invasive cancer in the large sessile colorectal polyps in the present study.

It is well known that initial colonoscopy has a significant miss rate of 24% for all types of adenomas^[24]. Yamaji *et al*^[25] reported recurrence rates for small adenomas and advanced lesions of 19.3% and 22.9%, respectively. In the present study, 80% of recurrences were identified at 3 mo post-EPMR, and the other recurrences were detected at 14 mo post-EPMR. Missed or metachronous adenomas detected at 3–6 mo, 1–3 years, and 3 years post-EPMR were detected in 32%, 46%, and 32% of cases, respective-

ly. The higher rate of metachronous adenomas may have been influenced by the fact that our patients had more advanced lesions (i.e. all had large sessile polyps), including invasive cancers. The present results support current guidelines which recommend that patients who undergo piecemeal resection of large sessile adenomas should have an initial follow-up colonoscopy within 3–6 mo, followed by an additional colonoscopy 1 year later^[10].

In conclusion, EPMR is a safe procedure for large sessile colorectal polyps, but should be applied carefully in malignant polyps because of high recurrence rate. Close follow-up endoscopic examinations are necessary for early detection of recurrence.

COMMENTS

Background

Endoscopic removal of colonic adenomatous polyps is a commonly used technique that reduces the incidence of colorectal cancer. However, endoscopic resection of large sessile colorectal polyps is still challenging because of its technical difficulty.

Research frontiers

Endoscopic piecemeal mucosal resection (EPMR) can be used for large sessile colorectal polyps. However, this approach remains controversial because of the high possibility of coexisting malignancy and the high recurrence rate associated with large sessile polyps. Few studies report the recurrence rate after EPMR, and the long-term outcomes of EPMR have yet to be established.

Innovations and breakthroughs

Several studies have shown that the incomplete removal of large sessile colorectal polyps, particularly by piecemeal resection, can contribute to a higher subsequent incidence of colorectal cancers. This study shows that EPMR is safe for benign colorectal polyps, but should be applied carefully in malignant polyps due to high recurrence rate.

Applications

By identifying the recurrence rate and long term follow-up results after EPMR, this study may contribute to the development of a therapeutic guideline for patients with large sessile colorectal polyps.

Terminology

EPMR, which is used to increase safety, is a technique for removing large sessile polyps. During EPMR, the polyp can be broken down into multiple pieces. However, this makes it difficult to evaluate the resection margin pathologically.

Peer review

This paper is devoted to obtaining knowledge on the outcome of piecemeal mucosal and large sessile polyps. The authors have concluded that EPMR should be carefully applied in malignant polyps.

REFERENCES

- Seitz U, Bohnacker S, Seewald S, Thonke F, Soehendra N. Long-term results of endoscopic removal of large colorectal adenomas. *Endoscopy* 2003; **35**: S41-S44
- Iishi H, Tatsuta M, Iseki K, Narahara H, Uedo N, Sakai N, Ishikawa H, Otani T, Ishiguro S. Endoscopic piecemeal resection with submucosal saline injection of large sessile colorectal polyps. *Gastrointest Endosc* 2000; **51**: 697-700
- Boix J, Lorenzo-Zúñiga V, Moreno de Vega V, Añãños FE, Domènech E, Ojanguren I, Gassull MA. Endoscopic removal of large sessile colorectal adenomas: is it safe and effective? *Dig Dis Sci* 2007; **52**: 840-844
- Salama M, Ormonde D, Quach T, Ee H, Yusoff I. Outcomes of endoscopic resection of large colorectal neoplasms: an Australian experience. *J Gastroenterol Hepatol* 2010; **25**: 84-89
- Fujishiro M. Perspective on the practical indications of endoscopic submucosal dissection of gastrointestinal neoplasms. *World J Gastroenterol* 2008; **14**: 4289-4295

- 6 **Saito Y**, Fukuzawa M, Matsuda T, Fukunaga S, Sakamoto T, Uraoka T, Nakajima T, Ikehara H, Fu KI, Itoi T, Fujii T. Clinical outcome of endoscopic submucosal dissection versus endoscopic mucosal resection of large colorectal tumors as determined by curative resection. *Surg Endosc* 2010; **24**: 343-352
- 7 **Tanaka S**, Oka S, Chayama K. Colorectal endoscopic submucosal dissection: present status and future perspective, including its differentiation from endoscopic mucosal resection. *J Gastroenterol* 2008; **43**: 641-651
- 8 **Walsh RM**, Ackroyd FW, Shellito PC. Endoscopic resection of large sessile colorectal polyps. *Gastrointest Endosc* 1992; **38**: 303-309
- 9 **Robertson DJ**, Greenberg ER, Beach M, Sandler RS, Ahnen D, Haile RW, Burke CA, Snover DC, Bresalier RS, McKeown-Eyssen G, Mandel JS, Bond JH, Van Stolk RU, Summers RW, Rothstein R, Church TR, Cole BF, Byers T, Mott L, Baron JA. Colorectal cancer in patients under close colonoscopic surveillance. *Gastroenterology* 2005; **129**: 34-41
- 10 **Winawer SJ**, Zauber AG, Fletcher RH, Stillman JS, O'Brien MJ, Levin B, Smith RA, Lieberman DA, Burt RW, Levin TR, Bond JH, Brooks D, Byers T, Hyman N, Kirk L, Thorson A, Simmang C, Johnson D, Rex DK. Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society. *CA Cancer J Clin* 2006; **56**: 143-159; quiz 184-185
- 11 **Han KS**, Sohn DK. Biopsy and nonlifting sign in endoscopically resectable colorectal cancers. *Gastrointest Endosc* 2008; **68**: 615
- 12 **Karita M**, Tada M, Okita K, Kodama T. Endoscopic therapy for early colon cancer: the strip biopsy resection technique. *Gastrointest Endosc* 1991; **37**: 128-132
- 13 **Conio M**, Repici A, Demarquay JF, Blanchi S, Dumas R, Filiberti R. EMR of large sessile colorectal polyps. *Gastrointest Endosc* 2004; **60**: 234-241
- 14 **Zlatanovic J**, Wayne JD, Kim PS, Baiocco PJ, Gleim GW. Large sessile colonic adenomas: use of argon plasma coagulator to supplement piecemeal snare polypectomy. *Gastrointest Endosc* 1999; **49**: 731-735
- 15 **Tanaka S**, Oka S, Kaneko I, Hirata M, Mouri R, Kanao H, Yoshida S, Chayama K. Endoscopic submucosal dissection for colorectal neoplasia: possibility of standardization. *Gastrointest Endosc* 2007; **66**: 100-107
- 16 **Brooker JC**, Saunders BP, Shah SG, Williams CB. Endoscopic resection of large sessile colonic polyps by specialist and non-specialist endoscopists. *Br J Surg* 2002; **89**: 1020-1024
- 17 **Regula J**, Wronska E, Polkowski M, Nasierowska-Guttmejer A, Pachlewski J, Rupinski M, Butruk E. Argon plasma coagulation after piecemeal polypectomy of sessile colorectal adenomas: long-term follow-up study. *Endoscopy* 2003; **35**: 212-218
- 18 **Repici A**, Triccerri R. Endoscopic polypectomy: techniques, complications and follow-up. *Tech Coloproctol* 2004; **8** Suppl 2: s283-s290
- 19 **Consolo P**, Luigiano C, Strangio G, Scaffidi MG, Giacobbe G, Di Giuseppe G, Zirilli A, Familiari L. Efficacy, risk factors and complications of endoscopic polypectomy: ten year experience at a single center. *World J Gastroenterol* 2008; **14**: 2364-2369
- 20 **Tolliver KA**, Rex DK. Colonoscopic polypectomy. *Gastroenterol Clin North Am* 2008; **37**: 229-251, ix
- 21 **Doniec JM**, Löhnert MS, Schniewind B, Bokelmann F, Kremer B, Grimm H. Endoscopic removal of large colorectal polyps: prevention of unnecessary surgery? *Dis Colon Rectum* 2003; **46**: 340-348
- 22 **Nivatvongs S**, Snover DC, Fang DT. Piecemeal snare excision of large sessile colon and rectal polyps: is it adequate? *Gastrointest Endosc* 1984; **30**: 18-20
- 23 **Christie JP**. Colonoscopic excision of large sessile polyps. *Am J Gastroenterol* 1977; **67**: 430-438
- 24 **Rex DK**, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, Lehman GA, Mark DG. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997; **112**: 24-28
- 25 **Yamaji Y**, Mitsuhashi T, Ikuma H, Watabe H, Okamoto M, Kawabe T, Wada R, Doi H, Omata M. Incidence and recurrence rates of colorectal adenomas estimated by annually repeated colonoscopies on asymptomatic Japanese. *Gut* 2004; **53**: 568-572

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Gastrointestinal symptoms in patients undergoing peritoneal dialysis: Multivariate analysis of correlated factors

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Abstract

AIM: To investigate gastrointestinal (GI) symptoms in peritoneal dialysis (PD) patients and to explore related factors contributing to GI symptoms.

METHODS: One hundred and twelve patients undergoing PD participated in the study. The gastrointestinal symptom rating scale was used for measuring GI symptoms. Information on age, height, weight, body mass index, disease leading to chronic renal failure, history of corticosteroid therapy, presence of predialytic GI symptoms, daily dosage of pills, and duration, type and daily dialysate volume of PD was obtained by interviewing patients and/or reviewing the medical records. Hemoglobin, albumin and Kt/V data were obtained from follow-up database. We used multiple regression analysis with stepwise backward variable selection to test for factors predicting GRS scores with significance level of selection entry at 0.05 and selection of stay at 0.10.

RESULTS: The prevalence of eating dysfunction, reflux and indigestion in the PD patients was 44.2%, 32.7%,

32.7%, respectively. A history of corticosteroid therapy ($b = 8.93, P < 0.001$) and all pills daily intake ($b = 0.16, P = 0.007$) were positively correlated to GI symptoms, while residual renal Kt/V ($b = -3.47, P = 0.009$) was negatively correlated to GI symptoms. Other factors were proven to be not associated with GI symptoms, with $P > 0.05$.

CONCLUSION: Eating dysfunction, reflux and indigestion were common in PD patients. Daily dosage of pills and corticosteroid history predicted GI symptoms, while residual renal function prevented them.

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Key words: Eating dysfunction; Gastroesophageal reflux; Dyspepsia; Peritoneal dialysis; Residual renal function

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Dong R, Guo ZY. Gastrointestinal symptoms in patients undergoing peritoneal dialysis: Multivariate analysis of correlated factors. *World J Gastroenterol* 2010; 16(22): 2812-2817 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v16/i22/2812.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i22.2812>

INTRODUCTION

Gastrointestinal (GI) symptoms are common in patients with chronic renal failure (CRF)^[1-3], especially in patients having continuous ambulatory peritoneal dialysis (CAPD)^[4]. A variety of GI symptoms in CAPD patients have been reported^[4,5], of which, gastroesophageal reflux symptoms (GERS), dyspepsia and eating dysfunction seem to be the most common ones^[4,5].

Anderson *et al*^[6] reported that 44.7% of PD patients

had frequent gastroesophageal reflux disease (GERD) and that age < 60 years, smoking, and body mass index (BMI) ≥ 27 predicted GERD; in contrast, sex, race, diabetes, PD, non steroidal anti-inflammatory drugs (NSAIDs), calcium channel blockers (CCBs), and coffee and alcohol use did not. Stojakowska *et al*^[7] have proven a negative correlation between GERD symptom score index and normalized protein catabolic rate (nPCR), and a positive correlation between GERD symptom score and the time from onset of CAPD, through observation in 43 patients. It is not clear whether PD *per se* is a risk factor for GERD^[6].

It is the high prevalence of GI symptoms in PD patients that raises questions about contributing factors and other possible factors, but previous studies obtained controversial results with a relatively small sample size. Whether the onset of these GI symptoms is related to the chronic renal failure itself, its treatment, or, alternatively, other factors, is still unknown.

The aim of this study was to investigate GI symptoms in CAPD patients and to explore the possible correlated factors contributing to these symptoms.

MATERIALS AND METHODS

Ethics

Patients gave informed consent and the study was approved by the Ethics Committee of Changhai Hospital, Shanghai, China.

Participants

The patients on active PD were recruited from the PD unit in Changhai Hospital. They consisted of in-patients and out-patients who maintained PD for at least three months. Patients with dementia, severe infectious illness, hepatocholecystopathy, peritonitis in the last three months, unstable blood pressure or glucose levels, and unwillingness to participate in the study were excluded.

Subjective gastrointestinal symptoms: the gastrointestinal symptom rating scale

To evaluate the presence of GI symptoms in PD patients, they were asked to complete the gastrointestinal symptom rating scale (GSRS) measuring GI symptoms in general.

The GSRS, a self-administered questionnaire, includes 15 items and uses a 7-grade Likert scale defined by descriptive anchors such that 1 = none, 2 = minor, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = severe, and 7 = very severe discomfort. The questionnaire was originally constructed as an interview-based rating scale designed to evaluate a wide range of GI symptoms^[8] and was later modified to become a self-administered questionnaire^[9]. The items can be grouped into five dimensions: abdominal pain syndrome (three items), reflux syndrome (two items), indigestion syndrome (four items), diarrhea syndrome (three items), and constipation syndrome (three items). One dimension, eating dysfunction, which was developed in a manner analogous to the GSRS^[10], was also considered relevant for the study and added to the original GSRS. Eating dysfunction dimension includes ques-

tions concerning early satiety, difficulties in eating normal portions, and postprandial pain. The questions concern symptom severity during the previous two weeks. A dimension score was calculated as the mean value of the items belonging to the specific syndrome with a minimum value of 1 and a maximum value of 7.

Patient information

By interviewing patients and/or reviewing the medical records we obtained information on age, height, weight, BMI, disease leading to CRF, history of corticosteroid therapy, presence of predialytic GI symptoms, daily dosage of pills and duration, type and daily dialysate volume of PD. The latest serum hemoglobin (HGB), albumin (ALB), and Kt/V urea, as an index of dialysis adequacy, were obtained from the follow-up database. Kt/V were calculated by Daugirdas Formula^[11].

Statistical analysis

Data were presented as mean (SD) for continuous variables that were approximately normally distributed, as median and interquartile range for skewed continuous variables, and as percentage for categorical variables. We used multiple regression analysis with stepwise variable selection to test for factors that predicted the GSRS scores with significance level of selection entry at 0.05 and selection of stay at 0.10. Results were considered significant when $P < 0.05$. All analyses were performed with SPSS for Windows, version 16.0.

RESULTS

Patient characteristics

In total, one hundred and twelve PD patients were approached to participate in the study and completed the questionnaires. Table 1 presents characteristics of the included PD patients. The sex distribution among PD patients was 61 men and 51 women. The mean age among PD patients was 59.67 (14.18) years and the mean BMI was 23.26 (4.27) kg/m². Patients with diabetes mellitus (DM) made up 24.1% of the study population in total and a majority (87.5%) of PD patients had no GI symptoms before the start of PD. The median duration of PD was 15.00 (8.00-33.00) mo and 53.6% of the patients underwent CAPD.

The gastrointestinal symptom rating scale scores

The prevalence of troublesome GI symptoms (GSRS > 1) was 61.6% for any dimension, 44.2% for eating dysfunction, 32.7% for reflux, 32.7% for indigestion, 18.6% for constipation, 6.2% for abdominal pain, and 5.3% for diarrhea (Figure 1). The mean GSRS scores for eating dysfunction were 1.57 (0.84), for reflux 1.71 (1.15), for indigestion 1.32 (0.56), for constipation 1.23 (0.58), for diarrhea 1.07 (0.35), for abdominal pain 1.04 (0.19).

GSRS scores and patient variables

There was no significant relationship between the GSRS scores and age, BMI, hemoglobin, albumin, presence of

Table 1 Clinical features of the study population *n* (%)

Clinical features	Patient (<i>n</i> = 112)
Age (mean ± SD) (yr)	59.67 ± 14.18
Sex	
Male	61 (54.5)
Female	51 (45.5)
BMI, mean (SD) (kg/m ²)	23.26 (4.27)
DM status	
DM	27 (24.1)
Non-DM	85 (75.9)
Disease leading to chronic renal failure	
Chronic glomerulonephritis	40 (35.7)
Primary hypertension	31 (27.7)
Diabetes mellitus	22 (19.6)
Polycystic kidney disease	7 (6.2)
Gout	3 (2.7)
Primary hypertension combined with gout	3 (2.7)
Obstructive nephropathy	2 (1.8)
Chronic interstitial nephritis	1 (0.9)
Nephrotic syndrome	1 (0.9)
Ischemic renal disease	1 (0.9)
Microscopic Polyarteritis	1 (0.9)
Steroid history	
Yes	8 (7.1)
No	104 (92.9)
Daily dosage of pills, median (interquartile ranges) ¹	12.00 (6.00-21.25)
Predialytic GI symptoms	
Yes	14 (12.5)
No	98 (87.5)
PD Duration, median (interquartile ranges) (mo) ²	15.00 (8.00-33.00)
Type of PD	
CAPD	60 (53.6)
IPD	50 (44.6)
Daily peritoneal dialysate volume (mean ± SD) (L) ³	7.83 ± 1.28
Albumin (mean ± SD) (g/L) ⁴	33.96 ± 4.77
Hemoglobin (mean ± SD) (g/L) ⁵	101.24 ± 20.06
Residual renal Kt/V, median (interquartile ranges) ⁶	0.39 (0.00-0.82)
Peritoneal Kt/V (mean ± SD) ⁶	1.51 ± 0.39
Total Kt/V (mean ± SD) ⁶	2.00 ± 0.51

¹Restricted to the 106 patients (94.64% of the overall sample) for whom complete information on the daily dosage of pills was available; ²Restricted to the 109 patients (97.32% of the overall sample) for whom complete information on peritoneal dialysis duration was available; ³Restricted to the 110 patients (98.21% of the overall sample) for whom complete information on peritoneal dialysate volume was available; ⁴Restricted to the 108 patients (96.43% of the overall sample) for whom complete information on serum albumin was available; ⁵Restricted to the 110 patients (98.21% of the overall sample) for whom complete information on serum hemoglobin was available; ⁶Restricted to the 81 patients (72.32% of the overall sample) for whom complete information on residual renal Kt/V, peritoneal Kt/V, and total Kt/V was available. BMI: Body mass index; DM: Diabetes mellitus; CAPD: Continuous ambulatory peritoneal dialysis; IPD: Intermittent peritoneal dialysis; GI: Gastrointestinal; PD: Peritoneal dialysis.

diabetes, presence of predialytic GI symptoms, peritoneal Kt/V, and duration, type and daily dialysate volume of PD, with $P > 0.05$ (Table 2).

There were three statistically significant predictors for the GSRS scores (Table 3). A history of corticosteroid therapy ($b = 8.93$, $P < 0.001$) was significantly related to the GSRS scores and its coefficient was positive, indicating that if a patient had a history of corticosteroid therapy, he/she would attain higher GSRS scores than a patient who had not. Next, the daily dosage of pills as patients

Table 2 Multiple stepwise regression of the variables not associated with the GSRS scores¹

Variables	<i>t</i>	<i>P</i> -value
Age	1.96	0.54
DM status	1.01	0.31
BMI	-0.50	0.62
Hemoglobin	0.82	0.42
Albumin	0.68	0.50
Predialytic GI symptoms	0.49	0.62
PD type	-0.89	0.38
Duration of PD	-0.58	0.57
Daily dialysate volume	-1.15	0.26
Peritoneal Kt/V	-0.21	0.84
Total Kt/V	-0.24	0.81

¹Restricted to the 81 patients (72.32% of the overall sample) for whom complete information on age, diabetes status, weight, height, serum hemoglobin, albumin levels, presence of predialytic gastrointestinal symptom, peritoneal dialysis type, duration of peritoneal dialysis, daily dialysate volume, history of corticosteroid use, daily dosage of pills, residual renal Kt/V, peritoneal Kt/V, total Kt/V were available. Adjusted for diabetic status (yes = 1, no = 0), presence of predialytic GI symptoms (yes = 1, no = 0), PD type (CAPD = 1, IPD = 0). GSRS: Gastrointestinal symptom rating scale.

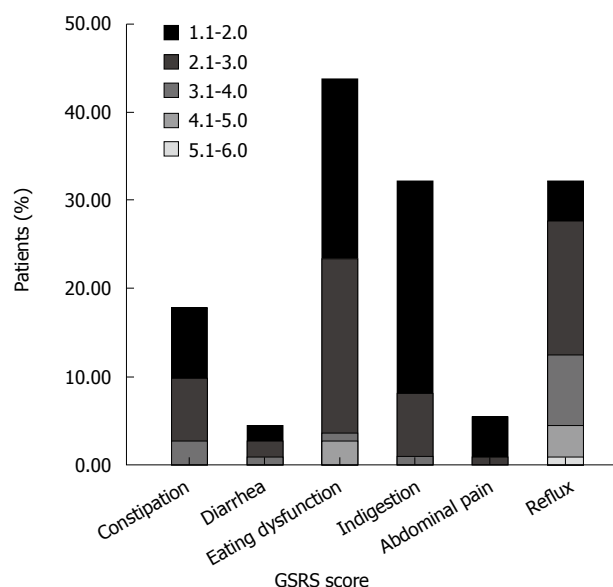


Figure 1 Prevalence and grading of gastrointestinal symptoms in peritoneal dialysis patients according to the gastrointestinal symptom rating scale.

daily intake ($b = 0.16$, $P = 0.007$) was significant and its coefficient was also positive, indicating that the more pills patients took daily, the higher the GSRS scores. Finally, the residual renal Kt/V ($b = -3.47$, $P = 0.009$) was significant and its coefficient was negative which would indicate that higher residual renal Kt/V was related to lower GSRS scores (Table 3).

DISCUSSION

The present study shows that gastrointestinal symptoms are common in PD patients, especially eating dysfunction (44.2%), reflux and indigestion (both 32.7%). A history of

Table 3 Coefficients of multiple stepwise regression model of the variables associated with GSRS scores¹

Variables	Unstandardized coefficients		Standardized coefficients	<i>t</i>	<i>P</i> value
	B	SD	β		
Constant	21.89	1.25	-	17.58	< 0.001
History of corticosteroids	8.93	2.13	0.39	4.20	< 0.001
Daily dosage of pills	0.16	0.06	0.26	2.80	0.007
Residual renal Kt/V	-3.47	1.30	-0.25	-2.68	0.009

¹Restricted to the 81 patients (72.32% of the overall sample) for whom complete information on age, diabetes status, weight, height, serum hemoglobin, albumin levels, presence of predialytic gastrointestinal symptoms, peritoneal dialysis type, duration of peritoneal dialysis, daily dialysate volume, history of corticosteroid use, daily dosage of pills, residual renal Kt/V, peritoneal Kt/V, total Kt/V were available. Adjusted for history of corticosteroid use (yes = 1, no = 0).

corticosteroid therapy ($b = 8.93$, $P < 0.001$) and the dosage of pills patients took daily ($b = 0.16$, $P = 0.007$) were positively related to GSRS scores, whereas residual renal Kt/V ($b = -3.47$, $P = 0.009$) was negatively correlated to GSRS scores. Other suspected factors were not statistically related to GSRS scores, including age, BMI, hemoglobin, albumin, presence of diabetes, presence of predialytic GI symptoms, peritoneal Kt/V, total Kt/V and duration, type and daily dialysate volume of PD.

More than half of the PD patients had various gastrointestinal complaints and patients who had a history of corticosteroid therapy seemed to be susceptible to gastrointestinal symptoms. The more pills patients took daily, the more complaints of GI symptoms. Otherwise, residual renal function seemed to be a protective factor for GI symptoms in PD patients since the higher the residual renal Kt/V, the less GI symptom complaints. In this study, PD specific factors including peritoneal Kt/V, total Kt/V, duration, type and daily dialysate volume of PD, were proven to contribute little to these symptoms.

GI symptoms are common in PD patients with a prevalence ranging from 43% to 58%^[5-7,12]. Our results with a high prevalence of eating dysfunction, indigestion and reflux symptoms are in line with these previous studies. Furthermore, these three symptoms were also shown to have the highest prevalence of all GI symptoms in PD patients in a recent study^[5]. The underlying pathophysiological mechanisms might be (1) Delayed gastric emptying, which is common in CRF patients. Strid *et al.*^[13] found that PD patients had longer gastric emptying time than predialytic state, but other studies found no obvious effect of dialysate on gastric emptying^[14-16]; (2) Increased intra-peritoneal pressure (IPP). Dejardin *et al.*^[17] found the occurrence of GERS was not different for patients with elevated day and night IPP; (3) Decreased lower esophageal sphincter pressure (LESP). Kim *et al.*^[18] demonstrated that CAPD patients with upper GI symptoms had lower LESP at 2000 mL of infused dialysate than patients without. In

contrast, Hylander *et al.*^[19] found no systematic changes in intragastric or LESPs at any time of CAPD; and (4) Other factors. Aguilera *et al.*^[20] discovered GI abnormalities were negatively associated with nutrition. Van *et al.*^[21] have demonstrated a glucose-based dialysate in the abdomen of PD patients with delayed gastric emptying.

As we know, PD patients are in a complicated clinical condition and clinical manifestations are affected by many factors, among which drug taking is an important one. However, drugs have some adverse effects. In general, patients who have a history of corticosteroid therapy are more susceptible to GI symptoms than patients who haven't, which also applies to PD patients. Not unexpectedly, we found corticosteroid taking history alone accounted for 18.9% of GSRS scores, which is probably due to injury of the digestive system caused by corticosteroids. High prevalence of GI symptoms was also found in a transplant population and a rheumatism population^[22,23], who both had a large consumption of corticosteroids.

Besides drug history, the daily dosage of pills patients take also plays an important role in causing GI symptoms. To our knowledge, this is the first study counting pills of patients' daily intake and looking for an association between the amount of pills and GI symptoms. We did find that the more pills taken, the more severe the GI symptoms. In our study, the average amount of pills a PD patient took daily was almost 15, and the maximum amount was 51, which consisted of CCBs, angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), statins, active vitamin D, iron agents, ketoacids, anti-platelet drugs and varieties of Chinese medicine, which were especially common in China. The underlying mechanisms are not fully understood.

Compared to hemodialysis (HD), PD is thought to provide better preservation of residual renal function (RRF), which in many studies partly accounts for reduction of mortality risk, decreasing inflammation factors, declining oxidative and carbonyl stress^[24-27]. In addition, in our study, another highlight of RRF we discovered was that it can prevent PD patients from developing GI symptoms. Apart from residual renal Kt/V, peritoneal Kt/V and total Kt/V were not proven to be correlated to GI symptom scores. These results indicated that the beneficial effect of residual renal clearance and peritoneal clearance were not equivalent with regard to patient outcome, and residual renal function may play a much more important role. Previous study has also shown that for each mL/min per 1.73 m² increase in residual renal glomerular filtration rate (GFR), a 12% reduction in mortality rate was found^[26]. In contrast, no significant effect of peritoneal clearance on patient survival was established^[26]. Regarding this, GI symptoms in PD patients were more associated with the uremia itself, therefore PD specific factors were not involved.

Previous study has concluded that age < 60 and BMI ≥ 27 predicted GERD in the general population, while diabetes and PD did not^[6]. However, we found neither age, BMI nor diabetes with PD predicted GI symptoms in PD patients. This may be due to the limitation in sample

size. Furthermore, albumin, as an index of nutrition, and duration of PD were not related to GI symptoms in our study. However, other studies obtained controversial results. A negative correlation between GERD symptom score and nPCR, and a positive correlation between GERD symptom score and the duration of CAPD, were found through observation in 43 patients^[7]. Moreover, analyses of 99 dialytic patients revealed that gastrointestinal symptom scores were not different in hypoalbuminemic and normoalbuminemic patients^[28]. In addition, gastrointestinal life quality was found not to be correlated with the duration of PD treatment^[29]. Factors which contributed to these conflicting results might be the sample size and the different ways used in evaluating the GI symptoms.

Our study included only CAPD and intermittent peritoneal dialysis (IPD) patients and neither of these groups was independently associated with GI symptom scores. Further evaluation in a larger population with different types of PD is needed for finding out the relation between PD type and GI symptoms.

Volume of dialysis fluid used was also a non-predictor. Another study recruited 61 PD patients and showed a strong linear correlation between IPP and intraperitoneal volume, but failed to find any influence of IPP on the occurrence of GERS except that patients with GERS had a higher BMI^[17]. The increased IPP was still not a rational explanation for the high prevalence of GI symptoms in the PD population.

One limitation of the present study is the relatively small number of samples, which may influence the power of statistical tests. Some factors, such as age, daily dosage and PD type showed a tendency to be correlated with GI symptoms, but correlation did not reach statistical significance because of the fact that 31 of 112 samples were excluded from the multiple regression model for the missing value of Kt/V and we only recruited patients with CAPD and IPD. Another limitation refers to the probable subjective bias in answering the questionnaire. Only one self-administered questionnaire was used in the study. Still another limitation refers to the lack of a control group consisting of patients undergoing HD.

In conclusion, the present study demonstrates a high prevalence of troublesome GI symptoms in PD patients, a positive correlation between history of corticosteroid use, the amount of pills patients take daily, and GI symptoms, and a negative correlation between residual renal function and GI symptoms. GI symptoms are more common in PD than in HD patients as well as compared to the predialytic population^[4] which strongly suggests that PD treatment is a putative cause of GI symptoms. However, the present study failed to prove a relationship between PD specific factors and GI symptoms. Therefore, further evaluation in a larger population of PD patients with a control group is needed.

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COMMENTS

Background

Gastrointestinal (GI) symptoms are more common in patients undergoing peritoneal dialysis (PD) than in patients with chronic renal failure undergoing hemodialysis, though the cause and the correlated factors are largely unknown. Uremia itself and the impaired digestive system caused by PD are the main suspected causes.

Research frontiers

PD patients are proven to have a higher prevalence of GI symptoms. Many studies have focused on the impact of dialysate on the gastrointestinal tract but obtained controversial results. In this study, the authors demonstrate that gastrointestinal complaints in PD patients are more related to a history of corticosteroid therapy, the number of pills taken daily and residual renal function rather than effects of the dialysate.

Innovations and breakthroughs

Recent reports have highlighted the high prevalence of GI symptoms in PD patients and their poor treatment. This is the first study exploring possible correlated factors from almost all details in the life of PD patients. In addition, this is the first study demonstrating that dialysate is not the main cause of GI symptoms in PD patients. Furthermore, this study suggests that GI symptoms in PD patients may be affected by residual renal function and drug history.

Applications

By understanding what factors are related to GI symptoms in PD patients, this study may represent a future strategy for prevention and intervention in the treatment of PD patients with GI symptoms.

Terminology

GSRS, Gastrointestinal Symptom Rating Scale, a self-administered questionnaire, includes 15 items and uses a 7-grade Likert scale defined by descriptive anchors such that 1 = none, 2 = minor, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = severe, and 7 = very severe discomfort. The items can be grouped into five dimensions: abdominal pain syndrome (three items), reflux syndrome (two items), indigestion syndrome (four items), diarrhea syndrome (three items), and constipation syndrome (three items). One dimension, eating dysfunction, which was developed in a manner analogous to the GSRS, was also considered relevant for the study and added to the original GSRS.

Peer review

This is a straightforward, well done study with straightforward conclusions. I hope the authors will take the next step and perform a more rigorous study with 2 control arms (chronic renal failure and HD) in a prospective fashion.

REFERENCES

- 1 **Cano AE**, Neil AK, Kang JY, Barnabas A, Eastwood JB, Nelson SR, Hartley I, Maxwell D. Gastrointestinal symptoms in patients with end-stage renal disease undergoing treatment by hemodialysis or peritoneal dialysis. *Am J Gastroenterol* 2007; **102**: 1990-1997
- 2 **Hammer J**, Oesterreicher C, Hammer K, Koch U, Traindl O, Kovarik J. Chronic gastrointestinal symptoms in hemodialysis patients. *Wien Klin Wochenschr* 1998; **110**: 287-291
- 3 **Fallone CA**, Mayrand S. Gastroesophageal reflux and hyperacidity in chronic renal failure. *Perit Dial Int* 2001; **21** Suppl 3: S295-S299
- 4 **Strid H**, Simrén M, Johansson AC, Svedlund J, Samuelsson O, Björnsson ES. The prevalence of gastrointestinal symptoms in patients with chronic renal failure is increased and associated with impaired psychological general well-being. *Nephrol Dial Transplant* 2002; **17**: 1434-1439
- 5 **Strid H**, Fjell A, Simrén M, Björnsson ES. Impact of dialysis on gastroesophageal reflux, dyspepsia, and proton pump inhibitor treatment in patients with chronic renal failure.

- Eur J Gastroenterol Hepatol* 2009; **21**: 137-142
- 6 **Anderson JE**, Yim KB, Crowell MD. Prevalence of gastroesophageal reflux disease in peritoneal dialysis and hemodialysis patients. *Adv Perit Dial* 1999; **15**: 75-78
 - 7 **Stojakowska M**, Blaut U, Smoleński O, Thor PJ. [Gastroesophageal reflux disease and its influence on nutritional status in patients treated with peritoneal dialysis] *Folia Med Cracov* 2005; **46**: 59-66
 - 8 **Svedlund J**, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988; **33**: 129-134
 - 9 **Dimenäs E**, Glise H, Hallerbäck B, Hernqvist H, Svedlund J, Wiklund I. Quality of life in patients with upper gastrointestinal symptoms. An improved evaluation of treatment regimens? *Scand J Gastroenterol* 1993; **28**: 681-687
 - 10 **Svedlund J**, Sullivan M, Liedman B, Lundell L. Long term consequences of gastrectomy for patient's quality of life: the impact of reconstructive techniques. *Am J Gastroenterol* 1999; **94**: 438-445
 - 11 **Daugirdas JT**. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol* 1993; **4**: 1205-1213
 - 12 **Lee SW**, Song JH, Kim GA, Yang HJ, Lee KJ, Kim MJ. Effect of dialysis modalities on gastric myoelectrical activity in end-stage renal disease patients. *Am J Kidney Dis* 2000; **36**: 566-573
 - 13 **Strid H**, Simrén M, Stotzer PO, Abrahamsson H, Björnsson ES. Delay in gastric emptying in patients with chronic renal failure. *Scand J Gastroenterol* 2004; **39**: 516-520
 - 14 **Hubalewska A**, Stompór T, Placzekiewicz E, Staszczak A, Huszno B, Sulowicz W, Szybiński Z. Evaluation of gastric emptying in patients with chronic renal failure on continuous ambulatory peritoneal dialysis using ^{99m}Tc-solid meal. *Nucl Med Rev Cent East Eur* 2004; **7**: 27-30
 - 15 **Fernström A**, Hylander B, Grybäck P, Jacobsson H, Hellström PM. Gastric emptying and electrogastrography in patients on CAPD. *Perit Dial Int* 1999; **19**: 429-437
 - 16 **Guz G**, Bali M, Poyraz NY, Bagdatoglu O, Yeğin ZA, Doğan I, Atasever T, Sert S, Sindel S. Gastric emptying in patients on renal replacement therapy. *Ren Fail* 2004; **26**: 619-624
 - 17 **Dejardin A**, Robert A, Goffin E. Intraperitoneal pressure in PD patients: relationship to intraperitoneal volume, body size and PD-related complications. *Nephrol Dial Transplant* 2007; **22**: 1437-1444
 - 18 **Kim MJ**, Kwon KH, Lee SW. Gastroesophageal reflux disease in CAPD patients. *Adv Perit Dial* 1998; **14**: 98-101
 - 19 **Hylander BI**, Dalton CB, Castell DO, Burkart J, Rössner S. Effect of intraperitoneal fluid volume changes on esophageal pressures: studies in patients on continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1991; **17**: 307-310
 - 20 **Aguilera A**, Bajo MA, Espinoza M, Oliveira A, Paiva AM, Codoceo R, Garca P, Sánchez S, Celadilla O, Castro MJ, Selgas R. Gastrointestinal and pancreatic function in peritoneal dialysis patients: their relationship with malnutrition and peritoneal membrane abnormalities. *Am J Kidney Dis* 2003; **42**: 787-796
 - 21 **Van V**, Schoonjans RS, Struijk DG, Verbanck JJ, Vanholder RC, Van B, Lefebvre RA, De V, Lameire NH. Influence of dialysate on gastric emptying time in peritoneal dialysis patients. *Perit Dial Int* 2002; **22**: 32-38
 - 22 **Herrero JI**, Benlloch S, Bernardos A, Bilbao I, Castells L, Castroagudin JF, González L, Irastorza I, Navasa M, Otero A, Pons JA, Rimola A, Suárez F, Casanovas T, Otero E, Rodríguez M, Serrano T, Otero S, López I, Miras M, Prieto M. Gastrointestinal complications in liver transplant recipients: MITOS study. *Transplant Proc* 2007; **39**: 2311-2313
 - 23 **Karateev AE**, Nasonova VA, Murav'ev IuV. [The assessment of the effect of glucocorticosteroid and nonsteroidal anti-inflammatory preparations on the development of an erosive-ulcerative lesion of the gastrointestinal tract in patients with rheumatic diseases] *Ter Arkh* 1999; **71**: 26-30
 - 24 **Sanabria M**, Muñoz J, Trillos C, Hernández G, Latorre C, Díaz CS, Murad S, Rodríguez K, Rivera A, Amador A, Ardila F, Caicedo A, Camargo D, Díaz A, González J, Leguizamón H, Lopera P, Marín L, Nieto I, Vargas E. Dialysis outcomes in Colombia (DOC) study: a comparison of patient survival on peritoneal dialysis vs hemodialysis in Colombia. *Kidney Int Suppl* 2008; **S165-S172**
 - 25 **Furuya R**, Kumagai H, Odamaki M, Takahashi M, Miyaki A, Hishida A. Impact of residual renal function on plasma levels of advanced oxidation protein products and pentosidine in peritoneal dialysis patients. *Nephron Clin Pract* 2009; **112**: c255-c261
 - 26 **Termorshuizen F**, Korevaar JC, Dekker FW, van Manen JG, Boeschoten EW, Krediet RT. The relative importance of residual renal function compared with peritoneal clearance for patient survival and quality of life: an analysis of the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD)-2. *Am J Kidney Dis* 2003; **41**: 1293-1302
 - 27 **Cueto-Manzano AM**, Rojas-Campos E, Martínez-Ramírez HR, Valera-González I, Medina M, Monteón F, Ruiz N, Becerra M, Palomeque MA, Cortés-Sanabria L. Can the inflammation markers of patients with high peritoneal permeability on continuous ambulatory peritoneal dialysis be reduced on nocturnal intermittent peritoneal dialysis? *Perit Dial Int* 2006; **26**: 341-348
 - 28 **Silang R**, Regalado M, Cheng TH, Wesson DE. Prokinetic agents increase plasma albumin in hypoalbuminemic chronic dialysis patients with delayed gastric emptying. *Am J Kidney Dis* 2001; **37**: 287-293
 - 29 **Heine GH**, Kastner CY, Jahnke T, Köhler H, Kuhlmann MK. Does a history of peritoneal dialysis result in an impaired gastrointestinal life quality? *Hemodial Int* 2007; **11**: 461-467

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Laparoscopic vs open left hepatectomy for hepatolithiasis

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Abstract

AIM: To explore the feasibility and therapeutic effect of total laparoscopic left hepatectomy (LLH) for hepatolithiasis.

METHODS: From June 2006 to October 2009, 61 consecutive patients with hepatolithiasis who met the inclusion criteria for LLH were treated in our institute. Of the 61 patients with hepatolithiasis, 28 underwent LLH (LLH group) and 33 underwent open left hepatectomy (OLH group). Clinical data including operation time, intraoperative blood loss, postoperative complication rate, postoperative hospital stay time, stone clearance and recurrence rate were retrospectively analyzed and compared between the two groups.

RESULTS: LLH was successfully performed in 28 patients. The operation time of LLH group was longer than that of OLH group (158 ± 43 min vs 132 ± 39 min, $P < 0.05$) and the hospital stay time of LLH group was shorter than that of OLH group (6.8 ± 2.8 d vs 10.2 ± 3.4 d, $P < 0.01$). No difference was found in intraoperative blood loss (180 ± 56 mL vs 184 ± 50 mL), postoperative complication rate (14.2% vs 15.2%), and stone residual rate (intermediate rate 17.9% vs 12.1% and final rate 0% vs 0%) between the two

groups. No perioperative death occurred in either group. Fifty-seven patients (93.4%) were followed up for 2-40 mo (mean 17 mo), including 27 in LLH group and 30 in OLH group. Stone recurrence occurred in 1 patient of each group.

CONCLUSION: LLH for hepatolithiasis is feasible and safe in selected patients with an equal therapeutic effect to that of traditional open hepatectomy.

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Key words: Hepatolithiasis; Laparoscopy; Hepatectomy; Complication; Therapeutic effect

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INTRODUCTION

Hepatolithiasis refers to the stone in branching bile ducts above the confluence of left and right hepatic ducts. It may occur alone or with extrahepatic bile duct stones and is a prevalent disease in Southeast Asia and its incidence is also higher in Chinese coast areas, southwest region, Hong Kong and Taiwan. Hepatectomy is a definite and effective approach for hepatolithiasis^[1,2]. With the refinement of laparoscopic instruments and accumulated experience in both laparoscopic surgery and hepatic surgery, laparoscopic hepatectomy has been used in treatment of hepatic benign and malignant tumors and donor hepatectomy of live donor liver transplantation^[3,4]. However, few studies are available on laparoscopic hepatectomy for hepatolithiasis^[5-8]. We successfully used total laparoscopic left hepatectomy (LLH) to treat hepatolithiasis in

8 patients between November 2003 and May 2006, and have preliminarily accumulated surgical experience with it and considered this operation safe and feasible^[9]. To further explore the therapeutic effect of total LLH on hepatolithiasis, clinical data about 61 consecutive patients with hepatolithiasis who underwent LLH or open left hepatectomy (OLH) were retrospectively analyzed and compared.

MATERIALS AND METHODS

Inclusion criteria and patients

The inclusion criteria for LLH for hepatolithiasis include (1) multiple stones in the left or left lateral intrahepatic ducts with fibrosis and atrophy of hepatic lobes or hepatic segments; (2) possibly combined with extrahepatic bile duct stones or a few stones in the right intrahepatic ducts, but with no extrahepatic bile duct stricture or stone incarceration in the lower part of common bile duct; and (3) liver function of Child A to B classification, with no portal hypertension, coagulation disorder, structural disease in the heart, lungs, liver and kidneys, intrahepatic biliary cancer, and abdominal surgical histories.

From June 2006 to October 2009, 61 consecutive patients with hepatolithiasis who met the inclusion criteria for LLH were treated in our institute. Of the 61 patients with hepatolithiasis, 28 underwent LLH (LLH group) and 33 underwent OLH (OLH group). Before operation, all patients had a complete medical evaluation, including liver function, renal function, electrocardiogram and chest X-ray. Preoperative ultrasonography, CT and MRCP were performed to identify the distribution of stones and changes in the bile duct tree. Of the 28 patients in LLH group, 10 were men and 18 women, with a mean age of 47 years (range 25-63 years). Twenty-one patients had left hepatolithiasis and 7 left and right hepatolithiasis. Twelve patients were accompanied with cholecystolithiasis, 13 with choledocholith, and 6 with mild jaundice. Liver function was classified as Child A and B in 22 and 6 patients, respectively. Two patients had a history of biliary surgery. Of the 33 patients in OLH group, 12 were men and 21 women with a mean age of 49 years (range 31-68 years). Twenty-three patients had left hepatolithiasis and 10 left and right hepatolithiasis. Twelve patients were accompanied with cholecystolithiasis, 15 with choledocholithiasis, and 9 with mild jaundice. Liver function was classified as Child A and B in 25 and 8 patients, respectively. Three patients had a history of biliary surgery. No significant difference was found in age, sex, stone distribution, liver function and surgical history between the two groups.

Operative techniques

Patients in LLH group, placed in supine position, underwent general anesthesia. The chief surgeon stood on the left of the patient and the first assistant on the right. Another surgeon who supported the mirror stood on the left of the chief surgeon. Two monitors were placed above each side of the patient's head. A 10-mm cut was

made below the umbilicus and a CO₂ pneumoperitoneum was established at a pressure of 14 mmHg. A 30° angled laparoscope was introduced. Under direct vision, two 12-mm trocars were respectively inserted below the xiphoid bone and the costal margin of the left mid-clavicular line, and a 5-mm trocar was also inserted below the costal margin of the right mid-clavicular line.

Left lateral segmentectomy: Laparoscopy was performed with the round and falciform ligaments transected using an ultrasound knife (Johnson and Johnson, USA). By meticulous dissection, the artery and vein of left lateral segment were visualized, clamped with absorbable clips and divided. Interrupted bile ducts were not clamped temporarily. Left triangular and coronary ligaments were divided with the trunk or branch of the left hepatic vein carefully dissected properly away from the second hepatic portal. If the confluence point of left hepatic veins and inferior vena cava was very close to the posterior border of the left liver, the left hepatic veins were clamped extremely near the posterior border of the left liver, but not divided until complete removal of Couinaud. If the confluence point of left hepatic veins and inferior vena cava was away from the liver parenchyma, the left hepatic vein was dissected, clamped with absorbable clips and divided (Figure 1). Liver parenchyma was transected from left of the round ligament to liver inferior border of sagittal portion, vessels and bile ducts in the transection plane were bluntly dissected, clamped and divided. Dilated intrahepatic bile ducts in the transection plane were opened. According to the size, number and position of residual stones, stone forceps were introduced below the xiphoid bone to directly remove stones, or a fiber choledochoscope (Olympus, Japan) was used to remove stones from the transection plane. If the stones were near the distal common bile duct or bigger, or combined with right hepatolithiasis, common bile duct exploration was performed to remove the stones. Cholecystectomy was done routinely. Continuous or interrupted suture was performed for the transection plane with 3-0 Vicryl. Water was injected *via* a T tube to determine whether the suture was tight. The transection plane was coagulated with fibrin glue to seal capillary vessels or covered with absorbable hemostatic gauze. The integral specimen was packed into a plastic bag and removed *via* an extended trocar hole. A drainage tube was left in vicinity of the transection plane through the costal margin of the left mid-clavicular line. Whether a drainage tube is left near the first hepatic portal depends on the intraoperative conditions.

Left hemihepatectomy: In the first hepatic portal, the left hepatic artery and the left branch of portal vein were dissected. The proximal end of left hepatic artery was clamped with two absorbable clips, the left hepatic artery was clamped with a mental soft clip 2-3 mm away from the absorbable clips, and the left hepatic artery was divided between mental and absorbable clips. The left branch of portal vein was treated with the same method

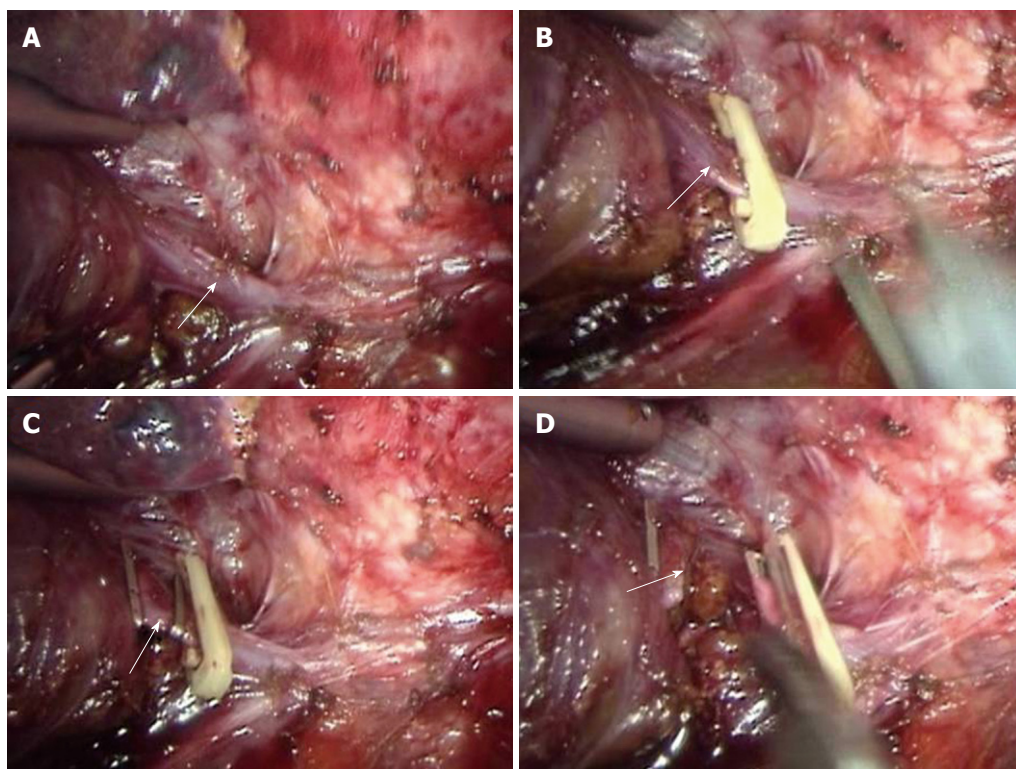


Figure 1 Laparoscopy showing dissected LHV (A, arrow), clamped LHV (B and C, arrow), and transected LHV (D, arrow). LHV: Left hepatic vein.

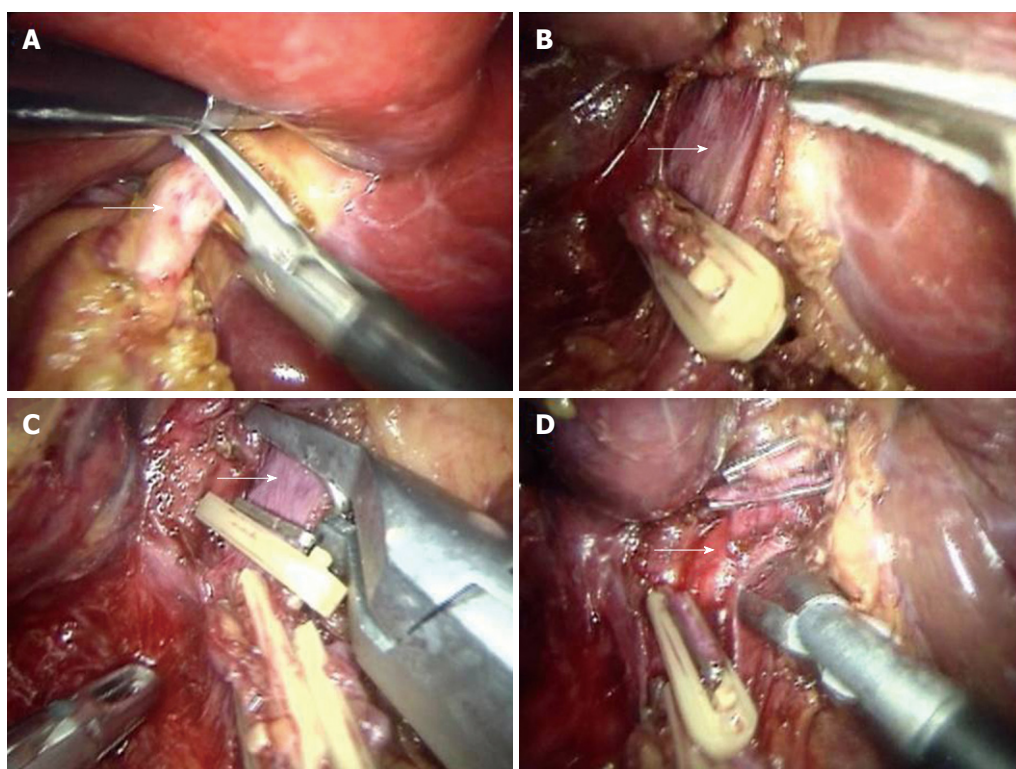


Figure 2 Laparoscopy showing dissected LBHA (A, arrow), exposed LBHV (B, arrow), dissected and clamped LBHV (C, arrow), and dissected left hepatic duct (D, arrow). LBHA: Left branch of hepatic artery; LBHV: Left branch of hepatic vein.

(Figure 2). Other surgical procedures were the same as those in left lateral segmentectomy.

In OLH group, an oblique incision was made along

the right costal margin or along the right rectus abdominis with the patients in supine position under general anesthesia. Cholecystectomy was done routinely. Common

Table 1 Surgical procedure for hepatolithiasis in 61 patients

Variables	LLH (n = 28)	OLH (n = 33)
Left hemihepatectomy	3	5
Left lateral segmentectomy	25	28
Combined with cholecystectomy	26	30
Combined with CBD exploration	22	31
T tube drainage	16	27
Primary suture of CBD	6	4
Intraoperative choledochoscope	28	28

LLH: Laparoscopic left hepatectomy; OLH: Open left hepatectomy; CBD: Common bile duct.

bile duct exploration was performed to remove stones. Couinaud was removed with a T drainage tube left.

Residual stones were completely removed by fiber choledochoscopy *via* the T tube sinus tract after operation in both groups.

Clinical data including operation time, intraoperative blood loss, postoperative complication rate, postoperative hospital stay time, and stone clearance and recurrence rate were compared between the two groups. Follow-up data were obtained from hospital charts or by telephone.

Statistical analysis

Categorical parameters of each group were compared by χ^2 test, and continuous parameters were compared using independent-sample *t* test. All analyses were performed using SPSS 12.0. *P* < 0.05 was considered statistically significant.

RESULTS

Laparoscopic left hepatectomy was successfully performed in 28 patients. Of the 28 patients, 3 underwent laparoscopic left hemihepatectomy, cholecystectomy, common bile duct exploration and T tube drainage, 18 underwent left lateral segmentectomy, cholecystectomy and common bile duct exploration. Of the 18 patients, 6 underwent primary suture of common bile duct, 12 underwent T tube drainage, 5 underwent left lateral segmentectomy with cholecystectomy, 1 underwent left lateral segmentectomy, common bile duct exploration and T tube drainage, and 1 underwent left lateral segmentectomy. All the 28 patients in LLH group underwent intraoperative cholangioscopic bile duct exploration or stone removal. Of the 33 patients in OLH group, 5 underwent open left hemihepatectomy, cholecystectomy, common bile duct exploration and T tube drainage, 23 underwent left lateral segmentectomy, cholecystectomy and common bile duct exploration. Of the 23 patients, 4 underwent primary suture of common bile duct, 19 underwent T tube drainage. Twenty-eight patients in OLH group underwent cholangioscopic bile duct exploration with stones removed (Table 1). Intraoperative findings and postoperative pathology displayed hepatolithiasis, cholangiectasis of Couinaud, chronic inflammation and fibration in all patients.

Table 2 Comparison between LLH and OLH for hepatolithiasis n (%)

Variables	LLH (n = 28)	OLH (n = 33)
Operating time (min)	158 ± 43	132 ± 39
Intraoperative blood loss (mL)	180 ± 56	184 ± 50
Intraoperative blood transfusion	0 (0)	1 (3.0)
Postoperative complications	4 (14.2)	5 (15.2)
Postoperative hospital stay (d)	6.8 ± 2.8	10.2 ± 3.4
Intermediate residual stone	5 (17.9)	4 (12.1)
Final residual stone	0 (0)	0 (0)
Stone recurrence	1 (3.6)	1 (3.0)
Perioperative mortality	0 (0)	0 (0)

The mean operation time was longer in LLH group than in OLH group (158 ± 43 min *vs* 132 ± 39 min, *P* < 0.05). The intraoperative blood loss in two groups was similar (180 ± 56 mL *vs* 184 ± 50 mL). One patient in OLH group was transfused with 2 units of concentrated red blood cells, no patient in LLH group received blood transfusion. Bile leakage occurred in 2 patients of LLH group, and healed automatically 3 and 5 d after operation. Pleural effusion, observed in 2 patients, disappeared after thoracentesis. In OLH group, seroperitoneum occurred in 1 patient, hepatic abscess in 1 patient and infection of incision in 3 patients. No significant difference was found in complication rate (14.2% *vs* 15.2%) and intermediate stone residual rate (17.9% *vs* 12.1%) between the two groups. The mean postoperative hospital stay time was shorter in LLH group than in OLH group (6.8 ± 2.8 d *vs* 10.2 ± 3.4 d, *P* < 0.01), and the serum transaminase was transiently increased and jaundice disappeared at discharge with no death occurred in both groups (Table 2).

Of the 16 patients in LLH group discharged with their T tubes, 11 underwent T extubation 28-35 d after operation, 5 underwent it 42-60 d after operation when residual stones were completely removed by choledochoscopy. Of the 27 patients in OLH group discharged with their T tubes, 23 underwent T extubation 28-40 d after operation, 4 underwent it 50-60 d after operation when residual stones were completely removed by choledochoscopy. Fifty-seven patients (93.4%) including 27 in LLH group and 30 in OLH group were followed up for 2-40 mo (mean 17 mo). Stone recurrence was found in 1 patient of LLH group and in 1 patient of OLH group. Intrahepatic biliary cancer occurred in 1 patient of OLH group 29 mo after operation, and was surgically removed in another hospital.

DISCUSSION

In 1991, Reich *et al*^[10] used laparoscope to remove a benign tumor located at the edge of liver, raising the curtain on laparoscopic hepatectomy. In 1993, Wayand *et al*^[11] performed laparoscopic segmentectomy (segments VI) for metastatic carcinoma. In 1996, Azagra *et al*^[12] first performed laparoscopic left lateral lobectomy (segments II and III) for hepatic adenoma in 1 patient. With the refinement of laparoscopic instruments and accumulated

experience in laparoscopic hepatectomy, indications for laparoscopic hepatectomy have gradually expanded from small, peripheral and benign diseases to large, central and malignant diseases^[13,14]. Lesions located in Couinaud including II, III, IVa, V and VI segments are the best indications for laparoscopic hepatectomy, and regular left lateral lobectomy is expected to become its gold standard^[18,15]. Most intrahepatic bile duct stones, especially left intrahepatic bile duct stones, manifested as a regional distribution, are usually combined with liver fibrosis and atrophy, which is also a good indication for laparoscopic hepatectomy^[8,16]. The inclusion criteria for LLH for hepatolithiasis in this study were (1) multiple stones in the left or left lateral intrahepatic ducts with fibrosis and atrophy of hepatic lobes or segments, possibly combined with extrahepatic bile duct stones or a few stones in the right intrahepatic ducts; and (2) except for lots of stones in the right intrahepatic ducts, severe acute cholangitis, hepatic abscess, stone incarceration in the lower part of common bile duct and bile duct neoplasms. We believe that only LLH rather than common bile duct exploration is required for simple left hepatolithiasis. Chen *et al*^[16] has described that endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy (EST) for left hepatolithiasis with extrahepatic bile duct stones, can completely remove common bile duct stones followed by laparoscopic hepatectomy. However, ERCP easily causes acute pancreatitis, thus making cholelithiasis heavier and EST destroys the integrity of duodenal papilla and sphincter Oddi, easily leading to biliary tract infection and difficulty to remove the stones with a diameter > 1.5 cm. Preoperative hospital stay time is significantly increased and secondary operation is required. In this study, common bile duct exploration was performed to remove stones followed by T-tube drainage or primary suture of common bile duct, which is suitable for different sizes of common bile duct stones, but is time-consuming and may lead to bile leakage. External drainage of bile may lead to electrolytical and digestive unbalance, which is not beneficial to postoperative recovery. Choledochoscopy can remove the stones through the stump of the left hepatic duct without cutting open the common bile duct, thus avoiding the above disadvantages. However, it is only suitable for a small number of patients. In this study, a few stones in the right hepatic bile duct were removed by intraoperative choledochoscopy through the common bile duct or by postoperative choledochoscopy *via* the T-tube sinus tract.

The liver possesses dual blood supply from hepatic artery and portal vein. Blood supply is abundant and bleeding easily occurs. During laparoscopic hepatectomy, since hepatic portal occlusion, hand pressure and saturation cannot be used for hemostasis, it is not easy to control intraoperative bleeding^[17]. Therefore, how to prevent, control and reduce intraoperative bleeding is a key to surgical success and postoperative recovery^[18]. In our study, full preoperative preparation was done for each patient. The patients with normal liver function and coagulation test were selected. Preoperative ultra-

sonography, CT or MRCP was performed to determine the distribution, location and size of stones and liver morphology. The stones were located in Couinauds II and III. The operators had rich experience in traditional hepatectomy and skilled laparoscopic technique. Excellent surgical instruments such as ultrasonic bleeding knife, vascular closure device and Ligasure vessel sealing system were provided. Each segment of the left liver was dissected in the first hepatic portal, and blocked with clips. The portal vascular structure of left liver was dissected outside Glisson sheath, and the branches of portal vein and hepatic artery were clamped with absorbable clips. The left hepatic vein trunk was dissected, clamped with clips and divided after transection of liver parenchyma. The transaction of liver parenchyma resulted in less tissue damage, less bleeding and good exposure of hepatic duct system. It was reported that Peng's multifunctional operative dissector can reduce bleeding and operation time^[19]. The ideal level of regular left lateral lobectomy is at the sagittal plane 1 cm away from the left of falciform ligament. The main vessels at this plane include superior and inferior segment branch of Couinaud of portal vein, trunk of left hepatic vein, left and upper branches of left hepatic vein, which are distributed in 2/3 of the superior part. Choledochoscope can enter the common bile duct or the right hepatic duct through the broken end of left hepatic duct or incisional anterior wall of the common bile duct to remove stones^[6,7]. If no biliary tract stricture is identified, small stones may not be completely removed, and then a T tube is left. The residual small stones can be removed by choledochoscopy 6 wk after operation.

Hepatolithiasis is common in China, accounting for about 10% of calculosis, or 40%-50% in some areas. Stone clearance rate for open hepatectomy can reach 81.7% with a reliable long-term therapeutic effect^[20]. Laparoscopic hepatectomy can achieve the same stone clearance rate in the left liver^[6]. Zhang *et al*^[8] reported that the therapeutic effect of laparoscopic left lateral lobectomy for left hepatolithiasis and choledocholith is better than that of traditional open stone removal. Machado *et al*^[21] reported one patient who underwent laparoscopic right hepatectomy for hepatolithiasis, showing that the learning curve can influence the feasibility and repeatability of laparoscopic hepatectomy^[22]. In this study, the therapeutic effects of laparoscopic and open hepatectomy on intrahepatic and extrahepatic bile duct stones were compared as previously described^[9]. Although randomized controlled trial data are lack, the blood loss, blood transfusion rate, complication rate and mortality in laparoscopic hepatectomy were equal to those in open hepatectomy. It was reported that the evacuating time, fasting time, use of analgesics, hospital stay time, time to return to work and degree of satisfaction are better in laparoscopic hepatectomy than in open hepatectomy, while operation time is longer and cost is higher in laparoscopic hepatectomy than in open hepatectomy^[5,23,24]. In our study, the mean operation time of LLH group was longer than that of OLH group, the mean blood

loss and postoperative complication rate were similar in the two groups, the mean hospital stay time was significantly shorter in LLH group (6.8 ± 2.8 d) than in OLH group (10.2 ± 3.4 d) possibly due to the mini-invasive advantages, such as small incision, postoperative light inflammatory response, less interference with immune function. In addition, intermediate and final stone clearance rate and long term stone recurrence rate for the two groups were also similar with no CO₂ gas embolism occurred in patients of LLH group. Air embolism is a potential risk factor for laparoscopic hepatectomy.

In summary, LLH for hepatolithiasis is safe and feasible in selected patients if it is performed by surgeons with experience in laparoscopic and hepatic surgery. Its therapeutic effect is equal to that of traditional open hepatectomy. Laparoscopic hepatectomy has the advantages such as mini-invasion, less postoperative pain and rapid recovery.

COMMENTS

Background

Hepatolithiasis is a prevalent disease in Southeast Asia with a high incidence in coast areas, southwest region, Hong Kong and Taiwan of China. Hepatectomy is a definite and effective procedure for hepatolithiasis. With the refinement of laparoscopic instruments and accumulated experience in laparoscopic and hepatic surgery, laparoscopic hepatectomy has been used in treatment of hepatic benign and malignant tumors and donor hepatectomy for live donor liver transplantation. However, few studies are available on laparoscopic hepatectomy for hepatolithiasis.

Research frontiers

The feasibility and therapeutic effect of total laparoscopic left hepatectomy (LLH) on hepatolithiasis are a hotspot.

Innovations and breakthroughs

This study explored the therapeutic effect of laparoscopic vs open left hepatectomy on hepatolithiasis and concluded that LLH for hepatolithiasis is feasible and safe in selected patients if it is performed by experienced surgeons. Its therapeutic effect is equal to that of traditional open hepatectomy.

Applications

Laparoscopic hepatectomy possesses the advantages such as mini-invasion, less postoperative pain and rapid recovery and worth of spreading.

Terminology

Hepatolithiasis: It refers to stones in the branching bile ducts above the confluence of left and right hepatic ducts, and may occur alone or in combination with extrahepatic bile duct stones.

Peer review

It is interesting and well-written. The results show that laparoscopic hepatectomy is worth of spreading due to its advantages such as mini-invasion, less postoperative pain and rapid recovery.

REFERENCES

- 1 Uenishi T, Hamba H, Takemura S, Oba K, Ogawa M, Yamamoto T, Tanaka S, Kubo S. Outcomes of hepatic resection for hepatolithiasis. *Am J Surg* 2009; **198**: 199-202
- 2 Lee TY, Chen YL, Chang HC, Chan CP, Kuo SJ. Outcomes of hepatectomy for hepatolithiasis. *World J Surg* 2007; **31**: 479-482
- 3 Zhang L, Chen YJ, Shang CZ, Zhang HW, Huang ZJ. Total laparoscopic liver resection in 78 patients. *World J Gastroenterol* 2009; **15**: 5727-5731
- 4 Nguyen KT, Gamblin TC, Geller DA. World review of laparoscopic liver resection-2,804 patients. *Ann Surg* 2009; **250**: 831-841
- 5 Lai EC, Ngai TC, Yang GP, Li MK. Laparoscopic approach of surgical treatment for primary hepatolithiasis: a cohort study. *Am J Surg* 2010; **199**: 716-721
- 6 Cai X, Wang Y, Yu H, Liang X, Peng S. Laparoscopic hepatectomy for hepatolithiasis: a feasibility and safety study in 29 patients. *Surg Endosc* 2007; **21**: 1074-1078
- 7 Di Giuro G, Balzarotti R, Lainas P, Franco D, Dagher I. Laparoscopic left hepatectomy with intraoperative biliary exploration for hepatolithiasis. *J Gastrointest Surg* 2009; **13**: 1147-1148
- 8 Zhang K, Zhang SG, Jiang Y, Gao PF, Xie HY, Xie ZH. Laparoscopic hepatic left lateral lobectomy combined with fiber choledochoscopic exploration of the common bile duct and traditional open operation. *World J Gastroenterol* 2008; **14**: 1133-1136
- 9 Jiang FZ, Tu JF, You HY, Han Y, Zheng XF, Zhang QY. [Laparoscopic left hepatectomy for hepatolithiasis]. *Gandanyi Waike Zazhi* 2007; **19**: 103-104
- 10 Reich H, McGlynn F, DeCaprio J, Budin R. Laparoscopic excision of benign liver lesions. *Obstet Gynecol* 1991; **78**: 956-958
- 11 Wayand W, Woisetschlager R. [Laparoscopic resection of liver metastasis] *Chirurg* 1993; **64**: 195-197
- 12 Azagra JS, Goergen M, Gilbert E, Jacobs D. Laparoscopic anatomical (hepatic) left lateral segmentectomy-technical aspects. *Surg Endosc* 1996; **10**: 758-761
- 13 Laurence JM, Lam VW, Langcake ME, Hollands MJ, Crawford MD, Pleass HC. Laparoscopic hepatectomy, a systematic review. *ANZ J Surg* 2007; **77**: 948-953
- 14 Tranchart H, Di Giuro G, Lainas P, Roudie J, Agostini H, Franco D, Dagher I. Laparoscopic resection for hepatocellular carcinoma: a matched-pair comparative study. *Surg Endosc* 2010; **24**: 1170-1176
- 15 McPhail MJ, Scibelli T, Abdelaziz M, Titi A, Pearce NW, Abu Hilal M. Laparoscopic versus open left lateral hepatectomy. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 345-351
- 16 Chen P, Bie P, Liu J, Dong J. Laparoscopic left hemihepatectomy for hepatolithiasis. *Surg Endosc* 2004; **18**: 717-718
- 17 Vibert E, Perniceni T, Levard H, Denet C, Shahri NK, Gayet B. Laparoscopic liver resection. *Br J Surg* 2006; **93**: 67-72
- 18 Di Giuro G, Lainas P, Franco D, Dagher I. Laparoscopic left hepatectomy with prior vascular control. *Surg Endosc* 2010; **24**: 697-699
- 19 Peng SY, Li JT, Mou YP, Liu YB, Wu YL, Fang HQ, Cao LP, Chen L, Cai XJ, Peng CH. Different approaches to caudate lobectomy with "curettage and aspiration" technique using a special instrument PMOD: a report of 76 cases. *World J Gastroenterol* 2003; **9**: 2169-2173
- 20 Yang T, Lau WY, Lai EC, Yang LQ, Zhang J, Yang GS, Lu JH, Wu MC. Hepatectomy for bilateral primary hepatolithiasis: a cohort study. *Ann Surg* 2010; **251**: 84-90
- 21 Machado MA, Makdissi FF, Surjan RC, Teixeira AR, Sepúlveda A Jr, Bacchella T, Machado MC. Laparoscopic right hemihepatectomy for hepatolithiasis. *Surg Endosc* 2008; **22**: 245
- 22 Viganò L, Laurent A, Tayar C, Tomatis M, Ponti A, Cherqui D. The learning curve in laparoscopic liver resection: improved feasibility and reproducibility. *Ann Surg* 2009; **250**: 772-782
- 23 Kazaryan AM, Pavlik Marangos I, Rosseland AR, Røskok BI, Mala T, Villanger O, Mathisen O, Giercksky KE, Edwin B. Laparoscopic liver resection for malignant and benign lesions: ten-year Norwegian single-center experience. *Arch Surg* 2010; **145**: 34-40
- 24 Ito K, Ito H, Are C, Allen PJ, Fong Y, DeMatteo RP, Jarnagin WR, D'Angelica MI. Laparoscopic versus open liver resection: a matched-pair case control study. *J Gastrointest Surg* 2009; **13**: 2276-2283

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Eight-year survival after advanced gastric cancer treated with S-1 followed by surgery

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INTRODUCTION

Improvements in early diagnosis of gastric cancer (GC) have led to an increase in the number of patients who are able to undergo curative resection^[1]. However, the prognosis for patients with unresectable or metastatic disease is very poor, and long-term survival, particularly for more than 5 years, is rare. S-1 is a novel oral fluoropyrimidine that was developed in Japan. It has been available for patients with GC in Japan since 1999 and is currently being investigated worldwide. S-1 consists of tegafur, 5-chloro-2,4-dihydropyridine (CDHP) and potassium oxonate at a fixed molar ratio of 1:0.4:1. Tegafur is a prodrug of fluorouracil (5-FU), which is the cytotoxic component of this combination. CDHP is a potent reversible inhibitor of dihydropyrimidine dehydrogenase (DPD), the chief catabolic enzyme of 5-FU. Potassium oxonate selectively inhibits orotate phosphoribosyltransferase, the enzyme responsible for 5-FU activation in the gastrointestinal tract, thus reducing the gastrointestinal toxicity of the combination. Many reports have indicated the efficacy of this antitumor agent against gastric cancer.

Abstract

We report a case of advanced gastric cancer, with cervical, axillary, and abdominal paraaortic lymph node metastases, that was successfully treated with chemotherapy and surgery. The disease was initially considered unresectable, and the patient was treated with orally administered S-1. Chemotherapy was effective, and all lymph node metastases disappeared after 6 courses. After 27 mo of chemotherapy, the patient underwent curative surgery, with subtotal gastrectomy and lymph node dissection. Histopathological examination revealed many viable poorly differentiated adenocarcinoma cells in the stomach, but no cancer cells in the lymph nodes. The patient is alive, without recurrence, 8 years later. This, therefore, is a case report of an 8-year survivor of advanced gastric cancer with distant lymph node metastasis.

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We report a case of advanced gastric cancer (AGC) with unresectable lymph node metastases treated with S-1.

CASE REPORT

A 71-year-old man visited a local hospital in 2002, complaining of upper abdominal discomfort of one-month duration. Endoscopy revealed a concave lesion in the stomach, and the patient was subsequently referred to our hospital. Endoscopy showed a diffuse concave lesion with ulceration on the lesser curvature in the middle of the stomach. A biopsy specimen showed signet-ring cell carcinoma. Physical examination revealed a few elastic-hard masses in the left supraclavicular area (approximately 7 cm in diameter) and left axillary space (approximately 6 cm in diameter), suggesting lymph node metastases from the GC. Abdominal computed tomography (CT) showed marked swelling of several paraaortic lymph nodes (Figure 1A). We could not find metastasis to the liver, lung, or peritoneum. The patient's diagnosis was AGC with extensive lymph node metastases (stage IV: cT2 cN3 cP0 cH0 cM1). His general condition was good, with The Eastern Cooperative Oncology Group (ECOG) performance status 0, and biochemical analysis of blood and urine specimens showed no abnormalities. Serum levels of the tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were not elevated (2.1 ng/mL and 12.0 U/mL, respectively). Since curative resection was impossible for this patient, chemotherapy with S-1 was started. As one course, 120 mg (80 mg/m²) of S-1 was orally administered daily for 28 d, followed by a 14-d rest period; administration occurred primarily at the outpatient clinic. After 2 courses, endoscopy showed no remarkable change in the gastric tumor. However, abdominal CT showed remarkable reductions in the size of regional and paraaortic lymph nodes (Figure 1B), and also in cervical and axillary lymph nodes (Figure 2A and B). This indicated a partial response (PR). The evaluation after 6 courses of S-1 showed that there was no change at the primary site, but CT showed complete disappearance of paraaortic, cervical and axillary lymph nodes. Therefore, this revealed a complete response (CR), and this condition was stable up to the subsequent surgery. The patient did not experience any critical adverse event during S-1 administration, despite developing hematological toxicity; including leucopenia (2100/ μ L at the minimum), thrombocytopenia, and non-hematological toxicity; including grade 2 hand-foot syndrome and grade 1 stomatitis, according to National Cancer Institute Common Toxicity Criteria Version 2.0. After 19 courses of S-1, the tumor was limited to the primary gastric site, with no metastasis detected from neck to abdomen by CT or positron emission tomography (PET); only the detection of gastric tumor by PET. Therefore, we concluded that curative resection could be achieved. In 2004, distal gastrectomy and lymph node dissection (D2) were performed. We did not find cancer cells in the intraoperative peritoneal lavage, or in the abdominal paraaortic lymph node specimens. Figure 3 shows the resected gastric specimen. A concave lesion of 45 mm \times 40 mm with an ulceration scar was located in the lesser curvature of the middle of the stomach (Figure 3). Histopathologi-

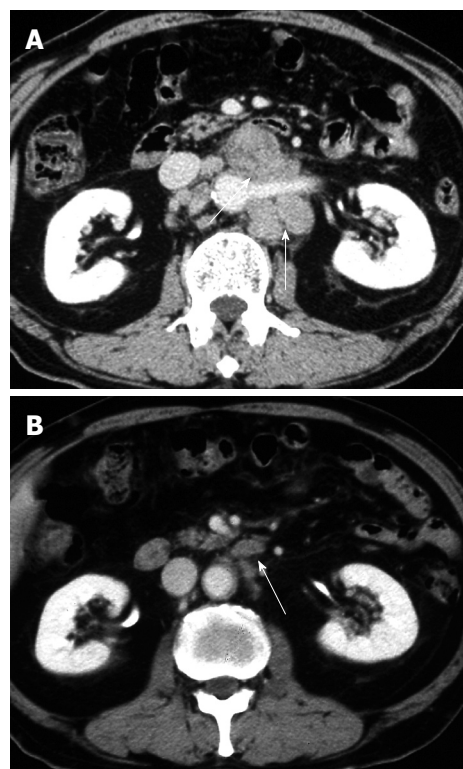


Figure 1 Abdominal computed tomography (CT). A: Marked swelling of paraaortic lymph nodes before treatment (arrow); B: After two courses of S-1, abdominal CT showed remarkable reductions (arrow).

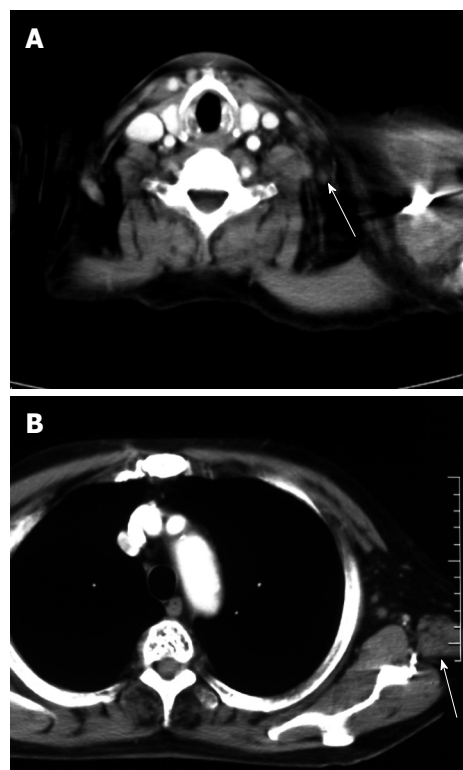


Figure 2 CT shows a Virchow's (A) and an axillary lymph node (B) which were reduced remarkably in size after treatment.

with an ulceration scar was located in the lesser curvature of the middle of the stomach (Figure 3). Histopathologi-

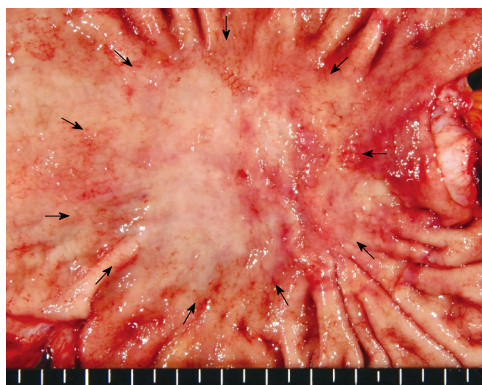


Figure 3 Resected specimen shows a concave lesion of 45 mm × 40 mm (arrows) with an ulceration scar which was located in the lesser curvature in the middle of the stomach.

cal examination showed numerous poorly differentiated adenocarcinoma cells in the mucosal layer (Figure 4A). There were only a small number of poorly differentiated adenocarcinoma cells, but severe fibrosis, in the submucosal layer (Figure 4B). The histological effect of chemotherapy on the primary tumor was limited, and according to the Japanese classification of gastric carcinoma (second English edition)^[2], it was classified as grade 1a. The only findings in the lymph nodes were remarkable fibrosis and giant cells, with no cancer cells seen; the histological effect on the lymph nodes metastasis was classified as grade 3 (Figure 4C). Therefore, a radical operation was considered to have been performed, and the final pathological stage assigned as Stage IA (pT1, pN0, sP0, sH0, sM0) according to the Japanese classification of gastric carcinoma (second English edition)^[2]. There have been no signs of recurrence since the surgery, and the patient has survived for 8 years after initially receiving chemotherapy.

DISCUSSION

There have been some series that reported high response rates with the use of newer combinations of chemotherapy regimens for unresectable or metastatic GC^[3]. However, standard regimens have not yet been established worldwide. S-1-based chemotherapy has shown survival benefits in recent randomized controlled trials. Boku *et al*^[4] reported that S-1 monotherapy showed a significant non-inferiority benefit compared to continuously infused 5-FU in unresectable or metastatic GC ($P < 0.001$). Moreover, Koizumi *et al*^[5] reported the results of a randomized controlled trial of S-1 *vs* S-1 with CDDP. The overall survival for S-1 with CDDP was superior to S-1 alone. The 1- and 2-year survival rates were 54.1% and 23.6%, respectively; whereas a 2-year survival rate of less than 10% was shown in the 5-FU+CDDP arm of a Japanese randomized controlled trial with a 10-year follow-up^[6].

Although recent phase III clinical trials indicated longer survival times^[7,8], few patients with unresectable GC have survived longer than 5 years. During the last 10 years at our hospital, only 9 of more than 500 patients treated with chemotherapy for metastatic or recurrent

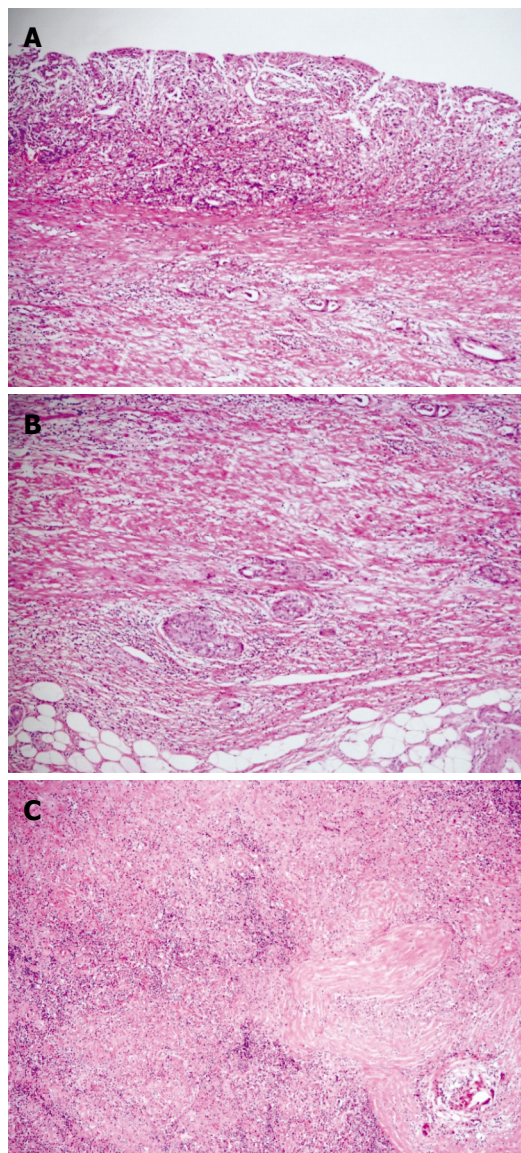


Figure 4 Histopathological examination. A: Histopathological examination showed numerous poorly differentiated adenocarcinoma cells in the mucosa; B: There were only a small number of poorly differentiated adenocarcinoma cells, but severe fibrosis, in the submucosal layer; C: The only findings in the lymph nodes were remarkable fibrosis and giant cells, with no cancer cells seen.

GC have survived for 5 years. Yoshida *et al*^[9] reported a 5-year survival rate of 2% for registered Japan Clinical Oncology Group (JCOG) clinical trials and mentioned certain characteristics of these survivors. Most of the 5-year survivors had good performance status, macroscopically non-scurrhous-type tumors, only one metastatic site, a paraaortic node metastasis as the only unresectable factor, and achieved a CR after the initial chemotherapy. However, it is unclear whether such long survivals are because of biological non-aggressiveness or due to a good response to chemotherapy. There have been only five cases reported in the literature of AGC with neck lymph node metastasis in which there was no residual tumor following chemotherapy and surgical resection^[10-14]. Ohyama *et al*^[10] reported a case of a patient treated with combination chemotherapy, including 5-FU, leucovorin, cisplatin,

and etoposide, in which there was a good response. Chemotherapy was followed by curative resection, including subtotal gastrectomy and inguinal, neck, and abdominal lymph node dissection. Pathological examination showed cancer cells only in the abdominal lymph nodes, but not in the stomach or neck lymph nodes. Iwazawa *et al.*^[11] reported a case of AGC with cervical lymph node metastasis, in which a CR for cervical lymph nodes was achieved with S-1 alone treatment, and a curative resection of the residual tumor was performed. However, further outcomes of these cases were not described. It remains unknown whether adjuvant surgery for residual tumor after effective chemotherapy is beneficial for GC patients with distant metastasis or not. In our experience, if there is a long-maintained disappearance of inoperable metastasis, adjuvant surgery is considered beneficial.

In our case, there was no confirmation of the neck and axillary lymphadenopathy from imaging examinations before treatment began, and therefore no evidence that this lymphadenopathy was due to metastasis from GC. This is a limitation of our report and we assume responsibility for not obtaining all available evidence of metastasis from GC, although surgeons agreed on the non-operability of this patient in the preoperative conference. However, in patients with histologically diffuse type GC that is greater than 3 cm in size and which has invaded the submucosal layer, 7.5% have lymph node metastasis beyond the perigastric site^[15]. Thus, our assumption that cervical and axillary lymphadenopathy was due to GC metastases was probably correct.

REFERENCES

- Hallisey MT, Dunn JA, Ward LC, Allum WH. The second British Stomach Cancer Group trial of adjuvant radiotherapy or chemotherapy in resectable gastric cancer: five-year follow-up. *Lancet* 1994; **343**: 1309-1312
- Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma, second English edition. *Gastric Cancer* 1998; **1**: 20-24
- Foukakis T, Lundell L, Gubanski M, Lind PA. Advances in the treatment of patients with gastric adenocarcinoma. *Acta Oncol* 2007; **46**: 277-285
- Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, Koizumi W, Saito H, Yamaguchi K, Takiuchi H, Nasu J, Ohtsu A. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. *Lancet Oncol* 2009; **10**: 1063-1069
- Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008; **9**: 215-221
- Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H, Yamamichi N, Miyata Y, Ikeda N, Yamamoto S, Fukuda H, Yoshida S. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol* 2003; **21**: 54-59
- Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 2006; **24**: 4991-4997
- Al-Batran SE, Hartmann JT, Probst S, Schmalenberg H, Hollerbach S, Hofheinz R, Rethwisch V, Seipelt G, Homann N, Wilhelm G, Schuch G, Stoecklacher J, Derigs HG, Hegewisch-Becker S, Grossmann J, Pauligk C, Atmaca A, Bokemeyer C, Knuth A, Jäger E. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. *J Clin Oncol* 2008; **26**: 1435-1442
- Yoshida M, Ohtsu A, Boku N, Miyata Y, Shirao K, Shimada Y, Hyodo I, Koizumi W, Kurihara M, Yoshida S, Yamamoto S. Long-term survival and prognostic factors in patients with metastatic gastric cancers treated with chemotherapy in the Japan Clinical Oncology Group (JCOG) study. *Jpn J Clin Oncol* 2004; **34**: 654-659
- Ohshima S, Komatsu O, Nakajima T, Ohta K, Takahashi T, Yanagisawa A. A case report of pathological complete remission with FLEP therapy. In: Nakajima T, Yamaguchi T, editors. Multimodality therapy for gastric cancer. Berlin, Heidelberg New York Tokyo: Springer-Verlag, 1999: 104-107
- Iwazawa T, Kinuta M, Yano H, Matsui S, Tamagaki S, Yasue A, Okada K, Kanoh T, Tono T, Nakano Y, Okamoto S, Monden T. An oral anticancer drug, TS-1, enabled a patient with advanced gastric cancer with Virchow's metastasis to receive curative resection. *Gastric Cancer* 2002; **5**: 96-101
- Kajihara K, Ishikawa H, Akama F, Ninomiya H, Shigeta K, Sano I, Nakamura Y, Iwasaki K. [A case of advanced gastric cancer with virchow's metastasis responding remarkably to combination chemotherapy of low-dose CDDP and 5-FU] *Gan To Kagaku Ryoho* 1998; **25**: 585-588
- Wada Y, Kamiya N, Asano S, Shinya F. [A case of advanced gastric cancer with Virchow's and paraaortic lymph node metastases successfully resected after combined chemotherapy of low-dose CDDP and 5-FU] *Gan To Kagaku Ryoho* 2001; **28**: 79-82
- Umehara Y, Okubo T, Sano Y, Sakamoto R, Nakamura T, Tsuchiya Y, Nagato Y, Moriyama R. [A case of advanced gastric remnant carcinoma with Virchow's metastasis treated with neoadjuvant chemotherapy (low dose CDDP + 5-FU) followed by surgical resection] *Gan To Kagaku Ryoho* 1995; **22**: 277-279
- Japanese Gastric Cancer Association. Gastric cancer treatment guideline (in Japanese). Tokyo: Kanehara, 2004: 22-38

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Treating bilio-duodenal obstruction: Combining new endoscopic technique with 6 Fr stent introducer

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Abstract

Periampullary cancer may cause not only biliary but also duodenal obstructions. In patients with concomitant duodenal obstructions, endoscopic biliary stenting remains technically difficult and may often require percutaneous transhepatic biliary drainage. We describe a method of metal stent placement *via* a thin forward-viewing endoscope in patients with simultaneous biliary and duodenal obstruction. In two consecutive patients with biliary and duodenal obstruction due to pancreatic cancer, a new biliary metal stent mounted in a slim delivery catheter was placed *via* a thin forward viewing endoscope after passage across the duodenal stenosis without balloon dilation. In both patients, with our new placement technique, metallic stents were successfully placed in a short time without adverse events. After biliary stenting, one patient received curative resection and the other received duodenal stenting for palliation. Metallic stent placement with a forward-viewing thin endoscope is a beneficial technique, which can avoid percutaneous drainage in patients with bilio-duodenal obstructions due to periampullary cancer.

INTRODUCTION

Patients with periampullary malignancies occasionally experience not only biliary but also duodenal obstructions. Although biliary obstruction usually occurs first and is followed by duodenal obstruction, the two occur simultaneously in some cases. These patients often require percutaneous biliary stenting because the endoscopic procedure is difficult.

A new commercially available self-expandable metallic stent (SEMS) mounted in an extra slim delivery catheter can be passed through the working channel of a thin endoscope. Here, we assessed the outcome of metal stent placement *via* a thin forward-viewing endoscope in two patients with simultaneous biliary and duodenal obstruction.

CASE REPORT

Two consecutive patients with simultaneous biliary and duodenal obstructions due to pancreatic cancer between No-

Table 1 Summary of studied patients

Case	Age/sex	Level of duodenal obstruction	Level of biliary obstruction	Comorbidity	Purpose of biliary stenting	Subsequent procedures
1	79/F	Pars II	Distal	None	Presurgical decompression	Pancreatico-duodenectomy
2	81/M	Pars II	Distal	Advanced esophageal cancer	Palliation	Duodenal and esophageal stenting

ember 2009 and January 2010 were investigated (Table 1). In both patients, duodenal obstructions did not allow the passage of a duodenoscope across the stricture without a dilating procedure. The patients underwent placement of SEMS *via* a slim forward-viewing gastroscope (GIF XP-240, Olympus, Tokyo, Japan) (a valid length of 1030 mm, an outer diameter of 7.7 mm, a working channel diameter of 2.2 mm). The SEMS used is a new uncovered SEMS, Zilver® 635 stent (10 mm in diameter) (Cook, Bloomington, IN, USA) which is a laser-cut nitinol stent mounted in an extra slim (6-Fr) delivery catheter. The study was approved by our institutional review board (OHASHI #21-017), and written informed consent was obtained from the patients.

A slim endoscope was inserted perorally and passed across the stricture. The ampulla was endoscopically visualized by retroflexing the endoscope in the distal second portion of duodenum. An endoscopic retrograde cholangiopancreatography (ERCP) catheter (PR-109Q, Olympus) was advanced into the bile duct using a wire-guided cannulation technique with a 0.025 "hydrophilic guidewire (Surf®, Piolax Medical Devices, Inc. Kanagawa, Japan). Then, the guidewire was replaced with a 0.035" Jagwire (Boston Scientific Inc., Natick, MA, USA), endoscopic papillary balloon dilation was performed with an 8-mm balloon dilator (Eliminator® PET biliary balloon dilator, ConMed, Utica, NY, USA), and the SEMS was released under endoscopic and fluoroscopic control.

Case 1

A 79-year-old woman was admitted to our hospital due to obstructive jaundice. Computed tomography (CT) revealed pancreatic cancer with duodenal invasion. The patient underwent ERCP for biliary stenting, during which a duodenoscope (JF-260V, 12.6 mm outer diameter, Olympus) could not be passed across the stricture in the second portion of duodenum. Despite the presence of duodenal stricture, she was able to take soft food orally.

The new stenting procedure with a thin forward-viewing endoscope was employed. In brief, a slim endoscope was inserted perorally and easily passed across the stricture (Figure 1). After successful biliary cannulation (Figure 2), a Zilver® 635 stent (8 cm in length) was placed and deployed at the optimal position (Figure 3). The entire procedure took 35 min, and no complications were encountered.

After biliary stenting, her cancer was considered resectable based on the assessment with detailed examinations. The patient successfully underwent pancreatoduodenectomy on day 14 after stenting when her jaundice

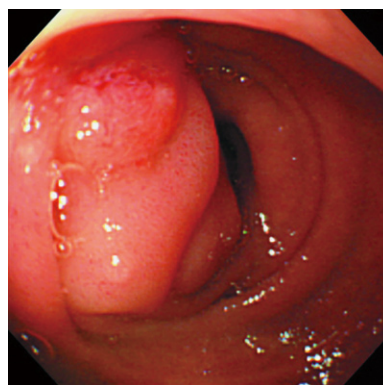


Figure 1 Endoscopic view of the forward-viewing endoscope showing duodenal stricture at the second portion of duodenum.

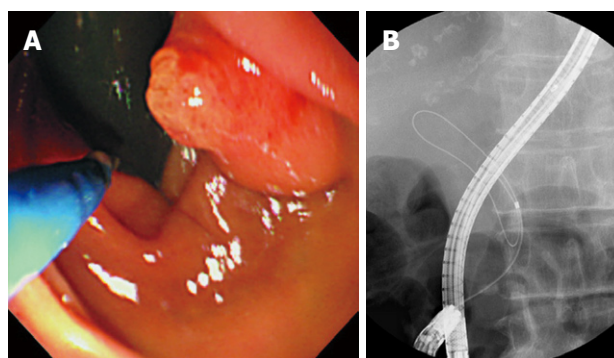


Figure 2 Selective biliary cannulation conducted with a thin forward-viewing endoscope. A: Endoscopic view showing endoscopic retrograde cholangiopancreatography (ERCP) catheter approaching to the papilla; B: X-ray picture showing common bile duct cannulation conducted with wire-guided technique without contrast injection.

was relieved. The resected specimen revealed duodenal invasion from pancreatic cancer. Since then, the patient has received adjuvant chemotherapy with S-1.

Case 2

An 81-year-old man was admitted for epigastric pain, nausea and vomiting. Ultrasonography and CT revealed a 40-mm pancreatic mass with invasion to the extrahepatic bile duct, duodenum and portal vein. In addition, esophagogastroduodenoscopy revealed an advanced cancer in the lower part of esophagus and a duodenal stenosis due to pancreatic cancer. The cancer was therefore considered unresectable. Because of these gastrointestinal obstructions, the patient could not take food orally and both biliary and duodenal stenting for palliation were scheduled. Liver function tests showed abnormal results, although

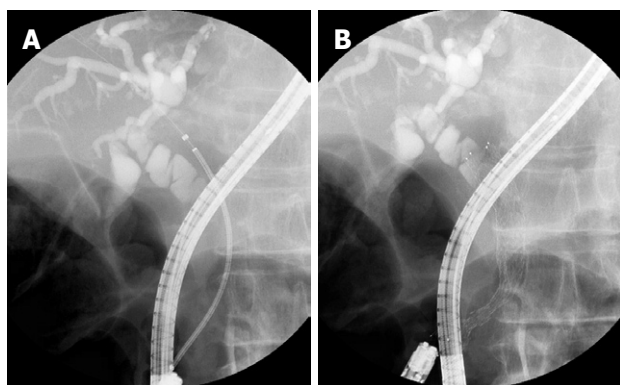


Figure 3 Biliary self-expandable metallic stent (SEMS) placement *via* a thin forward-viewing endoscope. A: An extra slim delivery catheter is inserted properly into the bile duct; B: SEMS is successfully placed.

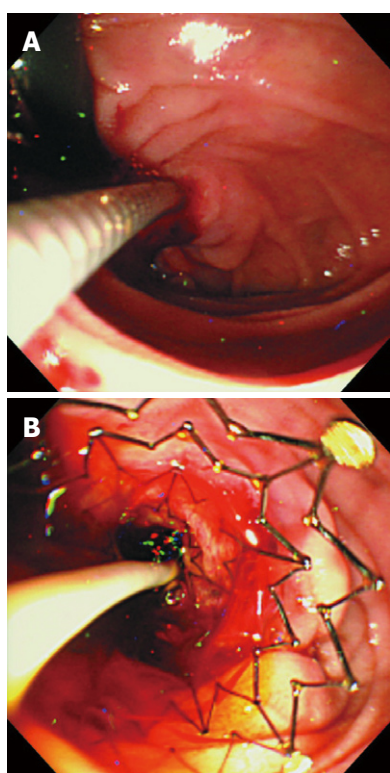


Figure 4 Endoscopic view showing biliary metallic stent placement *via* a thin forward-viewing endoscope. A: The delivery catheter is successfully introduced into the bile duct; B: SEMS is successfully placed.

jaundice was not identified. We carried out biliary stenting first in view of a treatment strategy based on scheduled palliative stenting, both biliary and duodenal^[1].

Because it was impossible to pass a large-caliber side-viewing duodenoscope (JF-260V, Olympus) through the duodenal stricture, we employed the new biliary stenting procedure with a thin forward-viewing endoscope as in Case 1 and subsequently placed a duodenal stent. A thin endoscope was easily passed across the duodenal as well as esophageal obstruction, after which a Zilver[®] 635 stent (8 cm in length) was successfully placed without difficulty, even though the endoscope was retroflexed as in Case 1 (Figure 4). The total procedure time was 22 min.



Figure 5 X-ray picture indicating successful placement of biliary, duodenal and esophageal stents.

No procedure-related complications were found.

On day 10 after biliary stent placement, the patient underwent stent placement at the duodenal stricture. A duodenal stent (Niti-S[®] Pyloric/Duodenal Uncovered Stent, Taewoong Medical Inc. Seoul, Korea) was successfully placed with a through-the-scope placement procedure at the optimal position. Two weeks after the duodenal stenting, the patient also underwent stent placement at the esophageal obstruction with an Ultraflex[™] (Boston Scientific) (Figure 5). Biliary, duodenal and esophageal stenting improved his dietary status, allowing intake of solid food.

DISCUSSION

We report here the successful endoscopic placement of a biliary SEMS using a thin forward-viewing endoscope in two patients with simultaneous biliary and duodenal obstructions. One patient underwent the procedure for presurgical decompression and the other for palliation.

The combination of biliary and duodenal obstruction in patients with periampullary cancer is relatively frequent, as 23% of the patients had both biliary and duodenal obstructions in a retrospective study of unresectable pancreatic head cancer^[2]. In most cases, duodenal obstruction occurs after biliary obstruction, but simultaneous obstruction also occasionally occurs. Because stent placement in these simultaneous cases is more complicated, these patients often require percutaneous biliary stenting. Even if a duodenoscope can be passed across the duodenal stricture when a duodenal stent is first placed, access to the ampulla may be hampered by stent mesh covering the papilla. Biliary stenting before duodenal stenting is therefore recommended for these patients^[1]. However, because the stricture is usually too narrow to allow passage of a large-caliber duodenoscope, hydrostatic balloon dilation is frequently required^[2,3]. Even after dilation, passage of a side-viewing duodenoscope across the obstruction is sometimes difficult, and is at a risk for duodenal perforation owing to the presence of acute angulation in the duodenum. Because the endoscope used in the present study is much thinner and forward-viewing, we were able to pass it easily and safely across the stricture under direct vision. The present technique thus has the great advantage of allowing the easier and safer emplacement of a biliary stent than a previously published technique of biliary stenting after a duodenal dilation with a side-viewing duo-

denoscopy in patients with combined biliary and duodenal obstruction^[2,3].

Although usually selected when endoscopic stenting is difficult, percutaneous stenting has two drawbacks, namely a compromised quality of life owing to the need for an indwelling percutaneous transhepatic biliary drainage (PTBD) catheter for at least one week before stent placement, and the risk of cancer implantation in the catheter tract. A retrospective analysis of patients who underwent PTBD before resection of extrahepatic cholangiocarcinoma showed that catheter tract cancer-implantation develops in 6%^[4]. Catheter tract cancer-implantation is reportedly observed in patients with pancreatic cancer as well as cholangiocarcinoma^[5]. In recent years, endoscopic ultrasound (EUS)-guided biliary drainage has been introduced as an alternative to PTBD when ERCP is unsuccessful. However, this procedure is associated with procedure-specific complications, such as bile leak-attributable peritonitis^[6,7], in addition to bleeding and cholangitis from stent dysfunction. Theoretically, it may cause cancer implantation in the peritoneal cavity and fistula track as in the case of PTBD. Transpapillary stenting is the only procedure conducted *via* a natural route and so must be the most desirable technique for presurgical as well as palliative decompression.

Recently, cholangiographic or cholangioscopic procedures with an ultra-slim endoscope have been reported^[8,9]. A prospective comparison of transnasal ERCP with an ultrathin forward-viewing endoscope *vs* conventional ERCP with a large caliber side-viewing duodenoscope has reported no statistically significant difference in the rates or times of cannulation, albeit that the success rate of cannulation with the transnasal method is lower. The endoscope used in the present study (7.7 mm in diameter) is slightly larger than that used in the previous study (5.9 mm in diameter)^[8]. Unlike the previous study, we introduced the endoscope into the duodenum perorally but were nevertheless able to accomplish biliary cannulation and stenting, without any difficulty. One previous case report of stent placement using an ultrathin forward-viewing endoscope *per os* has appeared^[10], in which a patient with choledocholithiasis who received placement of two 5-Fr hand-made stents, subsequently underwent sphincterotomy and stone removal using a peroral ultrathin endoscope after relief of cholangitis. With plastic stents, thin endoscopes with a narrower working channel are restricted to accepting only 6 Fr or thinner stents. However, larger stents are generally more favorable than smaller ones, particularly for palliative use. We consider that the use of the new SEMS mounted in an extra slim delivery catheter takes full advantage of the possibilities of a thin endoscope with a thinner working channel.

When duodenal tumor invasion extends to the major

papilla, endoscopic identification of the papilla is frequently difficult. Even if the papilla can be found, it is generally impossible to access the bile duct because of the difficulty of aligning the catheter with the bile duct axis. Under these conditions, therefore, stent placement would likely be difficult even with the present method and a percutaneous or EUS-guided transenteric procedure may be required instead. However, the number of such cases appears to be limited. The present report is a preliminary result with only two cases, and further study with more patients is warranted.

In conclusion, placement of the new SEMS mounted in an extra slim delivery catheter *via* a thin forward-viewing endoscope appears to be a safe and effective procedure for either presurgical or palliative decompression in patients with malignant biliary and duodenal obstruction. This study is preliminary, however, and further evaluation in a larger number of patients is warranted.

REFERENCES

- 1 **Baron TH.** Optimizing endoscopic placement of expandable stents throughout the GI tract. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 399-409
- 2 **Maire F, Hammel P, Ponsot P, Aubert A, O'Toole D, Hentic O, Levy P, Ruszniewski P.** Long-term outcome of biliary and duodenal stents in palliative treatment of patients with unresectable adenocarcinoma of the head of pancreas. *Am J Gastroenterol* 2006; **101**: 735-742
- 3 **Kaw M, Singh S, Gagneja H.** Clinical outcome of simultaneous self-expandable metal stents for palliation of malignant biliary and duodenal obstruction. *Surg Endosc* 2003; **17**: 457-461
- 4 **Sakata J, Shirai Y, Wakai T, Nomura T, Sakata E, Hatakeyama K.** Catheter tract implantation metastases associated with percutaneous biliary drainage for extrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; **11**: 7024-7027
- 5 **Fiori E, Galati G, Bononi M, De Cesare A, Binda B, Ciardi A, Volpino P, Cangemi V, Izzo L.** Subcutaneous metastasis of pancreatic cancer in the site of percutaneous biliary drainage. *J Exp Clin Cancer Res* 2003; **22**: 151-154
- 6 **Irisawa A, Hikichi T, Shibukawa G, Takagi T, Wakatsuki T, Takahashi Y, Imamura H, Sato A, Sato M, Ikeda T, Suzuki R, Obara K, Ohira H.** Pancreatobiliary drainage using the EUS-FNA technique: EUS-BD and EUS-PD. *J Hepatobiliary Pancreat Surg* 2009; **16**: 598-604
- 7 **Püspök A.** Biliary therapy: are we ready for EUS-guidance? *Minerva Med* 2007; **98**: 379-384
- 8 **Mori A, Ohashi N, Maruyama T, Tatebe H, Sakai K, Shibuya T, Inoue H, Takegoshi S, Okuno M.** Transnasal endoscopic retrograde cholangiopancreatography using an ultrathin endoscope: a prospective comparison with a routine oral procedure. *World J Gastroenterol* 2008; **14**: 1514-1520
- 9 **Larghi A, Waxman I.** Endoscopic direct cholangioscopy by using an ultra-slim upper endoscope: a feasibility study. *Gastrointest Endosc* 2006; **63**: 853-857
- 10 **Yan SL, Chen CH, Yeh YH, Yueh SK.** Successful biliary stenting and sphincterotomy using an ultrathin forward-viewing endoscope. *Endoscopy* 2009; **41** Suppl 2: E59-E60

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Impaction of a lithotripsy basket during endoscopic lithotomy of a common bile duct stone

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and the choledochotomy was closed. The postoperative course was uneventful. In conclusion, if the diameter of a CBD stone is more than 20 mm, then the risk of basket impaction increases, and surgery may be necessary as the initial treatment of the CBD stone.

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Key words: Common bile duct gallstones; Impacted biliary basket; Lithotripsy

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Abstract

The treatments for common bile duct (CBD) stones are being continually developed. Impaction of the lithotripsy basket during endoscopic removal of CBD stones was seen in 5.9% patients. We report the case of a 66-year-old woman who underwent surgery for the removal of an impacted biliary basket. She was admitted to our hospital with a complaint of right upper abdominal pain. Magnetic resonance cholangiopancreatography revealed a CBD stone (20 mm × 15 mm). We diagnosed her with choledocholithiasis and performed endoscopic retrograde cholangiopancreatography to remove the stone. However, unfortunately, the retrievable basket around the stone became impacted. An endotripter along with forceps could not be used owing to the entrapment of the basket, and thus we performed urgent surgery. The basket containing the stone was removed through a longitudinal choledochotomy. The wires leading to the basket were cut, and the basket containing the stone was removed *via* the incision. A T-tube was inserted,

INTRODUCTION

Endoscopic procedures for the removal of common bile duct (CBD) stones are well established^[1-3], and include endoscopic retrograde cholangiography (ERC), sphincterotomy, and basket or balloon extraction. However, endoscopic removal of large bile duct stones is difficult. The success or failure of lithotripsy depends on the size of the stone, number of stones, degree of jaundice, and presence or absence of gallbladder stone-induced cholecystitis. Here, we report the case of a patient with an impacted biliary basket.

CASE REPORT

A 66-year-old woman with right upper abdominal pain was admitted to our hospital. Laboratory analysis on admission

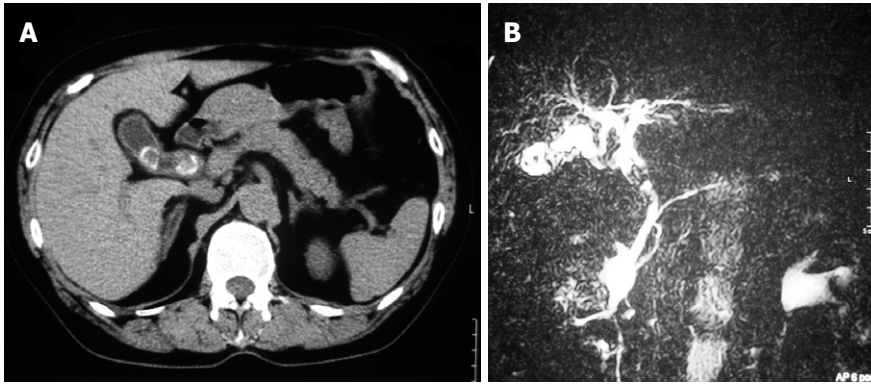


Figure 1 Computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP). A: Abdominal CT scan showing 2 stones in the gallbladder and a stone in the common bile duct; B: MRCP revealed a mass (20 mm × 15 mm in diameter) in the dilated common bile duct (20 mm in diameter).

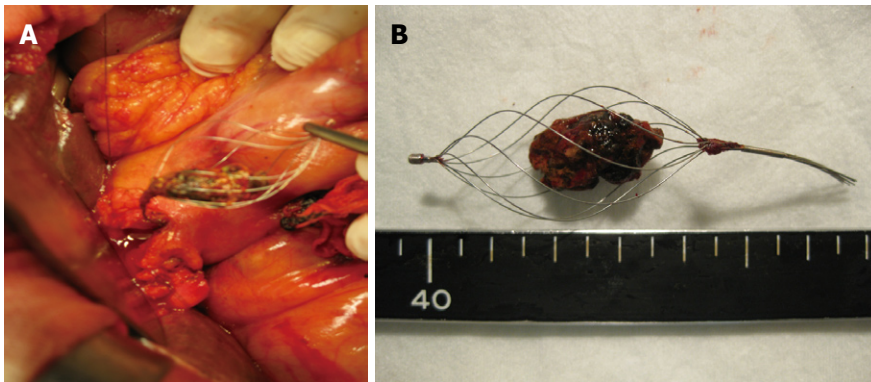


Figure 2 Intraoperative findings. A: During surgery, the impacted basket with stone were removed through a longitudinal choledochotomy; B: The size of the stone in the basket was 20 mm in diameter.

revealed elevated serum levels of the following biological parameters (the normal range for each parameter is in parentheses): alkaline phosphatase, 486 IU (109-335 IU); γ -glutamyl transpeptidase, 373 IU (12-48 IU); L-aspartate aminotransferase, 913 IU (12-32 IU); L-alanine aminotransferase, 449 IU (10-40 IU); total bilirubin, 2.3 mg/dL (0.2-1.4 mg/dL); and direct bilirubin, 1.3 mg/dL (0.0-0.3 mg/dL). The results of a complete blood count test and coagulation test were normal. The serum levels of tumor markers (carbohydrate antigen 19-9 and carcinoembryonic antigen) were within the normal ranges. Ultrasound examination and computed tomography revealed 2 stones in the gallbladder along with thickening of the gallbladder and a calcified stone in the CBD (Figure 1A). In addition, magnetic resonance cholangiopancreatography revealed a mass (20 mm × 15 mm in diameter) in the dilated CBD, which had a diameter of 20 mm (Figure 1B). We diagnosed the patient with choledocholithiasis-induced cholangitis and choledocholithiasis-induced cholecystitis. We planned endoscopic lithotripsy for the removal of the CBD stone as the initial treatment, followed by laparoscopic cholecystectomy.

ERC revealed the CBD stone as a large defect filling the upper part of the CBD. We performed endoscopic stone extraction, because the biliary tract was dilated after the endoscopic lithotripsy. The metal spiral sheath advanced to the basket containing the entrapped stone. However, unfortunately, the retrievable basket around the stone became impacted in the middle of the CBD. It was impossible to crush the stone because it had hardened owing to calcification; in addition, it was impossible to shut the opening wire and to replace the basket cath-

eter with an endotripter.

Subsequently, because the basket was trapped, we performed urgent surgery involving cholecystectomy and choledochotomy, because, in our department, we did not have an extracorporeal shock-wave lithotripsy.

During the operation, cholecystectomy was performed, and the CBD was explored. The basket containing the stones was removed through a longitudinal choledochotomy (Figure 2). The wires leading to the basket were cut, and the basket containing the stone was removed *via* the incision. Intraoperative choledochoscopy revealed no residual stones or fragments. A T-tube was inserted, and the choledochotomy was closed. The postoperative course was uneventful and the patient is clinically healthy.

DISCUSSION

The treatment of CBD stones has advanced from choledochotomy to endoscopic management, with a success rate of over 90% for the latter^[4,5]. The techniques used for the management of CBD stones are endoscopic sphincterotomy and endoscopic papillary balloon dilatation. However, the complications of endoscopic management are hemorrhage, pancreatitis, sepsis, cholangitis, and occasionally, impaction of the lithotripter basket during the endoscopic removal of a CBD stone. The reported incidence of impaction of a basket with an entrapped stone was 5.9%^[4,6,7], however, because of the developments in the therapeutic techniques for CBD stones, this incidence has decreased to 0.8%^[8].

The main factor responsible for impaction of the

basket was reported to be the large size of the stone^[4,6,7]. In the treatment of CBD stones with a diameter over 10-12 mm, a crush technique using endoscopic mechanical lithotripsy (EML) is employed, and the crushed stone is removed^[9].

EML has been successfully used in 80%-90% of cases to crush CBD stones that were too large to be removed using conventional methods^[10,11]. However, the management of very large stones with diameters of more than 25 mm, and of multiple stones in the ductus choledochus has failed^[5]. In 4 studies that reported impaction of the basket, the average size of the stone was 17 mm (range, 13-20 mm)^[4,7,12,13]. The success rate of EML for the treatment of large stones with diameters over 20 mm, was reported to be approximately 50%. However, the success rate is low if there are multiple stones and/or calcified stones. In our patient, the diameter of the stone was 20 mm: in addition, the stone was calcified and entrapped, and therefore could not be crushed and released in the lower part of the CBD. Impaction of the basket or CBD stone causes obstructive jaundice; furthermore, the likelihood of acute obstructive suppurative cholangitis, acute pancreatitis, and sepsis is great^[14]. In the worst cases, failure of stone removal may result in the death of the patient. Garg *et al.*^[15] reported that stone size alone may not be an important factor that decides the success of EML; CBD dilatation with adequate working space between the stone and the wall of the CBD is also an important factor. In our patient, the size of the CBD stone was 20 mm and the CBD was dilated 20 mm, thus, there was no adequate space between the stone and the CBD wall to open the wire basket fully, and this may be one of the causes of failure of EML. In addition, cholecystitis with gallbladder stones may induce inflammatory changes in the CBD, leading to stiffness of the CBD, and, as a result, no adequate working space between the stone and the wall of the CBD.

In conclusion, we determined that if the size of CBD stone is over 20 mm in diameter and is calcified, the risk of an impacted basket is increased. In addition, if the CBD is not adequately dilated, there is insufficient space between the stone and the CBD wall to open the wire basket fully. In these cases, surgery may be selected

as an initial treatment of CBD stones to prevent impaction of a basket.

REFERENCES

- 1 **Classen M**, Ossenberg FW. Non-surgical removal of common bile duct stones. *Gut* 1977; **18**: 760-769
- 2 **Safrany L**. Endoscopic treatment of biliary-tract diseases. An international study. *Lancet* 1978; **2**: 983-985
- 3 **Cotton PB**. Non-operative removal of bile duct stones by duodenoscopic sphincterotomy. *Br J Surg* 1980; **67**: 1-5
- 4 **Attila T**, May GR, Kortan P. Nonsurgical management of an impacted mechanical lithotripter with fractured traction wires: endoscopic intracorporeal electrohydraulic shock wave lithotripsy followed by extra-endoscopic mechanical lithotripsy. *Can J Gastroenterol* 2008; **22**: 699-702
- 5 **Fujita R**, Yamamura M, Fujita Y. Combined endoscopic sphincterotomy and percutaneous transhepatic cholangioscopic lithotripsy. *Gastrointest Endosc* 1988; **34**: 91-94
- 6 **Schneider MU**, Matek W, Bauer R, Domschke W. Mechanical lithotripsy of bile duct stones in 209 patients--effect of technical advances. *Endoscopy* 1988; **20**: 248-253
- 7 **Sauter G**, Sackmann M, Holl J, Pauletzki J, Sauerbruch T, Paumgartner G. Dormia baskets impacted in the bile duct: release by extracorporeal shock-wave lithotripsy. *Endoscopy* 1995; **27**: 384-387
- 8 **Schreurs WH**, Juttman JR, Stuijbergen WN, Oostvogel HJ, van Vroonhoven TJ. Management of common bile duct stones: selective endoscopic retrograde cholangiography and endoscopic sphincterotomy: short- and long-term results. *Surg Endosc* 2002; **16**: 1068-1072
- 9 **Demling L**, Seuberth K, Riemann JF. A mechanical lithotripter. *Endoscopy* 1982; **14**: 100-101
- 10 **Van Dam J**, Sivak MV Jr. Mechanical lithotripsy of large common bile duct stones. *Cleve Clin J Med* 1993; **60**: 38-42
- 11 **Hintze RE**, Adler A, Veltzke W. Outcome of mechanical lithotripsy of bile duct stones in an unselected series of 704 patients. *Hepatogastroenterology* 1996; **43**: 473-476
- 12 **Merrett M**, Desmond P. Removal of impacted endoscopic basket and stone from the common bile duct by extracorporeal shock waves. *Endoscopy* 1990; **22**: 92
- 13 **Payne WG**, Norman JG, Pinkas H. Endoscopic basket impaction. *Am Surg* 1995; **61**: 464-467
- 14 **Nuehaus B**, Safrany L. Complications of endoscopic sphincterotomy and their treatment. *Endoscopy* 1981; **13**: 197-199
- 15 **Garg PK**, Tandon RK, Ahuja V, Makharia GK, Batra Y. Predictors of unsuccessful mechanical lithotripsy and endoscopic clearance of large bile duct stones. *Gastrointest Endosc* 2004; **59**: 601-605

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Events Calendar 2010

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International Conference on Medical
Negligence and Litigation in Medical
Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology
Conference

January 28-30
Hong Kong, China
The 1st International Congress on
Abdominal Obesity

February 11-13
Fort Lauderdale, FL, United States
21th Annual International Colorectal
Disease Symposium

February 26-28
Carolina, United States
First Symposium of GI Oncology at
The Caribbean

March 04-06
Bethesda, MD, United States
8th International Symposium on
Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of
Gastroenterology & Endoscopy
Meeting

March 09-12
Brussels, Belgium
30th International Symposium on
Intensive Care and Emergency
Medicine

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian
National Association for Study of
the Liver

March 23-26
Cairo, Egypt
14th Pan Arab Conference on
Diabetes PACD14

March 25-28
Beijing, China
The 20th Conference of the Asian

Pacific Association for the Study of
the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in
Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology
and Hepatology Conference, EGHG
2010

April 14-17
Landover, Maryland, United States
12th World Congress of Endoscopic
Surgery

April 14-18
Vienna, Austria
The International Liver Congress™
2010

April 28-May 01
Dubrovnik, Croatia
3rd Central European Congress
of surgery and the 5th Croatian
Congress of Surgery

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual
Meeting

May 06-08
Munich, Germany
The Power of Programming:
International Conference on
Developmental Origins of Health
and Disease

May 15-19
Minneapolis, MN, United States
American Society of Colon and
Rectal Surgeons Annual Meeting

June 04-06
Chicago, IL, United States
American Society of Clinical
Oncologists Annual Meeting

June 09-12
Singapore, Singapore
13th International Conference on
Emergency Medicine

June 14
Kosice, Slovakia
Gastro-intestinal Models in
the Research of Probiotics and
Prebiotics-Scientific Symposium

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ILTS: International Liver
Transplantation Society ILTS Annual
International Congress

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Mannheim, Germany
16th World Congress for
Bronchoesophagology-WCBE

June 25-29
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Sessions

August 28-31
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10th OESO World Congress on
Diseases of the Oesophagus 2010

September 10-12
Montreal, Canada
International Liver Association's
Fourth Annual Conference

September 11-12
La Jolla, CA, United States
New Advances in Inflammatory
Bowel Disease

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference
on Antimicrobial Agents and
Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
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Controversies in Gastroenterology &
Liver Diseases

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Belgrade, Serbia
The 7th Biannual International
Symposium of Society of
Coloproctology

October 15-20
San Antonio, TX, United States
ACG 2010: American College of
Gastroenterology Annual Scientific
Meeting

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- 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 1, 274 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. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

Instructions to authors

Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary 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. Wiczorek 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

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Write as mean \pm SD or mean \pm 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 *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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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 $24.5 \mu\text{g/L}$; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; 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.

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Italics

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