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EDITORIAL

Ontogeny, growth and development of the small intestine: Understanding pediatric gastroenterology

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Abstract

Throughout our lifetime, the intestine changes. Some alterations in its form and function may be genetically determined, and some are the result of adaptation to diet, temperature, or stress. The critical period programming of the intestine can be modified, such as from subtle differences in the types and ratios of n3:m6 fatty acids in the diet of the pregnant mother, or in the diet of the weanlings. This early forced adaptation may persist in later life, such as the unwanted increased intestinal absorption of sugars, fatty acids and cholesterol. Thus, the ontogeny, early growth and development of the intestine is important for the adult gastroenterologist to appreciate, because of the potential for these early life events to affect the responsiveness of the intestine to physiological or pathological challenges in later life.

Key words: Intestinal development; Ontogeny; Pediatrics

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INTRODUCTION

The molecular mechanisms of fetal development of the intestine have been explored using transgenic and knockout mice, with the suggestion of the importance of Wnt, bone morphogenetic protein (BMP), PTEN/PI3K and Notch signaling[1]. After midgestation, the stratified cuboidal intestinal epithelium, derived from endoderm, begins to form villi as a result of epithelial-mesenchymal interactions[2]. Wnt and Indian hedgehog signaling interact to stimulate proliferation and to act as a morphogen, in turn acting on BMP signals in the mesenchyme to influence morphogenesis during the development of the intestine[3]. The cellular differentiation along the crypt-villus axis is maintained by Wnt pathway target genes[4,5]. Unphosphorylated active PTEN in turn controls the activation of the lipid kinase PI3 kinase pathway, where the PDK1 and especially the PKB (Akt) ser1Thr kinases act as the main effecter kinases of this proliferative pathway[6].

These complex processes are integrated to produce functionally important alterations during late intrauterine and early postnatal life of intestinal morphology and function, to prepare the infant for early feeding on high-fat milk, and then weaning onto lower fat-but higher carbohydrate-containing solid foods. Understanding these ontogenic events helps to understand the early age-dependent approach to nutritional disease state.

INTESTINAL MORPHOLOGY

At the time of birth, the human small intestine is morphologically and biochemically more mature than that of other mammals. Of interest though, since rodents are born at a more immature stage than humans, at least some of the brush border membrane (BBM) enzymatic maturation that occurs prenatally in humans only occurs after birth in rodents. This makes the rodent a useful model to better understand the process of intestinal maturation that occurs in premature infants.

The maturity of the small intestine is reflective of the length of the gestational period, with the developments of the human small intestine being largely completed in utero by the end of the first trimester[7]. Despite temporal differences in the ontogeny of the small intestine between species, the processes involved in the development of the small intestine remain similar. Thus, the human intestine goes through each of the stages that occur in rodents, so that animal studies may be used to better understand the development of the human intestine.

Development of the small intestine is comprised of three stages: (1) morphogenesis and cell proliferation, (2) cell differentiation, and (3) functional maturation[8]. Gastrulation is the process by which the primitive gut tube is formed. This consists of the endoderm, the precursor to the epithelial lining of the gastrointestinal (GI) tract, surrounded by mesenchyme. In humans, this process begins at three weeks gestation[7].

In the embryo, the GI system is one of the first to polarize by forming an entry and exit to the systems along the anterior and posterior axis. The *hox* genes are nuclear transcription factors that activate genes that encode secretory proteins. The *hox* genes play an important role in the formation of distinct regions of the brain and skeleton[7]. Through epithelial-mesenchyme interactions, these proteins may also be involved in determining anterior-posterior patterning in the fetal gut. Similarly, Sonic hedgehog and Indian hedgehog pathways mediate epithelial-mesenchymal interactions at early stages of gut formation[2].

Next, there is a transition into columnar epithelium, with the development of polarized enterocytes, and the formation of the BBM and basolateral membrane (BLM) of the enterocyte. The formation of nascent villi and microvilli occurs simultaneously, with cellular proliferation detectable along the villi. In humans, formation of the villus is initiated at 9-10 wk gestation, and proceeds in a cranial-caudal direction[7]. Villus and microvillus formation account for the approximate 100 000-fold increase in the intestinal surface area observed from the early first trimester period to birth[9].

The development of intestinal crypts then follows in humans, but in rodents, crypts do not develop until after birth[10]. The human fetus and the neonatal rat have transient villus-like structures in the proximal colon with properties similar to enterocytes, including the expression of BBM enzymes and transporters[11-13]. In later life, when premalignant changes occur in the colon in the form of development of colonic adenomatous polyps, the villous structure may recur. Interestingly, $CaCO_2$ cells derived from human colon cancer cells develop villi and villous functions, and are a good cell culture model for the assessment of, for example, intestinal absorption and metabolism.

The cells of the intestinal mucosa (the antagonists, enteroendocrine cells, Paneth and goblet cells) are compartmentalized within the crypt-villus unit. All four of the differentiated cell types of the intestinal mucosa are derived from one or more multipotent stem cells located in each intestinal crypt[14]. As cells move out of the crypt and up the villus or deeper into the crypts, "…differentiation occurs as progeny of the transit cell population migrate in vertically coherent bands…"[15]. Fibroblast growth factor receptor 3 (FGFR-3) is highly expressed in the undifferentiated crypt epithelial cells in the developing intestine, and FGFR-3 signaling through β-catenin/Tcf-4-dependent and independent pathways may regulate crypt epithelial stem cell expansion and crypt morphogenesis by the process of crypt bifurcation or fission[15]. Other growth factors such as Wnt(s) and FGF2 may cross talk with the β-catenin signaling pathway[16].

Cellular proliferation occurs in the crypts, differentiated cells populate the villi, and the dynamic balance between proliferation and differentiation is balanced by apoptosis of the senescent cells. Hepatocyte nuclear factor 4 α (HNF4α) belongs to the family of nuclear receptor transcription factors found in the liver, pancreas, kidney, and intestinal tract[17,18]. HNF4α may instruct "… cells to become specific to the intestinal epithelium"[19], as well as upregulating genes during epithelial cell differentiation such as Apo A-Ⅳ, intestinal alkaline phosphatase, liver and intestinal fatty acid binding proteins[20-23].

Bile acids regulate their own synthesis[24]. The luminal concentration of bile acids and the bile acid pool are low in the preterm and term infant, and rise as the animal ages[25,26]. These initially low values are associated with malabsorption of lipids[27]. The size of the bile acid pool increases with the activity of cholesterol 7α-hydroxylase (Cyp7a1) and oxysterol 7α-hydroxylase (Cyp7b1) by mechanisms that are independent of the farnesyl X receptor (FXR), and the short heterodimeric pathway (SHP)[24].

Increased bile acid absorption by the ileal apical sodium-dependent bile acid cotransporter (ASBT) also contributes to the expansion of the bile acid pool.

In mouse models of necrotizing enterocolitis (NEC)[28], the preinflammatory transcription factor NF-κβ mediates this intestinal injury as the result of platelet activating factor (PAF) converting p105 into p50. The p50 further upregulates proinflammatory cytokines which lead to a systemic inflammatory response and acute bowel injury[29].

Peroxisome proliferator-activated receptor-j (PPARj) is a nuclear receptor which associates with retinoid X receptor to "…suppress proliferation and promote differentiation of intestinal epithelial cells…", and to decrease the size of the proliferative zone of the intestinal crypts[30-32]. The thiazolidinedione drugs are PPARj ago-

nists which reduce cholera lexin mediated chloride secretion through the reduced expression of the apical CFTR channels, KCNQ1 K^+ channels as well as Na^+-K^+-$2Cl^-$ cotransporter-1 proteins in the BLM[33].

In addition to the enterocytes, four other small intestinal mucosal cell types develop: goblet cells, enteroendocrine cells, Paneth cells, and M cells. M cells are associated with Peyer's patches, and are detected by 17 wk of gestation[34]. In the human intestine, all epithelial cell types known to occur in the adult are present by the end of the first trimester[34]. The intestinal epithelium is able to maintain the differentiation programs of each lineage, depending on the location of the cells along the crypt-villous and proximal-distal gradients[35,36].

The regulation of GI development is complex, and involves a host of growth and transcription factors. Receptors for epidermal growth factor (EGF), transforming growth factor β (TGFβ), insulin-like growth factor Ⅱ (IGF-Ⅱ), hepatocyte growth factor (HGF), and GLP-2 are present in fetal human intestine[37,38]. Human fetal cortisone levels in the blood increase late in gestation[39]. Corticosterone (a glucocorticoid similar to cortisol) is thought to be the main factor involved in rat small intestinal maturation[40,41].

Studies investigating the development of human fetal small intestine xenografted to SCID mice demonstrate that the transplanted intestine normally undergoes differentiation in the absence of luminal and hormonal factors[42,43]. This finding, in conjunction with the observation that villus formation in rodents is autonomous[44], suggests that intestinal development may be "hard-wired", i.e. is regulated largely by intrinsic factors, with extrinsic factors playing only a secondary role. Indeed, several transcription factors including *N-myc*, HNF3β and C*dx-2* have been identified as potential intrinsic factors implicated in GI development. *N-myc* gene knockout animals demonstrate defects in GI development[45]. Homologous null mutants of HNF3β are lethal, as many structures, including the gut tube do not develop normally[46,47]. Cdx-2 expression is detected at the time of morphogenesis in mouse intestine, and is a known regulator of the expression of the small intestinal BBM enzymes sucrase-isomaltase (SI)[48].

The exogenous expression of Cdx-2 in a rat intestinal cell line induces the differentiation of goblet and absorptive cells from crypt cells. This suggests a possible role of Cdx-2 in the ontogeny of the GI tract. Several other signaling pathways (including the Notch, Wnt/β-catenin and BMP pathways) are also thought to play a role in patterning the gut during development, and in regulating epithelial differentiation through epithelial-mesenchymal interactions[49,50]. What then is the importance of the extracellular matrix (ECM)?

Indeed, in addition to regulation by transcriptional factors, intestinal development may be controlled through interactions with components of the ECM. Developmental changes in E-cadherins and integrins have been described[36,51,52], suggesting that the ECM may influence the ontogeny of epithelial cells. Cultures of human fetal enterocytes demonstrate enhanced differentiation, when they are grown on components of the ECM[53]. This suggests that a permissive rather than an instructive role may be attributed to the ECM in GI development. Indeed, when major components of the ECM have been deleted, in mice, they show no changes in GI morphogenesis, indicating that these components are not essential for GI development in this model[54].

FUNCTIONAL DEVELOPMENT

The functional development of the BBM enzyme activity has been well characterized[55-58].

BBM SI is first detected in the human fetus in the first trimester, but is not seen until weaning in the rat[58]. Both rat and human fetal small intestine demonstrate detectable BBM lactase-phlorizin hydrolase (LPH) activity, but LPH expression before and after birth varies depending on the species[59]. In humans, BBM enzyme activity has been correlated to morphogenesis, with the development of enzyme activity being associated with the formation of enterocytes[60]. Proximal-to-distal gradients of enzyme activity along the length of the small intestine are established early in gestation. In addition, crypt-villous gradients are evident, with LPH activity being highest at the villous tip, and SI activity maximal in the mid-villous region[61].

LPH

The earliest ingested nutrient in mammals is, of course, milk. The major carbohydrate in milk is disaccharide lactose. Lactose is cleaved by BBM LPH into glucose and galactose. LPH is therefore a crucial enzyme for neonates who are solely dependent on their mother's milk for nourishment.

Human LPH is first detected in the proximal small intestine at 8-9 wk of gestation, but later extends along the length of the small intestine[60]. In contrast, rat LPH is very low until 24 wk gestation, when its activity begins to increase. A rise in LPH activity in rodents occurs only late in the third trimester. In the human fetal jejunum, LPH activity correlated with the abundance of its mRNA[62], consistent with the proposal that LPH activity is regulated transcriptionally[63-65]. Nuclear transcription factors that have been shown to interact with the LPH promoter element CE-LPH1 include CDX-2[66], HOXC11[67]), GATA6[68], and HNF1[67].

Once weaning has occurred in nearly all species of mammals, both LPH activity and mRNA abundance decline[59,69,70]. Even in humans, the vast majority of the world's population experiences a decline in LPH activity sometime during childhood or adolescence. These lower values of LPH activity are 5%-10% of those values seen in early childhood[71]. In contrast, in geographical regions such as Western Europe and North America, where for thousands of years dairy cattle were raised as a continuing source of milk, LPH activity persists throughout adulthood[59,69,70], unless an adverse process affects the small

intestine. This is known as secondary lactose deficiency (i.e. the decline in LPH activity is secondary to a disease). The decline in LPH activity in early life in characteristic locations is primary, i.e. genetically determined. Thus, if an adult with northern European ancestry presents with new onset milk intolerance, lactose deficiency is suspected, and an underlying condition such as celiac disease or inflammatory bowel disease is looked for.

In humans, the correlation between mRNA abundance and activity of LPH suggest that transcriptional and post-transcriptional mechanisms are involved in the development of hypolactasia[72]. Post-translational mechanisms may also be involved in the decline in LPH activity, through the modulation of functional protein along the villus. Glycosylation of the protein results in the 225 kDa form, however, the mature BBM LPH enzyme represents a cleavage product of this glycosylated precursor[59,73,74]. The initial cleavage occurs intracellularly in a post-Golgi compartment[75]. This yields a protein which lacks LPH activity. Once LPH is inserted in enterocyte BBM, LPH is once again cleaved, but this time by extracellular trypsin, and this yields the mature and active 145 kDa form of the LPH and it is cleaved[76].

SI

SI is a bifunctional enterocyte BBM disaccharidase with sucrase, isomaltase and maltase activity. Sucrase hydrolyzes sucrose into glucose and fructose. In humans, SI is first detected at 9-10 wk gestation, and gradually increases until just prior to birth, when a marked increase in SI occurs. After birth, there is a rapid decline in SI levels to values comparable to those found in early gestation. Sucrase is not normally a part of the infant's diet, so it is not clear why SI activity is so high in the human fetus.

Fetal human SI protein is in the proSI form from 15-30 wk gestation, but after 30 wk most of the protein consists of sucrase and isomaltase subunits[77]. Enterokinase activity, which activates proteases that cleave proSI, appears at 26 wk, and coincides with the appearance of the sucrase and isomaltase subunits.

SI is transiently expressed in the colon of both humans and rodents[11,13,60,78] in association with the appearance of small intestinal-like morphology. The observation that SI is expressed in colorectal cancer cells suggests that the factors that normally repress SI expression in the colon may be lost in cancer cells.

In mice, low levels of SI mRNA abundance are detectable in the small intestine[78]. However, rat studies show that there is no BBM SI activity from birth until weaning[58]. Thus, even between different types of rodents there are variations in BBM SI development. At weaning, a dramatic increase in SI activity occurs, with adult rat levels being rapidly established. Expression of SI mRNA and protein is first detected in cells located at the crypt-villous junction, suggesting that the enterocytes containing SI are programmed in crypts. As these enterocytes migrate up the villus, the entire villus ultimately becomes populated with cells expressing SI. SI expression first appears in the proximal small intestine, and then proceeds distally to the ileum. This is a genetically programmed event that is not significantly affected by the animals' diet[70,79]. Premature SI induction can be induced by precocious stress, glucocorticosteroids, insulin, or thyroxine[70,79,80].

There is a correlaton between fetal SI activity and mRNA abundance, suggesting control at the level of either mRNA transcription or stability[81]. A number of regulatory elements (including SIF1, SIF2, and SIF3) have been identified within the promoter region of the *SI* gene, and are important for transcriptional induction. CDX-2 binds to the SIF1 element and transactivates the *SI* gene promoter[48]. CSX-2 appears to be the major regulator of SI transcription. A number of other potential transcription factors have been identified, such as HOXC11, which like CDX-2, bind to the SIF1 element of the SI promoter[67]. HNF1α interacts with the SIF3 element and to a lesser extent SIF2, to activate SI transcription[82]. GATA zinc-finger transcription factors interact with a region of the SI promoter upstream of the SIF1 element.

Glycosylation of the SI protein occurs in the endoplasmic reticulum (ER) and in the Golgi apparatus, yielding a 245 kDa protein[83]. Once the protein is inserted into the BBM, the post-translational processing is by the cleavage of the molecule into two subunits which occurs *via* trypsin digestion in the intestinal lumen[83]. The SI subunits remain associated by hydrostatic bonds. Defects in post-translational processing are thought to be responsible for inherited SI deficiency in humans[84].

Glucose transport

The ontogeny of intestinal nutrient transport is largely dependent on the species that is studied. In all mammals, sugar transporter protein does not appear until the intestine differentiates and forms crypts, villi and microvilli. The time at which this process occurs differs between species (please see above), and may be affected by the length of the gestational period. Differentiation of the mucosa alone, however, is not solely responsible for triggering the appearance of transporters, as many of them do not appear until after birth, or even after weaning.

Much of the research on the ontogeny of intestinal transport comes from rodent studies. Rodents are considered to be altricial, meaning that they are born "premature" as compared to humans. Indeed, many of the postnatal changes in the intestine seen in rats occurs parentally in humans, making neonatal rodents an ideal model for premature infants[85]. The pig is also a useful model of ontogeny, due to the similarities between the pig and the human small intestine[86].

The intestinal transport of nutrients, such as glucose, is first detected in the fetal small intestine of mammals, including humans[87]. Both placental nutrients[88,89] as well as the swallowing of amniotic fluid[90] contribute to fetal nutrient acquisition. In fact, the volumes of amniotic fluid which are swallowed in humans *in utero* at term are estimated to be approximately 500 mL/d[91]. Taste buds are detected early in gestation[92], and early experiments have shown that human fetal swallowing increases transamniotic saccharin infusion, and decreases following the

infusion of noxious substance[93]. Injection of galactose into the amniotic fluid of fetal rabbits increases intestinal mucosal weights, as well as the uptake of glucose[94]. Thus, even fetal rabbits are able to up-regulate intestinal transport capacity in response to nutrients. The importance of fetal swallowing in the development of the GI tact is also highlighted by experiments in which fetal sheep underwent esophageal ligation to prevent amniotic fluid from reaching the small intestine[95]. A decrease in small intestinal villous height, intestinal weight and body weight resulted.

Prenatal intestinal transporters are critical for the development of the fetus, as an estimated 10%-15% of fetal protein requirements in rhesus monkeys are met through nutrients that are present in the amniotic fluid[96]. The presence of growth factors released from the GI tract may also be important, as gastric infusion of epidermal growth factor (EGF) reversed the weight loss seen following esophageal ligation[90].

Once the epithelium lining the small intestine differentiates into columnar cells at 9-10 wk of gestation, transport BBM proteins including SGLT1 are expressed[97]. Significant levels of SGLT1 mRNA are also detected in fetal tissue, suggesting that carrier-mediated transport of glucose may be occurring[98]. Dramatic increases in the site density of SGLT1 are observed in fetal pigs between 74% of term and birth[98]. Between 17 and 30 wk gestation in humans, the duodenal-ileal gradient of glucose absorption is established[99]. In rats, glucose transport and SGLT1 protein and mRNA increase at weaning to levels higher than those seen in suckling or in adult animals[100,101]. Curiously, phloridzin does not block glucose transport by SGLT1 in suckling and mature animals to the same extent that it does in weanlings. This may suggest the presence of an age-specific alternative mechanism of glucose transport, or an age-related difference in the phloridzin binding site on SGLT1.

Kinetic analysis of glucose uptake rates in BBM vesicles from human fetal tissue suggests the presence of two transport systems. In addition to SGLT1, a low affinity, high capacity system is detected in the proximal small intestine[102,103]. This may represent GLUT2, which has been described in the BBM of adult rats exposed to high luminal sugar concentrations[104].

Human and rat fetal small intestine also express GLUT1 (as do erythrocytes and brain tissue), which appears earlier than GLUT2, and decreases gradually during fetal life[105,106]. Although the mechanism of this developmental regulation is unknown, GLUT1 may be involved in early cell growth proliferation. Intestinal BLM GLUT2 mRNA is expressed at high levels at birth[101], and GLUT2 transports glucose and fructose. In fact, GLUT2 mRNA is detected in fetal rats as early as day 16 following conception, even before intestinal villi are formed[106]. GLUT2 mRNA increases after weaning, and subsequently decreases to adult levels[101]. GLUT2 in the developing intestine is regulated by luminal glucose and fructose[107]. Luminal perfusion of 20 d old rat pups' intestine with fructose or glucose (100 mmol/L) increases GLUT2 mRNA. This enhancing effect of luminal glucose or fructose was blocked by the transcription inhibitor actinomycin D, but was not affected by the protein synthesis inhibitor cycloheximide. GLUT2 mRNA was also increased in bypassed intestinal loops, suggesting that systemic factors are involved in its regulation. Interestingly, GLUT2 mRNA abundance was even higher in the bypassed loop than in the section that was perfused, suggesting a possible compensatory mechanism due to perceived starvation.

Sugar uptake increases with the gestational age of the animal, and typically peaks immediately after birth, when the intestine takes over the burden of nutrient acquisition from the placenta. Studies done on pigs using the everted sleeve method demonstrate that the maximal transport rate (Vmax) for D-glucose was highest immediately after birth, with a subsequent decrease in the value of Vmax associated with the onset of suckling[86]. In contrast, in newborn pigs the onset of suckling appears to stimulate increases in BLM GLUT2 density[108]. It is not known if GLUT2 activity protein or mRNA can be modified by sugars in the intestinal tract of humans.

At birth, all enterocytes appear to have the capability to transport nutrients. As a result, uptake occurs in enterocytes from all along the villus, rather than just from the upper third, such as occurs in older rats[109]. This may contribute to the higher rate of sugar uptake. Soon after birth, the gradient of increasing transport as one moves from the crypt to the villus is established[109]. This may be responsible for the reduced uptake capacity of the intestine observed postnatally. The "dilution" of fetal enterocytes with new immature cells that do not express transporters may be responsible for this effect. Indeed, the subsequent age-related decline in transport observed in chickens was attributed to reductions in the site density of SGLT1[110].

Developmental changes in the intestinal transport of nutrients may also be non-specific (for example, changes in mucosal surface area, proliferation and migration of enterocytes, or changes in intestinal permeability). Indeed, the subsequent age-related decline in transport observed in chickens was attributed to reductions in the site density of SGLT1[110].

Studies on human premature neonates have used the urinary excretion of D-xylose and 3-O-methyl-glucose as measures as passive and active carrier-mediated monosaccharide absorption of these sugars, respectively, when compared to those born before 28 wk gestation[111]. The replacement of rat fetal enterocytes along the villus requires up to 2 wk, as compared to the 24-48 h required for the replacement of adult enterocytes. Non-specific changes are responsible for ontogenic alterations in mucosal weight, surface area and transport capacity. Postnatal development of enterocytes results in increases in the surface area of microvilli and the BLM[112,113]. Reduced turnover rates result in longer lifetimes of enterocytes, resulting in slower replacement of cells.

Reductions in BBM fluidity occur in post-weaning rabbits, in association with increases in the cholesterol-

to-phospholipid ratio in the BBM[114,115]. In general, reductions in fluidity result in reductions in permeability. Human neonates show decreases in intestinal permeability within the first 30 d of life, as assessed by lactulose/mannitol urinary excretion[116].

Fructose transport

Although SGLT1 and GLUT2 are expressed in enterocytes both in the fetus and at birth, the expression of BBM GLUT5 is only detected in post-weaning rats[101,117-119]. This contrasts with what is seen in pigs[86] and lambs[120]. In rats, GLUT5 protein and mRNA abundance parallel fructose transport, and therefore remain low throughout the suckling phase. GLUT5 protein and mRNA also remain low throughout weaning in rats, with higher levels detected in the post-weaning phase when fructose may first appear in the rat[101,118,119]. This increase in GLUT5 mRNA and protein coincides with the rise in fructose uptake seen at this period. Although there is a temporal association between the introduction of dietary fructose and the appearance of GLUT5, the expression of the transporter is "hard wired" and occurs at this time even in the absence of dietary stimuli[121]. However, the precocious introduction of fructose into the diet of 22 d old rat pups stimulates fructose transport and GLUT5 mRNA expression[121]. Jiang *et al*[122] showed that luminal perfusions of high concentrations (100 mmol/L) of fructose resulted in increases in GLUT5 mRNA and activity. This developmental reprogramming of fructose transport required *de novo* mRNA and protein synthesis, as both actinomycin D and cycloheximide (inhibitors of transcription and translation, respectively) abolished the effect.

In humans, the introduction of solid foods and fruit juice containing fructose at earlier stages of infancy, coupled with the increased use of fructose as a sweetener in dietary products, has resulted in increased exposure to fructose during infancy. Fructose has been implicated as the major cause of "toddler's diarrhea", largely because it is a late onset transporter that increases postnatally in human infants[123]. The infant intestine may not be equipped to absorb high amounts of fructose, resulting in fructose malabsorption. Fructose may then enter the colon, and the high osmolarity may cause osmotic diarrhea. Even in adults, the incidence of fructose intolerance may be increasing: in a recent study malabsorption may be over 70% in persons with persistent, unexplained, non-specific GI symptoms[124]. The dose of fructose used in this study was approximately equivalent to the amount of fructose found in two cans of pop.

Amino acid transport

Amino acid (AA) transporters appear prenatally in the intestines of chickens, rats, rabbits, and humans, and these transporters increase dramatically in the first days after birth[125]. In rats, rabbits, and pigs, the highest rates of BBM AA and peptide uptake are seen at birth, with decreases during suckling and post-weaning[126-128]. In rats, BBM AA transporters are expressed prenatally at the same time or shortly after SGLT1[129]. In 17-20 wk gestation human fetal small intestine, all of the AA transport systems studied (neutral, acidic, basic, and imino) were found to be functional, with a proximal-distal gradient established shortly after cypt-villus formation[130]. In human fetuses, AA transport occurs at 14 wk gestation, with glucose transport at 18 wk, and fatty acid absorption at 24 wk gestation[131].

AA transporters, including NBAT (which transports cationic and neutral AA) and EAAC1 (which transports glutamate), are expressed in suckling rats[132]. The intestinal absorption of peptides occurs *via* PepT1, which is distributed throughout the small intestine. The distribution of these transporters in suckling rats parallels that seen in adult animals. NBAT mRNA is highest in the proximal small intestine, while EAAC1 mRNA was highest in the more distal regions. While a marked crypt-villous gradient was found for PEPT1 and NBAT, EAAC1 immunoreactivity is confined to the lower third of the villus and to the crypts. Thus, the EAAC1 transporter of glutamate is the first AA transporter with decreased expression during epithelial cell differentiation[132].

There are developmental changes in AA and peptide transport. The ontogeny of AA transport is complicated due to the major species differences, and by the large number of AA transport systems, and the fact that some AA are essential or non-essential, depending on the age of the animal. Also protein requirements change throughout the lifespan of an animal, necessitating variations in either intake or uptake of protein or AA. For example, intestinal proline uptake per mg of tissue is maximal at birth in rats, decreases at the end of the suckling phase, and decreases further in older animals. This decline matches both the dietary protein levels and protein requirements[119]. In addition, the decline in uptake of essential AA is greater than that for non-essential AA[129]. This may be because young animals have a disproportionate need for essential AA in early life, due to their rapid growth at this age.

There are also different patterns of uptake for individual AA. For example, in cats, the basic AA transport declines more steeply than does neutral AA transport. In humans, lysine and phenylalanine transport appears later than does the transport of alanine, leucine, taurine and valine[133]. In rats, uptake declines at a similar rate with age from proline, methionine and lysine; however the decline in leucine uptake occurs twice as quickly[129]. Finally, in rats as transition occurs at weaning when the uptake of glucose, fructose, and lysine increase, this is coupled with decreases in proline and leucine uptake[119]. It is unknown why there are such complicated patterns of changes in AA uptake in early life.

Macromolecule transport

The uptake of macromolecules across the intestinal epithelial barrier is an important route by which immunoglobulins, growth factors and antigens are absorbed. This route of entry is especially important in neonates, who rely on it to obtain important immune factors from

maternal colostrum or milk. Most species (including rats, mice, and humans) are born hypoglobulinemic, and absorb IgG from maternal milk through proximal small intestine absorption[134,135].

The transport of macromolecules across the BBM may occur by receptor-mediated or non-specific transcytosis. The transport of macromolecules is facilitated by the presence of protease inhibitors in the maternal colostrum. Rodent studies demonstrate specific intestinal receptors that bind to the Fc portion of IgG[136]. These receptors are transcriptionally regulated, and are present in highest amounts in the duodenum[137]. In humans, IgG is transferred from the placenta to the fetus in the third trimester of pregnancy, with receptors for IgG being detected in the intestine of both the fetus and the neonate. Macromolecular movement across the BBM persists after birth, and then gradually decreases[136]. The initially high permeability of the intestine declines after birth, leading to a process commonly referred to as "gut closure". The time at which gut closure occurs and macromolecular transport ceases varies between species, with a rapid decrease in transport observed in pigs within the first few postnatal days, and a similar decrease seen around 21 d after birth in rats and rabbits[138]. In humans, the exact time that gut closure occurs is unknown, but intrinsic features as well as growth factors, hormones and breast milk may play a role in regulating this process. The decline in permeability may also be related to changes in the thickness and viscosity of the mucus gel layer which coats the BBM. The intervillus mucus gel is increased in weaned as compared to suckling rats. This would potentially increase the effective resistance of the unstirred water layer, and thereby contribute to the decrease in the uptake of macromolecules.

In human infants, uptake of macromolecules or lactoalbumin declines with advancing postconceptual and postnatal age[139]. The ability of the neonatal intestine to adapt to the presence or absence of luminal stimuli is apparent from studies demonstrating a delay in spontaneous closure of the intestine if breastfeeding is postponed beyond the first 30 h of life[140].

Pancreatic enzymes

The higher fecal fat losses in preterm infants compared with term infants is thought to be attributable to lower pancreatic and intestinal lipase activities. Despite the presence of lipase in breast milk and of lipases in the newborn tongue and stomach, micellar absorption of lipids appears later when pancreatic lipase and bile acid concentrations increase[141].

The human exocrine pancreas is functionally immature at birth, with substantial development occurring after birth. Proteolytic enzymes are detected early in the human fetus (20-25 wk gestation), with each enzyme developing in a unique manner. Trypsin increases during fetal life, to reach 90% of childhood levels at term[142]. In contrast, at birth chymotrypsin and carboxypeptidase B levels are less than 60% and 25% of childhood levels, respectively. Despite the differences in the temporal development of proteolytic enzymes, protein digestion in the preterm neonate is adequate, and may be supported by the early development of gastric pepsin and mucosal peptidases[143].

Pancreatic amylase activity is negligible in the human fetus, and is not detectable until one month after birth[144]. Salivary amylase is detected at 20 wk gestation, and while levels are low at birth, they increase to adult levels by the third month following birth[145]. Amylase is also present in human milk, and may aid in the digestion of starch contained in weaning foods[145]. The reduced levels of amylase activity may reflect the low levels of starch in the neonatal diet. At weaning, an increase in amylase activity is detected[144]. While this may be influenced by the appearance of starch in the infant's diet, animal studies demonstrate a persistent increase in amylase activity in rats subjected to prolonged nursing[146]. This suggests the presence of an inherent genetic program for the expression of amylase activity, not necessarily related to the starch content of the diet.

Pancreatic lipase activity in humans is detectable at 32 wk gestation. It remains low at birth, and increases 10 wk after birth[147]. Lingual and gastric lipases, however, are detected at 26 wk gestation[148]. At birth these lipases are able to hydrolyze 60%-70% of ingested fat, even in the absence of pancreatic lipase[149]. Lipase and esterase activity is present in human milk, and contributes to the increased fat absorption observed in breastfed infants[150]. Low lipolytic activity may be the rate-limiting step in the development of efficient fat absorption[143,151,152]. Authors propose that it is the ability to take up long chain fatty acids (LCFA) from the lumen that is the rate-limiting step[153].

The ontogeny of the intestine is "hard wired", and occurs even in the absence of luminal and hormonal factors[41,42]. Still, a number of studies have demonstrated that variations in maternal diets, as well as weaning diets, can influence the ontogeny of the intestine[154-156]. "Critical period programming" is a phenomenon by which a biological mechanism is irreversibly turned on or off once during a lifetime in response to prevailing conditions at a critical stage[157]. This concept, which has also been referred to as "metabolic programming" or "imprinting"[158,159], has been used to explain associations between prenatal/neonatal environment events, alterations in growth and development, and later pathophysiology[160,161]. Early exposure to a diet high in fructose during the suckling-weaning transition may contribute to modest dyslipidemia later in life[162]. Intestinal sugar uptake is also prone to critical period programming[163].

The role of dietary lipids in the programming of intestinal nutrient transport has been studied[154-156,164,165]. Thomson *et al*[164] demonstrated that feeding eight-week old rabbits a low cholesterol diet for two weeks reduced intestinal glucose uptake, and that this effect persisted for at least ten weeks after the animals returned to eating a normal diet. The response to diet depended on the duration of feeding, the age of the animals, and whether or not there was previous exposure to the diet. In this

study, effects on sugar transport were not explained by changes in food intake, body weight or intestinal weight. Furthermore, persistent changes were seen in the active transport of glucose, galactose, leucine and bile acids, while changes in the passive uptake of lipids were reversible.

When the ratio of polyunsaturated to saturated fatty acids in the diet of weanling rats is altered, diets enriched in saturated fatty acids increase hexose uptake, and these alterations were fast, progressive and irreversible[154]. Feeding the same diets to pregnant and lactating rats resulted in similar increases in sugar uptake in their weanling offspring[166]. Curiously, these changes were not seen in the suckling offspring, suggesting that the mechanisms responsible for adaptation may not be fully developed in these animals. Perin *et al*[165] confirmed that the weanling intestine was capable of adaptation, by continuing to feed the offspring of pregnant dams the same diet for three weeks post-weaning. Persistent alterations in sugar uptake were seen in response to variations in dietary lipids, once again emphasizing the importance of early exposure in the programming of intestinal nutrient transport. In addition to the differences between suckling and weanling offspring, the pattern of adaptation also appeared to differ between the jejunum and ileum.

Further studies went on to characterize the effect of diets enriched with arachidonic acid, docohexanoic acid and diets with different ratios of n6 to n3 fatty acids on intestinal nutrient transport[156]. As in the previous study by Perin *et al*[166], these maternal diets critically influenced the ontogeny of the intestine, with many of the changes in transport being irreversible. Furthermore, responsiveness to later dietary challenges depended on early-life feeding experiences, once again emphasizing the importance of early dietary exposure to the development and later adaptability of intestinal transport of nutrients.

Lipid and bile acid transport

Studies using human fetal jejunal explants (14-20 wk gestation) maintained in serum-free organ culture demonstrated increases in chylomicron, VLDL and HDL, paralleled by increases in triglycerides and cholesterol eaters. This demonstrates the ability of the fetal intestine to absorb fat in conjunction with ontogenic increases in lipid and lipoprotein synthesis[167]. Apolipoprotein B synthesis is developmentally regulated: fetal intestine synthesizes only apoB-100 at 11 wk, but both apo-48 and apo-B100 are synthesized at 16 wk, with apoB-46 being predominant in the mature intestine.

Pinocytosis of lipid globules is important after birth. The immature rat intestine is also able to absorb fatty acids and cholesterol[168]. Triglycerides (TG) are digested by gastric and lingual lipases into fatty acids and 2-monoacylglycerols, and their uptake is higher in the immature intestine than in adults[168,169]. Fatty acid binding proteins on the BBM are present in adults[170]. Lipid uptake is thought to be passive in sucklings (Meddings and Theisen, 1989). Once taken up into the enterocytes, lipids are resynthesized into TG, phospholipids (PL) and cholesterol esters (CE)[171].

Bile acids include solubilizing lipids in the intestinal lumen, which facilitate their diffusion through the intestinal unstirred water layer external to the BBM. Intestinal bile acid uptake is an important step in the enterohepatic circulation of bile acids (recently reviewed in Kullak-Ublick *et al*[172], and this uptake is therefore important in the overall process of lipid absorption. Bile acid transporters are curiously absent during suckling when fat intake is high and when bile acid secretion and recycling would be expected to be maximal. It is speculated that the malabsorption may allow bile acids to enter the colon and affect the development of the enteric flora. It is likely that passive absorption of bile acids during the suckling period may be the mechanism by which bile acids are recirculated[172].

Sodium-dependent bile acid transporters in the BBM or cytosol are detected in the rat at weaning[173,174]. Abrupt increases in bile acid transport at weaning occur in rat and human ileum, and are due to parallel increases in the steady state mRNA abundance and transporter number[175,176].

CONCLUSION

Thus, the ontogeny, early growth and development of the intestine is important for the adult gastroenterologist to appreciate, because of the potential for these early life events to affect the responsiveness of the intestine to physiological or pathological challenges in later life.

REFERENCES

1 **Scoville DH**, Sato T, He XC, Li L. Current view: intestinal stem cells and signaling. *Gastroenterology* 2008; **134**: 849-864
2 **de Santa Barbara P**, van den Brink GR, Roberts DJ. Development and differentiation of the intestinal epithelium. *Cell Mol Life Sci* 2003; **60**: 1322-1332
3 **Bitgood MJ**, McMahon AP. Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev Biol* 1995; **172**: 126-138
4 **van de Wetering M**, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 2002; **111**: 241-250
5 **Batlle E**, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, Clevers H. Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell* 2002; **111**: 251-263
6 **Cully M**, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006; **6**: 184-192
7 **Montgomery RK**, Mulberg AE, Grand RJ. Development of the human gastrointestinal tract: twenty years of progress. *Gastroenterology* 1999; **116**: 702-731
8 **Colony PC**. Successive phases of human fetal intestinal development. In: Kretchmer N, Miinkowski A, editors. Nutritional adaptation of the gastrointestinal tract of the newborn. New York: Vevey/Raven Press, 1983: 3-28
9 **Neu J**, Koldovsky O. Nutrient absorption in the preterm neonate. *Clin Perinatol* 1996; **23**: 229-243

10 **Hirano S**, Kataoka K. Histogenesis of the mouse jejunal mucosa, with special reference to proliferative cells and absorptive cells. *Arch Histol Jpn* 1986; **49**: 333-348
11 **Foltzer-Jourdainne C**, Kedinger M, Raul F. Perinatal expression of brush-border hydrolases in rat colon: hormonal and tissue regulations. *Am J Physiol* 1989; **257**: G496-G503
12 **Lacroix B**, Kedinger M, Simon-Assmann P, Rousset M, Zweibaum A, Haffen K. Developmental pattern of brush border enzymes in the human fetal colon. Correlation with some morphogenetic events. *Early Hum Dev* 1984; **9**: 95-103
13 **Zweibaum A**, Hauri HP, Sterchi E, Chantret I, Haffen K, Bamat J, Sordat B. Immunohistological evidence, obtained with monoclonal antibodies, of small intestinal brush border hydrolases in human colon cancers and foetal colons. *Int J Cancer* 1984; **34**: 591-598
14 **Vidrich A**, Buzan JM, Brodrick B, Ilo C, Bradley L, Fendig KS, Sturgill T, Cohn SM. Fibroblast growth factor receptor-3 regulates Paneth cell lineage allocation and accrual of epithelial stem cells during murine intestinal development. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G168-G178
15 **Holnthoner W**, Pillinger M, Groger M, Wolff K, Ashton AW, Albanese C, Neumeister P, Pestell RG, Petzelbauer P. Fibroblast growth factor-2 induces Lef/Tcf-dependent transcription in human endothelial cells. *J Biol Chem* 2002; **277**: 45847-45853
16 **Winton DJ**, Ponder BA. Stem-cell organization in mouse small intestine. *Proc Biol Sci* 1990; **241**: 13-18
17 **Duncan SA**, Manova K, Chen WS, Hoodless P, Weinstein DC, Bachvarova RF, Darnell JE Jr. Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. *Proc Natl Acad Sci USA* 1994; **91**: 7598-7602
18 **Babeu JP**, Darsigny M, Lussier CR, Boudreau F. Hepatocyte nuclear factor 4alpha contributes to an intestinal epithelial phenotype in vitro and plays a partial role in mouse intestinal epithelium differentiation. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G124-G134
19 **Taraviras S**, Mantamadiotis T, Dong-Si T, Mincheva A, Lichter P, Drewes T, Ryffel GU, Monaghan AP, Schütz G. Primary structure, chromosomal mapping, expression and transcriptional activity of murine hepatocyte nuclear factor 4gamma. *Biochim Biophys Acta* 2000; **1490**: 21-32
20 **Archer A**, Sauvaget D, Chauffeton V, Bouchet PE, Chambaz J, Pinçon-Raymond M, Cardot P, Ribeiro A, Lacasa M. Intestinal apolipoprotein A-IV gene transcription is controlled by two hormone-responsive elements: a role for hepatic nuclear factor-4 isoforms. *Mol Endocrinol* 2005; **19**: 2320-2334
21 **Klapper M**, Böhme M, Nitz I, Döring F. The human intestinal fatty acid binding protein (hFABP2) gene is regulated by HNF-4alpha. *Biochem Biophys Res Commun* 2007; **356**: 147-152
22 **Olsen L**, Bressendorff S, Troelsen JT, Olsen J. Differentiation-dependent activation of the human intestinal alkaline phosphatase promoter by HNF-4 in intestinal cells. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G220-G226
23 **Rowley CW**, Staloch LJ, Divine JK, McCaul SP, Simon TC. Mechanisms of mutual functional interactions between HNF-4alpha and HNF-1alpha revealed by mutations that cause maturity onset diabetes of the young. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G466-G475
24 **Burke KT**, Horn PS, Tso P, Heubi JE, Woollett LA. Hepatic bile acid metabolism in the neonatal hamster: expansion of the bile acid pool parallels increased Cyp7a1 expression levels. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G144-G151
25 **Russell DW**, Setchell KD. Bile acid biosynthesis. *Biochemistry* 1992; **31**: 4737-4749
26 **Boehm G**, Braun W, Moro G, Minoli I. Bile acid concentrations in serum and duodenal aspirates of healthy preterm infants: effects of gestational and postnatal age. *Biol Neonate* 1997; **71**: 207-214
27 **Heubi JE**, Balistreri WF, Suchy FJ. Bile salt metabolism in the first year of life. *J Lab Clin Med* 1982; **100**: 127-136
28 **De Plaen IG**, Liu SX, Tian R, Neequaye I, May MJ, Han XB, Hsueh W, Jilling T, Lu J, Caplan MS. Inhibition of nuclear factor-kappaB ameliorates bowel injury and prolongs survival in a neonatal rat model of necrotizing enterocolitis. *Pediatr Res* 2007; **61**: 716-721
29 **Liu Y**, Zhu L, Fatheree NY, Liu X, Pacheco SE, Tatevian N, Rhoads JM. Changes in intestinal Toll-like receptors and cytokines precede histological injury in a rat model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G442-G450
30 **Bajwa PJ**, Lee JW, Straus DS, Lytle C. Activation of PPARgamma by rosiglitazone attenuates intestinal Cl- secretion. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G82-G89
31 **Chen L**, Bush CR, Necela BM, Su W, Yanagisawa M, Anastasiadis PZ, Fields AP, Thompson EA. RS5444, a novel PPARgamma agonist, regulates aspects of the differentiated phenotype in nontransformed intestinal epithelial cells. *Mol Cell Endocrinol* 2006; **251**: 17-32
32 **Drori S**, Girnun GD, Tou L, Szwaya JD, Mueller E, Xia K, Shivdasani RA, Spiegelman BM. Hic-5 regulates an epithelial program mediated by PPARgamma. *Genes Dev* 2005; **19**: 362-375
33 **Thompson EA**. PPARgamma physiology and pathology in gastrointestinal epithelial cells. *Mol Cells* 2007; **24**: 167-176
34 **Moxey PC**, Trier JS. Specialized cell types in the human fetal small intestine. *Anat Rec* 1978; **191**: 269-285
35 **Roth KA**, Hermiston ML, Gordon JI. Use of transgenic mice to infer the biological properties of small intestinal stem cells and to examine the lineage relationships of their descendants. *Proc Natl Acad Sci USA* 1991; **88**: 9407-9411
36 **Simon TC**, Gordon JI. Intestinal epithelial cell differentiation: new insights from mice, flies and nematodes. *Curr Opin Genet Dev* 1995; **5**: 577-586
37 **Podolsky DK**. Regulation of intestinal epithelial proliferation: a few answers, many questions. *Am J Physiol* 1993; **264**: G179-G186
38 **Lovshin J**, Drucker DJ. Synthesis, secretion and biological actions of the glucagon-like peptides. *Pediatr Diabetes* 2000; **1**: 49-57
39 **Murphy BE**. Ontogeny of cortisol-cortisone interconversion in human tissues: a role for cortisone in human fetal development. *J Steroid Biochem* 1981; **14**: 811-817
40 **Marti A**, Fernandez-Otero MP. Prostaglandin E2 accelerates enzymatic and morphological maturation of the small intestine in suckling rats. *Biol Neonate* 1994; **65**: 119-125
41 **McDonald MC**, Henning SJ. Synergistic effects of thyroxine and dexamethasone on enzyme ontogeny in rat small intestine. *Pediatr Res* 1992; **32**: 306-311
42 **Winter HS**, Hendren RB, Fox CH, Russell GJ, Perez-Atayde A, Bhan AK, Folkman J. Human intestine matures as nude mouse xenograft. *Gastroenterology* 1991; **100**: 89-98
43 **Savidge TC**, Morey AL, Ferguson DJ, Fleming KA, Shmakov AN, Phillips AD. Human intestinal development in a severe-combined immunodeficient xenograft model. *Differentiation* 1995; **58**: 361-371
44 **Montgomery RK**, Sybicki MA, Grand RJ. Autonomous biochemical and morphological differentiation in fetal rat intestine transplanted at 17 and 20 days of gestation. *Dev Biol* 1981; **87**: 76-84
45 **Stanton BR**, Perkins AS, Tessarollo L, Sassoon DA, Parada LF. Loss of N-myc function results in embryonic lethality and failure of the epithelial component of the embryo to develop. *Genes Dev* 1992; **6**: 2235-2247

46 **Ang SL**, Rossant J. HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* 1994; **78**: 561-574
47 **Weinstein DC**, Ruiz i Altaba A, Chen WS, Hoodless P, Prezioso VR, Jessell TM, Darnell JE Jr. The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. *Cell* 1994; **78**: 575-588
48 **Suh E**, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. *Mol Cell Biol* 1994; **14**: 7340-7351
49 **Roberts DJ**, Johnson RL, Burke AC, Nelson CE, Morgan BA, Tabin C. Sonic hedgehog is an endodermal signal inducing Bmp-4 and Hox genes during induction and regionalization of the chick hindgut. *Development* 1995; **121**: 3163-3174
50 **Roberts DJ**, Smith DM, Goff DJ, Tabin CJ. Epithelial-mesenchymal signaling during the regionalization of the chick gut. *Development* 1998; **125**: 2791-2801
51 **Basora N**, Vachon PH, Herring-Gillam FE, Perreault N, Beaulieu JF. Relation between integrin alpha7Bbeta1 expression in human intestinal cells and enterocytic differentiation. *Gastroenterology* 1997; **113**: 1510-1521
52 **Rao M**, Manishen WJ, Maheshwari Y, Sykes DE, Siyanova EY, Tyner AL, Weiser MM. Laminin receptor expression in rat intestine and liver during development and differentiation. *Gastroenterology* 1994; **107**: 764-772
53 **Sanderson IR**, Ezzell RM, Kedinger M, Erlanger M, Xu ZX, Pringault E, Leon-Robine S, Louvard D, Walker WA. Human fetal enterocytes in vitro: modulation of the phenotype by extracellular matrix. *Proc Natl Acad Sci USA* 1996; **93**: 7717-7722
54 **Hynes RO**. Targeted mutations in cell adhesion genes: what have we learned from them? *Dev Biol* 1996; **180**: 402-412
55 **Seetharam B**, Yeh KY, Moog F, Alpers DH. Development of intestinal brush border membrane proteins in the rat. *Biochim Biophys Acta* 1977; **470**: 424-436
56 **Vaeth GF**, Henning SJ. Postnatal development of peptidase enzymes in rat small intestine. *J Pediatr Gastroenterol Nutr* 1982; **1**: 111-117
57 **Martin GR**, Henning SJ. Enzymic development of the small intestine: are glucocorticoids necessary? *Am J Physiol* 1984; **246**: G695-G699
58 **Leeper LL**, Henning SJ. Development and tissue distribution of sucrase-isomaltase mRNA in rats. *Am J Physiol* 1990; **258**: G52-G58
59 **Montgomery RK**, Büller HA, Rings EH, Grand RJ. Lactose intolerance and the genetic regulation of intestinal lactase-phlorizin hydrolase. *FASEB J* 1991; **5**: 2824-2832
60 **Goda T**, Yasutake H, Tanaka T, Takase S. Lactase-phlorizin hydrolase and sucrase-isomaltase genes are expressed differently along the villus-crypt axis of rat jejunum. *J Nutr* 1999; **129**: 1107-1113
61 **Lacroix B**, Kedinger M, Simon-Assmann P, Haffen K. Early organogenesis of human small intestine: scanning electron microscopy and brush border enzymology. *Gut* 1984; **25**: 925-930
62 **Villa M**, Ménard D, Semenza G, Mantei N. The expression of lactase enzymatic activity and mRNA in human fetal jejunum. Effect of organ culture and of treatment with hydrocortisone. *FEBS Lett* 1992; **301**: 202-206
63 **Escher JC**, de Koning ND, van Engen CG, Arora S, Büller HA, Montgomery RK, Grand RJ. Molecular basis of lactase levels in adult humans. *J Clin Invest* 1992; **89**: 480-483
64 **Wang Y**, Harvey C, Rousset M, Swallow DM. Expression of human intestinal mRNA transcripts during development: analysis by a semiquantitative RNA polymerase chain reaction method. *Pediatr Res* 1994; **36**: 514-521
65 **Fajardo O**, Naim HY, Lacey SW. The polymorphic expression of lactase in adults is regulated at the messenger RNA level. *Gastroenterology* 1994; **106**: 1233-1241
66 **Troelsen JT**, Mitchelmore C, Spodsberg N, Jensen AM, Norén O, Sjöström H. Regulation of lactase-phlorizin hydrolase gene expression by the caudal-related homoeodomain protein Cdx-2. *Biochem J* 1997; **322** (Pt 3): 833-838
67 **Mitchelmore C**, Troelsen JT, Sjöström H, Norén O. The HOXC11 homeodomain protein interacts with the lactase-phlorizin hydrolase promoter and stimulates HNF1alpha-dependent transcription. *J Biol Chem* 1998; **273**: 13297-13306
68 **Fitzgerald K**, Bazar L, Avigan MI. GATA-6 stimulates a cell line-specific activation element in the human lactase promoter. *Am J Physiol* 1998; **274**: G314-G324
69 **Galand G**. Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. Comparative development, effects of glucocorticoids, molecular mechanisms, and phylogenetic implications. *Comp Biochem Physiol B* 1989; **94**: 1-11
70 **Koldovsky O**. Developmental, dietary and hormonal control of intestinal disaccharidases in mammals (including man). In: Randle PJ, Steiner DF, Whelan WJ, editors. Carbohydrate metabolism and its disorders. London: Academic Press, 1981: 481-522
71 **Büller HA**, Grand RJ. Lactose intolerance. *Annu Rev Med* 1990; **41**: 141-148
72 **Rossi M**, Maiuri L, Fusco MI, Salvati VM, Fuccio A, Auricchio S, Mantei N, Zecca L, Gloor SM, Semenza G. Lactase persistence versus decline in human adults: multifactorial events are involved in down-regulation after weaning. *Gastroenterology* 1997; **112**: 1506-1514
73 **Naim HY**, Lentze MJ. Impact of O-glycosylation on the function of human intestinal lactase-phlorizin hydrolase. Characterization of glycoforms varying in enzyme activity and localization of O-glycoside addition. *J Biol Chem* 1992; **267**: 25494-25504
74 **Danielsen EM**, Skovbjerg H, Norén O, Sjöström H. Biosynthesis of intestinal microvillar proteins. Intracellular processing of lactase-phlorizin hydrolase. *Biochem Biophys Res Commun* 1984; **122**: 82-90
75 **Lottaz D**, Oberholzer T, Bähler P, Semenza G, Sterchi EE. Maturation of human lactase-phlorizin hydrolase. Proteolytic cleavage of precursor occurs after passage through the Golgi complex. *FEBS Lett* 1992; **313**: 270-276
76 **Wüthrich M**, Grünberg J, Hahn D, Jacob R, Radebach I, Naim HY, Sterchi EE. Proteolytic processing of human lactase-phlorizin hydrolase is a two-step event: identification of the cleavage sites. *Arch Biochem Biophys* 1996; **336**: 27-34
77 **Triadou N**, Zweibaum A. Maturation of sucrase-isomaltase complex in human fetal small and large intestine during gestation. *Pediatr Res* 1985; **19**: 136-138
78 **Tung J**, Markowitz AJ, Silberg DG, Traber PG. Developmental expression of SI is regulated in transgenic mice by an evolutionarily conserved promoter. *Am J Physiol* 1997; **273**: G83-G92
79 **Henning SJ**. Ontogeny of enzymes in the small intestine. *Annu Rev Physiol* 1985; **47**: 231-245
80 **Buts JP**, Duranton B, De Keyser N, Sokal EM, Maernhout AS, Raul F, Marandi S. Premature stimulation of rat sucrase-isomaltase (SI) by exogenous insulin and the analog B-Asp10 is regulated by a receptor-mediated signal triggering SI gene transcription. *Pediatr Res* 1998; **43**: 585-591
81 **Sebastio G**, Hunziker W, O'Neill B, Malo C, Ménard D, Auricchio S, Semenza G. The biosynthesis of intestinal sucrase-isomaltase in human embryo is most likely controlled at the level of transcription. *Biochem Biophys Res Commun* 1987; **149**: 830-839
82 **Wu GD**, Chen L, Forslund K, Traber PG. Hepatocyte nuclear factor-1 alpha (HNF-1 alpha) and HNF-1 beta regulate transcription via two elements in an intestine-specific promoter. *J Biol Chem* 1994; **269**: 17080-17085
83 **Naim HY**, Sterchi EE, Lentze MJ. Biosynthesis of the human

sucrase-isomaltase complex. Differential O-glycosylation of the sucrase subunit correlates with its position within the enzyme complex. *J Biol Chem* 1988; **263**: 7242-7253
84 **Fransen JA**, Hauri HP, Ginsel LA, Naim HY. Naturally occurring mutations in intestinal sucrase-isomaltase provide evidence for the existence of an intracellular sorting signal in the isomaltase subunit. *J Cell Biol* 1991; **115**: 45-57
85 **Koldovský O**. Search for role of milk-borne biologically active peptides for the suckling. *J Nutr* 1989; **119**: 1543-1551
86 **Puchal AA**, Buddington RK. Postnatal development of monosaccharide transport in pig intestine. *Am J Physiol* 1992; **262**: G895-G902
87 **Rubin DC**. Spatial analysis of transcriptional activation in fetal rat jejunal and ileal gut epithelium. *Am J Physiol* 1992; **263**: G853-G863
88 **Chabot JG**, Payet N, Hugon JS. Effects of epidermal growth factor (EGF) on adult mouse small intestine in vivo and in organ culture. *Comp Biochem Physiol A Comp Physiol* 1983; **74**: 247-252
89 **Chapman JF**, Phillips JC, Rosenthal MA, Herbert WN. Evaluation of the PG-Numeric assay for semi-automated analysis for phosphatidylglycerol in amniotic fluid. *Clin Chem* 1990; **36**: 1974-1977
90 **Mulvihill SJ**, Stone MM, Debas HT, Fonkalsrud EW. The role of amniotic fluid in fetal nutrition. *J Pediatr Surg* 1985; **20**: 668-672
91 **Pritchard JA**. Fetal swallowing and amniotic fluid volume. *Obstet Gynecol* 1966; **28**: 606-610
92 **Bradley RM**, Mistretta CM. Fetal sensory receptors. *Physiol Rev* 1975; **55**: 352-382
93 **Liley AW**. The foetus as a personality. *Aust N Z J Psychiatry* 1972; **6**: 99-105
94 **Buchmiller TL**, Fonkalsrud EW, Kim CS, Chopourian HL, Shaw KS, Lam MM, Diamond JM. Upregulation of nutrient transport in fetal rabbit intestine by transamniotic substrate administration. *J Surg Res* 1992; **52**: 443-447
95 **Trahair JF**, Harding R, Bocking AD, Silver M, Robinson PM. The role of ingestion in the development of the small intestine in fetal sheep. *Q J Exp Physiol* 1986; **71**: 99-104
96 **Pitkin RM**, Reynolds WA. Fetal ingestion and metabolism of amniotic fluid protein. *Am J Obstet Gynecol* 1975; **123**: 356-363
97 **Buddington RK**. Nutrition and ontogenetic development of the intestine. *Can J Physiol Pharmacol* 1994; **72**: 251-259
98 **Buddington RK**, Malo C. Intestinal brush-border membrane enzyme activities and transport functions during prenatal development of pigs. *J Pediatr Gastroenterol Nutr* 1996; **23**: 51-64
99 **Malo C**, Berteloot A. Proximo-distal gradient of Na+-dependent D-glucose transport activity in the brush border membrane vesicles from the human fetal small intestine. *FEBS Lett* 1987; **220**: 201-205
100 **Khan JM**, Wingertzahn MA, Teichberg S, Vancurova I, Harper RG, Wapnir RA. Development of the intestinal SGLT1 transporter in rats. *Mol Genet Metab* 2000; **69**: 233-239
101 **Shu R**, David ES, Ferraris RP. Dietary fructose enhances intestinal fructose transport and GLUT5 expression in weaning rats. *Am J Physiol* 1997; **272**: G446-G453
102 **Malo C**. Kinetic evidence for heterogeneity in Na+-D-glucose cotransport systems in the normal human fetal small intestine. *Biochim Biophys Acta* 1988; **938**: 181-188
103 **Malo C**. Separation of two distinct Na+/D-glucose cotransport systems in the human fetal jejunum by means of their differential specificity for 3-O-methylglucose. *Biochim Biophys Acta* 1990; **1022**: 8-16
104 **Kellett GL**, Helliwell PA. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem J* 2000; **350** Pt 1: 155-162
105 **Davidson NO**, Hausman AM, Ifkovits CA, Buse JB, Gould GW, Burant CF, Bell GI. Human intestinal glucose transporter expression and localization of GLUT5. *Am J Physiol* 1992; **262**: C795-C800
106 **Matsumoto K**, Takao Y, Akazawa S, Yano M, Uotani S, Kawasaki E, Takino H, Yamasaki H, Okuno S, Yamaguchi Y. Developmental change of facilitative glucose transporter expression in rat embryonal and fetal intestine. *Biochem Biophys Res Commun* 1993; **193**: 1275-1282
107 **Cui XL**, Jiang L, Ferraris RP. Regulation of rat intestinal GLUT2 mRNA abundance by luminal and systemic factors. *Biochim Biophys Acta* 2003; **1612**: 178-185
108 **Cherbuy C**, Darcy-Vrillon B, Posho L, Vaugelade P, Morel MT, Bernard F, Leturque A, Penicaud L, Duée PH. GLUT2 and hexokinase control proximodistal gradient of intestinal glucose metabolism in the newborn pig. *Am J Physiol* 1997; **272**: G1530-G1539
109 **Smith MW**. Expression of digestive and absorptive function in differentiating enterocytes. *Annu Rev Physiol* 1985; **47**: 247-260
110 **Barfull A**, Garriga C, Mitjans M, Planas JM. Ontogenetic expression and regulation of Na(+)-D-glucose cotransporter in jejunum of domestic chicken. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G559-G564
111 **Rouwet EV**, Heineman E, Buurman WA, ter Riet G, Ramsay G, Blanco CE. Intestinal permeability and carrier-mediated monosaccharide absorption in preterm neonates during the early postnatal period. *Pediatr Res* 2002; **51**: 64-70
112 **Morikawa Y**, Fujimoto K, Okada T. Morphometrical changes in the apical surface of the colonic absorptive cells in perinatal rats with special reference to the effect of fetal oral administration of milk in utero. *Lab Anim* 1991; **25**: 242-246
113 **Vagnerová R**, Kubínová L, Pácha J. Correlation of function and structure in developing rat distal colon. *Cell Tissue Res* 1997; **288**: 95-99
114 **Schwarz SM**, Bostwick HE, Danziger MD, Newman LJ, Medow MS. Ontogeny of basolateral membrane lipid composition and fluidity in small intestine. *Am J Physiol* 1989; **257**: G138-G144
115 **Schwarz SM**, Ling SD, Hostetler B, Draper JP, Watkins JB. Lipid composition and membrane fluidity in the small intestine of the developing rabbit. *Gastroenterology* 1984; **86**: 1544-1551
116 **Catassi C**, Bonucci A, Coppa GV, Carlucci A, Giorgi PL. Intestinal permeability changes during the first month: effect of natural versus artificial feeding. *J Pediatr Gastroenterol Nutr* 1995; **21**: 383-386
117 **Castelló A,** Gumá A, Sevilla L, Furriols M, Testar X, Palacín M, Zorzano A. Regulation of GLUT5 gene expression in rat intestinal mucosa: regional distribution, circadian rhythm, perinatal development and effect of diabetes. *Biochem J* 1995; **309** (Pt 1): 271-277
118 **Rand EB**, Depaoli AM, Davidson NO, Bell GI, Burant CF. Sequence, tissue distribution, and functional characterization of the rat fructose transporter GLUT5. *Am J Physiol* 1993; **264**: G1169-G1176
119 **Toloza EM**, Diamond J. Ontogenetic development of nutrient transporters in rat intestine. *Am J Physiol* 1992; **263**: G593-G604
120 **Char VC**, Rudolph AM. Digestion and absorption of carbohydrates by the fetal lamb in utero. *Pediatr Res* 1979; **13**: 1018-1023
121 **Shu R**, David ES, Ferraris RP. Luminal fructose modulates fructose transport and GLUT-5 expression in small intestine of weaning rats. *Am J Physiol* 1998; **274**: G232-G239
122 **Jiang L**, Ferraris RP. Developmental reprogramming of rat GLUT-5 requires de novo mRNA and protein synthesis. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G113-G120
123 **Hoekstra JH**, van Kempen AA, Bijl SB, Kneepkens CM. Fructose breath hydrogen tests. *Arch Dis Child* 1993; **68**: 136-138

124 **Choi YK**, Johlin FC Jr, Summers RW, Jackson M, Rao SS. Fructose intolerance: an under-recognized problem. *Am J Gastroenterol* 2003; **98**: 1348-1353
125 **Younoszai MK**, Lynch A. In vivo D-glucose absorption in the developing rat small intestine. *Pediatr Res* 1975; **9**: 130-133
126 **Navab F**, Winter CG. Effect of aging on intestinal absorption of aromatic amino acids in vitro in the rat. *Am J Physiol* 1988; **254**: G631-G636
127 **Himukai M**, Konno T, Hoshi T. Age-dependent change in intestinal absorption of dipeptides and their constituent amino acids in the guinea pig. *Pediatr Res* 1980; **14**: 1272-1275
128 **Guandalini S**, Rubino A. Development of dipeptide transport in the intestinal mucosa of rabbits. *Pediatr Res* 1982; **16**: 99-103
129 **Buddington RK**, Diamond JM. Ontogenetic development of intestinal nutrient transporters. *Annu Rev Physiol* 1989; **51**: 601-619
130 **Malo C**. Multiple pathways for amino acid transport in brush border membrane vesicles isolated from the human fetal small intestine. *Gastroenterology* 1991; **100**: 1644-1652
131 **Lebenthal A**, Lebenthal E. The ontogeny of the small intestinal epithelium. *JPEN J Parenter Enteral Nutr* 1999; **23**: S3-S6
132 **Rome S**, Barbot L, Windsor E, Kapel N, Tricottet V, Huneau JF, Reynes M, Gobert JG, Tomé D. The regionalization of PepT1, NBAT and EAAC1 transporters in the small intestine of rats are unchanged from birth to adulthood. *J Nutr* 2002; **132**: 1009-1011
133 **Moriyama IS**. [Development of fetal organs and adaptation to extrauterine life] *Nippon Sanka Fujinka Gakkai Zasshi* 1986; **38**: 1227-1237
134 **Israel EJ**, Taylor S, Wu Z, Mizoguchi E, Blumberg RS, Bhan A, Simister NE. Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology* 1997; **92**: 69-74
135 **Udall JN**, Bloch KJ, Vachino G, Feldman P, Walker WA. Development of the gastrointestinal mucosal barrier. IV. The effect of inhibition of proteolysis on the uptake of macromolecules by the intestine of the newborn rabbit before and after weaning. *Biol Neonate* 1984; **45**: 289-295
136 **Jakoi ER**, Cambier J, Saslow S. Transepithelial transport of maternal antibody: purification of IgG receptor from newborn rat intestine. *J Immunol* 1985; **135**: 3360-3364
137 **Martín MG**, Wu SV, Walsh JH. Ontogenetic development and distribution of antibody transport and Fc receptor mRNA expression in rat intestine. *Dig Dis Sci* 1997; **42**: 1062-1069
138 **Teichberg S**, Isolauri E, Wapnir RA, Roberts B, Lifshitz F. Development of the neonatal rat small intestinal barrier to nonspecific macromolecular absorption: effect of early weaning to artificial diets. *Pediatr Res* 1990; **28**: 31-37
139 **Jakobsson I**, Lindberg T, Lothe L, Axelsson I, Benediktsson B. Human alpha-lactalbumin as a marker of macromolecular absorption. *Gut* 1986; **27**: 1029-1034
140 **Vukavić T**. Timing of the gut closure. *J Pediatr Gastroenterol Nutr* 1984; **3**: 700-703
141 **Thomson AB**, Keelan M. The aging gut. *Can J Physiol Pharmacol* 1986; **64**: 30-38
142 **Lebenthal E**, Lee PC. Development of functional responses in human exocrine pancreas. *Pediatrics* 1980; **66**: 556-560
143 **Lindberg T**. Intestinal dipeptidases: characterization, development and distribution of intestinal dipeptidases of the human foetus. *Clin Sci* 1966; **30**: 505-515
144 **Sevenhuysen GP**, Holodinsky C, Dawes C. Development of salivary alpha-amylase in infants from birth to 5 months. *Am J Clin Nutr* 1984; **39**: 584-588
145 **Dewit O**, Dibba B, Prentice A. Breast-milk amylase activity in English and Gambian mothers: effects of prolonged lactation, maternal parity, and individual variations. *Pediatr Res* 1990; **28**: 502-506
146 **Lee PC**, Kim OK, Lebenthal E. Effect of early weaning and prolonged nursing on development of the rat pancreas. *Pediatr Res* 1982; **16**: 470-473
147 **Cleghorn G**, Durie P, Benjamin L, Dati F. The ontogeny of serum immunoreactive pancreatic lipase and cationic trypsinogen in the premature human infant. *Biol Neonate* 1988; **53**: 10-16
148 **Hamosh M**, Scanlon JW, Ganot D, Likel M, Scanlon KB, Hamosh P. Fat digestion in the newborn. Characterization of lipase in gastric aspirates of premature and term infants. *J Clin Invest* 1981; **67**: 838-846
149 **Sheldon W**. Congenital pancreatic lipase deficiency. *Arch Dis Child* 1964; **39**: 268-271
150 **Alemi B**, Hamosh M, Scanlon JW, Salzman-Mann C, Hamosh P. Fat digestion in very low-birth-weight infants: effect of addition of human milk to low-birth-weight formula. *Pediatrics* 1981; **68**: 484-489
151 **Hernell O**, Bläckberg L, Bernbäck S. Digestion of human milk fat in early infancy. *Acta Paediatr Scand Suppl* 1989; **351**: 57-62
152 **Boehm G**, Borte M, Müller H, Moro G, Minoli I. Activities of trypsin and lipase in duodenal aspirates of preterm infants: influence of dietary protein and fat composition. *Am J Clin Nutr* 1995; **61**: 524-527
153 **Rings EH**, Minich DM, Vonk RJ, Stellaard F, Fetter WP, Verkade HJ. Functional development of fat absorption in term and preterm neonates strongly correlates with ability to absorb long-chain Fatty acids from intestinal lumen. *Pediatr Res* 2002; **51**: 57-63
154 **Thomson AB**, Keelan M, Garg M, Clandinin MT. Evidence for critical-period programming of intestinal transport function: variations in the dietary ratio of polyunsaturated to saturated fatty acids alters ontogeny of the rat intestine. *Biochim Biophys Acta* 1989; **1001**: 302-315
155 **Perin NM**, Clandinin T, Thomson AB. Importance of milk and diet on the ontogeny and adaptation of the intestine. *J Pediatr Gastroenterol Nutr* 1997; **24**: 419-425
156 **Jarocka-Cyrta E**, Perin N, Keelan M, Wierzbicki E, Wierzbicki T, Clandinin MT, Thomson AB. Early dietary experience influences ontogeny of intestine in response to dietary lipid changes in later life. *Am J Physiol* 1998; **275**: G250-G258
157 **Karasov WH**, Solberg DH, Chang SD, Hughes M, Stein ED, Diamond JM. Is intestinal transport of sugars and amino acids subject to critical-period programming? *Am J Physiol* 1985; **249**: G770-G785
158 **Lucas A**. Programming by early nutrition: an experimental approach. *J Nutr* 1998; **128**: 401S-406S
159 **Lucas A**. Programming not metabolic imprinting. *Am J Clin Nutr* 2000; **71**: 602
160 **Barker DJ**, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993; **341**: 938-941
161 **Seckl J**. Mechanisms of fetal origins of health problems in adults need to be investigated. *BMJ* 1998; **317**: 210
162 **Bell RC**, Hoedl A, Turchinsky J. Fructose feeding in the suckling-weaning transition in rats: effects on hyperlipidemia in adulthood. *Arch Physiol Biochem* 2003; **111**: 17-22
163 **Keelan M**, Thomson AB, Garg ML, Clandinin MT. Critical period programming of intestinal glucose transport via alterations in dietary fatty acid composition. *Can J Physiol Pharmacol* 1990; **68**: 642-645
164 **Thomson AB**, Keelan M. Late effects of early feeding of a low cholesterol diet on the intestinal active and passive transport properties in the rabbit. *Mech Ageing Dev* 1987; **40**: 157-170
165 **Perin N**, Jarocka-Cyrta E, Keelan M, Clandinin T, Thomson A. Dietary lipid composition modifies intestinal morphology and nutrient transport in young rats. *J Pediatr Gastroenterol Nutr* 1999; **28**: 46-53

166 **Perin N**, Keelan M, Jarocka-Cyrta E, Clandinin MT, Thomson AB. Ontogeny of intestinal adaptation in rats in response to isocaloric changes in dietary lipids. *Am J Physiol* 1997; **273**: G713-G720
167 **Thibault L**, Ménard D, Loirdighi N, Levy E. Ontogeny of intestinal lipid and lipoprotein synthesis. *Biol Neonate* 1992; **62**: 100-107
168 **Meddings JB**, Theisen S. Development of rat jejunum: lipid permeability, physical properties, and chemical composition. *Am J Physiol* 1989; **256**: G931-G940
169 **Flores CA**, Hing SA, Wells MA, Koldovský O. Rates of triolein absorption in suckling and adult rats. *Am J Physiol* 1989; **257**: G823-G829
170 **Agellon LB**, Toth MJ, Thomson AB. Intracellular lipid binding proteins of the small intestine. *Mol Cell Biochem* 2002; **239**: 79-82
171 **Shiau YF**, Umstetter C, Kendall K, Koldovsky O. Development of fatty acid esterification mechanisms in rat small intestine. *Am J Physiol* 1979; **237**: E399-E403
172 **Kullak-Ublick GA**, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology* 2004; **126**: 322-342
173 **Stahl GE**, Mascarenhas MR, Fayer JC, Shiau YF, Watkins JB. Passive jejunal bile salt absorption alters the enterohepatic circulation in immature rats. *Gastroenterology* 1993; **104**: 163-173
174 **Christie DM**, Dawson PA, Thevananther S, Shneider BL. Comparative analysis of the ontogeny of a sodium-dependent bile acid transporter in rat kidney and ileum. *Am J Physiol* 1996; **271**: G377-G385
175 **Little JM**, Lester R. Ontogenesis of intestinal bile salt absorption in the neonatal rat. *Am J Physiol* 1980; **239**: G319-G323
176 **Barnard JA**, Ghishan FK, Wilson FA. Ontogenesis of taurocholate transport by rat ileal brush border membrane vesicles. *J Clin Invest* 1985; **75**: 869-873

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EDITORIAL

Is the control of dietary cholesterol intake sufficiently effective to ameliorate nonalcoholic fatty liver disease?

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Abstract

In our examination of the distribution of abdominal fat, dietary intake and biochemical data in patients with nonalcoholic fatty liver disease (NAFLD), non-obese NAFLD patients without insulin resistance presented a characteristic pattern of dietary intake. Dietary cholesterol intake was superabundant in non-obese patients compared with obese patients, although total energy and carbohydrate intake was not excessive. Namely, excess cholesterol intake appears to be one of the main factors associated with NAFLD development and liver injury. Therefore, the control of dietary cholesterol intake may lead to an improvement in NAFLD, and the NPC1L1 inhibitor ezetimibe might be a promising treatment for NAFLD. We review one pathogenic aspect of lipid metabolism dysregulation in NAFLD and survey new strategies for NAFLD treatment based on the modification of cholesterol metabolism.

Key words: Cholesterol; Ezetimibe; Nonalcoholic fatty liver disease; NPC1L1; Polyunsaturated fatty acids

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), which encompasses a broad spectrum of liver disorders ranging from simple hepatic steatosis to steatohepatitis and cirrhosis, is currently the most common cause of chronic liver disease and abnormal liver function tests in Western countries. The development of hepatic steatosis is considered to be associated with an excess intake of calories, visceral obesity and insulin resistance, which result in an increased release of free fatty acids from adipocytes and increased rates of fatty acid synthesis in the liver[1,2]. However, the mechanisms involved in the pathogenesis of NAFLD in humans have not been thoroughly investigated. Because of the associated triglyceride accumulation in hepatocytes, NAFLD has been mainly investigated as a lipogenic disorder. Indeed, fatty acid overload because of the acceleration of *de novo* synthesis and cellular uptake results in mitochondrial dysfunction, oxidative stress and impaired VLDL formation, which lead to disease progression[1,2]. These changes related to lipid metabolism were positively linked to transcriptomic and metabolomic profiles in rats with NAFLD induced by a high fat diet[3]. In addition, from our analyses of the expression profile of fatty acid metabolism-associated genes in

biopsy samples from NAFLD liver, a similar expression pattern was seen, which indicated that the expression of sterol regulatory element-binding protein-1c (SREBP-1c), a positive regulator of fatty acid synthesis, was still upregulated and the expression of AMP-activated protein kinase, a negative regulator of fatty acid synthesis, was down-regulated despite the increased uptake of free fatty acids and intracellular accumulation of fatty acids and triglycerides[4-9]. These results suggest a breakdown of the feedback regulation from the increased level of intracellular fatty acids. Recently, it has been considered that cholesterol metabolism has a significant role in the pathogenesis of NAFLD. In the examination of cholesterol metabolism-associated genes, despite cholesterol overload in hepatocytes, *de novo* synthesis of cholesterol is still activated in the NAFLD liver, meaning that cholesterol metabolism is dysregulated in NAFLD[10]. This review focuses on the intrahepatic cholesterol dysregulation in NAFLD and potential emerging therapies for NAFLD.

To understand the nature of NAFLD, a nutritional approach provided helpful information. Among NAFLD patients, a large percentage of patients have obesity with insulin resistance, however, many non-obese individuals are also included[11,12]. Considering visceral fat and insulin resistance, which are evident in obese patients, the distribution of abdominal fat, dietary intake and biochemical data were compared between obese (BMI > 25 kg/m^2) and non-obese patients (BMI < 25 kg/m^2) to identify potential nutritional factors that affect NAFLD[13,14]. Visceral fat and dietary intake of total energy and carbohydrates were at overtly higher levels in the obese group as a matter of course. In contrast, in non-obese patients, dietary cholesterol was significantly higher and dietary polyunsaturated fatty acids (PUFA) were significantly lower than those in obese patients. Mean concentrations of serum total cholesterol, LDL-cholesterol and triglycerides were near the upper limit of the normal range, and serum levels of adipocytokines were not in the abnormal range in either group.

Namely, superabundant dietary cholesterol and decreased dietary PUFA intake may contribute to NAFLD development without the presence of obesity or insulin resistance. These findings are supported by some animal models fed a high-cholesterol diet, which show hepatic steatosis without obesity[15-17]. However, these animals had obvious hypercholesterolemia in contrast to NAFLD patients. This might be because the dietary cholesterol levels are considerably higher (0.2%-1.25%) in animal models than in our examined NAFLD patients. Furthermore, in these patients, hypercholesterolemia might be masked by the overwork of hepatocytes, resulting in cholesterol overload in tissues. Cholesterol supply and fatty acid synthesis are associated on a stream of the liver X receptor α (LXRα)-SREBP-1c pathway. In hepatocytes, LXRα is a key regulator of cholesterol and fatty acid metabolism, and its endogenous agonistic ligands are oxysterols, which are metabolites of cholesterol. Surplus cholesterol produces increased levels of oxysterols, resulting in activation of the LXRα-SREBP-1c pathway and enhancement of fatty acid synthesis. Furthermore, upregulation of LXRα expression was more noticeable in non-obese than in obese NAFLD patients[8]. Also, in the study of PUFA, patients with NAFLD were found to have lower levels of hepatic n-3 and n-6 PUFA, and n-3 PUFA dietary intake had therapeutic effects on fatty liver in patients with NAFLD[18-20]. n-3 PUFAs, such as eicosapentaenoic acid, which function as suppressors of SREBP-1c, are considered to reduce hepatic levels of triglycerides. However, clinically, a drug containing eicosapentaenoic acid does not have a high enough efficacy in many cases to overcome NAFLD (our own data).

Until now, investigations of therapeutic interventions have largely focused on agents that modify oxidative stress and insulin sensitivity, but clearly, an effective therapy for NAFLD has not been proven. If excess cholesterol plays a key role in the onset and progression of NAFLD, the control of dietary cholesterol intake should be a beneficial treatment strategy. Niemann-Pick C1 like 1 (NPC1L1), found in the proximal jejunum and canalicular aspect of hepatocytes, is essential for the absorption/reabsorption of cholesterol from the intestines and liver. Accordingly, the NPC1L1 inhibitor ezetimibe is expected to decrease intracellular cholesterol levels and to down-regulate/inactivate the LXRα-SREBP-1c pathway, and may be a suitable candidate for NAFLD treatment. In animal models, knocking out NPC1L1 or treatment with a NPC1L1 inhibitor provides resistance against steatosis[21,22]. Clinically, we encountered and reported a patient with NAFLD in whom ezetimibe clearly provided an improvement against liver injury and steatosis[23]. In a clinical study, to reduce cholesterol load, ezetimibe was administered (10 mg/d, orally) to non-obese NAFLD patients (n = 12) without any other treatments and any lifestyle modifications (unpublished data). In fact, ezetimibe was effective for liver injury because significant improvements were seen in serum aminotransferase levels, with 75% of subjects normalizing their transaminases. Six months after the treatment, alanine aminotransferase levels decreased by nearly 60% on average. However, a steatotic appearance remained as determined by liver echotexture in many of the patients (9/12) after 12 mo of treatment, indicating that a significant attenuation of fat content was not necessarily found. Of note, suppression of dietary cholesterol absorption may be a feasible option to successfully treat NAFLD, particularly in non-obese patients.

Considering the above findings, cholesterol-modifying treatments are favorable for NAFLD patients, and ezetimibe is expected to show a prompt clinical effect on laboratory findings for at least non-obese patients. Hence, the following should be examined and determined: (1) Are HMG-CoA reductase (HMGR) inhibitors (statins), which suppress *de novo* cholesterol synthesis, effective for NAFLD as well as ezetimibe? In recent reports, some

affirm but some deny the effect[24-26]. However, statins, with the exception of pravastatin, have generally shown promising results with improved serum aminotransferase levels. Combination therapy with an HMGR inhibitor plus ezetimibe might be more effective than monotherapy, although several cases of hepatic injury as an adverse effect have been reported in patients with pre-existing chronic liver disease[27,28]; (2) Is the control of cholesterol levels also effective in obese NAFLD patients with insulin resistance? Because dietary cholesterol intake was also significantly higher in obese patients than in normal individuals[13], ezetimibe is possibly effective for obese patients. However, in obese patients, it is difficult to remove the impact of other factors such as lifestyle modifications and other baseline agents; therefore, a study in obese patients requires circumspection; (3) Does the control of cholesterol levels improve steatosis in long-term observations? In the studies of NAFLD treatment by statins, a consistent opinion has not been drawn on the matter of the improving effect in hepatic steatosis[24,25]; (4) Further studies are required to determine whether cholesterol modifications are effective for both types of NAFLD, simple steatosis and steatohepatitis; and (5) Does the control of cholesterol levels show an additive therapeutic effect with any other treatments such as antioxidants, hepatoprotective agents or insulin sensitizers?

CONCLUSION

According to our nutritional examinations, increased cholesterol intake may be one of the main causes of an increase in the prevalence of NAFLD. Therefore, as a potential treatment, cholesterol-lowering agents look promising. Indeed, several recent studies endorse the clinical indication of statin therapy for NAFLD. Ezetimibe has recently been viewed as an alternative to statin therapy in patients with hypercholesterolemia. Ezetimibe targets the cholesterol absorption/reabsorption step, and accordingly ezetimibe may be a suitable treatment for NAFLD. Larger trials are needed to confirm whether ezetimibe or statins are really efficacious as monotherapeutic agents and, to maximize clinical benefits while minimizing side effects, further trials may be required to investigate the best combination partners for the treatment of NAFLD.

REFERENCES

1 **Browning JD**, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004; **114**: 147-152
2 **Cheung O**, Sanyal AJ. Recent advances in nonalcoholic fatty liver disease. *Curr Opin Gastroenterol* 2009; **25**: 230-237
3 **Xie Z**, Li H, Wang K, Lin J, Wang Q, Zhao G, Jia W, Zhang Q. Analysis of transcriptome and metabolome profiles alterations in fatty liver induced by high-fat diet in rat. *Metabolism* 2009; Epub ahead of print
4 **Nakamuta M**, Kohjima M, Morizono S, Kotoh K, Yoshimoto T, Miyagi I, Enjoji M. Evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med* 2005; **16**: 631-635
5 **Kohjima M**, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Takayanagi R, Nakamuta M. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med* 2007; **20**: 351-358
6 **Kohjima M**, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N, Enjoji M, Takayanagi R, Nakamuta M. SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int J Mol Med* 2008; **21**: 507-511
7 **Higuchi N**, Kato M, Shundo Y, Tajiri H, Tanaka M, Yamashita N, Kohjima M, Kotoh K, Nakamuta M, Takayanagi R, Enjoji M. Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol Res* 2008; **38**: 1122-1129
8 **Nakamuta M**, Kohjima M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Yada M, Yada R, Takemoto R, Fukuizumi K, Harada N, Taketomi A, Maehara Y, Nakashima M, Enjoji M. The significance of differences in fatty acid metabolism between obese and non-obese patients with non-alcoholic fatty liver disease. *Int J Mol Med* 2008; **22**: 663-667
9 **Enjoji M**, Yada R, Fujino T, Yoshimoto T, Yada M, Harada N, Higuchi N, Kato M, Kohjima M, Taketomi A, Maehara Y, Nakashima M, Kotoh K, Nakamuta M. The state of cholesterol metabolism in the liver of patients with primary biliary cirrhosis: the role of MDR3 expression. *Hepatol Int* 2009; Epub ahead of print
10 **Nakamuta M**, Fujino T, Yada R, Yada M, Yasutake K, Yoshimoto T, Harada N, Higuchi N, Kato M, Kohjima M, Taketomi A, Maehara Y, Nakashima M, Kotoh K, Enjoji M. Impact of cholesterol metabolism and the LXRalpha-SREBP-1c pathway on nonalcoholic fatty liver disease. *Int J Mol Med* 2009; **23**: 603-608
11 **Kojima S**, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* 2003; **38**: 954-961
12 **Nonomura A**, Enomoto Y, Takeda M, Tamura T, Kasai T, Yoshikawa T, Nakamine H, Kojima H, Uemura M, Zen Y, Minato H. Clinical and pathological features of non-alcoholic steatohepatitis. *Hepatol Res* 2005; **33**: 116-121
13 **Yasutake K**, Nakamuta M, Shima Y, Ohyama A, Masuda K, Haruta N, Fujino T, Aoyagi Y, Fukuizumi K, Yoshimoto T, Takemoto R, Miyahara T, Harada N, Hayata F, Nakashima M, Enjoji M. Nutritional investigation of non-obese patients with non-alcoholic fatty liver disease: the significance of dietary cholesterol. *Scand J Gastroenterol* 2009; **44**: 471-477
14 **Allison SJ**. Liver: Dietary links to NAFLD in nonobese patients. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 320
15 **Kainuma M**, Fujimoto M, Sekiya N, Tsuneyama K, Cheng C, Takano Y, Terasawa K, Shimada Y. Cholesterol-fed rabbit as a unique model of nonalcoholic, nonobese, non-insulin-resistant fatty liver disease with characteristic fibrosis. *J Gastroenterol* 2006; **41**: 971-980
16 **Matsuzawa N**, Takamura T, Kurita S, Misu H, Ota T, Ando H, Yokoyama M, Honda M, Zen Y, Nakanuma Y, Miyamoto K, Kaneko S. Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. *Hepatology* 2007; **46**: 1392-1403
17 **Wouters K**, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, Kerksiek A, van Kruchten R, Maeda N, Staels B, van Bilsen M, Shiri-Sverdlov R, Hofker MH. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 474-486
18 **Allard JP**, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, Jalali P, Kandasamy T, Prayitno N, Sherman M, Guindi M, Ma DW, Heathcote JE. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 2008; **48**: 300-307
19 **Kajikawa S**, Harada T, Kawashima A, Imada K, Mizuguchi

K. Suppression of hepatic fat accumulation by highly purified eicosapentaenoic acid prevents the progression of d-galactosamine-induced hepatitis in mice fed with a high-fat/high-sucrose diet. *Biochim Biophys Acta* 2009; **1791**: 281-288
20 **Perez-Martinez P**, Perez-Jimenez F, Lopez-Miranda J. n-3 PUFA and lipotoxicity. *Biochim Biophys Acta* 2009; Epub ahead of print
21 **Davies JP**, Scott C, Oishi K, Liapis A, Ioannou YA. Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. *J Biol Chem* 2005; **280**: 12710-12720
22 **Deushi M**, Nomura M, Kawakami A, Haraguchi M, Ito M, Okazaki M, Ishii H, Yoshida M. Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome. *FEBS Lett* 2007; **581**: 5664-5670
23 **Enjoji M**, Tanaka M, Miyazaki M, Higuchi N, Kato M, Kotoh K, Matsunaka K, Nishinakagawa T, Machida K, Nakashima M, Nakamuta M. Clinical effectiveness of ezetimibe for a non-obese patients with nonalcoholic fatty liver disease. *Am J Case Rep* 2009; **10**: 145-148
24 **Hyogo H**, Tazuma S, Arihiro K, Iwamoto K, Nabeshima Y, Inoue M, Ishitobi T, Nonaka M, Chayama K. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism* 2008; **57**: 1711-1718
25 **Nelson A**, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *J Clin Gastroenterol* 2009; **43**: 990-994
26 **Kashi MR**, Torres DM, Harrison SA. Current and emerging therapies in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 396-406
27 **Bhardwaj SS**, Chalasani N. Lipid-lowering agents that cause drug-induced hepatotoxicity. *Clin Liver Dis* 2007; **11**: 597-613, vii
28 **Vuppalanchi R**, Chalasani N. Statins for hyperlipidemia in patients with chronic liver disease: are they safe? *Clin Gastroenterol Hepatol* 2006; **4**: 838-839

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TOPIC HIGHLIGHT

Luca Stocchi, MD, *Series Editor*

Current indications and role of surgery in the management of sigmoid diverticulitis

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Abstract

Sigmoid diverticulitis is a common disease which carries both a significant morbidity and a societal economic burden. This review article analyzes the current data regarding management of sigmoid diverticulitis in its variable clinical presentations. Wide-spectrum antibiotics are the standard of care for uncomplicated diverticulitis. Recently published data indicate that sigmoid diverticulitis does not mandate surgical management after the second episode of uncomplicated disease as previously recommended. Rather, a more individualized approach, taking into account frequency, severity of the attacks and their impact on quality of life, should guide the indication for surgery. On the other hand, complicated diverticular disease still requires surgical treatment in patients with acceptable comorbidity risk and remains a life-threatening condition in the case of free peritoneal perforation. Laparoscopic surgery is increasingly accepted as the surgical approach of choice for most presentations of the disease and has also been proposed in the treatment of generalized peritonitis. There is not sufficient evidence supporting any changes in the approach to management in younger patients. Conversely, the available evidence suggests that surgery should be indicated after one attack of uncomplicated disease in immunocompromised individuals. Uncommon clinical presentations of sigmoid diverticulitis and their possible association with inflammatory bowel disease are also discussed.

Key words: Sigmoid diverticulitis; Diverticulitis management; Diverticulitis surgery; Acute diverticulitis; Complicated diverticulitis; Perforated diverticulitis; Laparoscopic colectomy

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INTRODUCTION

Sigmoid diverticulitis is a common disease of the Western World and results in a significant number of hospital admissions[1] with considerable societal costs due to loss of productivity. The prevalence of diverticula in the sigmoid increases proportionally with aging and only rarely results in the inflammation referred to as sigmoid diverticulitis. Sigmoid diverticula may cause significant bleeding which is generally unrelated to diverticular inflammation and is generally referred to as diverticular bleeding or bleeding diverticulosis. Bleeding caused by diverticula will therefore not be included in this review article. The spectrum of sigmoid diverticulitis ranges from a single episode of mild sigmoid inflammation amenable to outpatient treatment to a life-threatening generalized peritonitis caused by acute diverticular perforation which

requires urgent surgical intervention.

The aim of this review article is to analyze the clinical presentation, treatment modalities for the various forms of sigmoid diverticulitis, the indications for elective and urgent surgery and the postoperative and functional outcomes reported in the literature.

RISK FACTORS AND PREVENTIVE STRATEGIES

There are few studies which present evidence of a causal relationship with preventable factors. The data obtained from a prospective cohort of 47 228 male health professionals who were free from diverticular disease in 1986 has been fundamental in providing evidence-based outcomes. Obesity is significantly associated with an increased incidence of both diverticular bleeding and diverticulitis, which have often been considered together in the studies from this large dataset. The relative risk of diverticulitis was found to be between 1.5 and 2, depending on whether body mass index (BMI), waist circumference or waist to hip ratio were considered[2]. Correspondingly, physical activity, particularly if vigorous, is associated with decreased incidence of sigmoid diverticulitis and diverticular bleeding[3]. A diet with an increased fiber intake, particularly cellulose, is also significantly associated with a decreased risk of diverticular disease[4]. On the other hand, the presumed correlation between incidence of sigmoid diverticulitis and the consumption of nut, corn and popcorn has not been confirmed when analyzing this large prospective cohort of men[5]. With respect to the use of medications, the regular and consistent use of nonsteroidal antiinflammatory drugs and acetaminophen is associated with symptoms of severe diverticular disease, particularly bleeding[6].

CLINICAL PRESENTATION AND DIAGNOSIS

Sigmoid diverticulitis generally presents with abdominal pain, typically located in the left lower quadrant and associated with a variable degree of peritoneal irritation, which can range from none to generalized peritonitis. Localized peritoneal reaction with guarding and rebound tenderness may be noted. Fever and elevation of the white blood cell count can aid in the diagnosis when present. A redundant sigmoid colon may reach the right lower quadrant, and sigmoid diverticulitis under these circumstances may resemble acute appendicitis. In cases of complicated diverticulitis a stricture may lead to obstructive symptoms with nausea and vomiting as the most noticeable symptoms. On the other hand, a history of recurrent urinary tract infection, dysuria with or without urgency, pneumaturia and fecaluria can suggest a colovesical fistula. When a patient reports passing stools per vagina, insertion of a vaginal speculum can reveal a fistulous opening at the vaginal apex, thus confirming a colovaginal fistula. A previous history of hysterectomy is a valuable clinical clue to the correct diagnosis as colovaginal and colovesical fistulas are rare in females with their uterus in place, as the uterus becomes a screen interposed between the inflamed colon and the bladder and vagina. Less commonly, sigmoid diverticulitis can involve other surrounding structures and cause coloenteric, colouterine or colocutaneous fistulas.

A full colonoscopy should be typically avoided during an episode of acute diverticulitis because of an increased risk of perforation. In select cases and experienced hands, a gentle flexible sigmoidoscopy can provide additional information and help rule out alternative diagnoses such as cancer, inflammatory bowel disease, or ischemic colitis. Computed tomography (CT) is the most commonly used imaging modality to determine the diagnosis of sigmoid diverticulitis. In this respect, CT has supplanted barium enema and gastrografin enema in the routine evaluation of the sigmoid colon[7]. It can also help establish a differential diagnosis with other conditions which might exhibit similar symptoms such as gynecologic or urinary tract disorders. Irritable bowel syndrome and diverticulitis may present with similar symptoms and physical findings. It is therefore important to confirm the diagnosis of sigmoid diverticulitis by imaging before recommending surgery.

CLASSIFICATIONS OF SIGMOID DIVERTICULITIS AND IMPLICATIONS FOR MANAGEMENT

It is appropriate to classify sigmoid diverticulitis into different categories as the morbidity and mortality of this condition are greatly variable. Traditionally, the Hinchey classification has been used to subdivide sigmoid diverticulitis into subgroups based on the degree and extent of the abdominal and pelvic disease identified at the time of surgery and associated with perforated diverticular disease of the colon[8]. Of note, Hinchey credited Hughes for the development of an earlier, similar classification in 1963[9]. The Hinchey classification, developed before the advent of routine CT imaging, remains the most widely used classification and a few updated modifications have therefore been proposed in recent years (Table 1). In fact, the original Hinchey classification might not be the most practical classification to help in the contemporary management of at least some cases of diverticular disease. For example, the Hinchey classification separates a pericolic abscess (Hinchey 1) from a distant abscess (Hinchey 2). However, larger pericolic abscesses and similarly sized distant abscesses might carry similar morbidity and require similar management. In these cases, more important factors in the clinical management of this complication of diverticular disease might instead be the abscess size, location in the pelvis or mesocolon and also the ability to percutaneously drain the abscess regardless of its vicinity to the sigmoid, and therefore maximize the feasibility of a subsequent one-stage operation. In this respect, some proposed modifications of the Hinchey classification spe-

Table 1 Hinchey classification and its modifications

	Original Hinchey classification	Sher[10], Kohler modification[11]	Wasvary modification[33]	Kaiser modification[71][1]
Stage Ⅰ	Pericolic abscess confined by the mesentery of the colon	Pericolic abscess	Ⅰa phlegmon	Ⅰa confined pericolic inflammation-phlegmon
			Ⅰb pericolic abscess	Ⅰb confined pericolic abscess
Stage Ⅱ	Pelvic abscess resulting from a local perforation of a pericolic abscess	ⅡA distant abscess amenable to percutaneous drainage ⅡB complex abscess associated with/without fistula	Pelvic abscess	Pelvic, distant intrabdominal or retroperitoneal abscess
Stage Ⅲ	Generalized peritonitis resulting from rupture of pericolic/pelvic abscess into the general peritoneal cavity	Generalized purulent peritonitis	Purulent peritonitis	Generalized purulent peritonitis
Stage Ⅳ	Fecal peritonitis results from the free perforation of a diverticulum	Fecal peritonitis	Fecal peritonitis	Fecal peritonitis

[1]This modification also includes stage 0, defined as mild clinical diverticulitis.

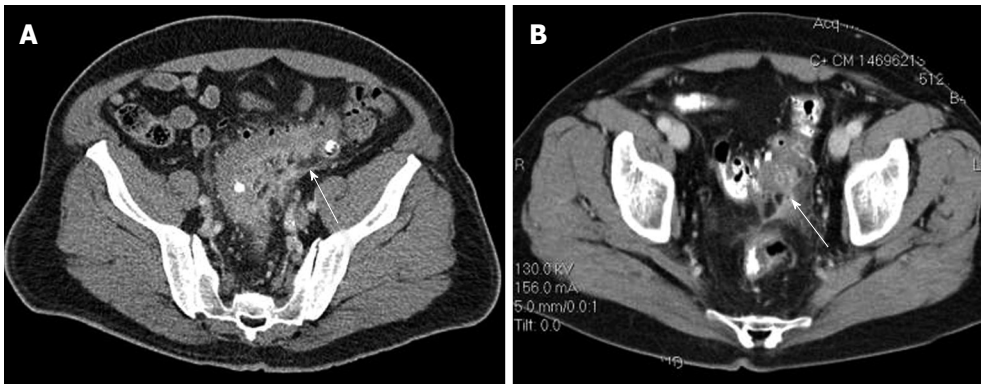

Figure 1 Diverticulitis. A: Uncomplicated sigmoid diverticulitis with colonic thickening and straining at CT (arrow), also referred to as "mild" CT diverticulitis. Two diverticula contain contrast medium without evidence of extravasation outside the sigmoid; B: "Severe" CT diverticulitis with extravasation of contrast and small amount of extraluminal air (arrow). This patient was initially managed non-operatively and eventually required surgery for recurrent disease.

Table 2 Ambrosetti classification[12]

Moderate diverticulitis	Severe diverticulitis
Localized sigmoid wall thickening (> 5 mm) Inflammation of pericolic fat	Same as mild diverticulitis plus one of the following: Abscess Extraluminal air Extraluminal contrast

cifically include the ability to percutaneously drain the abscess[10,11]. Furthermore, the Hinchey classification was developed based on the description of surgical findings and was not specifically designed to evaluate cases of sigmoid diverticulitis treated with antibiotics only. More recently, CT scanning has become the imaging modality of choice to diagnose sigmoid diverticulitis and has been proposed as being the imaging modality providing the most important and valuable indication as to the likelihood that medical treatment with antibiotics will fail. In this regard, Ambrosetti *et al*[12] have proposed a CT-based classification of sigmoid diverticulitis subdivided into "moderate disease" or "mild disease" in the case of localized sigmoid wall thickening (greater than 5 mm) and inflammation of the pericolic fat (Figure 1A). On the other hand, the term "severe disease" is used instead in the case of abscess, extraluminal air or extraluminal contrast extravasation (Figure 1B and Table 2).

UNCOMPLICATED DIVERTICULITIS

When the inflammatory process is limited to the sigmoid it is generally treated with antibiotics. If symptoms are not severe and the patient is otherwise healthy and compliant with medical treatment, wide spectrum antibiotic treatment can be administered orally on an outpatient basis and the patient followed with serial office visits. On the other hand, if the patient is systemically ill, elderly or has significant comorbidities, a hospital admission and treatment with intravenous antibiotics are warranted. Even when hospital admission is necessary, the appropriateness of an initially conservative approach with antibiotic management has been confirmed[13-17]. Most patients with uncomplicated sigmoid diverticulitis respond to medical treatment and generally experience significant decreases in their abdominal pain, temperature and white blood cell count within the first 48 h after initiation of antibiotic treatment[17,18].

In a minority of patients non-operative treatment fails, and symptoms either persist or worsen. In these cases,

urgent or semi-urgent surgery may become necessary during the same hospital stay. Among the remaining patients who successfully recover from their first episode of sigmoid diverticulitis, only a few eventually require elective sigmoid resection for recurrent disease and even more rarely are urgent operations necessary.

Following recovery from a new onset attack of uncomplicated diverticulitis the patient should undergo colonoscopy, or alternatively a barium enema, to rule out alternative diagnoses such as ischemic colitis, inflammatory bowel disease or, most importantly, a carcinoma.

INDICATIONS FOR ELECTIVE SURGERY

The indications for elective operation for sigmoid diverticulitis are evolving. For several years the traditional teaching has been that elective sigmoidectomy was warranted after 2 attacks of uncomplicated diverticulitis. This recommendation was based on the assumptions that after 2 attacks there was not only a very high probability of recurrent attacks of uncomplicated diverticulitis but also an increased risk of complicated diverticulitis including free perforation causing diffuse peritonitis. From this viewpoint surgery would therefore prevent the risk of complicated diverticulitis with its inherently increased morbidity and mortality. Recent studies have questioned this hypothesis[19] and suggest instead that most patients who have complicated diverticulitis experience this clinical presentation as their first manifestation of diverticular disease[20,21]. Other studies based on decision analysis models have indicated that the preferred timing of elective surgery to optimize life expectancy should be after the third[22] or fourth[23] attack of uncomplicated diverticulitis. This changed view on the indications for elective surgery has reduced the overall number of surgical procedures performed for diverticulitis. In a study of 685 390 hospital discharges for sigmoid diverticulitis, based on the Nationwide Inpatient Sample during the period 1991-2005, the ratio of hospital discharges for diverticulitis increased from 5.1 to 7.6 cases per 1000 inpatients. However, the proportion of patients who underwent surgery for uncomplicated diverticulitis declined from 17.9% to 13.7% ($P < 0.001$). In spite of these shifts, the percentage of patients with free perforation from diverticular disease remained stable throughout the study period at 1.5%[24]. With the limitation of a retrospective study based on administrative data, this study with a large number of patients also confirms that a less aggressive strategy for elective surgery did not result in any worrisome increase in the rate of presentation with diffuse peritonitis from diverticular perforation. Contemporary proponents of surgery after 2 attacks argue that earlier surgery favorably impacts patient symptoms[25] and that an increased number of diverticulitis attacks proportionally increases the conversion rates at the time of elective laparoscopic sigmoidectomy[26].

Overall, the recent data from the literature defining the natural history of uncomplicated diverticulitis has contributed to reducing the emphasis on the rule of surgery after the second attack. As a result of this shift, the most recent version of the Practice Parameters for Diverticulitis from the American Society of Colon and Rectal Surgery states that "the number of attacks of uncomplicated diverticulitis is not necessarily an overriding factor in defining the appropriateness of surgery"[27].

SURGICAL TREATMENT

The tenets of surgical treatment of diverticulitis are resection of the entire sigmoid and anastomosis between a soft and pliable area of descending colon and the upper rectum. The latter is generally recognized by the confluence of the teniae, which frequently occurs at the level of the sacral promontory. Failure to completely remove the sigmoid is associated with increased recurrence rates[28,29]. Some surgeons have emphasized preservation of the inferior mesenteric artery which might minimize the risk of anastomotic leakage[30], sexual dysfunction from intraoperative nerve injury[31], and optimize functional results[32]. Mobilization of the splenic flexure should be left to the discretion of the operating surgeon and is generally not necessary in the case of redundant left colon. The involvement of the tissue surrounding the sigmoid colon by the inflammatory process is variable. Often it is possible to identify the ureters intraoperatively and the required pelvic dissection can be limited to the upper rectum. However, there may be cases of complicated diverticulitis in which the extent and degree of inflammatory changes warrant the use of ureteral stents and/or the creation of a colorectal anastomosis in the more distal rectum. In such cases a difficult, prolonged dissection with significant blood loss may also justify the creation of a proximal diverting stoma. With respect to the required extent of resection, it is not necessary to remove the entire colonic segment bearing diverticula, which may actually be impossible in some cases due to the extent and density of diverticula throughout the colon. However, care should be taken to prevent inclusion of any diverticula into a stapled colorectal anastomosis. These principles are generally accepted and should apply equally to open or laparoscopic surgery. On the other hand, the timing of surgery in relation to the last diverticulitis attack has been the subject of controversy. The traditional practice entails a waiting period of 4-6 wk after a diverticulitis attack before performing an elective operation. Alternatively, some surgeons have suggested that early intervention for complicated diverticular disease may avoid the prolonged hospitalization and possibly multiple hospital admissions related to the traditional stepwise approach with initial antibiotic management and delayed elective surgery[33]. It has also been suggested that early surgery might obviate the creation of a stoma with its associated possible complications[34]. In addition, there is some evidence suggesting that an earlier timing of surgery, to within 30 d from the last diverticulitis attack, is not associated with increased morbidity when compared with operations performed between 30 and 60 d, or after 60 d following the last attack[35]. However, other investigators have reported less encouraging results. In the

case of laparoscopic surgery early surgical intervention has been associated with an increased conversion rate due to inflammation[36]. More importantly, a prospective study evaluating early elective sigmoid resection, carried out after 5-8 d of initial antibiotic treatment, has shown that this approach was associated with increased morbidity when compared with operations carried out 4-6 wk after the initial hospitalization[37]. While the data regarding the outcomes of early surgery following hospitalization for sigmoid diverticulitis remains controversial, there does not seem to be sufficiently consistent evidence at the moment to justify any anticipation of elective surgery before the traditional 4-6 wk waiting period.

INCREASED ROLE OF LAPAROSCOPIC SURGERY

While open surgery continues to be performed, especially in low volume centers and by low volume surgeons[38], laparoscopic surgery is increasingly preferred in the elective treatment of sigmoid diverticulitis. Several single-institutional series have confirmed feasibility and safety of the laparoscopic approach[39-42]. Laparoscopic sigmoidectomy is associated with reduced recovery time and return to bowel function, reduced hospital stay, and at least in some cases decreased morbidity[43-47] and costs[45,48]. Single-institutional series by experienced surgeons have reported conversion rates of as low as 2.8% and a median hospital stay of 4 d[42]. Minimally invasive sigmoidectomy can be performed using a straight laparoscopic technique or a laparoscopic hand-assisted technique[49,50]. A single-access sigmoidectomy has also been recently described[51]. The controversy persists as to whether the hand-assisted technique allows a reduction of operative times and conversion rates while extending the benefits of laparoscopic surgery to more difficult cases.

In general, the benefits of laparoscopic surgery have been confirmed by a large study based on data from the Nationwide Inpatient Sample during the years 1998-2000, which included 709 patients treated laparoscopically *vs* 17735 treated with the open technique. Laparoscopically completed patients had a mean reduction of hospital stay of almost 2 d and also reduction of postoperative morbidity when compared to their open counterparts. An important limitation of this study was that, due to the nature of the administrative database used, converted patients were not analyzed combined with the cases completed laparoscopically, which skews the results in favor of laparoscopic surgery[52]. However, a more recent study using the University Health System Consortium Database, in which converted patients were appropriately included in the laparoscopic group, confirmed a reduction in hospital stay, overall postoperative morbidity and total hospital cost in favor of laparoscopic sigmoidectomy for benign diseases[53]. In addition, there is further evidence of the benefits of laparoscopic surgery emerging from a prospective randomized trial, which has demonstrated reduction of major complications after laparoscopic surgery when compared with open sigmoidectomy[54]. This multicenter, randomized, double-blinded study accrued 104 patients in 5 centers from 2002 to 2006. Double-blinding was carried out by covering the patient abdomen with a large dressing at the time of surgery so that patients, as well as physicians in charge of patients discharge, were unaware of the surgical technique used. Eligible patients were randomized to open *vs* laparoscopic sigmoid resection. Patients were similar with respect to gender, age, BMI, comorbidities, indications for surgery and previous surgical procedures. Conversion rate was 19% and mortality 1%. Laparoscopic surgery resulted in expected recovery benefits including significant reduction of pain based on visual analog scores and systemic analgesia requirements, decreased hospital stay and improved quality of life based on short-term SF-36 questionnaires. In addition, laparoscopic surgery resulted in significant reduction of major complications, defined as a composite inclusive of intrabdominal abscess, anastomotic leakage, pulmonary embolism and myocardial infarction. Major complications combined for a 25% rate after open surgery *vs* 10% after laparoscopic procedures[54].

Based on the data from the last decade, it is reasonable to offer laparoscopic surgery in the surgical management of sigmoid diverticulitis and expect at least the recovery advantages reported after laparoscopic bowel resection.

RELATIONSHIP BETWEEN SURGICAL VOLUME AND OUTCOMES

A number of studies have investigated possible differences in outcomes related to the experience of the operators. With respect to the use of laparoscopic surgery, there is evidence that the volumes of both individual surgeons and hospitals are directly proportional to the likelihood of performing laparoscopic surgery for diverticular disease. Using National Inpatient Sample Data based on over 55000 patients, high-volume surgeons were almost 9 times more likely to perform laparoscopic surgery and high-volume hospitals were over 3 times more likely to perform laparoscopic surgery than their low-volume counterparts. These differences remained statistically significant when the data were stratified for age of the patient and timing of surgery; elective *vs* nonelective[38]. Volume/outcome studies have also been conducted within the subgroup of patients treated with laparoscopic surgery. In the multicenter, observational, German study from the Laparoscopic Colorectal Surgery Study Group of 1545 patients, the 52 participating institutions were divided into 3 groups according to the number of cases performed; greater than 100, between 30 and 100, and less than 30. While the percentage of patients with complicated diverticulitis was significantly increased in high-volume institutions (21% *vs* 8% in low-volume centers), operating times in these same institutions were shorter by approximately 30 min. Intraoperative complications, conversion rates and postoperative morbidity and mortality were numerically lowest in the high-volume centers, but these differences were not

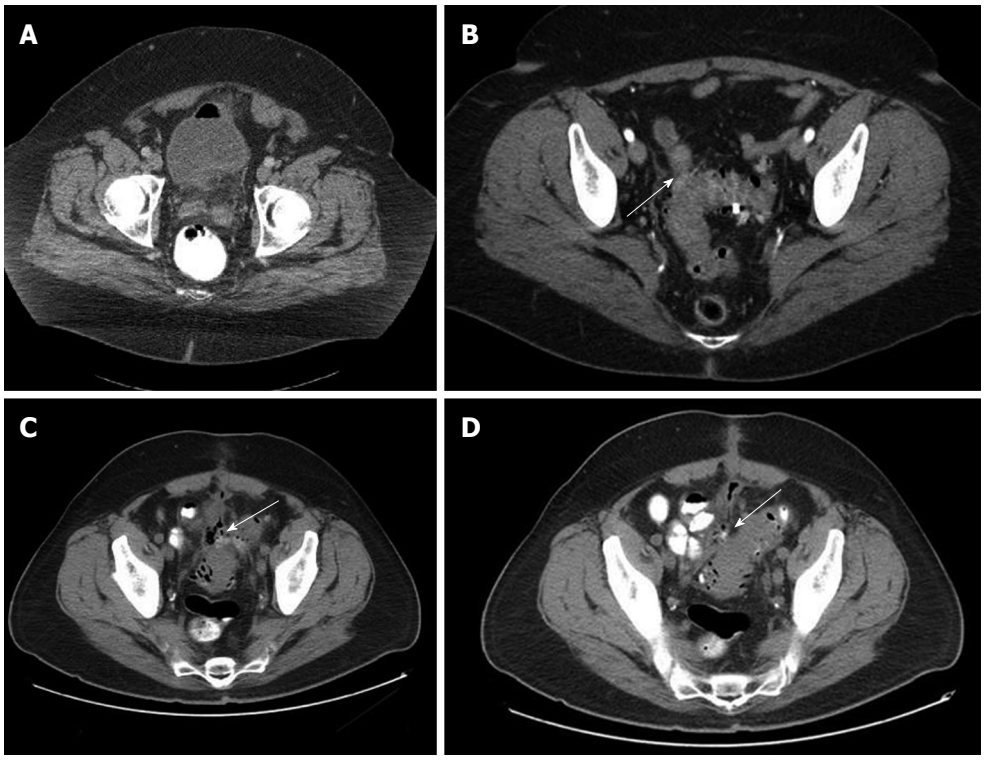

Figure 2 Fistula. A: Colovesical fistula as indicated by the presence of air in the bladder. This patient had symptoms and other CT findings consistent with sigmoid diverticulitis; B: Sigmoid diverticulitis and colovaginal fistula. This patient had undergone previous hysterectomy and complained of feculent discharge from her vagina. CT scan indicated inflamed sigmoid with adherent small bowel loop (arrow). The small bowel loop could be successfully separated from the sigmoid at the time of laparoscopic sigmoidectomy. There was no evidence of coloenteric fistula; Sigmoid diverticulitis with colocutaneous fistula (arrows) (C and D) (courtesy of Dr. Ravi Pokala Kiran, Department of Colorectal Surgery, Digestive Disease Institute, Cleveland Clinic, Cleveland, Ohio, USA).

statistically significant[55]. The results from this study seem to indicate that experienced surgeons in high-volume centers might be more facile at treating more complex cases with laparoscopic surgery. However, even low-volume centers can still achieve comparable postoperative outcomes and should therefore not be discouraged from performing laparoscopic surgery.

RESULTS OF SURGERY FOR DIVERTICULITIS

Contemporary surgical treatment of diverticulitis following the principles described above is considered curative with a less than 5% recurrence rate[29,56]. A suspicion of recurrent sigmoid diverticulitis following surgical resection should be confirmed by CT scan of the abdomen and pelvis after which antibiotic treatment should be initiated, as for a case of primary uncomplicated sigmoid diverticulitis. It is important to preoperatively discuss with the patient that the risk exists that surgery might not lead to resolution of the patient's complaints. When this is the case, an anastomotic stenosis should be ruled out as a possible source of the problem which can often be successfully treated[57]. However, persistent or recurrent symptoms can be more difficult to elucidate. At least one contemporary series has reported a 25% rate of persistent symptoms after surgery[58], which the authors felt could be only partially explained by an overlap with irritable bowel syndrome. One of the limitations of the assessment of symptoms and functional results after surgery is that sigmoid diverticulitis can cause a significant impairment of quality of life before surgery, a time at which quality of life is even more rarely assessed. The functional results of surgery should therefore be most accurately assessed when compared to the patient's preoperative status. A recent study has appropriately addressed this issue and reported a prospective evaluation of functional outcomes after laparoscopic sigmoid colectomy. A sample of 46 individuals underwent evaluation of their quality of life using the gastrointestinal quality of life indicator (GIQLI) administered before surgery and then at 3, 6, and 12 mo postoperatively. The quality of life significantly improved for the majority of the overall group, whereas it declined in only 5 patients. Urinary and sexual function were also tested using validated scores and did not change as a result of surgery[31]. When appropriately diagnosed by CT scan, sigmoid diverticulitis requiring surgery should be followed by improvement in symptoms and function in a substantial majority of cases.

COMPLICATED DIVERTICULITIS

There are several complications which may be associated with diverticular inflammation. These include fistula (Figure 2), phlegmon, stricture, abscess and free perforation. At times the definition of complicated disease may depend on the individual clinical judgment, as uncomplicated and complicated diseases are a continuum of in-

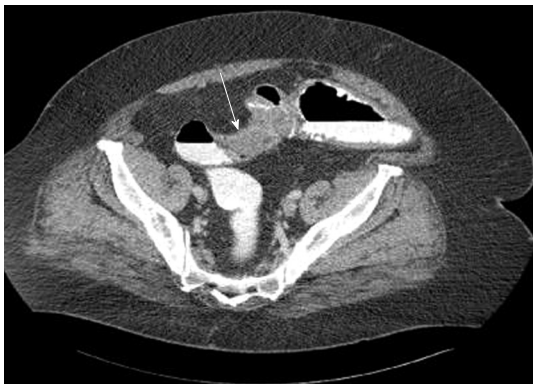

Figure 3 Sigmoid stricture (arrow) causing large bowel obstruction with proximal colonic dilatation. Clinical and imaging findings at presentation did not allow ruling out sigmoid carcinoma. This patient was treated with initial Hartmann procedure and the pathology report revealed sigmoid diverticulitis. He subsequently underwent Hartmann takedown after 3 mo.

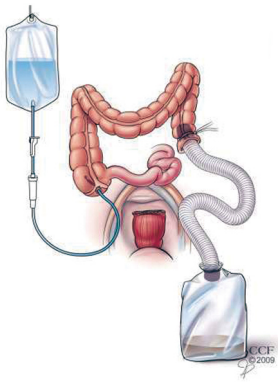

Figure 4 On-table intraoperative colonic lavage (see explanation in text).

creasingly severe inflammation which can cause a variable degree of stricture, intramural abscess or phlegmon. In the United States, complicated disease at presentation is more common in African-American patients and in individuals who lack medical insurance, based on an analysis from the Nationwide Inpatient Sample[59].

In general, surgery is recommended for complicated diverticulitis after the first episode as the risk of recurrent disease without surgery is very high. However, when age or comorbidities prohibitively increase perioperative risks, it may be appropriate to approach complicated diverticulitis with medical treatment alone[60].

Laparoscopic surgery remains feasible also for complicated diverticulitis[55,61,62] including cases with fistulas[63-66]. The morbidity after laparoscopic surgery for complicated diverticulitis might exceed that of uncomplicated disease, but this has not been uniformly proven[42].

It remains controversial whether the act of conversion, which is more likely for complicated diverticular disease[67], increases postoperative morbidity or not. It is generally accepted that when a conversion is necessary, an early conversion can minimize major complication so that it causes only minor morbidity[68] or does not result in any increased morbidity rate at all[69].

In general, a more selective use of laparoscopic surgery for more straightforward, uncomplicated cases of diverticular disease could minimize conversion rates and therefore capitalize on the advantages derived from the laparoscopic approach. On the other hand, a more liberal use of laparoscopic surgery, including for complicated cases in patients with a previous laparotomy, is likely to result in increased conversion rates. However, this less stringent patient selection could still offer the potential benefits of laparoscopic surgery to an increasing number of individuals requiring surgery for sigmoid diverticulitis without adverse effects on long-term patient outcomes[68].

STRICTURE

Sigmoid diverticulitis can present in the form of a stricture which may or may not be associated with typical symptoms. In the case of stricture, the indications for surgery may range from colonic obstruction requiring acute surgical intervention to the inability to rule out carcinoma as the cause of stricture (Figure 3). Sigmoid strictures can cause significant dilatation in the proximal colon, which can complicate the creation of a colorectal anastomosis after sigmoid resection. A staged procedure with sigmoidectomy and creation of a colostomy may therefore become necessary. A possible option in the surgical management of severe sigmoid stricture causing significant fecal loading is a resection with on-table colonic lavage and primary anastomosis (Figure 4). This is carried out by inserting a large Foley catheter through an appendicostomy or distal ileal enterotomy secured with a purse-string suture with the tip of the catheter placed into the cecum. This Foley catheter is connected to a bag of warm saline solution which is typically used for irrigation. A large corrugated tube, such as an anesthesia ventilator tube, is then placed in the open end of the descending colon and secured with umbilical tape or large suture to the bowel wall. The distal end of the tubing is placed into a bucket on the floor where the effluent is collected. It is frequently necessary to mobilize both the hepatic and splenic flexures and manually propel solid stools towards the distal end which can significantly increase operative times. A proximal stoma diversion in addition to a colorectal anastomosis may be a prudent adjunct to the operative procedure, with or without intraoperative colonic lavage. Alternatively, a stricture can be treated with placement of endoluminal metallic stents to correct the obstruction, reduce the discrepancy in bowel diameter and allow a subsequent one-stage surgical procedure consisting of sigmoid resection and primary colorectal anastomosis[70]. Other options in the management of large bowel obstruction related to diverticular disease are subtotal colectomy and primary ileorectal anastomosis, and, in the most difficult cases, creation of a decompressive colostomy proximal to the strictured sigmoid followed by delayed sigmoid resection. The choice among these various options depends on both the individual patient and the surgeon's level of confidence in performing each of the approaches described above.

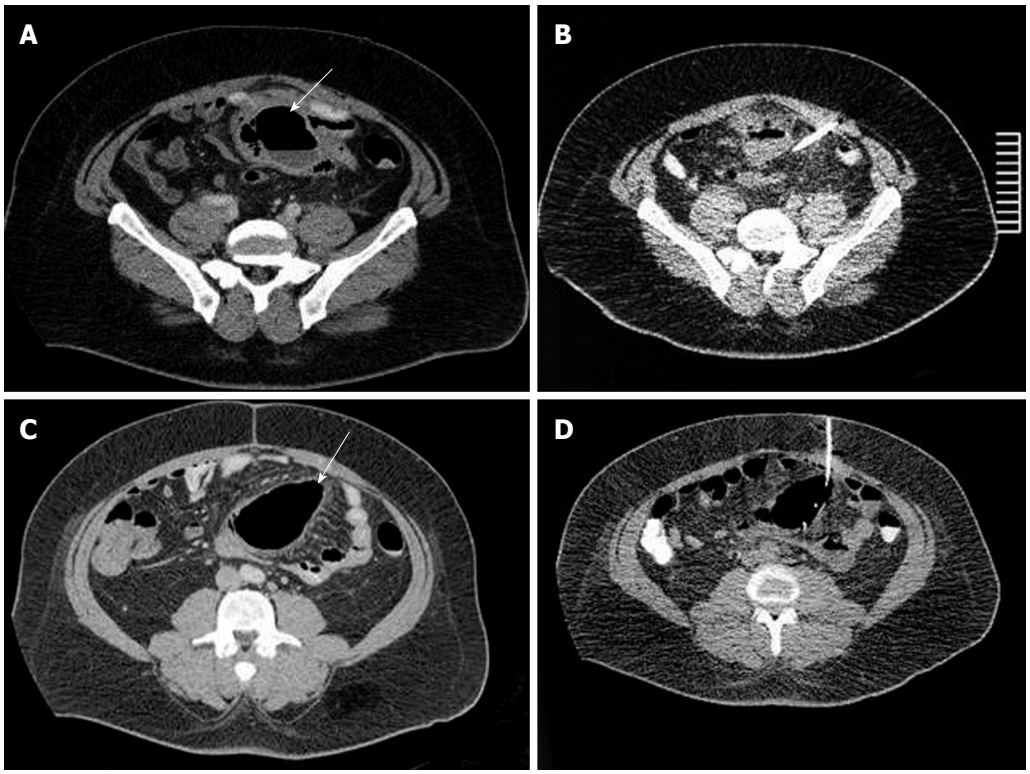

Figure 5 Sigmoid diverticulitis complicated by pericolic abscesses (A and C, arrows) requiring treatment by placement of two separate CT-guided percutaneous drains (B and D). This patient underwent laparoscopic sigmoidectomy with primary colorectal anastomosis and removal of both drains 6 wk after percutaneous drain placement.

PERIDIVERTICULAR ABSCESS

There is evidence suggesting that clinical presentation of sigmoid diverticulitis as peridiverticular abscess has increased in recent years[24]. It is generally acknowledged that elective surgery should be performed after percutaneous drainage of peridiverticular abscess (Figure 5) due to the high risk of recurrent diverticulitis[13,71]. In these cases surgery is generally performed 4-6 wk after initial percutaneous drainage. Some surgeons prefer to leave the drain in place until surgery, others remove the drain if the output becomes minimal and a drain contrast study rules out an existing sigmoid fistula. An accepted exception is the use of percutaneous drainage alone to obviate the need of surgery in poor risk patients[72].

The safety and effectiveness of percutaneous drainage in controlling the immediate symptoms of diverticular disease presenting with an abscess have been reported by several authors[14,73-77]. A number of variables have been examined as possible factors associated with the success rate of non-operative management.

Firstly, the size of the abscess seems to be an important indicator for success of non-operative management, especially when antibiotics alone are considered as first line treatment. A diameter of approximately 3-4 cm or less is more likely to be associated with successful antibiotic treatment[14,76,77]. Based on the ability of antibiotics alone to control smaller abscesses, some authors have suggested that the role of CT-guided drainage of diverticular-related abscesses should be re-evaluated and percutaneous drainage should be utilized less often[14]. Another factor with a possible impact on management is the abscess location. In fact, there is evidence suggesting that an abscess located in the mesocolon might be more responsive to non-operative treatment than a pelvic abscess[15,71]. In this regard, in a study analyzing 73 patients initially treated with antibiotics and undergoing CT-guided drainage only in case of failure of medical treatment, 71% of patients with a pelvic abscess ultimately required surgery *vs* 51% after percutaneous drainage of a mesocolic abscess. Based on these results, the authors suggested that sigmoid colectomy should be recommended after drainage of a pelvic abscess but not necessarily after percutaneous drainage of a mesocolic abscess[74]. The success of non-operative treatment in at least some patients has prompted other investigators to question the role of routine surgery after successful drainage of pericolonic abscess[20].

It remains difficult to critically evaluate the results of the various treatment options available for abdominal and pelvic abscesses related to diverticulitis because of both variability in clinical practices and data reporting. In some institutions percutaneous drainage is the preferred approach whenever technically feasible, which generally requires an abscess diameter of at least 3 cm. On the other hand, in other institutions the initial treatment of diverticular abscesses includes antibiotics alone and only after failure of antibiotic treatment is a percutaneous drainage considered. In addition, the data regarding the effectiveness of percutaneous drainage alone without

subsequent surgery remain limited, because of both small sample sizes and short follow-up. Further studies will be necessary before the standard of care of elective surgery after initial percutaneous drainage is abandoned.

GENERALIZED PERITONITIS FROM PERFORATED SIGMOID DIVERTICULITIS

A perforation of a sigmoid diverticulum into the free peritoneum is a life-threatening condition requiring immediate surgical intervention. The standard of care in most of these cases is a resection of the colonic segment including the perforation and creation of a proximal colostomy. Several authors refer to this operation as a Hartmann procedure, which by definition involves the resection of the sigmoid, closure of the rectal stump and creation of an end-descending colostomy, and which has also been performed laparoscopically[78,79]. Other surgeons have suggested that especially when the patient is severely septic and hemodynamically unstable the initial goal should be an expedited resection limited to the involved segment[80], sometimes referred to as a "perforectomy", in which at least some of the sigmoid should be left intact until the patient completes his or her recovery from the initial operation. In this case, a completion sigmoid resection would be typically performed at the time of colostomy takedown several months later so that the patient ultimately receives appropriate surgical treatment for sigmoid diverticulitis[81]. The morbidity and mortality from Hartmann procedure for free diverticular perforation remain substantial. The aggregate mortality in a total of 1051 patients reported in 54 combined studies conducted between 1966 and 2003 was almost 19% and was associated with a 24% incidence of wound infection and a 10% incidence of stoma complications[82]. In spite of advancements in intensive care, imaging and medical treatments, the mortality for this condition has remained stable over time[83]. Intestinal continuity can generally be reestablished 3-6 mo after the initial operation[84] although it has been reported that between approximately 30% and 70% of patients never have their colostomy closed[81,85-87]. In addition, a Hartmann takedown remains a difficult elective procedure[88] fraught with significant morbidity[89].

Considering the significant morbidity and mortality associated with a Hartmann procedure and its sequelae, some authors have suggested that in select circumstances it might be possible to resect the perforated segment and primarily reestablish intestinal continuity[90,91], which some surgeons feel can benefit from intraoperative colonic lavage as described above[92,93] (Figure 4). This view remains controversial and most surgeons would not recommend a resection and primary colorectal anastomosis for generalized peritonitis from diverticular perforation. However, in select circumstances it is possible to perform a colorectal anastomosis and proximal diverting loop ileostomy. This approach seems to be preferable to a Hartmann resection when the degree of intraoperative contamination and the underlying patient condition allow this approach. In these cases, the use of a defunctioning stoma in addition to colorectal anastomosis might result in a good compromise between postoperative morbidity, quality of life and probability of permanent stoma[94].

LAPAROSCOPIC LAVAGE, A NOVEL SURGICAL APPROACH TO GENERALIZED PERITONITIS

The advent of laparoscopic surgery and the increased use of the laparoscopic approach to treat perforated peptic ulcers and appendicitis have led to the development of laparoscopic strategies for the treatment of perforated diverticulitis. In this regard, laparoscopic lavage is a recently proposed treatment option which would potentially save the patient from both a major bowel resection and the creation of a stoma. The initial experiences of laparoscopic lavage have been promising with respect to perioperative mortality and complications[95]. In addition, while most proponents of initial laparoscopic lavage have decided in favor of an elective, delayed sigmoidectomy[96-100], a multicenter study from Ireland has reported encouraging results following a policy of lavage followed by continued observation. In fact, Myers *et al*[101] noted recurrence of sigmoid diverticulitis in 4 out of 92 treated patients, none of whom required surgery after a median follow-up of 36 mo. These data from different centers suggest that laparoscopic lavage has the potential to become, at least in select cases, the definitive treatment for perforated diverticulitis. However, the data on laparoscopic lavage for diverticular peritonitis remains limited and further investigations into this option are warranted to confirm these initial, promising results.

YOUNGER PATIENTS: SHOULD THE INDICATIONS FOR SURGERY CHANGE?

The indication for surgery in younger patients, generally defined as those who are 50 years old or younger, has been the subject of controversy. It has been reported that younger patients more frequently require surgery for diverticulitis[102] or are prone to recurrent disease[103]. Based on the presumed association between younger age and more virulent disease, some surgeons have suggested that elective surgery should be recommended in patients younger than 50 years old after their first attack of uncomplicated diverticulitis[104,105]. However, other retrospective series have not confirmed a correlation between younger age and more severe disease[106-109]. In addition, prospective data do not support a more aggressive surgical approach for younger patients. In this regard, Guzzo and Hyman[110] examined 762 patients admitted to their institution with sigmoid diverticulitis between 1990 and 2001, including 259 individuals younger than 50. The risk of requiring surgery during the first admission was comparable between older and younger patients. In addition,

out of 196 younger patients who were treated medically at the time of their initial admission, only one (0.5%) presented with perforation during a median follow-up of 5.2 years. In another prospective study with a median follow-up of 9.5 years, 118 patients were followed after their initial attack of diverticulitis, 28 of whom were 50 years old or younger. Age and findings at initial CT scan were analyzed as possible predictive factors for risk of poor outcome during the follow-up period, defined as recurrent, persistent or complicated diverticulitis. The probability of poor outcome at 5 years was 54% in younger patients with initially severe CT diverticulitis *vs* 19% for older patients with mild disease, based on CT imaging. At univariate analysis, age was a predictive factor for poor outcome. However, after stratification for severity of disease, age was no longer a significant factor[111]. Based on the available contemporary data there does not seem to be sufficient justification to recommend elective surgery after one attack of sigmoid diverticulitis in younger patients and rather the disease should be treated similarly in both younger and older patients depending on its severity and inclination to recurrence.

IMMUNOSUPPRESSED OR IMMUNOCOMPROMISED PATIENTS

Transplant recipients or patients with chronic diseases affecting the immune system are at increased risk of more aggressive and complicated diverticulitis[112-114], including initial presentation as free peritoneal perforation[115,116]. Chronic use of steroids is also associated with increased postoperative mortality after surgery for diverticulitis[20].

Therefore, it is generally recommended that surgery should be offered to this subset of patients after their first documented episode of diverticulitis. The studies supporting this practice are generally retrospective with small sample sizes[114]. On the other hand, there is no data presenting evidence against this practice. Therefore it seems reasonable to continue offering surgery after one episode of uncomplicated diverticulitis in immunocompromised patients. In this respect, some surgeons have emphasized that surgery should be carried out after the diverticulitis attack during the same hospital stay and a proximal diversion should be considered[117]. Other authors have even suggested that patients with one episode of uncomplicated diverticulitis who are transplant candidates should undergo prophylactic sigmoidectomy before their transplant. The evidence in favor of this practice remains scant, based on earlier studies and generally restricted to renal transplant recipients[118,119]. On the other hand, patients awaiting liver, heart and lung transplant are typically in poor health from their primary disease and generally should not be considered for prophylactic sigmoidectomy prior to their transplantation.

With respect to HIV infection and AIDS, there is no substantial data specific for sigmoid diverticulitis[120]. In general, the outcome of major abdominal surgery in HIV-positive individuals without AIDS is not significantly different from the general population. However, when a patient develops diverticulitis in the presence of AIDS or other causes of acute immunosuppression, postoperative infections are more likely. If surgery becomes necessary in these cases, a Hartmann procedure or a primary sigmoid resection with anastomosis and proximal diversion should be therefore preferable.

EVOLVING CONCEPTS IN DIVERTICULAR DISEASE

Sigmoid diverticulitis may have clinical manifestations which are difficult to accurately characterize. Its symptoms may overlap in some cases with the conditions collectively referred to as irritable bowel syndrome. Our understanding and therapeutic approach for this condition are evolving. From a surgical perspective it is imperative to minimize unnecessary surgery if diverticulitis cannot be documented radiologically, especially with a concurrent clinical history suggestive of irritable bowel syndrome. However, if irritable bowel syndrome can be ruled out, there seem to be a group of patients with chronic left lower quadrant abdominal pain and occasional alteration of bowel habits, but without fever or leukocytosis, who might still benefit from surgery. The condition of this subgroup of patients has been referred to as "smoldering diverticulitis". Horgan and colleagues identified smoldering diverticulitis in 47 patients, corresponding to approximately 5% out of their denominator of 930 patients undergoing sigmoid resection for diverticulitis. A total of 88% of these patients remained pain-free after at least 12 mo of follow-up following sigmoidectomy and primary anastomosis[121]. Atypical sigmoid diverticulitis should be part of the differential diagnosis in the patient with left lower quadrant pain, as surgery is curative in the majority of these cases.

An additional, novel clinical syndrome recently proposed as a separate entity within the realm of diverticular disease is referred to as segmental colitis associated with diverticulosis (SCAD)[122-124]. This is a non-specific, localized inflammatory process associated with diverticulosis involving the sigmoid but not the rectum or the proximal colon, generally presenting in middle-aged or elderly patients with rectal bleeding, diarrhea and abdominal pain variably combined. It most commonly affects males. Histology indicates inflammation without granulomas and serology should be negative for anti-neutrophil cytoplasmic antibodies (ANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA). Treatment with 5-aminosalicylate is generally effective in resolving the inflammation both symptomatically and endoscopically[122].

The pathogenesis of SCAD and its relationship with inflammatory bowel disease remain controversial[122,125]. Regardless, SCAD is becoming increasingly accepted as a separate entity from the traditional sigmoid diverticulitis and its known complications. While anti-inflammatory agents have been effective in the management of SCAD,

their role in the more common forms of diverticular disease remain unproven.

Another area of investigation concerns the potential causal relationship between sigmoid diverticulitis and colorectal cancer, which has been suggested based on comparisons with patients having diverticulosis without diverticulitis[126]. This association has not yet been validated and will therefore require further study. At the moment, sigmoid diverticulitis is not considered a precancerous or high-risk condition for the development of colorectal cancer and the recommended screening modalities do not differ from the guidelines accepted for the average-risk population.

CONCLUSION

Sigmoid diverticulitis is a condition ranging from mild inflammation of the sigmoid to life-threatening colonic perforation. Antibiotics are generally effective in mild forms of the disease while surgery is indicated in cases of multiple recurrences or complicated disease. Based on recent data, the systematic indication for surgery after 2 attacks should be abandoned in favor of a more individualized approach. Laparoscopic surgery is gaining favor in the surgical treatment of sigmoid diverticulitis. A subset of patients with atypical presentation presents a significant challenge in management; some may benefit from surgery whereas others could benefit from anti-inflammatory agent treatment.

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REFERENCES

1 **Etzioni DA**, Mack TM, Beart RW Jr, Kaiser AM. Diverticulitis in the United States: 1998-2005: changing patterns of disease and treatment. *Ann Surg* 2009; **249**: 210-217
2 **Strate LL**, Liu YL, Aldoori WH, Syngal S, Giovannucci EL. Obesity increases the risks of diverticulitis and diverticular bleeding. *Gastroenterology* 2009; **136**: 115-122.e1
3 **Strate LL**, Liu YL, Aldoori WH, Giovannucci EL. Physical activity decreases diverticular complications. *Am J Gastroenterol* 2009; **104**: 1221-1230
4 **Aldoori WH**, Giovannucci EL, Rimm EB, Wing AL, Willett WC. Use of acetaminophen and nonsteroidal antiinflammatory drugs: a prospective study and the risk of symptomatic diverticular disease in men. *Arch Fam Med* 1998; **7**: 255-260
5 **Strate LL**, Liu YL, Syngal S, Aldoori WH, Giovannucci EL. Nut, corn, and popcorn consumption and the incidence of diverticular disease. *JAMA* 2008; **300**: 907-914
6 **Aldoori W**, Ryan-Harshman M. Preventing diverticular disease. Review of recent evidence on high-fibre diets. *Can Fam Physician* 2002; **48**: 1632-1637
7 **Ambrosetti P**, Jenny A, Becker C, Terrier TF, Morel P. Acute left colonic diverticulitis--compared performance of computed tomography and water-soluble contrast enema: prospective evaluation of 420 patients. *Dis Colon Rectum* 2000; **43**: 1363-1367
8 **Hinchey EJ**, Schaal PG, Richards GK. Treatment of perforated diverticular disease of the colon. *Adv Surg* 1978; **12**: 85-109
9 **Hughes ES**, Cuthbertson AM, Carden AB. The surgical management of acute diverticulitis. *Med J Aust* 1963; **50**(1): 780-782
10 **Sher ME**, Agachan F, Bortul M, Nogueras JJ, Weiss EG, Wexner SD. Laparoscopic surgery for diverticulitis. *Surg Endosc* 1997; **11**: 264-267
11 **Kohler L**, Sauerland S, Neugebauer E. Diagnosis and treatment of diverticular disease: results of a consensus development conference. The Scientific Committee of the European Association for Endoscopic Surgery. *Surg Endosc* 1999; **13**: 430-436
12 **Ambrosetti P**, Grossholz M, Becker C, Terrier F, Morel P. Computed tomography in acute left colonic diverticulitis. *Br J Surg* 1997; **84**: 532-534
13 **Mueller MH**, Glatzle J, Kasparek MS, Becker HD, Jehle EC, Zittel TT, Kreis ME. Long-term outcome of conservative treatment in patients with diverticulitis of the sigmoid colon. *Eur J Gastroenterol Hepatol* 2005; **17**: 649-654
14 **Brandt D**, Gervaz P, Durmishi Y, Platon A, Morel P, Poletti PA. Percutaneous CT scan-guided drainage vs. antibiotherapy alone for Hinchey II diverticulitis: a case-control study. *Dis Colon Rectum* 2006; **49**: 1533-1538
15 **Alvarez JA**, Baldonedo RF, Bear IG, Otero J, Pire G, Alvarez P, Jorge JI. Presentation, management and outcome of acute sigmoid diverticulitis requiring hospitalization. *Dig Surg* 2007; **24**: 471-476
16 **Shaikh S**, Krukowski ZH. Outcome of a conservative policy for managing acute sigmoid diverticulitis. *Br J Surg* 2007; **94**: 876-879
17 **Evans J**, Kozol R, Frederick W, Voytavich A, Pennoyer W, Lukianoff A, Lardner J. Does a 48-hour rule predict outcomes in patients with acute sigmoid diverticulitis? *J Gastrointest Surg* 2008; **12**: 577-582
18 **Sra HK**, Shipman K, Virk HS. Does a 48-hour rule predict outcomes in patients with acute sigmoid diverticulitis? *J Gastrointest Surg* 2009; **13**: 1892
19 **Chapman JR**, Dozois EJ, Wolff BG, Gullerud RE, Larson DR. Diverticulitis: a progressive disease? Do multiple recurrences predict less favorable outcomes? *Ann Surg* 2006; **243**: 876-830; discussion 880-883
20 **Chapman J**, Davies M, Wolff B, Dozois E, Tessier D, Harrington J, Larson D. Complicated diverticulitis: is it time to rethink the rules? *Ann Surg* 2005; **242**: 576-581; discussion 581-583
21 **Salem TA**, Molloy RG, O'Dwyer PJ. Prospective study on the management of patients with complicated diverticular disease. *Colorectal Dis* 2006; **8**: 173-176
22 **Richards RJ**, Hammitt JK. Timing of prophylactic surgery in prevention of diverticulitis recurrence: a cost-effectiveness analysis. *Dig Dis Sci* 2002; **47**: 1903-1908
23 **Salem L**, Veenstra DL, Sullivan SD, Flum DR. The timing of elective colectomy in diverticulitis: a decision analysis. *J Am Coll Surg* 2004; **199**: 904-912
24 **Ricciardi R**, Baxter NN, Read TE, Marcello PW, Hall J, Roberts PL. Is the decline in the surgical treatment for diverticulitis associated with an increase in complicated diverticulitis? *Dis Colon Rectum* 2009; **52**: 1558-1563
25 **Makela JT**, Kiviniemi HO, Laitinen ST. Elective surgery for recurrent diverticulitis. *Hepatogastroenterology* 2007; **54**: 1412-1416
26 **Cole K**, Fassler S, Suryadevara S, Zebley DM. Increasing the number of attacks increases the conversion rate in laparoscopic diverticulitis surgery. *Surg Endosc* 2009; **23**: 1088-1092
27 **Rafferty J**, Shellito P, Hyman NH, Buie WD. Practice parameters for sigmoid diverticulitis. *Dis Colon Rectum* 2006; **49**: 939-944
28 **Benn PL**, Wolff BG, Ilstrup DM. Level of anastomosis and recurrent colonic diverticulitis. *Am J Surg* 1986; **151**: 269-271

29 **Thaler K**, Baig MK, Berho M, Weiss EG, Nogueras JJ, Arnaud JP, Wexner SD, Bergamaschi R. Determinants of recurrence after sigmoid resection for uncomplicated diverticulitis. *Dis Colon Rectum* 2003; **46**: 385-388
30 **Tocchi A**, Mazzoni G, Fornasari V, Miccini M, Daddi G, Tagliacozzo S. Preservation of the inferior mesenteric artery in colorectal resection for complicated diverticular disease. *Am J Surg* 2001; **182**: 162-167
31 **Forgione A**, Leroy J, Cahill RA, Bailey C, Simone M, Mutter D, Marescaux J. Prospective evaluation of functional outcome after laparoscopic sigmoid colectomy. *Ann Surg* 2009; **249**: 218-224
32 **Sarli L**, Pavlidis C, Cinieri FG, Regina G, Sansebastiano G, Veronesi L, Ferro M, Morari S, Violi V, Roncoroni L. Prospective comparison of laparoscopic left hemicolectomy for colon cancer with laparoscopic left hemicolectomy for benign colorectal disease. *World J Surg* 2006; **30**: 446-452
33 **Wasvary H**, Turfah F, Kadro O, Beauregard W. Same hospitalization resection for acute diverticulitis. *Am Surg* 1999; **65**: 632-635; discussion 636
34 **Zdichavsky M**, Granderath FA, Blumenstock G, Kramer M, Kuper MA, Konigsrainer A. Acute laparoscopic intervention for diverticular disease (AIDD): a feasible approach. *Langenbecks Arch Surg* 2010; **395**: 41-48
35 **Natarajan S**, Ewings EL, Vega RJ. Laparoscopic sigmoid colectomy after acute diverticulitis: when to operate? *Surgery* 2004; **136**: 725-730
36 **Zingg U**, Pasternak I, Guertler L, Dietrich M, Wohlwend KA, Metzger U. Early vs. delayed elective laparoscopic-assisted colectomy in sigmoid diverticulitis: timing of surgery in relation to the acute attack. *Dis Colon Rectum* 2007; **50**: 1911-1917
37 **Reissfelder C**, Buhr HJ, Ritz JP. What is the optimal time of surgical intervention after an acute attack of sigmoid diverticulitis: early or late elective laparoscopic resection? *Dis Colon Rectum* 2006; **49**: 1842-1848
38 **Weber WP**, Guller U, Jain NB, Pietrobon R, Oertli D. Impact of surgeon and hospital caseload on the likelihood of performing laparoscopic vs open sigmoid resection for diverticular disease: a study based on 55,949 patients. *Arch Surg* 2007; **142**: 253-259; discussion 259
39 **Pugliese R**, Di Lernia S, Sansonna F, Scandroglio I, Maggioni D, Ferrari C, Costanzi A, Chiara O. Laparoscopic treatment of sigmoid diverticulitis: a retrospective review of 103 cases. *Surg Endosc* 2004; **18**: 1344-1348
40 **Schwandner O**, Farke S, Fischer F, Eckmann C, Schiedeck TH, Bruch HP. Laparoscopic colectomy for recurrent and complicated diverticulitis: a prospective study of 396 patients. *Langenbecks Arch Surg* 2004; **389**: 97-103
41 **Garrett KA**, Champagne BJ, Valerian BT, Peterson D, Lee EC. A single training center's experience with 200 consecutive cases of diverticulitis: can all patients be approached laparoscopically? *Surg Endosc* 2008; **22**: 2503-2508
42 **Jones OM**, Stevenson AR, Clark D, Stitz RW, Lumley JW. Laparoscopic resection for diverticular disease: follow-up of 500 consecutive patients. *Ann Surg* 2008; **248**: 1092-1097
43 **Faynsod M**, Stamos MJ, Arnell T, Borden C, Udani S, Vargas H. A case-control study of laparoscopic versus open sigmoid colectomy for diverticulitis. *Am Surg* 2000; **66**: 841-843
44 **Dwivedi A**, Chahin F, Agrawal S, Chau WY, Tootla A, Tootla F, Silva YJ. Laparoscopic colectomy vs. open colectomy for sigmoid diverticular disease. *Dis Colon Rectum* 2002; **45**: 1309-1314; discussion 1314-1315
45 **Lawrence DM**, Pasquale MD, Wasser TE. Laparoscopic versus open sigmoid colectomy for diverticulitis. *Am Surg* 2003; **69**: 499-503; discussion 503-504
46 **Gonzalez R**, Smith CD, Mattar SG, Venkatesh KR, Mason E, Duncan T, Wilson R, Miller J, Ramshaw BJ. Laparoscopic vs open resection for the treatment of diverticular disease. *Surg Endosc* 2004; **18**: 276-280
47 **Alves A**, Panis Y, Slim K, Heyd B, Kwiatkowski F, Mantion G. French multicentre prospective observational study of laparoscopic versus open colectomy for sigmoid diverticular disease. *Br J Surg* 2005; **92**: 1520-1525
48 **Senagore AJ**, Duepree HJ, Delaney CP, Dissanaike S, Brady KM, Fazio VW. Cost structure of laparoscopic and open sigmoid colectomy for diverticular disease: similarities and differences. *Dis Colon Rectum* 2002; **45**: 485-490
49 **Anderson J**, Luchtefeld M, Dujovny N, Hoedema R, Kim D, Butcher J. A comparison of laparoscopic, hand-assist and open sigmoid resection in the treatment of diverticular disease. *Am J Surg* 2007; **193**: 400-403; discussion 403
50 **Lee SW**, Yoo J, Dujovny N, Sonoda T, Milsom JW. Laparoscopic vs. hand-assisted laparoscopic sigmoidectomy for diverticulitis. *Dis Colon Rectum* 2006; **49**: 464-469
51 **Leroy J**, Cahill RA, Asakuma M, Dallemagne B, Marescaux J. Single-access laparoscopic sigmoidectomy as definitive surgical management of prior diverticulitis in a human patient. *Arch Surg* 2009; **144**: 173-179; discussion 179
52 **Guller U**, Jain N, Hervey S, Purves H, Pietrobon R. Laparoscopic vs open colectomy: outcomes comparison based on large nationwide databases. *Arch Surg* 2003; **138**: 1179-1186
53 **Hinojosa MW**, Murrell ZA, Konyalian VR, Mills S, Nguyen NT, Stamos MJ. Comparison of laparoscopic vs open sigmoid colectomy for benign and malignant disease at academic medical centers. *J Gastrointest Surg* 2007; **11**: 1423-1429; discussion 1429-1430
54 **Klarenbeek BR**, Veenhof AA, Bergamaschi R, van der Peet DL, van den Broek WT, de Lange ES, Bemelman WA, Heres P, Lacy AM, Engel AF, Cuesta MA. Laparoscopic sigmoid resection for diverticulitis decreases major morbidity rates: a randomized control trial: short-term results of the Sigma Trial. *Ann Surg* 2009; **249**: 39-44
55 **Scheidbach H**, Schneider C, Rose J, Konradt J, Gross E, Bärlehner E, Pross M, Schmidt U, Köckerling F, Lippert H. Laparoscopic approach to treatment of sigmoid diverticulitis: changes in the spectrum of indications and results of a prospective, multicenter study on 1,545 patients. *Dis Colon Rectum* 2004; **47**: 1883-1888
56 **Bergamaschi R**, Arnaud JP. Anastomosis level and specimen length in surgery for uncomplicated diverticulitis of the sigmoid. *Surg Endosc* 1998; **12**: 1149-1151
57 **Ambrosetti P**, Francis K, De Peyer R, Frossard JL. Colorectal anastomotic stenosis after elective laparoscopic sigmoidectomy for diverticular disease: a prospective evaluation of 68 patients. *Dis Colon Rectum* 2008; **51**: 1345-1349
58 **Egger B**, Peter MK, Candinas D. Persistent symptoms after elective sigmoid resection for diverticulitis. *Dis Colon Rectum* 2008; **51**: 1044-1048
59 **Lidor AO**, Gearhart SL, Wu AW, Chang DC. Effect of race and insurance status on presentation, treatment, and mortality in patients undergoing surgery for diverticulitis. *Arch Surg* 2008; **143**: 1160-1165; discussion 1165
60 **Nelson RS**, Ewing BM, Wengert TJ, Thorson AG. Clinical outcomes of complicated diverticulitis managed nonoperatively. *Am J Surg* 2008; **196**: 969-972; discussion 973-974
61 **Reissfelder C**, Buhr HJ, Ritz JP. Can laparoscopically assisted sigmoid resection provide uncomplicated management even in cases of complicated diverticulitis? *Surg Endosc* 2006; **20**: 1055-1059
62 **Zapletal C**, Woeste G, Bechstein WO, Wullstein C. Laparoscopic sigmoid resections for diverticulitis complicated by abscesses or fistulas. *Int J Colorectal Dis* 2007; **22**: 1515-1521
63 **Nguyen SQ**, Divino CM, Vine A, Reiner M, Katz LB, Salky B. Laparoscopic surgery for diverticular disease complicated by fistulae. *JSLS* 2006; **10**: 166-168
64 **Laurent SR**, Detroz B, Detry O, Degauque C, Honoré P, Meurisse M. Laparoscopic sigmoidectomy for fistulized diverticulitis. *Dis Colon Rectum* 2005; **48**: 148-152
65 **Pokala N**, Delaney CP, Brady KM, Senagore AJ. Elective

laparoscopic surgery for benign internal enteric fistulas: a review of 43 cases. *Surg Endosc* 2005; **19**: 222-225
66 **Bartus CM**, Lipof T, Sarwar CM, Vignati PV, Johnson KH, Sardella WV, Cohen JL. Colovesical fistula: not a contraindication to elective laparoscopic colectomy. *Dis Colon Rectum* 2005; **48**: 233-236
67 **Vargas HD**, Ramirez RT, Hoffman GC, Hubbard GW, Gould RJ, Wohlgemuth SD, Ruffin WK, Hatter JE, Kolm P. Defining the role of laparoscopic-assisted sigmoid colectomy for diverticulitis. *Dis Colon Rectum* 2000; **43**: 1726-1731
68 **Hassan I**, Cima RR, Larson DW, Dozois EJ, O'Byrne MM, Larson DR, Pemberton JH. The impact of uncomplicated and complicated diverticulitis on laparoscopic surgery conversion rates and patient outcomes. *Surg Endosc* 2007; **21**: 1690-1694
69 **Le Moine MC**, Fabre JM, Vacher C, Navarro F, Picot MC, Domergue J. Factors and consequences of conversion in laparoscopic sigmoidectomy for diverticular disease. *Br J Surg* 2003; **90**: 232-236
70 **Small AJ**, Young-Fadok TM, Baron TH. Expandable metal stent placement for benign colorectal obstruction: outcomes for 23 cases. *Surg Endosc* 2008; **22**: 454-462
71 **Kaiser AM**, Jiang JK, Lake JP, Ault G, Artinyan A, Gonzalez-Ruiz C, Essani R, Beart RW Jr. The management of complicated diverticulitis and the role of computed tomography. *Am J Gastroenterol* 2005; **100**: 910-917
72 **Neff CC**, vanSonnenberg E, Casola G, Wittich GR, Hoyt DB, Halasz NA, Martini DJ. Diverticular abscesses: percutaneous drainage. *Radiology* 1987; **163**: 15-18
73 **Bahadursingh AM**, Virgo KS, Kaminski DL, Longo WE. Spectrum of disease and outcome of complicated diverticular disease. *Am J Surg* 2003; **186**: 696-701
74 **Ambrosetti P**, Chautems R, Soravia C, Peiris-Waser N, Terrier F. Long-term outcome of mesocolic and pelvic diverticular abscesses of the left colon: a prospective study of 73 cases. *Dis Colon Rectum* 2005; **48**: 787-791
75 **Durmishi Y**, Gervaz P, Brandt D, Bucher P, Platon A, Morel P, Poletti PA. Results from percutaneous drainage of Hinchey stage II diverticulitis guided by computed tomography scan. *Surg Endosc* 2006; **20**: 1129-1133
76 **Kumar RR**, Kim JT, Haukoos JS, Macias LH, Dixon MR, Stamos MJ, Konyalian VR. Factors affecting the successful management of intra-abdominal abscesses with antibiotics and the need for percutaneous drainage. *Dis Colon Rectum* 2006; **49**: 183-189
77 **Siewert B**, Tye G, Kruskal J, Sosna J, Opelka F, Raptopoulos V, Goldberg SN. Impact of CT-guided drainage in the treatment of diverticular abscesses: size matters. *AJR Am J Roentgenol* 2006; **186**: 680-686
78 **Chouillard E**, Maggiori L, Ata T, Jarbaoui S, Rivkine E, Benhaim L, Ghiles E, Etienne JC, Fingerhut A. Laparoscopic two-stage left colonic resection for patients with peritonitis caused by acute diverticulitis. *Dis Colon Rectum* 2007; **50**: 1157-1163
79 **Agaba EA**, Zaidi RM, Ramzy P, Aftab M, Rubach E, Gecelter G, Ravikumar TS, DeNoto G. Laparoscopic Hartmann's procedure: a viable option for treatment of acutely perforated diverticultis. *Surg Endosc* 2009; **23**: 1483-1486
80 **Church JM**. Surgical treatment of sigmoid diverticulitis. *Schweiz Med Wochenschr* 1991; **121**: 744-748
81 **Salem L**, Anaya DA, Roberts KE, Flum DR. Hartmann's colectomy and reversal in diverticulitis: a population-level assessment. *Dis Colon Rectum* 2005; **48**: 988-995
82 **Salem L**, Flum DR. Primary anastomosis or Hartmann's procedure for patients with diverticular peritonitis? A systematic review. *Dis Colon Rectum* 2004; **47**: 1953-1964
83 **Chandra V**, Nelson H, Larson DR, Harrington JR. Impact of primary resection on the outcome of patients with perforated diverticulitis. *Arch Surg* 2004; **139**: 1221-1224
84 **Oomen JL**, Cuesta MA, Engel AF. Reversal of Hartmann's procedure after surgery for complications of diverticular disease of the sigmoid colon is safe and possible in most patients. *Dig Surg* 2005; **22**: 419-425
85 **Elliott TB**, Yego S, Irvin TT. Five-year audit of the acute complications of diverticular disease. *Br J Surg* 1997; **84**: 535-539
86 **Wedell J**, Banzhaf G, Chaoui R, Fischer R, Reichmann J. Surgical management of complicated colonic diverticulitis. *Br J Surg* 1997; **84**: 380-383
87 **Maggard MA**, Zingmond D, O'Connell JB, Ko CY. What proportion of patients with an ostomy (for diverticulitis) get reversed? *Am Surg* 2004; **70**: 928-931
88 **Wigmore SJ**, Duthie GS, Young IE, Spalding EM, Rainey JB. Restoration of intestinal continuity following Hartmann's procedure: the Lothian experience 1987-1992. *Br J Surg* 1995; **82**: 27-30
89 **Aydin HN**, Remzi FH, Tekkis PP, Fazio VW. Hartmann's reversal is associated with high postoperative adverse events. *Dis Colon Rectum* 2005; **48**: 2117-2126
90 **Richter S**, Lindemann W, Kollmar O, Pistorius GA, Maurer CA, Schilling MK. One-stage sigmoid colon resection for perforated sigmoid diverticulitis (Hinchey stages III and IV). *World J Surg* 2006; **30**: 1027-1032
91 **Abbas S**. Resection and primary anastomosis in acute complicated diverticulitis, a systematic review of the literature. *Int J Colorectal Dis* 2007; **22**: 351-357
92 **Biondo S**, Perea MT, RPerea MT, Rague JM, Jaurrieta E. One-stage procedure in non-elective surgery for diverticular disease complications. *Colorectal Dis* 2001; **3**: 42-45
93 **Regenet N**, Tuech JJ, Pessaux P, Ziani M, Rouge C, Hennekinne S, Arnaud JP. Intraoperative colonic lavage with primary anastomosis vs. Hartmann's procedure for perforated diverticular disease of the colon: a consecutive study. *Hepatogastroenterology* 2002; **49**: 664-667
94 **Constantinides VA**, Heriot A, Remzi F, Darzi A, Senapati A, Fazio VW, Tekkis PP. Operative strategies for diverticular peritonitis: a decision analysis between primary resection and anastomosis versus Hartmann's procedures. *Ann Surg* 2007; **245**: 94-103
95 **O'Sullivan GC**, Murphy D, O'Brien MG, Ireland A. Laparoscopic management of generalized peritonitis due to perforated colonic diverticula. *Am J Surg* 1996; **171**: 432-434
96 **Faranda C**, Barrat C, Catheline JM, Champault GG. Two-stage laparoscopic management of generalized peritonitis due to perforated sigmoid diverticula: eighteen cases. *Surg Laparosc Endosc Percutan Tech* 2000; **10**: 135-138; discussion 139-141
97 **Taylor CJ**, Layani L, Ghusn MA, White SI. Perforated diverticulitis managed by laparoscopic lavage. *ANZ J Surg* 2006; **76**: 962-965
98 **Bretagnol F**, Pautrat K, Mor C, Benchellal Z, Huten N, de Calan L. Emergency laparoscopic management of perforated sigmoid diverticulitis: a promising alternative to more radical procedures. *J Am Coll Surg* 2008; **206**: 654-657
99 **Franklin ME Jr**, Portillo G, Trevino JM, Gonzalez JJ, Glass JL. Long-term experience with the laparoscopic approach to perforated diverticulitis plus generalized peritonitis. *World J Surg* 2008; **32**: 1507-1511
100 **Karoui M**, Champault A, Pautrat K, Valleur P, Cherqui D, Champault G. Laparoscopic peritoneal lavage or primary anastomosis with defunctioning stoma for Hinchey 3 complicated diverticulitis: results of a comparative study. *Dis Colon Rectum* 2009; **52**: 609-615
101 **Myers E**, Hurley M, O'Sullivan GC, Kavanagh D, Wilson I, Winter DC. Laparoscopic peritoneal lavage for generalized peritonitis due to perforated diverticulitis. *Br J Surg* 2008; **95**: 97-101
102 **Pautrat K**, Bretagnol F, Huten N, de Calan L. Acute diverticulitis in very young patients: a frequent surgical mana-

gement. *Dis Colon Rectum* 2007; **50**: 472-477
103 **Lahat A**, Menachem Y, Avidan B, Yanai H, Sakhnini E, Bardan E, Bar-Meir S. Diverticulitis in the young patient--is it different ? *World J Gastroenterol* 2006; **12**: 2932-2935
104 **Konvolinka CW**. Acute diverticulitis under age forty. *Am J Surg* 1994; **167**: 562-565
105 **Cunningham MA**, Davis JW, Kaups KL. Medical versus surgical management of diverticulitis in patients under age 40. *Am J Surg* 1997; **174**: 733-735; discussion 735-736
106 **Vignati PV**, Welch JP, Cohen JL. Long-term management of diverticulitis in young patients. *Dis Colon Rectum* 1995; **38**: 627-629
107 **Spivak H**, Weinrauch S, Harvey JC, Surick B, Ferstenberg H, Friedman I. Acute colonic diverticulitis in the young. *Dis Colon Rectum* 1997; **40**: 570-574
108 **Biondo S**, Pares D, Marti Rague J, Kreisler E, Fraccalvieri D, Jaurrieta E. Acute colonic diverticulitis in patients under 50 years of age. *Br J Surg* 2002; **89**: 1137-1141
109 **Nelson RS**, Velasco A, Mukesh BN. Management of diverticulitis in younger patients. *Dis Colon Rectum* 2006; **49**: 1341-1345
110 **Guzzo J**, Hyman N. Diverticulitis in young patients: is resection after a single attack always warranted? *Dis Colon Rectum* 2004; **47**: 1187-1190; discussion 1190-1191
111 **Chautems RC**, Ambrosetti P, Ludwig A, Mermillod B, Morel P, Soravia C. Long-term follow-up after first acute episode of sigmoid diverticulitis: is surgery mandatory?: a prospective study of 118 patients. *Dis Colon Rectum* 2002; **45**: 962-966
112 **Perkins JD**, Shield CF 3rd, Chang FC, Farha GJ. Acute diverticulitis. Comparison of treatment in immunocompromised and nonimmunocompromised patients. *Am J Surg* 1984; **148**: 745-748
113 **Tyau ES**, Prystowsky JB, Joehl RJ, Nahrwold DL. Acute diverticulitis. A complicated problem in the immunocompromised patient. *Arch Surg* 1991; **126**: 855-858; discussion 858-859
114 **Qasabian RA**, Meagher AP, Lee R, Dore GJ, Keogh A. Severe diverticulitis after heart, lung, and heart-lung transplantation. *J Heart Lung Transplant* 2004; **23**: 845-849
115 **Alexander P**, Schuman E, Vetto RM. Perforation of the colon in the immunocompromised patient. *Am J Surg* 1986; **151**: 557-561
116 **Church JM**, Fazio VW, Braun WE, Novick AC, Steinmuller DR. Perforation of the colon in renal homograft recipients. A report of 11 cases and a review of the literature. *Ann Surg* 1986; **203**: 69-76
117 **Aydin HN**, Remzi FH. Diverticulitis: when and how to operate? *Dig Liver Dis* 2004; **36**: 435-445
118 **Carson SD**, Krom RA, Uchida K, Yokota K, West JC, Weil R 3rd. Colon perforation after kidney transplantation. *Ann Surg* 1978; **188**: 109-113
119 **Sawyerr OI**, Garvin PJ, Codd JE, Graff RJ, Newton WT, Willman VL. Colorectal complications of renal allograft transplantation. *Arch Surg* 1978; **113**: 84-86
120 **Sachar DB**. Diverticulitis in immunosuppressed patients. *J Clin Gastroenterol* 2008; **42**: 1154-1155
121 **Horgan AF**, McConnell EJ, Wolff BG, The S, Paterson C. Atypical diverticular disease: surgical results. *Dis Colon Rectum* 2001; **44**: 1315-1318
122 **Freeman HJ**. Natural history and long-term clinical behavior of segmental colitis associated with diverticulosis (SCAD syndrome). *Dig Dis Sci* 2008; **53**: 2452-2457
123 **Peppercorn MA**. The overlap of inflammatory bowel disease and diverticular disease. *J Clin Gastroenterol* 2004; **38**: S8-S10
124 **Ludeman L**, Shepherd NA. What is diverticular colitis? *Pathology* 2002; **34**: 568-572
125 **Harpaz N**, Sachar DB. Segmental colitis associated with diverticular disease and other IBD look-alikes. *J Clin Gastroenterol* 2006; **40** Suppl 3: S132-S135
126 **Stefansson T**, Ekbom A, Sparen P, Pahlman L. Association between sigmoid diverticulitis and left-sided colon cancer: a nested, population-based, case control study. *Scand J Gastroenterol* 2004; **39**: 743-747

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REVIEW

Vascular invasion in pancreatic cancer: Imaging modalities, preoperative diagnosis and surgical management

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Abstract

Pancreatic cancer is associated with a poor prognosis, and surgical resection remains the only chance for curative therapy. In the absence of metastatic disease, which would preclude resection, assessment of vascular invasion is an important parameter for determining resectability of pancreatic cancer. A frequent error is to misdiagnose an involved major vessel. Obviously, surgical exploration with pathological examination remains the "gold standard" in terms of evaluation of resectability, especially from the point of view of vascular involvement. However, current imaging modalities have improved and allow detection of vascular invasion with more accuracy. A venous resection in pancreatic cancer is a feasible technique and relatively reliable. Nevertheless, a survival benefit is not achieved by curative resection in patients with pancreatic cancer and vascular invasion. Although the discovery of an arterial invasion during the operation might require an aggressive management, discovery before the operation should be considered as a contraindication. Detection of vascular invasion remains one of the most important challenges in pancreatic surgery. The aim of this article is to provide a complete review of the different imaging modalities in the detection of vascular invasion in pancreatic cancer.

Key words: Vascular invasion; Cancer; Pancreas; Management

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INTRODUCTION

The incidence of pancreatic cancer has gradually increased over the 20th century and in the early years of this century[1,2]. Cancer of the pancreas is the sixth most common cancer and fourth cause of death from cancer (22% of deaths among gastrointestinal cancers)[1-3].

Pancreatic cancer is associated with a poor prognosis, with less than 5% of patients surviving 5 years after the diagnosis[4]. Surgical resection remains the only chance for curative therapy in these patients[5-7]. Accurate preoperative staging of pancreatic cancer is essential to avoid unnecessary surgery in those with unresectable disease and, at the same time, in order not to deny the opportunity for cure in patients with resectable disease[5,6,8].

Only 16% of patients initially present a disease confined to the pancreas (stage I)[6,7]. Thus, of patients seen, 85%-90% have surgically unresectable tumors at the time of diagnosis[6,7,9-11].

There is no evidence-based consensus on the optimal preoperative imaging assessment of patients with suspected pancreatic cancer[6,8,12].

The criteria of unresectability are numerous[7,13-23]. However, in the absence of metastatic disease which precludes resection, assessment of vascular invasion is an important parameter for determining resectability for pancreatic cancer[5]. A frequent error is to misdiagnose an involved major vessel[11]. Vascular invasion is a relatively frequent discovery in pancreatic cancer; found in 21%-64% of patients, depending on the population studied[7,24].

From the point of view of arterial vessels, a tumoral infiltration of a large trunk (celiac axis, superior mesenteric artery, or hepatic artery) must be carefully analyzed because it constitutes a contraindication to surgery[25-27]. However, isolated involvement of smaller branches such as the gastro-duodenal artery will not preclude surgical resection[25]. The superior mesenteric vessels are the most frequently involved vessels in this cancer, due to their intimate relationship with the head, the uncinate process, and body of the pancreas[25,28].

Limited venous invasion does not represent an absolute contraindication for surgery[4,26,27,29-31]. Obviously, surgical exploration with pathological examination remains the "gold standard" in terms of evaluation of resectability, especially from the point of view of vascular involvement. However, current imaging modalities have improved and allow detection of vascular invasion with more accuracy. Detection is the key to the surgeon's preoperative planning, because the posterior and lateral surfaces of the portal and superior mesenteric vein can be evaluated only after the surgical procedure is well advanced[14]. Thus, the management of a suspicious tumoral adhesion to a vessel is one of the most important challenges in a Whipple type procedure.

In this review, the current imaging modalities for assessing vascular involvement of pancreatic cancer will be discussed. Subsequently, the management and outcome of vascular invasion in patients with pancreatic cancer will also be reviewed briefly.

COMPUTED TOMOGRAPHY

Computer tomography (CT) gives information about localization, size and extension of tumor[8,18], while being non-invasive[32]. A recent meta-analysis showed CT to be 91% sensitive and 85% specific for tumoral detection[33]. Phoa *et al*[34] showed that, with regard to tumor convexity towards a vessel, Grades D (concave contour of the tumor towards vessel) or E (circumferential involvement of vessel) have a risk of invasion of 88%; and a possibility of resection of 7% for the type D and of 0% for the type E[35]. Loyer *et al*[35] found that Grades A (fat plane separating the tumor from the vessel) and B (normal pancreatic tissue between tumor and vessel) had a resection rate of 95%, therefore these two grades are factors of better prognosis.

On the other hand, the length of tumor contact with the vessel (if it is greater than 5 mm) is a relatively good predictive factor for vascular invasion (78% for portal vein and 81% for superior mesenteric vein)[34].

A circumferential contact of more than 180 degrees has been shown to have a good correlation with unresectability[34,36,37]. For this criterion, Lu *et al*[38] found a sensitivity of 84%, a specificity of 98%, a positive predictive value (PPV) of 95%, and a negative predictive value (NPV) of 93%, for unresectability. Furthermore, Phoa *et al*[34] reported a sensitivity of 60%, and a specificity of 90%, if tumor convexity Grades D or E were combined with circumferential involvement of > 90 degrees. In addition, a strongly narrowed vessel also has an important risk of being invaded[34,36], but prudence is essential, especially for a vein, due to the mass effect of the tumor without the presence of vascular invasion[10,39,40]. In addition, an artery may be completely invaded, with no apparent change in vessel caliber[36,39].

Concerning the irregularity of the vascular wall, Li *et al*[36] reported a sensitivity and a specificity of 45% and 99%, respectively, for tumor detection in arteries, and 63% and 100% in the case of veins.

Regarding the rare superior mesenteric vein teardrop sign, Hough *et al*[41] found a sensitivity of this CT sign of 91% and a specificity of 98%; similar findings were reported in other series[36].

Consequently, Li *et al*[36] reported that the CT criteria for arterial invasion might be: an arterial embedment in tumor, or the combination of tumor involvement of more than one-half of the circumference of the arteries with artery wall irregularity or with artery stenosis (sensitivity of 79%, specificity of 99%). The criteria for venous invasion might be venous occlusion, tumor involvement of more than one-half of the circumference of the veins, vein wall irregularity, vein caliber stenosis, and teardrop superior mesenteric vein sign (sensitivity of 92%, specificity of 100%).

From the point of view of the detection of vascular invasion, many studies have evaluated CT (Table 1). CT has improved much these last years. Technology has developed multi-slice with 4-64 detector rows, allowed thin-sections and dual-phase, with faster time of acquisition, and numerous possibilities of image post-processing (3D reconstructions, multiplanar reconstructions)[19,29,40,42-45].

Fourteen years ago, Yoshimi *et al*[46] reported one of the first cases of 3D vascular reconstruction, allowing the evaluation of portal invasion with a higher accuracy than angiography alone. Currently, pancreatic section thickness of 1 mm is obtained in approximately 20 s, allowing true volume acquisition, with vascular details better than angiography[28,47,48] useful when assessing vascular invasion[44]. Furthermore, CT angiography allows anatomical study of small pancreatic vessels with a remarkable degree of accuracy[49,50].

Moreover, dilation of the peri-pancreatic veins with no visualization of inferior branches on CT suggests tumor invasion of peri-pancreatic tissue[50].

Several studies have highlighted the importance of

Table 1 CT performance in the detection of vascular invasion in more than 50 patients with pancreatic cancer

Studies (yr)	*n*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Megibow *et al*[24] (1995)	118	47	69	89	28
Raptopoulos *et al*[208] (1997)	82	NA	NA	NA	96
Sugiyama *et al*[91] (1997)	73	65[1]	77	NA	NA
McCarthy *et al*[16] (1998)	67	NA	NA	55/94[2]	95/94
Diehl *et al*[209] (1998)	89	86	NA	NA	NA
Böttger *et al*[10] (1998)	255	22.2[3]	96.4	72.7	74.1
Sugiyama *et al*[88] (1999)	91	64[4]	79	NA	NA
Nakao *et al*[105] (1999)	55	82.1[5]	74.1	76.7	80
Pietrabissa *et al*[130] (1999)	50	82	53	NA	NA
Gress *et al*[89] (1999)	151	15	100	100	60
Squillaci *et al*[69] (2003)	50	97	100	100	95
House *et al*[210] (2004)	115	85-87[6]	95-99	83-93	92-98
Soriano *et al*[8] (2004)	62	67	94	89	80
Li *et al*[36] (2005)	54	92/79[7]	100/99	NA	NA
Buchs *et al*[98] (2007)	153	54.5[8]	91.2	66.7	86.1

[1,3,4,5]Only evaluated for portal vein invasion; [2]PPV of 55% for venous invasion and 94% for arterial invasion; NPV of 95% for venous invasion and 94% for arterial invasion; [6]Sensitivity of 85% for the superior mesenteric and portal vein invasion, 86% for the superior mesenteric artery invasion, 87% for the celiac trunk invasion; specificity of 95% for the superior mesenteric vein and portal vein involvement, 97% for the superior mesenteric artery invasion, 99% for celiac trunk involvement; PPV of 90% for the superior mesenteric vein and portal vein invasion, 83% for the superior mesenteric artery involvement, 93% for celiac trunk invasion; NPV of 92% for the superior mesenteric vein and portal vein involvement, 98% for the superior mesenteric artery and celiac trunk invasion; [7]Sensitivity of 92% for venous invasion and 79% for arterial invasion; specificity of 100% for the veins and 99% for the arteries; [8]For multi-slice CT. CT: Computer tomography; PPV: Positive predictive value; NPV: Negative predictive value; NA: Not available.

the moment of image acquisition. With regard to the pancreas, it seems that a portal venous phase (60 s after intravenous administration of iodinated contrast medium) or that a pancreatic phase (40-70 s) provides more information than an arterial phase (18 s) or that of a hepatic phase (70 to 100 s)[19,29,51-54]. McNulty *et al*[51] reported that an arterial phase can be reserved for patients in whom CT angiography is required.

Lastly, Imbriaco *et al*[55] showed that dual-phase helical CT (arterial: 20 s, and pancreatic late: 70 s) was interesting but was comparable with single-phase helical CT (pancreatic early: 50 s).

In conclusion, CT is the assessment of choice in first intention, permitting in one non-invasive examination a TNM staging evaluation.

From the vascular point of view, many criteria exist (especially circumferential involvement of vessel of more than 180 degrees, radiological absence of a fat plane between tumor and vessel, vascular occlusion with collaterals, teardrop sign) which allow accuracy in diagnosing vascular invasion. Development of new radiological techniques (3D reconstructions, multiplanar reconstructions) has improved accuracy of assessment of vascular invasion.

MAGNETIC RESONANCE IMAGING (MRI)

MRI with cholangiopancreatography gives much information for the evaluation of primary tumor and metastatic dissemination, improved by the use of gadolinium or mangafodipir trisodium[1,13,47,56-58]. Currently, the use of MRI in an "all-in-one" staging method (MRI, coupled with angiography and cholangiopancreatography) is a subject under deliberation[58-60].

MRI criteria for vascular invasion are: (1) occlusion of the vessel, with or without collaterals, (2) tumoral infiltration of peri-vascular fat tissue, (3) circumferential contact of more than 180 degrees between the tumor and the vessel, and (4) mass effect along one side of the vessel for more than 2 cm[7,56,60,61].

As regards the detection of vascular invasion, MRI has an accuracy of approximately 94% for enhanced T1-weighted imaging[62]. Romijn *et al*[58] found in their study an accuracy of 81% with mangafodipir trisodium (definitely higher than MRI without contrast medium).

Other studies have attempted to analyze the performance of MRI in the detection of vascular invasion. They found a sensitivity of 47%-83%[24,60], a specificity of more than 95%[7,59], a PPV of more than 70%[7,8], and a NPV of 23%-96%[24,60].

Modern MRI technology makes it possible to obtain 3D reconstructions, facilitating the study of the peri-pancreatic vessels[61,63,64]. Some series have also demonstrated the adequate time for vascular pancreatic image acquisition: biphasic imaging at 15 and 45 s after arrival of contrast material (gadolinium) in the abdominal aorta[65].

Accuracy of MRI for vascular visualization is quite similar to that of CT[56,66,67]. It consequently seems logical to reserve this expensive and time-consuming technology for those patients not able to benefit from CT (allergy to iodine, renal insufficiency, pregnancy) or if CT findings are inconclusive[68].

ANGIOGRAPHY

Currently, conventional angiography is no longer part of the diagnostic protocol in most centers[13], because this examination does not permit the detection of the tumor itself[1], and can easily be replaced by other less invasive methods which give more information on tumoral extension.

On the other hand, preoperative arteriography may visualize vascular abnormalities (anatomical variations, acquired stenosis), allowing a possible modification of surgical strategy (revascularisation, replacement hepatic artery, embolization of an aneurism)[17,69,70].

With regard to vascular invasion, angiographic criteria are: (1) vascular stenosis or occlusion, with or without collaterals, (2) thrombosis of a vessel, (3) acute angle appearing in the venous wall, and (4) envelopment of the vessel within tumor[69,71-74].

In at least 20% of cases, angiography misses the vascular invasion[10], because it gives only information about the lumen of the vessel[72]. Angiography depends upon displacement of vessels and distortion of vascular contours unless clear vessel occlusion is present. Furthermore, the tumor may completely encase and invade the small amount of fat surrounding the vessel, and yet not cause a distortion of the contour of the vascular lumen, which is required for detection on angiography. This feature can be visualized during endoscopic ultrasonography or CT. Thus, angiography requires more extensive vascular involvement in order for it to be detected[5,74,75].

The results reported for detection of vascular invasion by pancreatic cancer using angiography are: a sensitivity between 21%[5,8] and more than 80%[10,76], a specificity between 50%[72] and 100%[8,69], a PPV more than 60%[5,72], and a NPV between 50%[72] and 83%[10]. Late angiographic times allow visualization of the portal vein, and possible invasions. In addition, it is possible to inject contrast medium directly into the portal vein by a transhepatic access, for example at the time of intravascular ultrasonography (see below).

In conclusion, studies show that angiography is paradoxically relatively poor in the detection of vascular invasion. On the other hand, it permits the visualization of arterial and venous anomalies, allowing a change in surgical strategy.

ABDOMINAL ULTRASONOGRAPHY (US)

Abdominal US is often the first line examination for a patient presenting with jaundice and pain[13].

From the vascular point of view, US coupled with Doppler gives a reasonably reliable measure of vascular patency and can improve accuracy in assessing vascular invasion[13,77,78]. Its sensitivity ranges between 60%[79] and more than 90%[80]; its specificity has been reported to be higher than 90%[79,80], the PPV is higher than 90%[81], and the NPV is higher than 75%[82,83]. Very recently, authors reported US to be 93% accurate in detecting portal vein invasion, by using 3D vascular reconstruction technology[84].

Color Doppler sonographic criteria for vascular invasion are: (1) absence of hyperechoic tissue between the tumor and the vessel, (2) more than 2 cm continuity between tumor and vessel, (3) circumferential contact between the tumor and the vessel, (4) circumferential narrowing of vessel lumen, and (5) vascular occlusion or thrombosis[81-83,85-87].

In addition, perioperative US has been reported as 100% sensitive in identifying tumors, and 92% sensitive and specific in detecting portal invasion[88]. In 22% of patients with pancreatic neoplasms, US-Doppler makes it possible to modify therapeutic strategy[86].

In conclusion, US coupled with Doppler is a relatively accurate, cheap, and non-ionizing imaging modality for initial screening of patients with suspicion of tumors of the pancreas. However, US has demonstrated weakness in recognition of deeper localizations.

With regard to the detection of vascular invasion, studies have shown that US coupled with Doppler is a reliable method. However, these series evaluated almost exclusively the portal vein and its tributaries. Recent improvement in US imaging, allowing 3D reconstruction, offers new potential for this technology in the assessment of tumoral vascular involvement.

ENDOSCOPIC ULTRASONOGRAPHY (EUS)

EUS is a relatively new technique, providing direct ultrasonic imaging of the pancreas through the gastrointestinal lumen[2,13]. However, the probes are expensive and EUS requires a trained endoscopist[13,63].

EUS has been shown to be accurate in diagnosing and staging pancreatic cancer[89], with the help of fine needle aspiration (FNA), with 96.6% sensitivity, 99.0% specificity, 96.2% NPV, and 99.1% PPV[90].

EUS criteria for vascular invasion are: (1) loss of the hyperechoic vessel wall/tumor interface, (2) direct visualization of tumor within the vessel lumen, (3) vascular encasement or occlusion, (4) non-visualization of a major vessel, in the presence of collaterals, (5) proximity of the tumor (< 3 mm) to the vessel, and (6) irregularity of the vascular wall[5,8,11,89,91-96].

Sugiyama *et al*[91] reported that EUS is more accurate than CT, US, and angiography for the detection of portal invasion; similar findings were shown in other series[97,98]. In addition, Brugge *et al*[93] showed that EUS was highly sensitive in the detection of portal and splenic vein invasions.

Arterial invasion is assessed with more difficulty by EUS[92,98-100]. Globally, the sensitivity is 50%-100%[92,95,101,102], the specificity 58%-100%[92,102], the PPV 28%-100%[92,96], and the NPV 18%-93%[89,94].

Very recently, Fritscher-Ravens *et al*[103] reported the use of 3D linear EUS in the assessment of vascular involvement with very interesting results compared with classical EUS. Linear 3D EUS enhanced the evaluation

of vascular involvement of pancreatic lesions, especially in chronic pancreatitis.

In conclusion, it is appropriate to incorporate EUS in the preoperative assessment when there is suspicion of pancreatic cancer. From the point of view of the detection of vascular invasion, EUS has shown good accuracy, especially for venous invasion.

INTRAVASCULAR ULTRASONOGRAPHY (IVUS)

When a tumor appears to be contiguous with the portal vein or with the superior mesenteric vein, the diagnosis of vascular invasion can be difficult. Some limited reports have suggested that IVUS might allow the distinction between a simple compression by mass effect and invasion[71].

Moreover, IVUS makes it possible to detect intraportal thrombus, sometimes missed by CT[71]. IVUS is performed either by a transhepatic access, or by a transmesenteric catheterization (during operative time)[71,104-108]. Complications are rare[72,104-106].

IVUS criteria for vascular invasion are: (1) obliteration of the echoic band of the portal vein by the hypoechoic tumor, (2) tumor mass blended with the venous wall, and (3) tumor protrusion into the vascular lumen[71,72,76,104-106,109].

One of the limitations of IVUS is the lack of specificity in the case of pancreatitis[71,105]. Moreover, IVUS has a limited penetration, allowing only localised investigations. Another weakness remains the lack of spatial orientation, making the interpretation of the images difficult[72,106].

There are few studies concerning IVUS in detection of vascular invasion in pancreatic cancer. Moreover, they report only portal and superior mesenteric vein results, not evaluating arterial invasion. The results are: sensitivity more than 95%[71,76], specificity more than 90%[71,76], PPV more than 90%[105], and NPV more than 95%[105].

Kaneko *et al*[109], has pioneered the use of IVUS in staging of pancreatic cancer, recently using 3D reconstructions of IVUS with a high degree of accuracy. Tezel *et al*[110] also reported that a contact of more than 18 mm between the tumor and the portal or the superior mesenteric vein was a factor of poor prognosis. The use of IVUS allows stent placement[111], a possibility in the palliative treatment of portal stenosis.

In conclusion, studies show that IVUS is probably superior to CT and portography for the detection of vascular invasion. However, data is available only for the portal vein and for the superior mesenteric vein. To our knowledge, there are no data concerning the utility of IVUS in detecting tumoral arterial invasion.

Because IVUS is expensive and invasive, Nakao *et al*[105] recommend performing this examination only in cases in which the distinction between compression and invasion cannot be made by conventional imaging techniques.

LAPAROSCOPY AND LAPAROSCOPIC ULTRASONOGRAPHY (LUS)

For almost 30 years[112], laparoscopic examination of the abdominal cavity has offered an excellent, although invasive, visualization of peritoneum and the liver[13,47,63,113,114].

From the vascular point of view, incision of the gastrohepatic omentum allows a direct access to the underlying vessels[47,115]. However, it seems certain that laparoscopy alone cannot detect vascular invasion, in particular mesenteric, without help of perioperative ultrasonography[116].

Currently, routine laparoscopy is not recommended in cases of cancer of the head of the pancreas, because it influences further surgical strategy in only 14%-19% of cases[116,117]. On the other hand, a study showed that in the case of cancer of the body or the tail of the pancreas, laparoscopy could avoid up to 50% of the operations, because of metastases not identified during staging[116].

Obviously, laparoscopy can also be used with a palliative aim (double derivations), if the tumor is unresectable[21,117-120]. Laparoscopy has its limits: it only allows visualization of the liver surface; impossibility of analyzing the retroperitoneum and its vessels; technical problems due to adherences[21,47,63,120,121].

LUS was subsequently developed, and this allows detailed study of the liver, the lymphatic area, and the corresponding vessels[47,121-126]. Vascular structures can be accurately visualized by LUS in approximately 95% of patients with tumors in the head of the pancreas[126].

LUS criteria for vascular invasion are: (1) loss of the hyperechoic vessel - tumor interface, (2) obliteration or thrombosis of a vessel, (3) a fixed stenosis, (4) vessel encasement by tumor encirclement and rigidity, and (5) presence of invading tumor within the vessel lumen[122,127-129].

There are numerous studies evaluating resectability by LUS, but to our knowledge few have focused on vascular invasion. They have found a sensitivity of more than 50%[129], a specificity of more than 80%[130], a PPV of 93%[127], and a NPV of 73%[128].

Despite these encouraging results, several authors do not recommend systematic use of laparoscopy or LUS. They prefer to recommend this technique for doubtful cases[21,121,131-133].

POSITRON EMISSION TOMOGRAPHY (PET) AND POSITRON EMISSION TOMOGRAPHY COUPLED WITH COMPUTED TOMOGRAPHY

PET is a non-invasive imaging method, which gives information about cellular metabolic activity.

Currently, 18F-fluorodeoxyglucose (FDG) is injected and taken up preferentially by malignant tumors, and secondary localizations, rather than by healthy tissue[13,17,18,47,63,134-136]. The FDG is not metabolized and is trapped inside the cell[47], allowing it to be imaged in contrast to surrounding tissue[18].

PET is accurate in diagnosing small tumors (< 2 cm), as well as peritoneal implants and metastases[13,47,63,102,135,137-141]. In addition, PET is able to differentiate

inflammatory pathologies from tumoral ones[47,135,139,142,143]. PET differentiates malignant and benign pathologies with a sensitivity of 85%-100% and a specificity of 67%-99%; often higher than that of CT[135, 141,144-146].

In addition, false negatives exist in the case of strongly differentiated tumors, small periampullary tumors or in cases of hyperglycemia[63,135,146,147]. In the case of normoglycemic patients, PET has a sensitivity for tumoral detection of 93%-98%[135,137,146,148,149], although in the case of hyperglycemic patients, this falls to 63%, or even less[135,137,146,149], in parallel with the NPV which falls from 96% to 38%[146].

Concerning lymphatic invasion, PET detection has proved poor, probably due to the proximity of regional lymph nodes to the primary tumor[102,134,135,137,150], and the lack of anatomic detail[13,18,139]. PET alone is unable to visualize vessels and cannot assess vascular invasion[63,135,151]. Thus, the association of PET with CT (PET/CT) seems promising[139,152].

Heinrich *et al*[139] showed recently that PET/CT has a PPV for the differentiation between a benign and a malignant pathology of 91%, whereas its NPV is 64%. PET/CT detects a cancer of the pancreas with a sensitivity of 93%, and is more specific than CT alone (69% *vs* 21%, respectively, $P = 0.07$). However, data are lacking regarding the assessment of vascular involvement. The use of multislice CT coupled with PET, and angio-CT protocols, might allow better visualization of the vessels.

SURGICAL MANAGEMENT OF VASCULAR INVASION

Frequently, vascular invasion may be assessed only when the operation is already quite advanced (section of the pancreas, digestive transection)[22,27,153-156]. Palpation at the time of the Kocher maneuver (maneuver which permits exposure of structures behind duodenum and pancreatic head) is commonly performed to assess the relationship of a pancreatic head tumor to the superior mesenteric artery. However, if the tumor is large, if there is associated pancreatitis, or if the patient is undergoing reoperation, palpation is an inaccurate way to assess this critical tumor-vessel relationship prior to gastric and pancreatic transection[22].

The management of a suspicious tumoral adhesion to a vessel is one of the most important challenges in a Whipple procedure. In such a case, the surgeon is confronted with three options: (1) leave tumor attached to the vessel, resulting in a grossly positive margin of resection; (2) try to separate the tumor from the vessel, with a considerable hemorrhagic risk; and (3) or perform a partial or segmental resection of the portion of invaded vessel with reconstruction[22].

Arterial invasion

If the invasion of the superior mesenteric or portal vein is not in itself a criterion of unresectability[4,154,155,157], arterial invasion is a more controversial issue. Many authors regard this invasion as a contraindication to surgery[27,154,158], because of the high morbidity and mortality rates associated with arterial resection and reconstruction[159]. Furthermore, arterial invasion usually includes extensive involvement of the mesenteric neural plexus[160], rendering radical resection oncologically unsound because of the frequent finding of positive margins[154].

However, in many cases, the preoperative assessment cannot diagnose such an invasion. The surgeon must then adapt his surgical strategy. Fortner[161] recommended the resection of invaded arterial segment, if a reconstruction seemed possible.

From the arterial point of view, celiac or hepatic invasion, discovered during the operation, can be the object of a resection and a reconstruction, either by direct anastomosis, by interposition of a venous graft (for example reverse saphenous or internal jugular vein), or with a prosthesis[156,161-163]. An arterial graft (for example the splenic artery) can also be used[156,163]. These techniques seemed relatively reliable, with a mortality of 5%, in a recent study[164].

Regarding the modified Appleby's operation (*en-bloc* resection of the celiac trunk with distal pancreatectomy and total gastrectomy) for advanced cancers of body and tail of the pancreas, several Japanese groups propose an extended resection of the celiac trunk, splenic artery, common hepatic artery, and/or superior mesenteric artery, resulting in 5-6 mo of average survival. Hepatic vascularization must be maintained and evaluated during the whole operation, and if necessary, compensated, in order to avoid an acute hepatic insufficiency[163,165-168].

Recently, Gagandeep *et al*[169] reported their experience using celiac axis resection for pancreatic cancer with a prolonged survival, and proposed the consideration of this technique for central and distal pancreatic cancer invading the celiac trunk.

Hirano *et al*[170] reported a high R0 resectability rate (91%) with distal pancreatectomy with *en bloc* celiac axis resection.

When the superior mesenteric artery is invaded, an arterial jejunal branch is isolated. Heparin is injected there, in order to allow the clamping of the superior mesenteric artery with full safety. The artery is then reconstructed either by direct anastomosis, or by anastomosis to the aorta[161].

In the case of an invasion of the hepatic artery, techniques of reconstruction require a venous graft (jugular, reverse saphenous, gonadic veins) or prosthesis, or an arterial graft (splenic, gastro-epiploic, gastro-duodenal)[22,163,171-173].

In some cases of cancer of the body of the pancreas, with invasion of the common hepatic artery and celiac trunk, Kondo *et al*[174] tried to embolize the hepatic artery, obtaining a collateral pathway from the superior mesenteric artery. This allowed a distal pancreatectomy with *en bloc* resection of the celiac trunk, without hepatic ischemia.

Other authors have described more traditional techniques of resection-reconstruction, using the gastroduodenal artery[175]. Combined resection of the celiac trunk with a distal pancreatectomy has been found to improve the overall prognosis of patients with locally advanced cancer of the body and tail of the pancreas[176].

Venous invasion

Contrary to arterial involvement, the invasion of the superior mesenteric vein or portal vein is not in itself a criterion of unresectability[4,154,155,157,177].

In uncommon cases, the pancreatic tumor infiltrates the anterior surface of the inferior vena cava. It is possible to excise the invaded part, and to replace it with a synthetic prosthesis. Often, autologous tissues are preferred (jugular, saphenous veins)[22].

When the portal vein is involved, it is legitimate to attempt a resection, especially if the vein is invaded by more than 2 cm, in order to obtain negative margins (Table 2)[4,178-180]. Portal invasion is not a predictor of aggressive tumor biology, but rather a reflection of tumor size and location[153,157,177,179]. Up to 50% of tumors thought to have vascular invasion intraoperatively have been found subsequently to have only inflammatory adhesions to the portal vein after histologic examination[157,181,182]. This finding underlines the difficulty in determining tumoral venous invasion before and during surgery, since peritumoral inflammation may simulate true tumor infiltration[178]. Very recently, Fukuda *et al*[183] reported that the depth of portal vein invasion significantly alters survival after curative pancreatic resection combined with portal vein resection. The survival rate was similar for patients with no portal invasion and those with superficial invasion. However, a deeper portal invasion was associated with a poorer survival rate, similar to that of patients undergoing non-curative resection.

The excision is done either by a segmentary resection, or by a tangential resection[22,184,185]. The reconstruction requires an end-to-end anastomosis either by direct suture or by using an interposition venous or prosthetic graft[22,74,154,156,157,161,162,184-189]. The technical limit of portal vein resection without graft is 4 cm in the hepatic hilus and 7 cm after pancreatic resection[189]. For minimal tumor invasion into the portal vein, autologous saphenous vein patch has been described[27]. Wide resection of the portal vein may require transection of the splenic vein. To avoid segmental portal hypertension, end-to-side reanastomosis of the splenic vein to the interposition graft is recommended[184].

If the portal clampage lasts longer than 30 min, it is recommended to clamp also the superior mesenteric artery, in order to prevent intestinal congestion[22,189]. If the portal clampage lasts longer than 60 min, it is necessary to consider a bypass between the superior mesenteric vein and femoral vein[189,190].

Resection of the portal vein is associated with a higher morbidity rate (bleeding, infections, cardiopulmonary complications), than when this is not performed[4,185,191].

Table 2 Recent results of portal resections in pancreatic cancer

Studies (yr)	*n*	Mortality (%)	Survival at 1 year (%)	Median survival (mo)
Sindelar *et al*[159] (1989)	20	20[1]	50	12
Tashiro *et al*[189] (1991)	27	8.4	51.9	NA
Ishikawa *et al*[74] (1992)	35	5.7	NA	9+/-5
Launois *et al*[193] (1993)	9	0	NA	6.1
Takahashi *et al*[156] (1994)	79	16.5	17-61.5[2]	6-14
Allema *et al*[192] (1994)	20	15	30%	7
Nakao *et al*[211] (1995)	89	8	5.5-39.6[3]	NA
Nakao *et al*[190] (1995)	104	8	NA	NA
Roder *et al*[27] (1996)	31	0	39	8
Fuhrman *et al*[154] (1996)	23	4	NA	NA
Harrison *et al*[157] (1996)	58	5	59	13
Leach *et al*[196] (1998)	31	0	NA	22
Launois *et al*[188] (1999)	14	0	23	5
Bachellier *et al*[195] (2001)	21	3.2	NA	13
van Geenen *et al*[185] (2001)	34	0	55	14
Shibata *et al*[197] (2001)	23	4	31	6.8-20.6[4]
Hartel *et al*[212] (2002)	68	4	[5]	NA
Aramaki *et al*[194] (2003)	22	4.5	NA	NA
Nakagohri *et al*[213] (2003)	33	6	35-81	15
Li *et al*[164] (2004)	79	5[6]	NA	NA
Tseng *et al*[206] (2004)	110	1	85	23.4
Wagner *et al*[4] (2004)	51	7.7	NA	NA
Shimada *et al*[177] (2006)	86	1	[7]	14
Carrère *et al*[182] (2006)	45	4.4	[8]	15
Riediger *et al*[181] (2006)	53	3.8	[9]	NA
Fukuda *et al*[183] (2007)	37	2.4	47.7	NA

[1]Included 3 arterial reconstructions. 17 patients benefited from adjunctive radiotherapy; [2]17% survival at 1 year if margins were positive (median survival: 6 mo) and 61.5% if margins were negative (median survival: 14 mo); [3]Survival at 1 year: 39.6% if the vessel was not invaded, 11.3% if the media was invaded, and 5.5% if the intima was invaded; [4]Median survival was 6.8 mo if the intima was invaded, 15.3 mo if the intima was spared, and 20.6 mo if there was no true vascular invasion; [5]5-year survival rate: 23%; [6]This mortality also includes arterial reconstructions (11 patients); [7]5-year survival rate: 12%; [8]3-year survival rate: 22%; [9]5-year survival rate: 17.9%.

In addition, Fuhrman *et al*[154] reported an operative time, an operative blood loss, and perioperative transfusion requirements of greater magnitude in patients who required venous resection. The mortality rate is also higher after portal vein resection but this value is not always significant[4,188,192,193]. These findings are not confirmed by other series[22,27,157,181,182,185,187,191,194-201]. Numerous authors have reported a mortality rate below 5%, similar to that of standard pancreatoduodenectomy[27,154,157,164,178,181,182,185,188,194-197].

In 62%-85% of cases, the vascular margins are found to be positive[27,31,185,192], explaining a very poor median survival. However, recently, Siriwardana *et al*[202] reported, in a systematic review of synchronous portal-superior mesenteric vein resection during pancreatectomy for cancer, a high rate (67.4%) of nodal involvement during the procedure. For the authors, this implied that by the time a pancreatic tumor involves the portal vein the risk of metastases is high, rendering the possibility of cure by surgery improbable[202].

If the tumor invades the superior mesenteric vein, it is not a criterion of unresectability. Various techniques exist to allow complete resection of the tumor,

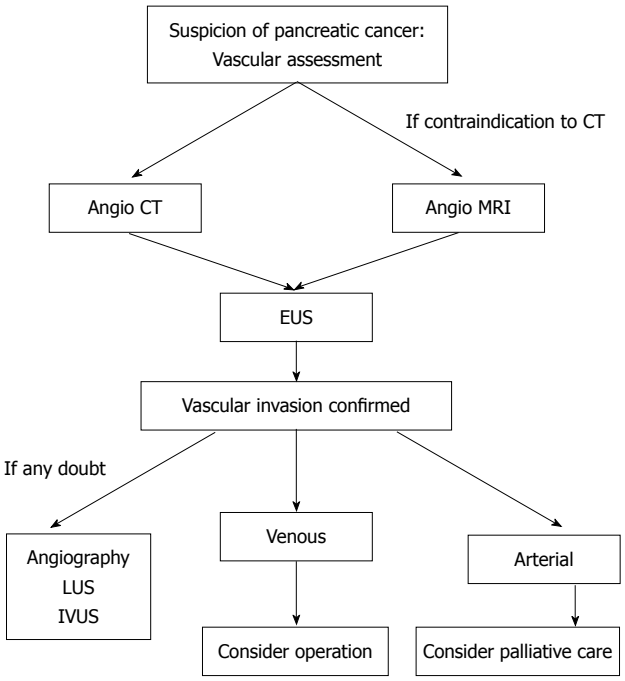

Figure 1 Proposed algorithm for the management of suspected vascular invasion in pancreatic cancer.

either by tangential excision, or by excision-reconstruction[153,155,161,162,185,197,203,204].

In conclusion, various studies show that venous resection in pancreatic cancer is a feasible technique and relatively reliable, at least with regard to mortality, but (importantly) at the price of a higher morbidity. However, a survival benefit is not achieved by curative resection in patients with pancreatic cancer and vascular invasion[205,206]. On the other hand, the discovery of an arterial invasion during the operation might require an aggressive management, using vascular reconstruction. Furthermore, neoadjuvant treatment (combination of 5-fluorouracil/cisplatin chemoradiation) showed only limited impact on survival but appeared to be associated with improved local control[207].

CONCLUSION

In the absence of metastatic disease, assessment of vascular invasion is a key aspect in the evaluation of resectability for pancreatic cancer. A frequent error is to misdiagnose an involved major vessel. Obviously, surgical exploration with pathological examination remains the "gold standard" in terms of evaluation of resectability, especially from the point of view of vascular involvement. However, current imaging modalities have improved and now allow detection of vascular invasion with more accuracy. Multi-slice CT has become the best imaging modality for this purpose, and the adjunction of PET might be a means to improve results further. EUS is useful, but it remains very operator-dependant. Data are still lacking for the exact role of MRI regarding this issue (Figure 1). Detection of vascular invasion remains one of the most important challenges in pancreatic surgery.

REFERENCES

1 Guidelines for the management of patients with pancreatic cancer periampullary and ampullary carcinomas. *Gut* 2005; **54** Suppl 5: v1-16
2 **Warshaw AL**, Fernández-del Castillo C. Pancreatic carcinoma. *N Engl J Med* 1992; **326**: 455-465
3 **Connolly MM**, Dawson PJ, Michelassi F, Moossa AR, Lowenstein F. Survival in 1001 patients with carcinoma of the pancreas. *Ann Surg* 1987; **206**: 366-373
4 **Wagner M**, Redaelli C, Lietz M, Seiler CA, Friess H, Büchler MW. Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. *Br J Surg* 2004; **91**: 586-594
5 **Ahmad NA**, Kochman ML, Lewis JD, Kadish S, Morris JB, Rosato EF, Ginsberg GG. Endosonography is superior to angiography in the preoperative assessment of vascular involvement among patients with pancreatic carcinoma. *J Clin Gastroenterol* 2001; **32**: 54-58
6 **DeWitt J**, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004; **141**: 753-763
7 **Arslan A**, Buanes T, Geitung JT. Pancreatic carcinoma: MR, MR angiography and dynamic helical CT in the evaluation of vascular invasion. *Eur J Radiol* 2001; **38**: 151-159
8 **Soriano A**, Castells A, Ayuso C, Ayuso JR, de Caralt MT, Ginès MA, Real MI, Gilabert R, Quintó L, Trilla A, Feu F, Montanyà X, Fernández-Cruz L, Navarro S. Preoperative staging and tumor resectability assessment of pancreatic cancer: prospective study comparing endoscopic ultrasonography, helical computed tomography, magnetic resonance imaging, and angiography. *Am J Gastroenterol* 2004; **99**: 492-501
9 **Yeo CJ**, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. *Ann Surg* 1997; **226**: 248-257; discussion 257-260
10 **Böttger TC**, Boddin J, Düber C, Heintz A, Küchle R, Junginger T. Diagnosing and staging of pancreatic carcinoma-what is necessary? *Oncology* 1998; **55**: 122-129
11 **Snady H**, Bruckner H, Siegel J, Cooperman A, Neff R, Kiefer L. Endoscopic ultrasonographic criteria of vascular invasion by potentially resectable pancreatic tumors. *Gastrointest Endosc* 1994; **40**: 326-333
12 **Scaglione M**, Pinto A, Romano S, Scialpi M, Volterrani L, Rotondo A, Romano L. Using multidetector row computed tomography to diagnose and stage pancreatic carcinoma: the problems and the possibilities. *JOP* 2005; **6**: 1-5
13 **Takhar AS**, Palaniappan P, Dhingsa R, Lobo DN. Recent developments in diagnosis of pancreatic cancer. *BMJ* 2004; **329**: 668-673
14 **Vargas R**, Nino-Murcia M, Trueblood W, Jeffrey RB Jr. MDCT in Pancreatic adenocarcinoma: prediction of vascular invasion and resectability using a multiphasic technique with curved planar reformations. *AJR Am J Roentgenol* 2004; **182**: 419-425
15 **Saldinger PF**, Reilly M, Reynolds K, Raptopoulos V, Chuttani R, Steer ML, Matthews JB. Is CT angiography sufficient for prediction of resectability of periampullary neoplasms? *J Gastrointest Surg* 2000; **4**: 233-237; discussion 238-239

16 **McCarthy MJ**, Evans J, Sagar G, Neoptolemos JP. Prediction of resectability of pancreatic malignancy by computed tomography. *Br J Surg* 1998; **85**: 320-325
17 **Balci NC**, Semelka RC. Radiologic diagnosis and staging of pancreatic ductal adenocarcinoma. *Eur J Radiol* 2001; **38**: 105-112
18 **Saisho H**, Yamaguchi T. Diagnostic imaging for pancreatic cancer: computed tomography, magnetic resonance imaging, and positron emission tomography. *Pancreas* 2004; **28**: 273-278
19 **Catalano C**, Laghi A, Fraioli F, Pediconi F, Napoli A, Danti M, Reitano I, Passariello R. Pancreatic carcinoma: the role of high-resolution multislice spiral CT in the diagnosis and assessment of resectability. *Eur Radiol* 2003; **13**: 149-156
20 **Fishman EK**, Horton KM, Urban BA. Multidetector CT angiography in the evaluation of pancreatic carcinoma: preliminary observations. *J Comput Assist Tomogr* 2000; **24**: 849-853
21 **Pisters PW**, Lee JE, Vauthey JN, Charnsangavej C, Evans DB. Laparoscopy in the staging of pancreatic cancer. *Br J Surg* 2001; **88**: 325-337
22 **Bold RJ**, Charnsangavej C, Cleary KR, Jennings M, Madray A, Leach SD, Abbruzzese JL, Pisters PW, Lee JE, Evans DB. Major vascular resection as part of pancreaticoduodenectomy for cancer: radiologic, intraoperative, and pathologic analysis. *J Gastrointest Surg* 1999; **3**: 233-243
23 **Beger HG**, Rau B, Gansauge F, Poch B, Link KH. Treatment of pancreatic cancer: challenge of the facts. *World J Surg* 2003; **27**: 1075-1084
24 **Megibow AJ**, Zhou XH, Rotterdam H, Francis IR, Zerhouni EA, Balfe DM, Weinreb JC, Aisen A, Kuhlman J, Heiken JP. Pancreatic adenocarcinoma: CT versus MR imaging in the evaluation of resectability--report of the Radiology Diagnostic Oncology Group. *Radiology* 1995; **195**: 327-332
25 **Horton KM**, Fishman EK. Multidetector CT angiography of pancreatic carcinoma: part I, evaluation of arterial involvement. *AJR Am J Roentgenol* 2002; **178**: 827-831
26 **Valls C**, Andía E, Sanchez A, Fabregat J, Pozuelo O, Quintero JC, Serrano T, Garcia-Borobia F, Jorba R. Dual-phase helical CT of pancreatic adenocarcinoma: assessment of resectability before surgery. *AJR Am J Roentgenol* 2002; **178**: 821-826
27 **Roder JD**, Stein HJ, Siewert JR. Carcinoma of the periampullary region: who benefits from portal vein resection? *Am J Surg* 1996; **171**: 170-174; discussion 174-175
28 **Horton KM**, Fishman EK. Volume-rendered 3D CT of the mesenteric vasculature: normal anatomy, anatomic variants, and pathologic conditions. *Radiographics* 2002; **22**: 161-172
29 **Gritzmann N**, Macheiner P, Hollerweger A, Hübner E. CT in the differentiation of pancreatic neoplasms--progress report. *Dig Dis* 2004; **22**: 6-17
30 **Horton KM**, Fishman EK. Multidetector CT angiography of pancreatic carcinoma: part 2, evaluation of venous involvement. *AJR Am J Roentgenol* 2002; **178**: 833-836
31 **Poen JC**, Ford JM, Niederhuber JE. Chemoradiotherapy in the management of localized tumors of the pancreas. *Ann Surg Oncol* 1999; **6**: 117-122
32 **Cunningham JD**, Glajchen N, Brower ST. The use of spiral computed tomography in the evaluation of vessel encasement for pancreatic cancer. *Int J Pancreatol* 1996; **19**: 9-14
33 **Bipat S**, Phoa SS, van Delden OM, Bossuyt PM, Gouma DJ, Laméris JS, Stoker J. Ultrasonography, computed tomography and magnetic resonance imaging for diagnosis and determining resectability of pancreatic adenocarcinoma: a meta-analysis. *J Comput Assist Tomogr* 2005; **29**: 438-445
34 **Phoa SS**, Reeders JW, Stoker J, Rauws EA, Gouma DJ, Laméris JS. CT criteria for venous invasion in patients with pancreatic head carcinoma. *Br J Radiol* 2000; **73**: 1159-1164
35 **Loyer EM**, David CL, Dubrow RA, Evans DB, Charnsangavej C. Vascular involvement in pancreatic adenocarcinoma: reassessment by thin-section CT. *Abdom Imaging* 1996; **21**: 202-206
36 **Li H**, Zeng MS, Zhou KR, Jin DY, Lou WH. Pancreatic adenocarcinoma: the different CT criteria for peripancreatic major arterial and venous invasion. *J Comput Assist Tomogr* 2005; **29**: 170-175
37 **Phoa SS**, Tilleman EH, van Delden OM, Bossuyt PM, Gouma DJ, Laméris JS. Value of CT criteria in predicting survival in patients with potentially resectable pancreatic head carcinoma. *J Surg Oncol* 2005; **91**: 33-40
38 **Lu DS**, Reber HA, Krasny RM, Kadell BM, Sayre J. Local staging of pancreatic cancer: criteria for unresectability of major vessels as revealed by pancreatic-phase, thin-section helical CT. *AJR Am J Roentgenol* 1997; **168**: 1439-1443
39 **Lepanto L**, Arzoumanian Y, Gianfelice D, Perreault P, Dagenais M, Lapointe R, Létourneau R, Roy A. Helical CT with CT angiography in assessing periampullary neoplasms: identification of vascular invasion. *Radiology* 2002; **222**: 347-352
40 **Brügel M**, Rummeny EJ, Dobritz M. Vascular invasion in pancreatic cancer: value of multislice helical CT. *Abdom Imaging* 2004; **29**: 239-245
41 **Hough TJ**, Raptopoulos V, Siewert B, Matthews JB. Teardrop superior mesenteric vein: CT sign for unresectable carcinoma of the pancreas. *AJR Am J Roentgenol* 1999; **173**: 1509-1512
42 **Baek SY**, Sheafor DH, Keogan MT, DeLong DM, Nelson RC. Two-dimensional multiplanar and three-dimensional volume-rendered vascular CT in pancreatic carcinoma: interobserver agreement and comparison with standard helical techniques. *AJR Am J Roentgenol* 2001; **176**: 1467-1473
43 **Kopka L**, Rogalla P, Hamm B. [Multislice CT of the abdomen--current indications and future trends] *Rofo* 2002; **174**: 273-282
44 **Nino-Murcia M**, Tamm EP, Charnsangavej C, Jeffrey RB Jr. Multidetector-row helical CT and advanced postprocessing techniques for the evaluation of pancreatic neoplasms. *Abdom Imaging* 2003; **28**: 366-377
45 **Li H**, Zeng MS, Zhou KR, Jin DY, Lou WH. Pancreatic adenocarcinoma: signs of vascular invasion determined by multi-detector row CT. *Br J Radiol* 2006; **79**: 880-887
46 **Yoshimi F**, Hasegawa H, Koizumi S, Amemiya R, Ono H, Kobayashi H, Matsueda K, Itabashi M. Application of three-dimensional spiral computed tomographic angiography to pancreatoduodenectomy for cancer. *Br J Surg* 1995; **82**: 116-117
47 **Riker A**, Libutti SK, Bartlett DL. Advances in the early detection, diagnosis, and staging of pancreatic cancer. *Surg Oncol* 1997; **6**: 157-169
48 **Fishman EK**, Horton KM. Imaging pancreatic cancer: the role of multidetector CT with three-dimensional CT angiography. *Pancreatology* 2001; **1**: 610-624
49 **Chong M**, Freeny PC, Schmiedl UP. Pancreatic arterial anatomy: depiction with dual-phase helical CT. *Radiology* 1998; **208**: 537-542
50 **Yamada Y**, Mori H, Kiyosue H, Matsumoto S, Hori Y, Maeda T. CT assessment of the inferior peripancreatic veins: clinical significance. *AJR Am J Roentgenol* 2000; **174**: 677-684
51 **McNulty NJ**, Francis IR, Platt JF, Cohan RH, Korobkin M, Gebremariam A. Multi--detector row helical CT of the pancreas: effect of contrast-enhanced multiphasic imaging on enhancement of the pancreas, peripancreatic vasculature, and pancreatic adenocarcinoma. *Radiology* 2001; **220**: 97-102
52 **Graf O**, Boland GW, Warshaw AL, Fernandez-del-Castillo C, Hahn PF, Mueller PR. Arterial versus portal venous helical CT for revealing pancreatic adenocarcinoma: conspicuity of tumor and critical vascular anatomy. *AJR Am J Roentgenol* 1997; **169**: 119-123
53 **Lu DS**, Vedantham S, Krasny RM, Kadell B, Berger WL, Reber HA. Two-phase helical CT for pancreatic tumors: pancreatic versus hepatic phase enhancement of tumor, pancreas, and vascular structures. *Radiology* 1996; **199**: 697-701
54 **Vedantham S**, Lu DS, Reber HA, Kadell B. Small peripancreatic veins: improved assessment in pancreatic cancer patients using thin-section pancreatic phase helical CT. *AJR*

Am J Roentgenol 1998; **170**: 377-383
55 **Imbriaco M**, Megibow AJ, Camera L, Pace L, Mainenti PP, Romano M, Selva G, Salvatore M. Dual-phase versus single-phase helical CT to detect and assess resectability of pancreatic carcinoma. *AJR Am J Roentgenol* 2002; **178**: 1473-1479
56 **Schima W**, Függer R, Schober E, Oettl C, Wamser P, Grabenwöger F, Ryan JM, Novacek G. Diagnosis and staging of pancreatic cancer: comparison of mangafodipir trisodium-enhanced MR imaging and contrast-enhanced helical hydro-CT. *AJR Am J Roentgenol* 2002; **179**: 717-724
57 **Adamek HE**, Albert J, Breer H, Weitz M, Schilling D, Riemann JF. Pancreatic cancer detection with magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography: a prospective controlled study. *Lancet* 2000; **356**: 190-193
58 **Romijn MG**, Stoker J, van Eijck CH, van Muiswinkel JM, Torres CG, Laméris JS. MRI with mangafodipir trisodium in the detection and staging of pancreatic cancer. *J Magn Reson Imaging* 2000; **12**: 261-268
59 **Trede M**, Rumstadt B, Wendl K, Gaa J, Tesdal K, Lehmann KJ, Meier-Willersen HJ, Pescatore P, Schmoll J. Ultrafast magnetic resonance imaging improves the staging of pancreatic tumors. *Ann Surg* 1997; **226**: 393-405; discussion 405-407
60 **Lopez Hänninen E**, Amthauer H, Hosten N, Ricke J, Böhmig M, Langrehr J, Hintze R, Neuhaus P, Wiedenmann B, Rosewicz S, Felix R. Prospective evaluation of pancreatic tumors: accuracy of MR imaging with MR cholangiopancreatography and MR angiography. *Radiology* 2002; **224**: 34-41
61 **Catalano C**, Pavone P, Laghi A, Panebianco V, Scipioni A, Fanelli F, Brillo R, Passariello R. Pancreatic adenocarcinoma: combination of MR imaging, MR angiography and MR cholangiopancreatography for the diagnosis and assessment of resectability. *Eur Radiol* 1998; **8**: 428-434
62 **Sironi S**, De Cobelli F, Zerbi A, Angeli E, Balzano G, Taccagni G, Di Carlo V, Del Maschio A. Pancreatic adenocarcinoma: assessment of vascular invasion with high-field MR imaging and a phased-array coil. *AJR Am J Roentgenol* 1996; **167**: 997-1001
63 **Sarmiento JM**, Sarr MG. Staging strategies for pancreatic adenocarcinoma: what the surgeon really wants to know. *Curr Gastroenterol Rep* 2003; **5**: 117-124
64 **Ito K**, Blasbalg R, Hussain SM, Mitchell DG. Portal vein and its tributaries: evaluation with thin-section three-dimensional contrast-enhanced dynamic fat-suppressed MR imaging. *Radiology* 2000; **215**: 381-386
65 **Kanematsu M**, Shiratori Y, Hoshi H, Kondo H, Matsuo M, Moriwaki H. Pancreas and peripancreatic vessels: effect of imaging delay on gadolinium enhancement at dynamic gradient-recalled-echo MR imaging. *Radiology* 2000; **215**: 95-102
66 **Gabata T**, Matsui O, Kadoya M, Yoshikawa J, Miyayama S, Takashima T, Nagakawa T, Kayahara M, Nonomura A. Small pancreatic adenocarcinomas: efficacy of MR imaging with fat suppression and gadolinium enhancement. *Radiology* 1994; **193**: 683-688
67 **Ichikawa T**, Haradome H, Hachiya J, Nitatori T, Ohtomo K, Kinoshita T, Araki T. Pancreatic ductal adenocarcinoma: preoperative assessment with helical CT versus dynamic MR imaging. *Radiology* 1997; **202**: 655-662
68 **Semelka RC**, Kelekis NL, Molina PL, Sharp TJ, Calvo B. Pancreatic masses with inconclusive findings on spiral CT: is there a role for MRI? *J Magn Reson Imaging* 1996; **6**: 585-588
69 **Squillaci E**, Fanucci E, Sciuto F, Masala S, Sodani G, Carlani M, Simonetti G. Vascular involvement in pancreatic neoplasm: a comparison between spiral CT and DSA. *Dig Dis Sci* 2003; **48**: 449-458
70 **Biehl TR**, Traverso LW, Hauptmann E, Ryan JA Jr. Preoperative visceral angiography alters intraoperative strategy during the Whipple procedure. *Am J Surg* 1993; **165**: 607-612
71 **Kaneko T**, Nakao A, Inoue S, Harada A, Nonami T, Itoh S, Endo T, Takagi H. Intraportal endovascular ultrasonography in the diagnosis of portal vein invasion by pancreatobiliary carcinoma. *Ann Surg* 1995; **222**: 711-718
72 **Hannesson PH**, Lundstedt C, Dawiskiba S, Stridbeck H, Ihse I. Transhepatic intravascular ultrasound for evaluation of portal venous involvement in patients with cancer of the pancreatic head region. *Eur Radiol* 2002; **12**: 1150-1154
73 **Dooley WC**, Cameron JL, Pitt HA, Lillemoe KD, Yue NC, Venbrux AC. Is preoperative angiography useful in patients with periampullary tumors? *Ann Surg* 1990; **211**: 649-654; discussion 654-655
74 **Ishikawa O**, Ohigashi H, Imaoka S, Furukawa H, Sasaki Y, Fujita M, Kuroda C, Iwanaga T. Preoperative indications for extended pancreatectomy for locally advanced pancreas cancer involving the portal vein. *Ann Surg* 1992; **215**: 231-236
75 **Snady H**, Cooperman A, Siegel J. Endoscopic ultrasonography compared with computed tomography with ERCP in patients with obstructive jaundice or small periampullary mass. *Gastrointest Endosc* 1992; **38**: 27-34
76 **Kaneko T**, Nakao A, Inoue S, Endo T, Itoh S, Harada A, Nonami T, Takagi H. Portal venous invasion by pancreatobiliary carcinoma: diagnosis with intraportal endovascular US. *Radiology* 1994; **192**: 681-686
77 **Ueno N**, Tomiyama T, Tano S, Wada S, Miyata T. Color Doppler ultrasonography in the diagnosis of portal vein invasion in patients with pancreatic cancer. *J Ultrasound Med* 1997; **16**: 825-830
78 **Clarke DL**, Thomson SR, Madiba TE, Sanyika C. Preoperative imaging of pancreatic cancer: a management-oriented approach. *J Am Coll Surg* 2003; **196**: 119-129
79 **Tomiyama T**, Ueno N, Tano S, Wada S, Kimura K. Assessment of arterial invasion in pancreatic cancer using color Doppler ultrasonography. *Am J Gastroenterol* 1996; **91**: 1410-1416
80 **Casadei R**, Ghigi G, Gullo L, Moretti CC, Greco VM, Salizzoni E, Canini R, Marrano D. Role of color Doppler ultrasonography in the preoperative staging of pancreatic cancer. *Pancreas* 1998; **16**: 26-30
81 **Wren SM**, Ralls PW, Stain SC, Kasiraman A, Carpenter CL, Parekh D. Assessment of resectability of pancreatic head and periampullary tumors by color flow Doppler sonography. *Arch Surg* 1996; **131**: 812-817; discussion 817-818
82 **Angeli E**, Venturini M, Vanzulli A, Sironi S, Castrucci M, Salvioni M, Zerbi A, Di Carlo V, Del Maschio A. Color Doppler imaging in the assessment of vascular involvement by pancreatic carcinoma. *AJR Am J Roentgenol* 1997; **168**: 193-197
83 **Ishida H**, Konno K, Hamashima Y, Naganuma H, Komatsuda T, Sato M, Ishida J, Masamune O. Assessment of resectability of pancreatic carcinoma by color Doppler sonography. *Abdom Imaging* 1999; **24**: 295-298
84 **Kobayashi A**, Yamaguchi T, Ishihara T, Ohshima T, Ohno I, Seza K, Shirai Y, Sudo K, Nakagawa A, Tadenuma H, Nakamura K, Saisho H. Assessment of portal vein invasion in pancreatic cancer by fusion 3-dimensional ultrasonography. *J Ultrasound Med* 2005; **24**: 363-369
85 **Morrin MM**, Kruskal JB, Raptopoulos V, Weisinger K, Farrell RJ, Steer ML, Kane RA. State-of-the-art ultrasonography is as accurate as helical computed tomography and computed tomographic angiography for detecting unresectable periampullary cancer. *J Ultrasound Med* 2001; **20**: 481-490
86 **Ralls PW**, Wren SM, Radin R, Stain SC, Yang J, Parekh D. Color flow sonography in evaluating the resectability of periampullary and pancreatic tumors. *J Ultrasound Med* 1997; **16**: 131-140
87 **Minniti S**, Bruno C, Biasiutti C, Tonel D, Falzone A, Falconi M, Procacci C. Sonography versus helical CT in identification and staging of pancreatic ductal adenocar-

cinoma. *J Clin Ultrasound* 2003; **31**: 175-182
88 **Sugiyama M**, Hagi H, Atomi Y. Reappraisal of intraoperative ultrasonography for pancreatobiliary carcinomas: assessment of malignant portal venous invasion. *Surgery* 1999; **125**: 160-165
89 **Gress FG**, Hawes RH, Savides TJ, Ikenberry SO, Cummings O, Kopecky K, Sherman S, Wiersema M, Lehman GA. Role of EUS in the preoperative staging of pancreatic cancer: a large single-center experience. *Gastrointest Endosc* 1999; **50**: 786-791
90 **Krishna NB**, LaBundy JL, Saripalli S, Safdar R, Agarwal B. Diagnostic value of EUS-FNA in patients suspected of having pancreatic cancer with a focal lesion on CT scan/MRI but without obstructive jaundice. *Pancreas* 2009; **38**: 625-630
91 **Sugiyama M**, Hagi H, Atomi Y, Saito M. Diagnosis of portal venous invasion by pancreatobiliary carcinoma: value of endoscopic ultrasonography. *Abdom Imaging* 1997; **22**: 434-438
92 **Aslanian H**, Salem R, Lee J, Andersen D, Robert M, Topazian M. EUS diagnosis of vascular invasion in pancreatic cancer: surgical and histologic correlates. *Am J Gastroenterol* 2005; **100**: 1381-1385
93 **Brugge WR**, Lee MJ, Kelsey PB, Schapiro RH, Warshaw AL. The use of EUS to diagnose malignant portal venous system invasion by pancreatic cancer. *Gastrointest Endosc* 1996; **43**: 561-567
94 **Rösch T**, Dittler HJ, Strobel K, Meining A, Schusdziarra V, Lorenz R, Allescher HD, Kassem AM, Gerhardt P, Siewert JR, Höfler H, Classen M. Endoscopic ultrasound criteria for vascular invasion in the staging of cancer of the head of the pancreas: a blind reevaluation of videotapes. *Gastrointest Endosc* 2000; **52**: 469-477
95 **Tierney WM**, Francis IR, Eckhauser F, Elta G, Nostrant TT, Scheiman JM. The accuracy of EUS and helical CT in the assessment of vascular invasion by peripapillary malignancy. *Gastrointest Endosc* 2001; **53**: 182-188
96 **Yusoff IF**, Mendelson RM, Edmunds SE, Ramsay D, Cullingford GL, Fletcher DR, Zimmerman AM. Preoperative assessment of pancreatic malignancy using endoscopic ultrasound. *Abdom Imaging* 2003; **28**: 556-562
97 **Kahl S**, Glasbrenner B, Zimmermann S, Malfertheiner P. Endoscopic ultrasound in pancreatic diseases. *Dig Dis* 2002; **20**: 120-126
98 **Buchs NC**, Frossard JL, Rosset A, Chilcott M, Koutny-Fong P, Chassot G, Fasel JH, Poletti PA, Becker CD, Mentha G, Bühler L, Morel P. Vascular invasion in pancreatic cancer: evaluation of endoscopic ultrasonography, computed tomography, ultrasonography, and angiography. *Swiss Med Wkly* 2007; **137**: 286-291
99 **Rösch T**, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992; **102**: 188-199
100 **Yasuda K**, Mukai H, Nakajima M, Kawai K. Staging of pancreatic carcinoma by endoscopic ultrasonography. *Endoscopy* 1993; **25**: 151-155
101 **Rivadeneira DE**, Pochapin M, Grobmyer SR, Lieberman MD, Christos PJ, Jacobson I, Daly JM. Comparison of linear array endoscopic ultrasound and helical computed tomography for the staging of periampullary malignancies. *Ann Surg Oncol* 2003; **10**: 890-897
102 **Schwarz M**, Pauls S, Sokiranski R, Brambs HJ, Glasbrenner B, Adler G, Diederichs CG, Reske SN, Möller P, Beger HG. Is a preoperative multidiagnostic approach to predict surgical resectability of periampullary tumors still effective? *Am J Surg* 2001; **182**: 243-249
103 **Fritscher-Ravens A**, Knoefel WT, Krause C, Swain CP, Brandt L, Patel K. Three-dimensional linear endoscopic ultrasound-feasibility of a novel technique applied for the detection of vessel involvement of pancreatic masses. *Am J Gastroenterol* 2005; **100**: 1296-1302
104 **Kaneko T**, Nakao A, Takagi H. Intraportal endovascular ultrasonography for pancreatic cancer. *Semin Surg Oncol* 1998; **15**: 47-51
105 **Nakao A**, Kaneko T. Intravascular ultrasonography for assessment of portal vein invasion by pancreatic carcinoma. *World J Surg* 1999; **23**: 892-895
106 **Hannesson PH**, Stridbeck H, Lundstedt C, Dawiskiba S, Andrén-Sandberg A, Ihse I. Intravascular ultrasound for evaluation of portal venous involvement in pancreatic cancer. *Eur Radiol* 1997; **7**: 21-25
107 **Kaneko T**, Nakao A, Harada A, Nomami T, Takagi H. Intraportal endovascular ultrasonography in pancreatic cancer--a new technique for the diagnosis of portal vein invasion: a preliminary report. *Surgery* 1994; **115**: 438-444
108 **Stein M**, Schneider PD, Ho HS, Eckert R, Urayama S, Bold RJ. Percutaneous transhepatic portography with intravascular ultrasonography for evaluation of venous involvement of hepatobiliary and pancreatic tumors. *J Vasc Interv Radiol* 2002; **13**: 805-814
109 **Kaneko T**, Inoue S, Sugimoto H, Takeda S, Harada A, Nakao A. Intraoperative diagnosis of pancreatic cancer extension using IVUS. *Hepatogastroenterology* 2001; **48**: 944-948
110 **Tezel E**, Kaneko T, Takeda S, Inoue S, Nagasaka T, Nakao A. Intraportal endovascular ultrasound for portal vein resection in pancreatic carcinoma. *Hepatogastroenterology* 2005; **52**: 237-242
111 **Manninen HI**, Räsänen H. Intravascular ultrasound in interventional radiology. *Eur Radiol* 2000; **10**: 1754-1762
112 **Cuschieri A**, Hall AW, Clark J. Value of laparoscopy in the diagnosis and management of pancreatic carcinoma. *Gut* 1978; **19**: 672-677
113 **Cuschieri A**. Laparoscopy for pancreatic cancer: does it benefit the patient? *Eur J Surg Oncol* 1988; **14**: 41-44
114 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057
115 **Conlon KC**, Dougherty E, Klimstra DS, Coit DG, Turnbull AD, Brennan MF. The value of minimal access surgery in the staging of patients with potentially resectable peripancreatic malignancy. *Ann Surg* 1996; **223**: 134-140
116 **Barreiro CJ**, Lillemoe KD, Koniaris LG, Sohn TA, Yeo CJ, Coleman J, Fishman EK, Cameron JL. Diagnostic laparoscopy for periampullary and pancreatic cancer: what is the true benefit? *J Gastrointest Surg* 2002; **6**: 75-81
117 **Potter MW**, Shah SA, McEnaney P, Chari RS, Callery MP. A critical appraisal of laparoscopic staging in hepatobiliary and pancreatic malignancy. *Surg Oncol* 2000; **9**: 103-110
118 **Edwin B**, Mala T, Mathisen Ø, Gladhaug I, Buanes T, Lunde OC, Søreide O, Bergan A, Fosse E. Laparoscopic resection of the pancreas: a feasibility study of the short-term outcome. *Surg Endosc* 2004; **18**: 407-411
119 **Menack MJ**, Spitz JD, Arregui ME. Staging of pancreatic and ampullary cancers for resectability using laparoscopy with laparoscopic ultrasound. *Surg Endosc* 2001; **15**: 1129-1134
120 **Merchant NB**, Conlon KC. Laparoscopic evaluation in pancreatic cancer. *Semin Surg Oncol* 1998; **15**: 155-165
121 **Nieveen van Dijkum EJ**, Romijn MG, Terwee CB, de Wit LT, van der Meulen JH, Lameris HS, Rauws EA, Obertop H, van Eyck CH, Bossuyt PM, Gouma DJ. Laparoscopic staging and subsequent palliation in patients with peripancreatic carcinoma. *Ann Surg* 2003; **237**: 66-73
122 **Doran HE**, Bosonnet L, Connor S, Jones L, Garvey C, Hughes M, Campbell F, Hartley M, Ghaneh P, Neoptolemos JP, Sutton R. Laparoscopy and laparoscopic ultrasound in the evaluation of pancreatic and periampullary tumours. *Dig Surg* 2004; **21**: 305-313
123 **John TG**, Greig JD, Carter DC, Garden OJ. Carcinoma of the pancreatic head and periampullary region. Tumor staging with laparoscopy and laparoscopic ultrasonography. *Ann*

Surg 1995; **221**: 156-164
124 **Minnard EA**, Conlon KC, Hoos A, Dougherty EC, Hann LE, Brennan MF. Laparoscopic ultrasound enhances standard laparoscopy in the staging of pancreatic cancer. *Ann Surg* 1998; **228**: 182-187
125 **Murugiah M**, Paterson-Brown S, Windsor JA, Miles WF, Garden OJ. Early experience of laparoscopic ultrasonography in the management of pancreatic carcinoma. *Surg Endosc* 1993; **7**: 177-181
126 **Rau B**, Hünerbein M, Schlag PM. Is there additional information from laparoscopic ultrasound in tumor staging? *Dig Surg* 2002; **19**: 479-483
127 **Bemelman WA**, de Wit LT, van Delden OM, Smits NJ, Obertop H, Rauws EJ, Gouma DJ. Diagnostic laparoscopy combined with laparoscopic ultrasonography in staging of cancer of the pancreatic head region. *Br J Surg* 1995; **82**: 820-824
128 **John TG**, Wright A, Allan PL, Redhead DN, Paterson-Brown S, Carter DC, Garden OJ. Laparoscopy with laparoscopic ultrasonography in the TNM staging of pancreatic carcinoma. *World J Surg* 1999; **23**: 870-881
129 **Vollmer CM**, Drebin JA, Middleton WD, Teefey SA, Linehan DC, Soper NJ, Eagon CJ, Strasberg SM. Utility of staging laparoscopy in subsets of peripancreatic and biliary malignancies. *Ann Surg* 2002; **235**: 1-7
130 **Pietrabissa A**, Caramella D, Di Candio G, Carobbi A, Boggi U, Rossi G, Mosca F. Laparoscopy and laparoscopic ultrasonography for staging pancreatic cancer: critical appraisal. *World J Surg* 1999; **23**: 998-1002; discussion 1003
131 **Holzman MD**, Reintgen KL, Tyler DS, Pappas TN. The role of laparoscopy in the management of suspected pancreatic and periampullary malignancies. *J Gastrointest Surg* 1997; **1**: 236-243; discussion 243-244
132 **Maire F**, Sauvanet A, Trivin F, Hammel P, O'Toole D, Palazzo L, Vilgrain V, Belghiti J, Ruszniewski P, Levy P. Staging of pancreatic head adenocarcinoma with spiral CT and endoscopic ultrasonography: an indirect evaluation of the usefulness of laparoscopy. *Pancreatology* 2004; **4**: 436-440
133 **Rumstadt B**, Schwab M, Schuster K, Hagmüller E, Trede M. The role of laparoscopy in the preoperative staging of pancreatic carcinoma. *J Gastrointest Surg* 1997; **1**: 245-250
134 **Annovazzi A**, Peeters M, Maenhout A, Signore A, Dierckx R, Van De Wiele C. 18-fluorodeoxyglucose positron emission tomography in nonendocrine neoplastic disorders of the gastrointestinal tract. *Gastroenterology* 2003; **125**: 1235-1245
135 **Delbeke D**, Pinson CW. Pancreatic tumors: role of imaging in the diagnosis, staging, and treatment. *J Hepatobiliary Pancreat Surg* 2004; **11**: 4-10
136 **Kalra MK**, Maher MM, Boland GW, Saini S, Fischman AJ. Correlation of positron emission tomography and CT in evaluating pancreatic tumors: technical and clinical implications. *AJR Am J Roentgenol* 2003; **181**: 387-393
137 **Diederichs CG**, Staib L, Vogel J, Glasbrenner B, Glatting G, Brambs HJ, Beger HG, Reske SN. Values and limitations of 18F-fluorodeoxyglucose-positron-emission tomography with preoperative evaluation of patients with pancreatic masses. *Pancreas* 2000; **20**: 109-116
138 **Goh BK**, Tan YM, Chung YF. Utility of fusion CT-PET in the diagnosis of small pancreatic carcinoma. *World J Gastroenterol* 2005; **11**: 3800-3802
139 **Heinrich S**, Goerres GW, Schäfer M, Sagmeister M, Bauerfeind P, Pestalozzi BC, Hany TF, von Schulthess GK, Clavien PA. Positron emission tomography/computed tomography influences on the management of resectable pancreatic cancer and its cost-effectiveness. *Ann Surg* 2005; **242**: 235-243
140 **Jadvar H**, Fischman AJ. Evaluation of pancreatic carcinoma with FDG PET. *Abdom Imaging* 2001; **26**: 254-259
141 **Rose DM**, Delbeke D, Beauchamp RD, Chapman WC, Sandler MP, Sharp KW, Richards WO, Wright JK, Frexes ME, Pinson CW, Leach SD. 18Fluorodeoxyglucose-positron emission tomography in the management of patients with suspected pancreatic cancer. *Ann Surg* 1999; **229**: 729-737; discussion 737-738
142 **Nakamoto Y**, Higashi T, Sakahara H, Tamaki N, Kogire M, Doi R, Hosotani R, Imamura M, Konishi J. Delayed (18)F-fluoro-2-deoxy-D-glucose positron emission tomography scan for differentiation between malignant and benign lesions in the pancreas. *Cancer* 2000; **89**: 2547-2554
143 **Rajput A**, Stellato TA, Faulhaber PF, Vesselle HJ, Miraldi F. The role of fluorodeoxyglucose and positron emission tomography in the evaluation of pancreatic disease. *Surgery* 1998; **124**: 793-797; discussion 797-798
144 **Syrota A**, Duquesnoy N, Paraf A, Kellershohn C. The role of positron emission tomography in the detection of pancreatic disease. *Radiology* 1982; **143**: 249-253
145 **Delbeke D**, Rose DM, Chapman WC, Pinson CW, Wright JK, Beauchamp RD, Shyr Y, Leach SD. Optimal interpretation of FDG PET in the diagnosis, staging and management of pancreatic carcinoma. *J Nucl Med* 1999; **40**: 1784-1791
146 **Zimny M**, Bares R, Fass J, Adam G, Cremerius U, Dohmen B, Klever P, Sabri O, Schumpelick V, Buell U. Fluorine-18 fluorodeoxyglucose positron emission tomography in the differential diagnosis of pancreatic carcinoma: a report of 106 cases. *Eur J Nucl Med* 1997; **24**: 678-682
147 **Sendler A**, Avril N, Helmberger H, Stollfuss J, Weber W, Bengel F, Schwaiger M, Roder JD, Siewert JR. Preoperative evaluation of pancreatic masses with positron emission tomography using 18F-fluorodeoxyglucose: diagnostic limitations. *World J Surg* 2000; **24**: 1121-1129
148 **Sperti C**, Pasquali C, Decet G, Chierichetti F, Liessi G, Pedrazzoli S. F-18-fluorodeoxyglucose positron emission tomography in differentiating malignant from benign pancreatic cysts: a prospective study. *J Gastrointest Surg* 2005; **9**: 22-82; discussion 28-29
149 **Diederichs CG**, Staib L, Glatting G, Beger HG, Reske SN. FDG PET: elevated plasma glucose reduces both uptake and detection rate of pancreatic malignancies. *J Nucl Med* 1998; **39**: 1030-1033
150 **Lemke AJ**, Niehues SM, Hosten N, Amthauer H, Boehmig M, Stroszczynski C, Rohlfing T, Rosewicz S, Felix R. Retrospective digital image fusion of multidetector CT and 18F-FDG PET: clinical value in pancreatic lesions--a prospective study with 104 patients. *J Nucl Med* 2004; **45**: 1279-1286
151 **Mertz HR**, Sechopoulos P, Delbeke D, Leach SD. EUS, PET, and CT scanning for evaluation of pancreatic adenocarcinoma. *Gastrointest Endosc* 2000; **52**: 367-371
152 **Hosten N**, Lemke AJ, Wiedenmann B, Böhmig M, Rosewicz S. Combined imaging techniques for pancreatic cancer. *Lancet* 2000; **356**: 909-910
153 **Cusack JC Jr**, Fuhrman GM, Lee JE, Evans DB. Managing unsuspected tumor invasion of the superior mesenteric-portal venous confluence during pancreaticoduodenectomy. *Am J Surg* 1994; **168**: 352-354
154 **Fuhrman GM**, Leach SD, Staley CA, Cusack JC, Charnsangavej C, Cleary KR, El-Naggar AK, Fenoglio CJ, Lee JE, Evans DB. Rationale for en bloc vein resection in the treatment of pancreatic adenocarcinoma adherent to the superior mesenteric-portal vein confluence. Pancreatic Tumor Study Group. *Ann Surg* 1996; **223**: 154-162
155 **Sakorafas GH**, Farnell MB, Nagorney DM, Farley DR, Que FG, Donohue JH, Thompson GG, Sarr MG. Management of peri-pancreatic vasculature during pancreatoduodenectomy: tips to avoid severe haemorrhage. *Eur J Surg Oncol* 1999; **25**: 524-528
156 **Takahashi S**, Ogata Y, Tsuzuki T. Combined resection of the pancreas and portal vein for pancreatic cancer. *Br J Surg* 1994; **81**: 1190-1193
157 **Harrison LE**, Klimstra DS, Brennan MF. Isolated portal vein involvement in pancreatic adenocarcinoma. A contraindication for resection? *Ann Surg* 1996; **224**: 342-347; discussion

347-349
158 **Imamura M**, Doi R. Treatment of locally advanced pancreatic cancer: should we resect when resectable? *Pancreas* 2004; **28**: 293-295
159 **Sindelar WF**. Clinical experience with regional pancreatectomy for adenocarcinoma of the pancreas. *Arch Surg* 1989; **124**: 127-132
160 **Nagakawa T**, Mori K, Nakano T, Kadoya M, Kobayashi H, Akiyama T, Kayahara M, Ohta T, Ueno K, Higashino Y. Perineural invasion of carcinoma of the pancreas and biliary tract. *Br J Surg* 1993; **80**: 619-621
161 **Fortner JG**. Technique of regional subtotal and total pancreatectomy. *Am J Surg* 1985; **150**: 593-600
162 **Fortner JG**. Regional resection of cancer of the pancreas: a new surgical approach. *Surgery* 1973; **73**: 307-320
163 **Konishi M**, Kinoshita T, Nakagori T, Inoue K, Oda T, Kimata T, Kikuchi H, Ryu M. Distal pancreatectomy with resection of the celiac axis and reconstruction of the hepatic artery for carcinoma of the body and tail of the pancreas. *J Hepatobiliary Pancreat Surg* 2000; **7**: 183-187
164 **Li B**, Chen FZ, Ge XH, Cai MZ, Jiang JS, Li JP, Lu SH. Pancreatoduodenectomy with vascular reconstruction in treating carcinoma of the pancreatic head. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 612-615
165 **Hirai I**, Kimura W, Kamiga M, Mizutani M, Takeshita A, Watanabe T, Fuse A. The significance of intraoperative Doppler ultrasonography in evaluating hepatic arterial flow when assessing the indications for the Appleby procedure for pancreatic body cancer. *J Hepatobiliary Pancreat Surg* 2005; **12**: 55-60
166 **Horiguchi A**, Miyakawa S, Mizuno K, Ishihara S, Miura K. Portal vein resection without reconstruction during Appleby operation in a patient with pancreatic body carcinoma with cavernous transformation. *Hepatogastroenterology* 1999; **46**: 2628-2630
167 **Kimura W**, Han I, Furukawa Y, Sunami E, Futakawa N, Inoue T, Shinkai H, Zhao B, Muto T, Makuuchi M, Komatsu H. Appleby operation for carcinoma of the body and tail of the pancreas. *Hepatogastroenterology* 1997; **44**: 387-393
168 **Yamaguchi K**, Nakano K, Kobayashi K, Ogura Y, Konomi H, Sugitani A, Tanaka M. Appleby operation for pancreatic body-tail carcinoma: report of three cases. *Surg Today* 2003; **33**: 873-878
169 **Gagandeep S**, Artinyan A, Jabbour N, Mateo R, Matsuoka L, Sher L, Genyk Y, Selby R. Extended pancreatectomy with resection of the celiac axis: the modified Appleby operation. *Am J Surg* 2006; **192**: 330-335
170 **Hirano S**, Kondo S, Hara T, Ambo Y, Tanaka E, Shichinohe T, Suzuki O, Hazama K. Distal pancreatectomy with en bloc celiac axis resection for locally advanced pancreatic body cancer: long-term results. *Ann Surg* 2007; **246**: 46-51
171 **Kusano T**, Tamai O, Miyazato H, Isa T, Shiraishi M, Muto Y. Vascular reconstruction of the hepatic artery using the gastroepiploic artery: a case report. *Hepatogastroenterology* 1999; **46**: 2278-2280
172 **Ohwada S**, Ogawa T, Ohya T, Kawashima Y, Nakamura S, Satoh Y, Saitoh A, Takeyoshi I, Yokoe T, Morishita Y. Gonadal vein graft for hepatic artery reconstruction. *Hepatogastroenterology* 1999; **46**: 1823-1826
173 **Sarmiento JM**, Panneton JM, Nagorney DM. Reconstruction of the hepatic artery using the gastroduodenal artery. *Am J Surg* 2003; **185**: 386-387
174 **Kondo S**, Katoh H, Shimizu T, Omi M, Hirano S, Ambo Y, Okushiba S, Morikawa T. Preoperative embolization of the common hepatic artery in preparation for radical pancreatectomy for pancreas body cancer. *Hepatogastroenterology* 2000; **47**: 1447-1449
175 **Makary MA**, Fishman EK, Cameron JL. Resection of the celiac axis for invasive pancreatic cancer. *J Gastrointest Surg* 2005; **9**: 503-507
176 **Mayumi T**, Nimura Y, Kamiya J, Kondo S, Nagino M, Kanai M, Miyachi M, Hamaguchi K, Hayakawa N. Distal pancreatectomy with en bloc resection of the celiac artery for carcinoma of the body and tail of the pancreas. *Int J Pancreatol* 1997; **22**: 15-21
177 **Shimada K**, Sano T, Sakamoto Y, Kosuge T. Clinical implications of combined portal vein resection as a palliative procedure in patients undergoing pancreaticoduodenectomy for pancreatic head carcinoma. *Ann Surg Oncol* 2006; **13**: 1569-1578
178 **Schäfer M**, Müllhaupt B, Clavien PA. Evidence-based pancreatic head resection for pancreatic cancer and chronic pancreatitis. *Ann Surg* 2002; **236**: 137-148
179 **Strasberg SM**, Drebin JA, Soper NJ. Evolution and current status of the Whipple procedure: an update for gastroenterologists. *Gastroenterology* 1997; **113**: 983-994
180 **Takahashi S**, Ogata Y, Aiura K, Kitajima M, Hiramatsu K. Combined resection of the portal vein for pancreatic cancer: preoperative diagnosis of invasion by portography and prognosis. *Hepatogastroenterology* 2000; **47**: 545-549
181 **Riediger H**, Makowiec F, Fischer E, Adam U, Hopt UT. Postoperative morbidity and long-term survival after pancreaticoduodenectomy with superior mesenterico-portal vein resection. *J Gastrointest Surg* 2006; **10**: 1106-1115
182 **Carrère N**, Sauvanet A, Goere D, Kianmanesh R, Vullierme MP, Couvelard A, Ruszniewski P, Belghiti J. Pancreaticoduodenectomy with mesentericoportal vein resection for adenocarcinoma of the pancreatic head. *World J Surg* 2006; **30**: 1526-1535
183 **Fukuda S**, Oussoultzoglou E, Bachellier P, Rosso E, Nakano H, Audet M, Jaeck D. Significance of the depth of portal vein wall invasion after curative resection for pancreatic adenocarcinoma. *Arch Surg* 2007; **142**: 172-179; discussion 180
184 **Clavien PA**, Rüdiger HA. A simple technique of portal vein resection and reconstruction during pancreaticoduodenectomy. *J Am Coll Surg* 1999; **189**: 629-634
185 **van Geenen RC**, ten Kate FJ, de Wit LT, van Gulik TM, Obertop H, Gouma DJ. Segmental resection and wedge excision of the portal or superior mesenteric vein during pancreatoduodenectomy. *Surgery* 2001; **129**: 158-163
186 **Chae YS**, Choi JS, Kim KS, Seong JS, Lee WJ, Kim BR. Preoperative chemoradiation and pancreaticoduodenectomy with portal vein resection for localized advanced pancreatic cancer. *Yonsei Med J* 2003; **44**: 551-556
187 **Harrison LE**, Brennan MF. Portal vein resection for pancreatic adenocarcinoma. *Surg Oncol Clin N Am* 1998; **7**: 165-181
188 **Launois B**, Stasik C, Bardaxoglou E, Meunier B, Campion JP, Greco L, Sutherland F. Who benefits from portal vein resection during pancreaticoduodenectomy for pancreatic cancer? *World J Surg* 1999; **23**: 926-929
189 **Tashiro S**, Uchino R, Hiraoka T, Tsuji T, Kawamoto S, Saitoh N, Yamasaki K, Miyauchi Y. Surgical indication and significance of portal vein resection in biliary and pancreatic cancer. *Surgery* 1991; **109**: 481-487
190 **Nakao A**, Harada A, Nonami T, Kaneko T, Takagi H. Regional vascular resection using catheter bypass procedure for pancreatic cancer. *Hepatogastroenterology* 1995; **42**: 734-739
191 **Howard TJ**, Villanustre N, Moore SA, DeWitt J, LeBlanc J, Maglinte D, McHenry L. Efficacy of venous reconstruction in patients with adenocarcinoma of the pancreatic head. *J Gastrointest Surg* 2003; **7**: 1089-1095
192 **Allema JH**, Reinders ME, van Gulik TM, van Leeuwen DJ, de Wit LT, Verbeek PC, Gouma DJ. Portal vein resection in patients undergoing pancreatoduodenectomy for carcinoma of the pancreatic head. *Br J Surg* 1994; **81**: 1642-1646
193 **Launois B**, Franci J, Bardaxoglou E, Ramee MP, Paul JL, Malledant Y, Campion JP. Total pancreatectomy for ductal adenocarcinoma of the pancreas with special reference to resection of the portal vein and multicentric cancer. *World J Surg* 1993; **17**: 122-126; discussion 126-127

194 **Aramaki M**, Matsumoto T, Etoh T, Ishio T, Himeno Y, Sasaki A, Yada K, Kawano K, Kitano S. Clinical significance of combined pancreas and portal vein resection in surgery for pancreatic adenocarcinoma. *Hepatogastroenterology* 2003; **50**: 263-266

195 **Bachellier P**, Nakano H, Oussoultzoglou PD, Weber JC, Boudjema K, Wolf PD, Jaeck D. Is pancreaticoduodenectomy with mesentericoportal venous resection safe and worthwhile? *Am J Surg* 2001; **182**: 120-129

196 **Leach SD**, Lee JE, Charnsangavej C, Cleary KR, Lowy AM, Fenoglio CJ, Pisters PW, Evans DB. Survival following pancreaticoduodenectomy with resection of the superior mesenteric-portal vein confluence for adenocarcinoma of the pancreatic head. *Br J Surg* 1998; **85**: 611-617

197 **Shibata C**, Kobari M, Tsuchiya T, Arai K, Anzai R, Takahashi M, Uzuki M, Sawai T, Yamazaki T. Pancreatectomy combined with superior mesenteric-portal vein resection for adenocarcinoma in pancreas. *World J Surg* 2001; **25**: 1002-1005

198 **Pedrazzoli S**, Pasquali C, Sperti C. General aspects of surgical treatment of pancreatic cancer. *Dig Surg* 1999; **16**: 265-275

199 **Machado MC**, Penteado S, Cunha JE, Jukemura J, Herman P, Bacchella T, Machado MA, Montagnini AL. Pancreatic head tumors with portal vein involvement: an alternative surgical approach. *Hepatogastroenterology* 2001; **48**: 1486-1487

200 **Zhou GW**, Wu WD, Xiao WD, Li HW, Peng CH. Pancreatectomy combined with superior mesenteric-portal vein resection: report of 32 cases. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 130-134

201 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Pancreaticoduodenectomy with en bloc portal vein resection for pancreatic carcinoma with suspected portal vein involvement. *World J Surg* 2004; **28**: 602-608

202 **Siriwardana HP**, Siriwardena AK. Systematic review of outcome of synchronous portal-superior mesenteric vein resection during pancreatectomy for cancer. *Br J Surg* 2006; **93**: 662-673

203 **Koniaris LG**, Staveley-O'Carroll KF, Zeh HJ, Perez E, Jin XL, Maley WR, Zabari G, Bartlett DL, Khanna A, Franceschi D, Schoeniger LO. Pancreaticoduodenectomy in the presence of superior mesenteric venous obstruction. *J Gastrointest Surg* 2005; **9**: 915-921

204 **Moore GE**, Sako Y, Thomas LB. Radical pancreatoduodenectomy with resection and reanastomosis of the superior mesenteric vein. *Surgery* 1951; **30**: 550-553

205 **Park DI**, Lee JK, Kim JE, Hyun JG, Shim SG, Lee KT, Palk SW, Rhee JC, Choi KW, Lim JH, Kim YI. The analysis of resectability and survival in pancreatic cancer patients with vascular invasion. *J Clin Gastroenterol* 2001; **32**: 231-234

206 **Tseng JF**, Raut CP, Lee JE, Pisters PW, Vauthey JN, Abdalla EK, Gomez HF, Sun CC, Crane CH, Wolff RA, Evans DB. Pancreaticoduodenectomy with vascular resection: margin status and survival duration. *J Gastrointest Surg* 2004; **8**: 935-949; discussion 949-950

207 **Turrini O**, Viret F, Moureau-Zabotto L, Guiramand J, Moutardier V, Lelong B, de Chaisemartin C, Giovannini M, Delpero JR. Neoadjuvant 5 fluorouracil-cisplatin chemoradiation effect on survival in patients with resectable pancreatic head adenocarcinoma: a ten-year single institution experience. *Oncology* 2009; **76**: 413-419

208 **Raptopoulos V**, Steer ML, Sheiman RG, Vrachliotis TG, Gougoutas CA, Movson JS. The use of helical CT and CT angiography to predict vascular involvement from pancreatic cancer: correlation with findings at surgery. *AJR Am J Roentgenol* 1997; **168**: 971-977

209 **Diehl SJ**, Lehmann KJ, Sadick M, Lachmann R, Georgi M. Pancreatic cancer: value of dual-phase helical CT in assessing resectability. *Radiology* 1998; **206**: 373-378

210 **House MG**, Yeo CJ, Cameron JL, Campbell KA, Schulick RD, Leach SD, Hruban RH, Horton KM, Fishman EK, Lillemoe KD. Predicting resectability of periampullary cancer with three-dimensional computed tomography. *J Gastrointest Surg* 2004; **8**: 280-288

211 **Nakao A**, Harada A, Nonami T, Kaneko T, Inoue S, Takagi H. Clinical significance of portal invasion by pancreatic head carcinoma. *Surgery* 1995; **117**: 50-55

212 **Hartel M**, Niedergethmann M, Farag-Soliman M, Sturm JW, Richter A, Trede M, Post S. Benefit of venous resection for ductal adenocarcinoma of the pancreatic head. *Eur J Surg* 2002; **168**: 707-712

213 **Nakagohri T**, Kinoshita T, Konishi M, Inoue K, Takahashi S. Survival benefits of portal vein resection for pancreatic cancer. *Am J Surg* 2003; **186**: 149-153

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REVIEW

Diffusion-weighted MRI in abdominal oncology: Clinical applications

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Abstract

Diffusion-weighted magnetic resonance imaging (DWI) provides image contrast that is different from that obtained by conventional magnetic resonance techniques. Although previously, DWI has been used to evaluate various diseases of the central nervous system, several technical advances have expanded the clinical applications of DWI beyond the central nervous system. As a result, many reports have been published on the use of DWI in abdominal diseases. Particularly, abdominal DWI has now being focused on evaluation of patients with abdominal cancer. DWI can be used for pretreatment tumor detection, characterization including predicting tumor response to therapy, monitoring tumor response during therapy, and follow-up study after treatment to detect possible tumor recurrence.

Key words: Diffusion weighted magnetic resonance imaging; Abdominal neoplasms

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INTRODUCTION

Diffusion-weighted magnetic resonance imaging (DWI) has enabled us to obtain additional information derived from the microscopic motion of water protons, which is not possible using conventional magnetic resonance imaging (MRI). Previously, DWI has been used to evaluate various diseases of the central nervous system. The most established clinical application of DWI for the central nervous system is evaluation of acute stroke[1].

DWI has many advantages. First, it is completely noninvasive, does not require exposure to ionizing radiation or injection of contrast material, and does not cause patient discomfort. Second, because it is derived from a well-established MRI technique, DWI does not require expert technicians with sophisticated technical skills or expensive equipment, such as a cyclotron that is required for positron emission tomography. Another advantage of DWI is that it can be added easily to a routine MRI protocol because it requires only a very short prolongation of examination time[2].

Recently, several technical advances have expanded the clinical applications of DWI beyond the central nervous system, and many studies have been published on the use of DWI in abdominal diseases. Particularly, abdominal DWI has now being focused on evaluating patients with abdominal cancer[3-9]. In this article, the application of DWI in abdominal oncology is described.

HOW TO INTERPRET DWI

DWI can offer qualitative and quantitative information

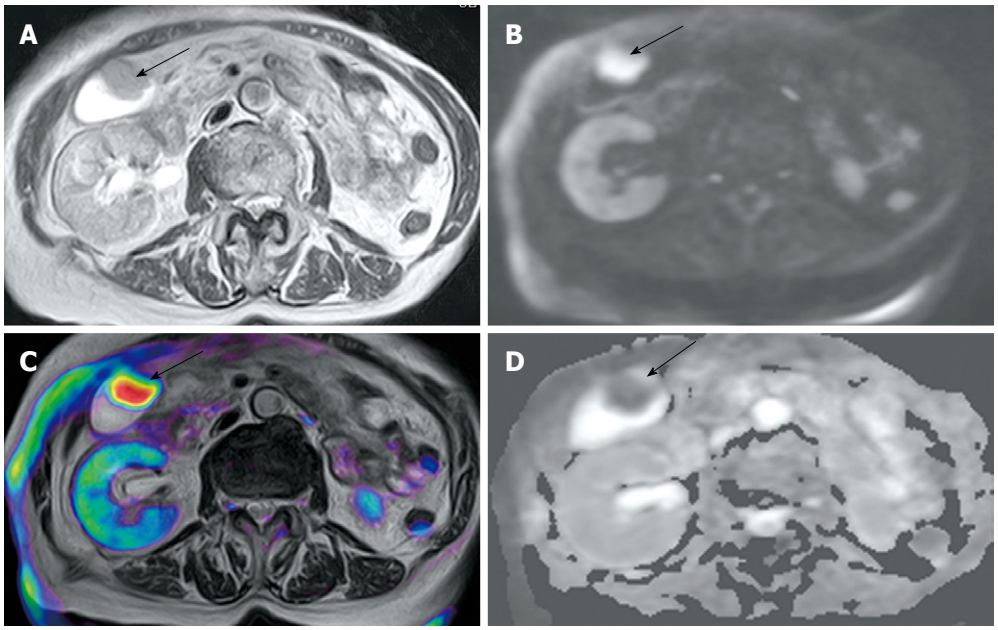

Figure 1 MRI of a patient with gallbladder carcinoma. A: Axial T2-weighted MRI of a patient with gallbladder carcinoma shows a mass (arrow) protruding into the gallbladder lumen; B: Corresponding axial DW image shows high intensity (arrow); C: Corresponding color fusion image of T2-weighted image and DW image shows gallbladder carcinoma (arrow). On color fusion images, the red area corresponds to high signal intensity on DW images and blue correspond to low intensity; D: Corresponding ADC map shows low intensity (arrow).

that can be helpful for tumor assessment (Figure 1). The former assesses visual differences in signal intensity between a tumor and its surrounding normal tissue, and the latter enables calculation of values (apparent diffusion coefficient, ADC) obtained from DWI, such as a computed tomography value.

Qualitative assessment in DWI

Visual assessment of relative tissue signal intensity on DWI is being used for tumor detection and characterization[3]. Tumors generally tend to block diffusion more than the tissue from which they originate and show relative high signal intensity on DWI (Figure 1B); however, some normal organs, such as the spleen, adrenal gland and seminal vesicle, also show high signal intensity on DWI. Moreover, DWI has a pitfall known as "T2 shine-through". DWI is obtained by adding a diffusion-weighting gradient (known as an MPG) to T2-weighted images, the basic sequence of conventional MRI. Thus, because DWI shows signal intensity that depends on diffusion and T2 signal intensity, a region with a high T2 signal retains the high signal on DWI, and may be mistaken for restricted diffusion. Therefore, special care must be taken with these pitfalls in diagnosing with DWI. DWI is usually interpreted by superimposing DWI and conventional morphological T2-weighted images because DWI cannot show minute morphological structures (Figure 1C).

Quantitative analysis in DWI

Quantitative tumor assessment is possible by calculating ADC after performing DWI with changed parameters (known as *b* values). ADC values in various malignant lesions generally tend to decrease, probably due to increased tissue cellularity or cell density, because the latter correlates with malignancy (Figure 1D). In addition to the cellular membranes, intracellular cytoskeleton, organelles, matrix fibers and soluble macromolecules contribute to diffusion restrictions in tumors[10]; therefore, ADC values are expected to reflect histopathological tissue characteristics. ADC is calculated for each pixel of the image and is displayed as a map. By setting regions of interest within tumors on these maps, ADCs of the tumor can be measured.

CLINICAL APPLICATIONS OF DWI IN ABDOMINAL ONCOLOGY

Tumor detection and characterization

Tumors generally tend to show relative high signal intensity on DWI. Using qualitative assessment, Nasu *et al*[11] have shown that DWI is superior to superparamagnetic iron oxide (SPIO)-enhanced MRI in detecting liver metastases, which had been the best available examination technique. They have reported that the sensitivity and specificity of DWI was 82% and 94%, respectively. Koh *et al*[12] also have reported that the sensitivity and specificity of DWI for detecting liver metastases was 78% and 95%. Thus, qualitative assessment with DWI has superior ability for assessing liver metastasis.

In colorectal tumors, Ichikawa *et al*[7] have shown that DWI has high sensitivity and specificity for detecting tumors, and several authors have shown that DWI has high sensitivity and specificity for detecting tumors even in the pancreatico-biliary system[6,9].

In quantitative assessment of DWI, ADC measurement has the potential to differentiate benign and malignant liver tumors. In many studies, malignancy has a lower ADC value than benignity. Taouli *et al*[13] have shown that metastatic liver tumors have the lowest ADC in malignant and benign focal lesions of the liver, and have revealed a significant difference between benign and malignant lesions. Chan *et al*[14] have shown that DWI can be used to distinguish between hepatic abscess and cystic or necrotic malignant liver tumor; ADC of abscess

cavities has a lower value than that of cystic or necrotic malignant liver tumors. Also in abdominal tumors other than in the liver, ADCs of malignant lesions have shown lower values[9,15,16]. However, most studies have reported that ADC measurement has no clear threshold to discriminate malignant and benign tumors because of substantial overlapping[9,13,15-18].

Predicting and monitoring response to therapy

Conventional criteria using morphological images have been used to evaluate antitumor therapy; however, measuring tumor size is often not adequate when tumors are treated with cytotoxic therapy and molecular targeting agents, because changes in tumor size after therapy with these drugs are not expected[4,19]; therefore, a new method for evaluating tumor response is required that can precisely reflects the clinical outcome, earlier than conventional imaging modalities.

The ability of DWI to predict therapy outcome has been shown in many clinical studies. Several authors have reported that tumors with low pretreatment ADC values show a better response to various therapies than those with high ADC[20-24]. However, studies of areas other than the abdomen have addressed that the relationship between pretreatment ADC and prognosis yield, with different results: patients suffering from a tumor with high pretreatment ADC show better long-term post-treatment prognosis than those with low ADC[25,26].

Many researchers have reported that DWI has the potential for evaluating tumor response during treatment. The results of animal studies have proved that ADC increases can be depicted in those responding to treatment[27]. In clinical studies, researchers have reported that an early increase in the ADC value after starting therapy suggests a better treatment outcome[20,28-32].

Monitoring response to therapy by visual assessment of DWI has been reported in brain tumors and bone metastasis, but not in the abdominal region[21,33]. Studies on bone metastasis have revealed that the treatment response after therapy could be assessed as a decrease in signal intensity[33]. Several authors have shown that tumors demonstrate an increase in ADC after treatment before a change in tumor size occurs, which heralds later diminution of the tumor size[18,22,34-38]. Chen *et al*[34] have reported that patients with hepatocellular carcinoma show a significant rise in ADC value when they respond to treatment. Koh *et al*[22] also have reported that patients with colorectal hepatic metastases show an increase in ADC, at least in those who show a partial response to treatment, but not in non-responders. A decrease in ADC during follow-up suggests tumor recurrence[27].

FUTURE DEVELOPMENT

Several studies have indicated that DWI may be useful for tumor staging, including lymph node and distant metastases[21,39-42]. For tumor staging, whole-body imaging is desirable. Takahara *et al* have shown that whole-body DWI is promising[43-45] using their method to examine the whole body by composite construction of segmented imaging. The images are processed using maximum intensity projection and 3D display[43-45]. More clinical research on this technique is needed because their study was preliminary.

The most important issue regarding DWI is non-standardization among MRI manufactures and researchers. Substantial differences in the ADC values of the same normal and diseased organs have been presented[5] by researchers using a different imaging technique; therefore, standardization of the imaging protocol is fundamental.

Currently, spatial resolution of DWI is not high enough. In order to compensate for such limited resolution, qualitative assessment might need superimposition of DWI on corresponding T2-weighted images, and quantitative assessment may require meticulous ADC measurements for small lesions. Utilization of high-field MRI may be able to solve the issue of limited spatial resolution.

CONCLUSION

DWI is a promising imaging technique to evaluate abdominal tumors. This technique can be used for pretreatment tumor detection, characterization including predicting tumor response to therapy, monitoring tumor response during therapy, and follow-up study after treatment to detect possible tumor recurrence. Standardization of the imaging protocol and large clinical trials regarding the usefulness of DWI are needed.

REFERENCES

1 **Schaefer PW**, Grant PE, Gonzalez RG. Diffusion-weighted MR imaging of the brain. *Radiology* 2000; **217**: 331-345
2 **Thoeny HC**, De Keyzer F. Extracranial applications of diffusion-weighted magnetic resonance imaging. *Eur Radiol* 2007; **17**: 1385-1393
3 **Koh DM**, Collins DJ. Diffusion-weighted MRI in the body: applications and challenges in oncology. *AJR Am J Roentgenol* 2007; **188**: 1622-1635
4 **Hamstra DA**, Rehemtulla A, Ross BD. Diffusion magnetic resonance imaging: a biomarker for treatment response in oncology. *J Clin Oncol* 2007; **25**: 4104-4109
5 **Padhani AR**, Liu G, Koh DM, Chenevert TL, Thoeny HC, Takahara T, Dzik-Jurasz A, Ross BD, Van Cauteren M, Collins D, Hammoud DA, Rustin GJ, Taouli B, Choyke PL. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations. *Neoplasia* 2009; **11**: 102-125
6 **Ichikawa T**, Erturk SM, Motosugi U, Sou H, Iino H, Araki T, Fujii H. High-b value diffusion-weighted MRI for detecting pancreatic adenocarcinoma: preliminary results. *AJR Am J Roentgenol* 2007; **188**: 409-414
7 **Ichikawa T**, Erturk SM, Motosugi U, Sou H, Iino H, Araki T, Fujii H. High-B-value diffusion-weighted MRI in colorectal cancer. *AJR Am J Roentgenol* 2006; **187**: 181-184
8 **Naganawa S**, Sato C, Kumada H, Ishigaki T, Miura S, Takizawa O. Apparent diffusion coefficient in cervical cancer of the uterus: comparison with the normal uterine cervix. *Eur Radiol* 2005; **15**: 71-78
9 **Sugita R**, Yamazaki T, Furuta A, Itoh K, Fujita N, Takahashi

S. High b-value diffusion-weighted MRI for detecting gallbladder carcinoma: preliminary study and results. *Eur Radiol* 2009; **19**: 1794-1798
10 **Lyng H**, Haraldseth O, Rofstad EK. Measurement of cell density and necrotic fraction in human melanoma xenografts by diffusion weighted magnetic resonance imaging. *Magn Reson Med* 2000; **43**: 828-836
11 **Nasu K**, Kuroki Y, Nawano S, Kuroki S, Tsukamoto T, Yamamoto S, Motoori K, Ueda T. Hepatic metastases: diffusion-weighted sensitivity-encoding versus SPIO-enhanced MR imaging. *Radiology* 2006; **239**: 122-130
12 **Koh DM**, Brown G, Riddell AM, Scurr E, Collins DJ, Allen SD, Chau I, Cunningham D, deSouza NM, Leach MO, Husband JE. Detection of colorectal hepatic metastases using MnDPDP MR imaging and diffusion-weighted imaging (DWI) alone and in combination. *Eur Radiol* 2008; **18**: 903-910
13 **Taouli B**, Vilgrain V, Dumont E, Daire JL, Fan B, Menu Y. Evaluation of liver diffusion isotropy and characterization of focal hepatic lesions with two single-shot echo-planar MR imaging sequences: prospective study in 66 patients. *Radiology* 2003; **226**: 71-78
14 **Chan JH**, Tsui EY, Luk SH, Fung AS, Yuen MK, Szeto ML, Cheung YK, Wong KP. Diffusion-weighted MR imaging of the liver: distinguishing hepatic abscess from cystic or necrotic tumor. *Abdom Imaging* 2001; **26**: 161-165
15 **Kim T**, Murakami T, Takahashi S, Hori M, Tsuda K, Nakamura H. Diffusion-weighted single-shot echoplanar MR imaging for liver disease. *AJR Am J Roentgenol* 1999; **173**: 393-398
16 **Yamada I**, Aung W, Himeno Y, Nakagawa T, Shibuya H. Diffusion coefficients in abdominal organs and hepatic lesions: evaluation with intravoxel incoherent motion echo-planar MR imaging. *Radiology* 1999; **210**: 617-623
17 **van Rijswijk CS**, Kunz P, Hogendoorn PC, Taminiau AH, Doornbos J, Bloem JL. Diffusion-weighted MRI in the characterization of soft-tissue tumors. *J Magn Reson Imaging* 2002; **15**: 302-307
18 **Einarsdóttir H**, Karlsson M, Wejde J, Bauer HC. Diffusion-weighted MRI of soft tissue tumours. *Eur Radiol* 2004; **14**: 959-963
19 **Thoeny HC**, De Keyzer F, Vandecaveye V, Chen F, Sun X, Bosmans H, Hermans R, Verbeken EK, Boesch C, Marchal G, Landuyt W, Ni Y. Effect of vascular targeting agent in rat tumor model: dynamic contrast-enhanced versus diffusion-weighted MR imaging. *Radiology* 2005; **237**: 492-499
20 **Cui Y**, Zhang XP, Sun YS, Tang L, Shen L. Apparent diffusion coefficient: potential imaging biomarker for prediction and early detection of response to chemotherapy in hepatic metastases. *Radiology* 2008; **248**: 894-900
21 **Mardor Y**, Roth Y, Ochershvilli A, Spiegelmann R, Tichler T, Daniels D, Maier SE, Nissim O, Ram Z, Baram J, Orenstein A, Pfeffer R. Pretreatment prediction of brain tumors' response to radiation therapy using high b-value diffusion-weighted MRI. *Neoplasia* 2004; **6**: 136-142
22 **Koh DM**, Scurr E, Collins D, Kanber B, Norman A, Leach MO, Husband JE. Predicting response of colorectal hepatic metastasis: value of pretreatment apparent diffusion coefficients. *AJR Am J Roentgenol* 2007; **188**: 1001-1008
23 **DeVries A**, Kremser C, Hein P, Griebel J, Krezcy A, Öfner D, Pfeiffer K, Lukas P, Judmaier W. Tumor microcirculation and diffusion predict therapy outcome for primary rectal carcinoma1. *Int J Radiat Oncol Biol Phys* 2003; **56**: 958-965
24 **Dzik-Jurasz A**, Domenig C, George M, Wolber J, Padhani A, Brown G, Doran S. Diffusion MRI for prediction of response of rectal cancer to chemoradiation. *Lancet* 2002; **360**: 307-308
25 **Higano S**, Yun X, Kumabe T, Watanabe M, Mugikura S, Umetsu A, Sato A, Yamada T, Takahashi S. Malignant astrocytic tumors: clinical importance of apparent diffusion coefficient in prediction of grade and prognosis. *Radiology* 2006; **241**: 839-846
26 **Murakami R**, Sugahara T, Nakamura H, Hirai T, Kitajima M, Hayashida Y, Baba Y, Oya N, Kuratsu J, Yamashita Y. Malignant supratentorial astrocytoma treated with postoperative radiation therapy: prognostic value of pretreatment quantitative diffusion-weighted MR imaging. *Radiology* 2007; **243**: 493-499
27 **Thoeny HC**, De Keyzer F, Chen F, Ni Y, Landuyt W, Verbeken EK, Bosmans H, Marchal G, Hermans R. Diffusion-weighted MR imaging in monitoring the effect of a vascular targeting agent on rhabdomyosarcoma in rats. *Radiology* 2005; **234**: 756-764
28 **Moffat BA**, Chenevert TL, Lawrence TS, Meyer CR, Johnson TD, Dong Q, Tsien C, Mukherji S, Quint DJ, Gebarski SS, Robertson PL, Junck LR, Rehemtulla A, Ross BD. Functional diffusion map: a noninvasive MRI biomarker for early stratification of clinical brain tumor response. *Proc Natl Acad Sci USA* 2005; **102**: 5524-5529
29 **Chenevert TL**, McKeever PE, Ross BD. Monitoring early response of experimental brain tumors to therapy using diffusion magnetic resonance imaging. *Clin Cancer Res* 1997; **3**: 1457-1466
30 **Kremser C**, Judmaier W, Hein P, Griebel J, Lukas P, de Vries A. Preliminary results on the influence of chemoradiation on apparent diffusion coefficients of primary rectal carcinoma measured by magnetic resonance imaging. *Strahlenther Onkol* 2003; **179**: 641-649
31 **Theilmann RJ**, Borders R, Trouard TP, Xia G, Outwater E, Ranger-Moore J, Gillies RJ, Stopeck A. Changes in water mobility measured by diffusion MRI predict response of metastatic breast cancer to chemotherapy. *Neoplasia* 2004; **6**: 831-837
32 **Lee KC,** Bradley DA, Hussain M, Meyer CR, Chenevert TL, Jacobson JA, Johnson TD, Galban CJ, Rehemtulla A, Pienta KJ, Ross BD. A feasibility study evaluating the functional diffusion map as a predictive imaging biomarker for detection of treatment response in a patient with metastatic prostate cancer to the bone. *Neoplasia* 2007; **9**: 1003-1011
33 **Byun WM**, Shin SO, Chang Y, Lee SJ, Finsterbusch J, Frahm J. Diffusion-weighted MR imaging of metastatic disease of the spine: assessment of response to therapy. *AJNR Am J Neuroradiol* 2002; **23**: 906-912
34 **Chen CY**, Li CW, Kuo YT, Jaw TS, Wu DK, Jao JC, Hsu JS, Liu GC. Early response of hepatocellular carcinoma to transcatheter arterial chemoembolization: choline levels and MR diffusion constants--initial experience. *Radiology* 2006; **239**: 448-456
35 **Kamel IR**, Bluemke DA, Eng J, Liapi E, Messersmith W, Reyes DK, Geschwind JF. The role of functional MR imaging in the assessment of tumor response after chemoembolization in patients with hepatocellular carcinoma. *J Vasc Interv Radiol* 2006; **17**: 505-512
36 **Deng J**, Miller FH, Rhee TK, Sato KT, Mulcahy MF, Kulik LM, Salem R, Omary RA, Larson AC. Diffusion-weighted MR imaging for determination of hepatocellular carcinoma response to yttrium-90 radioembolization. *J Vasc Interv Radiol* 2006; **17**: 1195-1200
37 **Kamel IR**, Reyes DK, Liapi E, Bluemke DA, Geschwind JF. Functional MR imaging assessment of tumor response after 90Y microsphere treatment in patients with unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 49-56
38 **Kamel IR**, Bluemke DA, Ramsey D, Abusedera M, Torbenson M, Eng J, Szarf G, Geschwind JF. Role of diffusion-weighted imaging in estimating tumor necrosis after chemoembolization of hepatocellular carcinoma. *AJR Am J Roentgenol* 2003; **181**: 708-710
39 **Choi EK**, Kim JK, Choi HJ, Park SH, Park BW, Kim N, Kim JS, Im KC, Cho G, Cho KS. Node-by-node correlation between MR and PET/CT in patients with uterine cervical cancer: diffusion-weighted imaging versus size-based

criteria on T2WI. *Eur Radiol* 2009; **19**: 2024-2032
40 **Herneth AM**, Philipp MO, Naude J, Funovics M, Beichel RR, Bammer R, Imhof H. Vertebral metastases: assessment with apparent diffusion coefficient. *Radiology* 2002; **225**: 889-894
41 **Baur A**, Stäbler A, Brüning R, Bartl R, Krödel A, Reiser M, Deimling M. Diffusion-weighted MR imaging of bone marrow: differentiation of benign versus pathologic compression fractures. *Radiology* 1998; **207**: 349-356
42 **Baur A**, Huber A, Ertl-Wagner B, Dürr R, Zysk S, Arbogast S, Deimling M, Reiser M. Diagnostic value of increased diffusion weighting of a steady-state free precession sequence for differentiating acute benign osteoporotic fractures from pathologic vertebral compression fractures. *AJNR Am J Neuroradiol* 2001; **22**: 366-372
43 **Takahara T**, Imai Y, Yamashita T, Yasuda S, Nasu S, Van Cauteren M. Diffusion weighted whole body imaging with background body signal suppression (DWIBS): technical improvement using free breathing, STIR and high resolution 3D display. *Radiat Med* 2004; **22**: 275-282
44 **Kwee TC**, Takahara T, Ochiai R, Nievelstein RA, Luijten PR. Diffusion-weighted whole-body imaging with background body signal suppression (DWIBS): features and potential applications in oncology. *Eur Radiol* 2008; **18**: 1937-1952
45 **Li S**, Sun F, Jin ZY, Xue HD, Li ML. Whole-body diffusion-weighted imaging: technical improvement and preliminary results. *J Magn Reson Imaging* 2007; **26**: 1139-1144

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ORIGINAL ARTICLE

Analgesic effects of JCM-16021 on neonatal maternal separation-induced visceral pain in rats

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Author contributions: Bian ZX made contributions to the study design, data interpretation, and wrote the manuscript; Zhang M performed the majority of experiments and data analysis; Han QB conducted the majority of quality control for the formula; Xu HX and Sung JJY made contributions to the study design; Xu HX contributed to the quality control of herbs and products.
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Abstract

AIM: To investigate the pharmacological effect of JCM-16021, a Chinese herbal formula, and its underlying mechanisms.

METHODS: JCM-16021 is composed of seven herbal plant materials. All raw materials of the formula were examined according to the quality control criteria listed in the Chinese Pharmacopeia (2005). In a neonatal maternal separation (NMS) model, male Sprague-Dawley rats were submitted to daily maternal separation from postnatal day 2 to day 14, or no specific handling (NH). Starting from postnatal day 60, rats were administered JCM-16021 (2, 4, 8 g/kg per day) orally twice a day for 28 d. Pain threshold pressure and electromyographic activities of external oblique muscles in response to colorectal distention recorded with a Power Lab System (AD Instruments International), were tested as pain indices. Changes in serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the colon of rats were analyzed; the enterochromaffin cell numbers and serotonin transporter in the colon of rats were also evaluated with an immunohistochemistry method.

RESULTS: NMS treatment significantly reduced pain threshold pressure (37.4 ± 1.4 mmHg), as compared to that of NH rats (57.7 ± 1.9 mmHg, $P < 0.05$). After JCM-16021 treatment, the pain threshold pressure significantly increased when compared to that before treatment (34.2 ± 0.9 mmHg *vs* 52.8 ± 2.3 mmHg in the high dose group, 40.2 ± 1.6 mmHg *vs* 46.5 ± 1.3 mmHg in the middle dose group, and 39.3 ± 0.7 mmHg *vs* 46.5 ± 1.6 mmHg in the low dose group, $P < 0.05$). Also JCM-16021 significantly and dose-dependently decreased electromyographic activity to the graded colorectal distension (CRD), (the mean ΔAUC values were: 0.17 ± 0.03, 0.53 ± 0.15, 1.06 ± 0.18, 1.22 ± 0.24 in the high dose group; 0.23 ± 0.04, 0.68 ± 0.17, 1.27 ± 0.26, 1.8 ± 0.3 in the middle dose group; and 0.29 ± 0.06, 0.8 ± 0.16, 1.53 ± 0.24, 2.1 ± 0.21 in the low dose group for the pressures 20, 40, 60, 80 mmHg), as compared to the NMS vehicle group. The mean ΔAUC values were: 0.57 ± 0.12, 1.33 ± 0.18, 2.57 ± 0.37, 3.08 ± 0.37 for the pressures 20, 40, 60, 80 mmHg ($P < 0.05$). JCM-16021 treatment significantly reduced the 5-HT concentrations (from high, middle and low dosage groups: 60.25 ± 5.98 ng/100 mg, 60.32 ± 4.22 ng/100 mg, 73.31 ± 7.65 ng/100 mg), as compared to the NMS vehicle groups (93.11 ± 9.85 ng/100 mg, $P < 0.05$); and increased the 5-HIAA concentrations (after treatment, from high, middle and low dosage groups: 54.24 ± 3.27 ng/100 mg, 50.34 ± 1.26 ng/100 mg, 51.37 ± 2.13 ng/100 mg) when compared to that in the NMS vehicle group (51.75 ± 1.98 ng/100 mg, $P < 0.05$); but did not change the enterochromaffin cell numbers in the colon of rats. In addition, NMS rats had higher SERT expression ($n = 10$) than NH rats ($n = 8$,

$P < 0.05$). JCM-16021 treatment significantly decreased SERT expression when compared to the NMS group ($P < 0.01$-0.001).

CONCLUSION: JCM-16021 can attenuate visceral hypersensitivity, and this analgesic effect may be mediated through the serotonin signaling pathway in the colon of rats.

Key words: Analgesia effect; Neonatal maternal separation; Visceral hyperalgesia; Herbal medicine; Serotonin pathway

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INTRODUCTION

Irritable bowel syndrome (IBS) is characterized by chronic abdominal pain and altered bowel movements such as diarrhea and constipation[1,2]. Although conventional therapies (e.g. laxatives, antidepressants, antispasmodics, and bulking agents) are used to relieve the symptoms of IBS, the overall efficacy of these agents is poor[3,4]; and these agents have not been proven to be more effective than placebo in providing overall relief of symptoms in randomized, controlled clinical trials. Therefore, increasing numbers of IBS sufferers are seeking help from complementary and alternative medicines. Recently, our research group showed that JCM-16021, an herbal formula composed of seven herbs, can relieve symptoms in IBS patients[5]. In this randomized, double-blinded, and placebo-controlled trial, 84 diarrhea-predominant IBS patients received treatment (28 patients in each arm). At the end of the 8 wk treatment, 52% of participants in the JCM-16021 plus Holopon (hyoscine methobromide)-placebo group (Group A), 32% in the Holopon plus herbal-placebo group (Group B), and 42.7% in the double placebo group (Group C) experienced overall symptom improvements. Patients in Group A had the highest percentage improvement (Group A *vs* Group B *vs* Group C: 52% *vs* 32% *vs* 42.7%), but the mechanism of this effect remains unclear.

Serotonin (5-HT), an important neurotransmitter and paracrine signaling molecule, alters visceral perception and motor function by influencing the sympathetic, parasympathetic, and enteric nervous systems[6]. The majority of 5-HT is synthesized and stored in enterochromaffin (EC) cells in the gastrointestinal tract. Previous studies have shown that the changes in EC cells and the increased 5-HT concentrations in the human colon are associated with the generation of IBS symptoms and in other gastrointestinal functional disorders[7-10]. Furthermore, novel serotonergic agents, such as the 5-HT_3 antagonist alosetron and the 5-HT_4 agonist tegaserod, have significant impacts on IBS symptoms through their visceral analgesic properties and diverse effects on motor functions in the lower gastrointestinal tract[11]. Therefore, the 5-HT signaling pathway represents a promising target for IBS treatment.

A neonatal maternal separation (NMS)-induced visceral hyperalgesia rat model was previously established[12]. Because its characteristics mimic the symptoms of IBS patients, it is often used to study the mechanism of visceral hyperalgesia and to evaluate the pharmacological effects of potential IBS therapies[13,14].

Considering the effects of JCM-16021 in IBS patients and the function of serotonin in visceral hyperalgesia, this study aimed to investigate the analgesic effect of JCM-16021 on NMS-induced visceral hyperalgesia in rats, and its potential underlying mechanism. We hypothesized that JCM-16021 could attenuate visceral pain through the 5-HT signaling pathway in the colon of rats. These results were previously presented as a poster at the 16th United European Gastroenterology Week in October 2008 in Vienna, Austria[15].

MATERIALS AND METHODS

Herb materials

JCM-16021 is composed of seven plant materials, which are listed in Table 1. Purchasing, authentication and quality control of all seven herbs were performed based on the requirements of the Chinese Pharmacopoeia[16]. The authenticated voucher specimens (the voucher numbers are CMED-0043-02, CMED-0018-17, CMED0024-02, CMED-0044-02, CMED-0180-02, CMED-0179-02, CMED-0118-02) were stored in the Research Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China.

Reagents

Chloral hydrate was purchased from Fluka. Hematoxylin, 5-HT, and 5-HIAA were purchased from Sigma (Sigma-Aldrich Co., St. Louis, MO, USA). Holopon (hyoscine methobromide, 99%) was purchased from GSK Hong Kong.

Preparation and quality analysis of JCM-16021

JCM-16021 was prepared in the form of granules as follows: mixed medicinal materials weighing approximately 110 g (equal to the total amount of raw materials in one day's dosage of JCM-16021 formula for IBS patients) were macerated for 30 min, subsequently decocted for 60 min three times, and rinsed 10 times (v/w) with distilled water. The filtrates were combined and dried in a vacuum at 40℃. A water-soluble pale yellow powder, approximately 22 g,

Table 1 Composition of JCM-16021 and related quality analysis results

Composition and samples	Atractylone[1]	Gallic acid[1]	Corilagin[2]	Paeoniflorin[3]	Magnolol[4]	Honokiol[5]	Tetrahydropalmatine[6]	Quercitrin[7]	Heavy metal & pesticide residues
Fructus Terminaliae Chebulae (*Terminalia chebula Retz.*) 9%		+	2.18%						Pass
Radix Paeoniae Lactiflorae (*Paeonia lactiflora Pall.*) 14%				0.45%					Pass
Cortex Magnoliae Officinalis (*Magnolia officinalis Rehd. et Wils.*) 9%					1.76%	0.89%			Pass
Rhizoma Corydalis Yanhusuo (*Corydalis yanhusuo W. T. Wang*) 14%							0.10%		Pass
Herba Polygoni Chinensis (*Polygonum chinense L*) 18%								0.04%	Pass
Rhizoma Atractylodis Macrocephalae (*Atractylodes macrocephala Koidz*) 18%	+								Pass
Semen coicis Lachryma-jobi [*Coix lacryma-jobi L. var. ma-yuan (Roman.) Stapf*] 18%									Pass
Final product	-	+	2.43%	0.70%	ND	ND	0.01%	ND	Pass

This table presents the composition of formula and its ratio of each component in whole formula. [1]TLC test, + means detected, - means not detected; [2]Y = 4309.9x + 11.158, R^2 = 1, 0.155-7.75 μg; [3]Y = 1141.8x + 54.916, R^2 = 0.9999, 0.322-18.343 μg; [4]Y = 7822.7x + 1167.9, R^2 = 0.9994, 0.375-3.75 μg; [5]Y=7947.5x + 1465.9, R^2 = 0.9982, 0.34-3.4 μg; [6]Y = 5782.6x - 11.063, R^2 = 1, 0.028-1.4 μg; [7]Y=2612.4x + 1.6674, R^2 = 1, 0.0228-1.14 μg; ND means that the chemical marker was detected but not determined due to peak area being too small.

was obtained. To ensure the quality of the final product, all raw materials were examined according to the quality control criteria listed in the Chinese Pharmacopeia 2005[16]. As recommended by the Chinese Pharmacopeia 2005[16], paeoniflorin, magnolol, honokiol, and tetrahydropalmatine were selected as the chemical markers for *Radix Paeoniae Lactiflorae*, *Cortex Magnoliae Officinalis*, and *Rhizoma Corydalis Yanhusuo*, respectively. Corilagin, a constituent of *Fructus Terminaliae Chebulae* was also selected since it is a major component of the final product. Quercitrin was used as the chemical marker of *Herba Polygoni Chinensis*. In addition, gallic acid and atractylone were qualitatively checked in *Fructus Terminaliae Chebulae* and *Rhizoma Atractylodis Macrocephalae*, respectively. Heavy metals and pesticide residues were monitored to ensure safety.

Animals and neonatal maternal separation

Primiparous timed-pregnant Sprague-Dawley female rats were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong, on gestational day 13-14. Dams were housed individually in macrolon cages and maintained in rooms with temperature kept at 23 ± 2℃ and an alternating 12: 12 h light-dark cycle. All of the experimental protocols were carried out with the approval of the Committee on Use of Human-Animal Subjects in Teaching and Research of the Hong Kong Baptist University and according to the Regulations of the Department of Health, Hong Kong, China.

The neonatal maternal separation (NMS) rat model was established based on a previous report[12]. Briefly, pups in the NMS group were separated from their mothers and placed into individual cages in another room 180 min daily from postnatal day 2 to day 14, whereas normally-handled (NH) pups remained undisturbed in their home cage with the dam. All pups were weaned on postnatal day 22, and only male pups were used in the present study to avoid hormonal cycle induced variations. Male rats on postnatal day 60 were used in a series of three experiments.

Experimental design

This experiment involved three sets of studies: The first series of experiments aimed to evaluate the pharmacological effects of JCM-16021 on visceral pain by assessing changes in pain threshold pressure before and after JCM-16021 treatment. Six groups of rats were used. Group 1 (n = 10) with NH rats and Group 2 (n = 10) with NMS rats were given distilled water as a control. Groups 3, 4 and 5 (n = 10, 9, 9) with NMS rats received JCM-16021 at 8, 4 and 2 g/kg per day, respectively. Group 6 received 0.3 mg/kg per day Holopon (hyoscine methobromide, 99%, GSK Hong Kong) as an active control (n = 8). All pain threshold pressure detection tests were conducted in the morning between 9 am and 12 pm.

The second series of experiments aimed to test the analgesic effect of JCM-16021 through assessing electromyographic (EMG) activities of the left external abdominal oblique muscles to colorectal distension (CRD) before and after treatment with JCM-16021. Grouping (n = 7-10) was the same as that in the first series, with surgeries performed on treatment day 23, and EMG recording conducted on treatment day 28. The pain threshold test was not performed in this set of rats.

A third series of experiments with five groups (46 rats, n = 8-10 each group) of rats aimed to test the effects of JCM-16021 on the concentration of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), EC cell number and expression of serotonin transporter (SERT). Group

1 with NH rats and Group 2 with NMS rats received distilled water as a control. Groups 3, 4 and 5 with NMS rats received JCM-16021 at dosages of 8, 4, and 2 g/kg per day respectively. When the treatment course finished, the rats were deeply anesthetized with an overdose of midazolam hydrochloride, and 4 cm of the colon (5-6 cm from the anus) was harvested immediately. A piece of the colon was immediately fixed in 4% neutral-buffered paraformaldehyde for immunostaining. The rest was frozen with liquid nitrogen, and stored at -80℃ for later analysis of 5-HT/5-HIAA content.

Drug administration

Starting at postnatal day 60 (body weight around 250 g), rats were daily treated orally with different dosages of JCM-16021 (8, 4 and 2 g/kg per day body weight, in 10 mL/kg distilled water), Holopon at a dosage of 0.3 mg/kg per day body weight, or vehicle (distilled water at 10 mL/kg body weight). The dosage of JCM-16021 was set according to the clinical trial and 4 g/kg per day was determined to be equivalent to the clinical dosage[5].

Abdominal withdrawal reflex (AWR) test

AWR tests were performed as previously described[17]. Briefly, a flexible latex balloon (medical finger glove, 4 cm long, 2.3 cm diameter flaccid) was inserted into the rat colon. Rats were allowed to adapt in a transparent box alone for 30 min after insertion of the colorectal balloon. CRD was then applied in increments of 5 mmHg, maintained for 2 s at each step to observe. The pain threshold pressure was defined as the intensity of CRD that induced a sudden and persistent abdominal muscle contraction in rats with abdomen lift off the platform. The experiments were repeated three times with at least 5 min intervals for recovery. During the test, the observers were blinded to the treatment groups of the rats.

EMG recording test

To measure rat's visceral sensitivity, the visceral motor response (VMR) to CRD was studied by recording the EMG as previously described with modification[12,18]. Briefly, a pair of Teflon-coated stainless wires (Cooner Wire, Chatsworth, CA) was surgically implanted into the left external abdominal oblique muscles on treatment day 23 and EMG recording tests were conducted on treatment day 28. On the test day, animals were subjected to CRD. A flexible latex balloon (medical finger glove, 4 cm long, 2.3 cm diameter flaccid) tied around a urethral catheter (3 mm diameter) was lubricated with liquid paraffin oil and inserted intrarectally in its descending colon with the distal tip 1 cm from the anal verge and secured to the base of the tail under short ether anesthesia. CRD was initiated by Barostat (Distender Series Ⅱ R, G&J electronics, Canada). The EMG recording signal was amplified and filtered (50-5000 Hz) by Power Lab System (AD Instruments International). Three cycles of graded CRD (20, 40, 60, and 80 mmHg; 20 s duration; 2 min inter-stimulus interval) were applied to each rat. During the five day recovery from surgery, the treatments were continued. The overall effect of any given reagents was determined by calculating the changes of the area under the curve (AUC) of the raw EMG amplitude response after treatment, based on the formula ΔAUC% baseline = (AUC during CRD - AUC before CRD)/AUC before CRD.

5-HT and 5-HIAA content assays

5-HT and 5-HIAA concentrations in the colon were analyzed following a procedure with a slight modification[19]. Fluorescence of the handled sample was measured at an activation wavelength of 365 nm and an emission wavelength of 470 nm.

Immunohistochemistry assay for EC cells and SERT

Immunohistochemical detection of EC cells in the colon of rats was performed using a routine streptavidin-biotin peroxidase technique employing Chr-A antibody (1:250, Santa Cruz Biotechnology, Santa Cruz, CA, USA)[20] and mouse monoclonal anti-SERT antibody (1:250, Advanced targeting system, AB-N09) as previously described[19]. The immunoreaction products were observed under a light NIKON microscope equipped with a NIKON color digital camera system. A NIKON 20 × objective was used to collect images of colon sections. The mean densities of the positive immunoreaction of serotonin transporter receptors and the positive cell numbers of Chr-A in at least 6 serial slides from the colonic sections of each rat were analyzed.

Statistical analysis

All data are expressed as mean ± SE. The changes in visceral pain threshold pressure were analyzed by comparing the values before and after treatment for each group using a paired *t*-test, and the differences between before and after treatment in a group using a one-way analysis of variance (ANOVA). EMG activity data were analyzed by one-way ANOVA between different groups to determine whether the overall change was significant, in a similar way to the analysis of the changes of concentrations of 5-HT and 5-HIAA, EC cell numbers and SERT in rat colon. $P < 0.05$ was considered statistically significant.

RESULTS

Quality control of JCM-16021

To ensure the quality of JCM-16021, eight chemical markers were qualitatively and quantitatively tracked from the raw materials to the final product, and heavy metals and pesticide residues were also examined. The results are listed in Table 1[5].

NMS induced visceral hyperalgesia

As shown in Figure 1, there is a significant decrease in pain threshold pressure in the NMS vehicle rats before the treatment, when comparing with that of the NH vehicle group before the treatment ($P < 0.001$). The pain threshold pressure values were 37.4 ± 4.4 mmHg

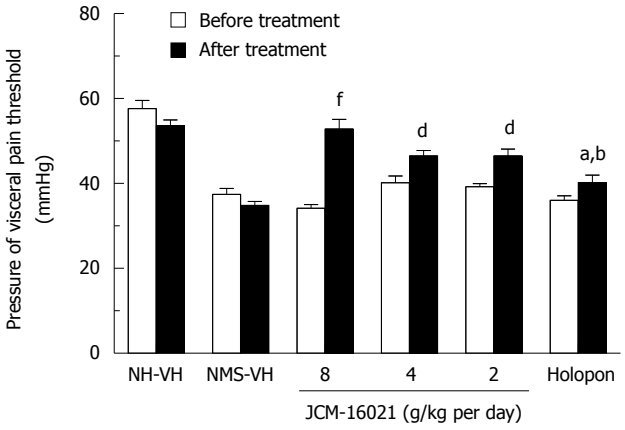

Figure 1 Pain threshold pressure assessment in different groups. Data are presented as the mean ± SE. Before treatment, the pain threshold of NMS groups was significantly decreased, as compared with that of the NH-VH group ($^{b}P < 0.001$). After treatment, JCM-16021 and Holopon significantly increased the pain threshold pressure ($^{a}P < 0.05$, $^{d}P < 0.01$, $^{f}P < 0.001$) compared with that before treatment of each group.

and 57.7 ± 5.9 mmHg in NMS vehicle and NH vehicle groups before treatment, respectively.

In EMG tests, the visceromotor response to CRD, which was reflected as AUC changes over the baseline in the NMS vehicle group after vehicle treatment (0.57 ± 0.12, 1.33 ± 0.18, 2.57 ± 0.37, 3.08 ± 0.37 under the pressures 20, 40, 60, 80 mmHg) was significantly increased compared to that of the NH vehicle group after vehicle treatment (0.11 ± 0.04, 0.58 ± 0.19, 0.96 ± 0.3, 1.39 ± 0.39 under the pressures 20, 40, 60, 80 mmHg, Figure 2) ($P < 0.05$). These results indicate that NMS induces allodynia (20 mmHg) and visceral hyperalgesia (40-80 mmHg) in rats.

Analgesic effect of JCM-16021 in NMS rats

As shown in Figure 1, JCM-16021 can significantly reduce the pain threshold pressure in three dosage groups (from high dose to low dose: 52.8 ± 2.3 mmHg, 46.5 ± 1.3 mmHg, and 46.5 ± 1.6 mmHg) comparing with that of the NMS vehicle group (34.8 ± 0.9 mmHg, $P < 0.05$). After treatment the pain threshold pressure in three JCM-16021 groups also significantly decreased when compared to that before treatment (from high dose to low dose: 52.8 ± 2.3 mmHg *vs* 34.2 ± 0.9 mmHg, 46.5 ± 1.3 mmHg *vs* 40.2 ± 1.6 mmHg and 46.5 ± 1.6 mmHg *vs* 39.3 ± 0.7 mmHg, $P < 0.01$). Holopon also had a similar analgesic effect to JCM-16021 when comparing the pain threshold pressure values either with that of NMS vehicle group or the value before the Holopon treatment.

In the EMG test, as shown in Figure 2A-D, the EMG activity to the graded CRD, which was reflected as AUC changes over the baseline, significantly and dose-dependently decreased after JCM-16021 treatment compared to that of the NMS vehicle group ($P < 0.05$). The mean ΔAUC significantly fell in the high dose group (0.17 ± 0.03, 0.53 ± 0.15, 1.06 ± 0.18, 1.22 ± 0.24 for the pressures 20, 40, 60, 80 mmHg), middle dose group (0.23 ± 0.04, 0.68 ± 0.17, 1.27 ± 0.26, 1.8 ± 0.3 for the pressures 20, 40, 60, 80 mmHg), and low dose group (0.29 ± 0.06, 0.8 ± 0.16,

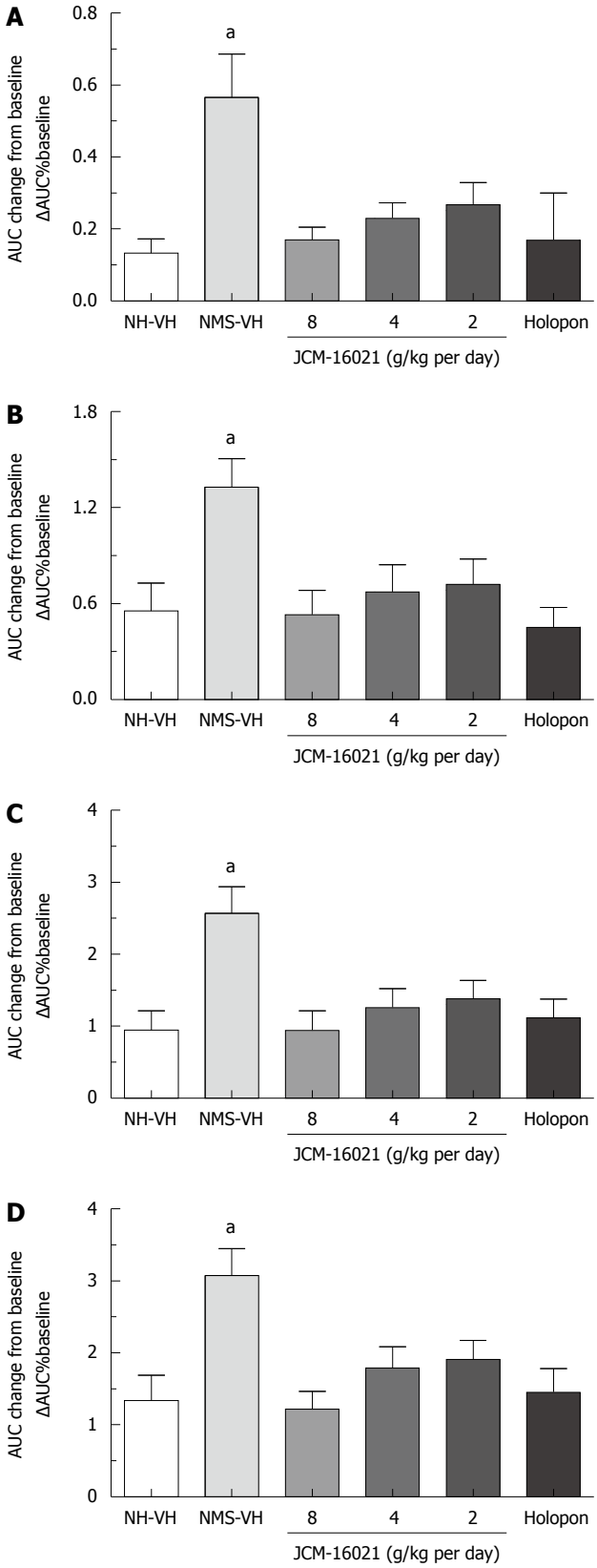

Figure 2 JCM-16021 effect on the electromyographic activity in response to graded CRD at pressures of 20 mmHg (A), 40 mmHg (B), 60 mmHg (C), 80 mmHg (D). Data are presented as mean ± SE (n = 7-10). Significant difference is indicated by $^{a}P < 0.05$ when compared with the NMS-VH group.

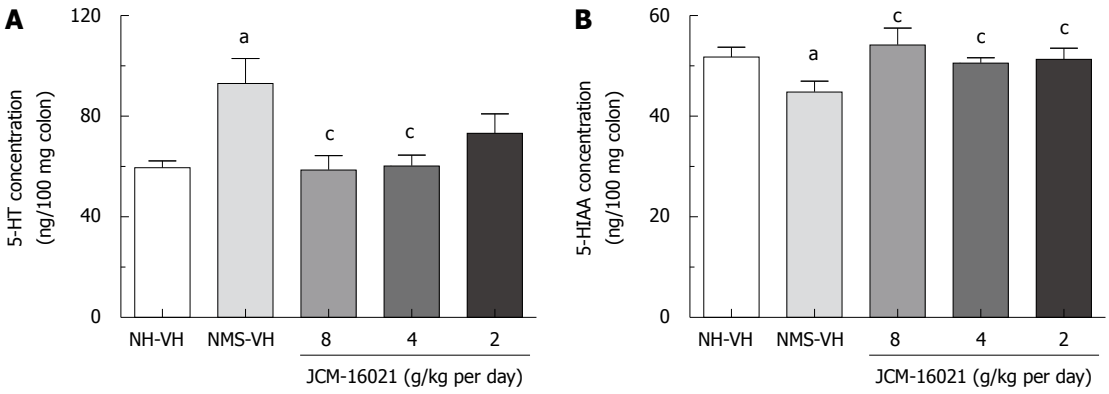

Figure 3 JCM-16021 effects on the concentrations of 5-HT and 5-HIAA in the colons of rats. A: JCM-16021 significantly decreased 5-HT concentration in neonatal maternal separation (NMS) rats; B: JCM-16021 significantly increased 5-HIAA concentration in NMS rats. Data are presented as mean ± SE (ng/100 mg colon tissue, n = 8-10). Significant difference is indicated by $^{a}P < 0.05$ when compared with the NH control group and by $^{c}P < 0.05$ when compared with the NMS control group.

1.53 ± 0.24, 2.1 ± 0.21 for the pressures 20, 40, 60, 80 mmHg), compared to that of the NMS vehicle group. The mean ΔAUC values were: 0.57 ± 0.12, 1.33 ± 0.18, 2.57 ± 0.37, 3.08 ± 0.37 for the pressures 20, 40, 60, 80 mmHg, $P < 0.05$. Also Holopon significantly reduced the EMG activity compared to the NMS vehicle group ($P < 0.05$).

JCM-16021 decreases the 5-HT concentration in the colon of rats

As shown in Figure 3A, 5-HT concentration in NMS vehicle groups (n = 9, 93.11 ± 9.85 ng/100 mg) was significantly higher than that in NH vehicle groups (n = 8, 59.53 ± 7.57 ng/100 mg, $P < 0.01$). JCM-16021 treatment at the high and middle dosage, but not the low dosage, significantly decreased the 5-HT concentration in the colon of rats ($P < 0.01$), when compared to the NMS vehicle group. After treatment with JCM-16021, the 5-HT concentrations in high, middle and low dosage groups (n = 9, 10, 10) were 60.25 ± 5.98 ng/100 mg, 60.32 ± 4.22 ng/100 mg, 73.31 ± 7.65 ng/100 mg, respectively. Further, although there was no significant difference in 5-HT concentration between the low dosage group and NMS vehicle group, the value was still lower than that of the NMS-VH group (93.11 ± 9.85 ng/100 mg). Clearly, there is a tendency that JCM-16021 treatment could reduce the 5-HT concentration.

JCM-16021 significantly increases the 5-HIAA concentration in the colon of rats

NMS treatment significantly decreased 5-HIAA concentration in the colon of rats. 5-HIAA concentration in NMS vehicle groups (n = 9, 44.86 ± 2.13 ng/100 mg) was significantly higher than that in NH vehicle groups (n = 8, 51.75 ± 1.98 ng/100 mg, $P < 0.05$). JCM-16021 treatment significantly increased the 5-HIAA concentration in the colon of rats when compared with that of the NMS control group ($P < 0.05$). After treatment with JCM-16021, the 5-HIAA concentrations were 54.24 ± 9.81 ng/100 mg in the high dosage group, 50.61 ± 1.26 ng/100 mg in the middle dosage group, and 51.37 ± 2.13 ng/100 mg in the low dosage group (Figure 3B).

JCM-16021 does not change EC cell numbers in the colon of rats

As shown in Figure 4, EC cell number in NMS vehicle groups (n = 9, 12.97 ± 1.17) was significantly higher than that in NH vehicle groups (n = 8, 9.70 ± 0.92, $P < 0.05$). JCM-16021 treatment did not significantly change the EC cell number in the three dose groups.

JCM-16021 decreased SERT expression in the colon of rats

As shown in Figure 5, NMS rats had higher SERT expression (0.61 ± 0.03, n = 10) than NH rats (0.56 ± 0.05, n = 8, $P < 0.05$). JCM-16021 treatment at different dosages (n = 10 in each group) significantly decreased SERT expression in the colon. The mean gray indexes among JCM-16021 groups were 0.59 ± 0.03 in the high dose group, 0.54 ± 0.02 in the middle dose group and 0.53 ± 0.03 in the low dose group ($P < 0.01$-0.001).

DISCUSSION

This study demonstrated that JCM-16021 can dose-dependently attenuate the visceromotor response to CRD in NMS rats. Moreover, it decreases 5-HT concentration and increases 5-HIAA concentration in the colon of rats. These findings indicate that JCM-16021 has an analgesic effect on visceral hyperalgesia, and this effect may be mediated through the serotonin signaling pathway in the colon of rats.

Chronic visceral hyperalgesia is an important and characteristic feature of IBS and other functional bowel disorders[21]. In order to investigate the mechanism of visceral hyperalgesia, animal models have been developed, such as the early life colon irritation model[17], neonatal maternal separation model[12], and adult repeated stress model in rodents[22]. The current study showed that NMS induced a lower pain threshold pressure than that seen in NH rats, and increased EMG activity in response to CRD, thus confirming that NMS induces visceral hyperalgesia in adulthood[12]. Further, our results also showed that even in 20 mmHg CRD stimulation, NMS rats still have

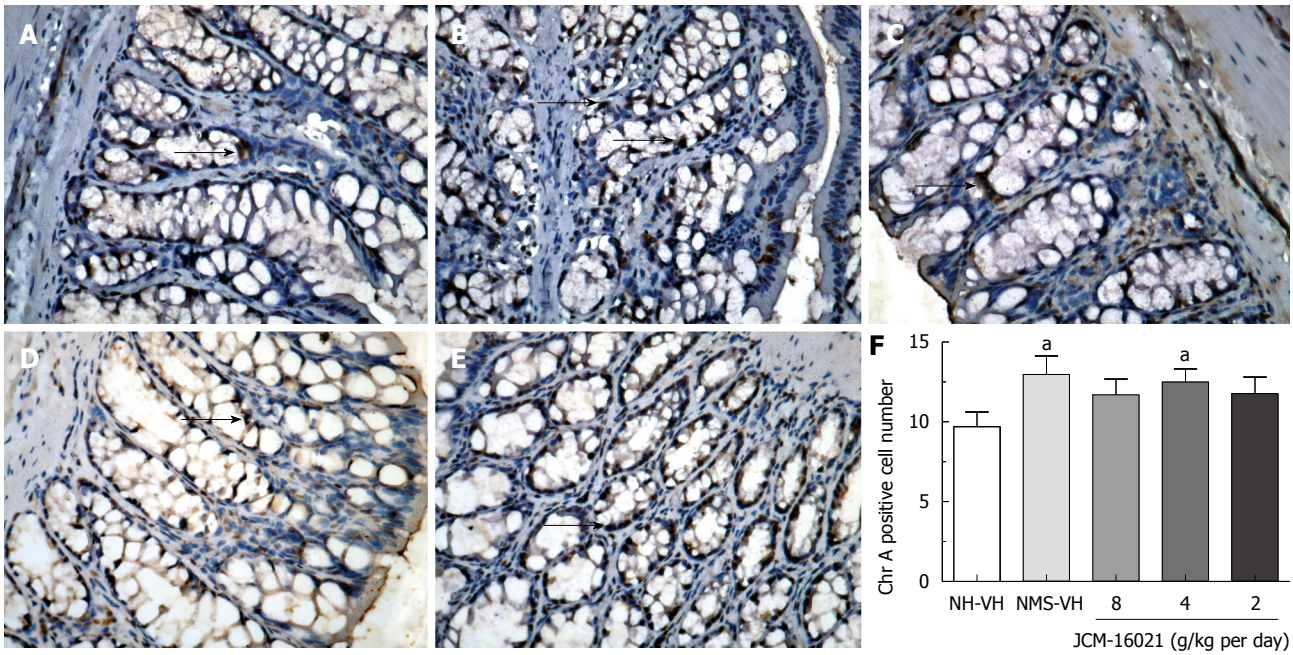

Figure 4 JCM-16021 effect on enterochromaffin cell number (Chr A positive cell number) in the colons of rats. Chr A staining cells (arrows) are present in colon tissues in the normal control group (A), the NMS control group (B), the NMS with high dosage (C), the NMS with middle dosage (D), and the NMS with low dosage of JCM-16021 (E). NMS significantly increased the number of Chr A positive cells in the colons of rats, and JCM-16021 did not change the number of Chr A positive cells (F). Data are presented as mean ± SE (n = 8-10). Significant difference is indicated by $^{a}P < 0.05$ compared with the normal control group.

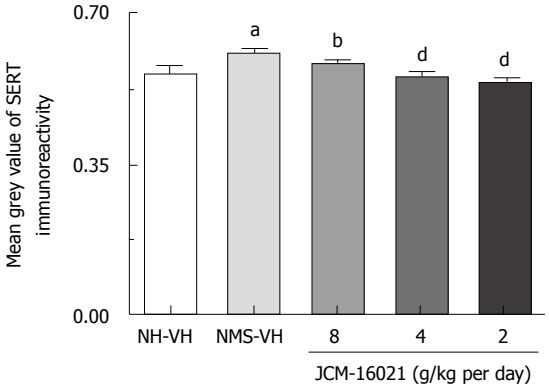

Figure 5 SERT expression in rat colon by immunohistochemistry assay. SERT expression in the colonic tissues of NMS rats was significantly increased over that of NH rats. JCM-16021 treatment decreased SERT expression. Results are expressed as mean ± SE, n = 8-10 in each group. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.001$ *vs* NH.

significant EMG changes compared to NH rats, thus NMS induces not only visceral hyperalgesia, but also allodynia in adulthood. Our results also showed that JCM-16021 increased the pain threshold pressure with a dose-related effect, and dose-dependently reduced the EMG activity to CRD in NMS rats. Therefore, these results indicate that JCM-16021 has an analgesic effect which can attenuate allodynia and visceral pain in NMS rats. Interestingly, the results from the AWR test showed that there is no dose-related response, but the results from the EMG test did show a dose-dependent response. The difference may originate from the objectivity of the two pain indexes; the EMG data, as a quantitative value, is more reliable than that of pain threshold pressure.

As for the mechanism of visceral hyperalgesia, it is believed that the up-regulation of visceral pain perception results, at least in part, from profound and long-lasting changes in the development of the central nervous system, including systems that regulate stress responsiveness[23,24]. With regard to the 5-HT effect in visceral hyperalgesia, previous data is not consistent. Our previous study reported that the amount of colonic 5-HT in rats with visceral hyperalgesia induced by mechanical colorectal irritation significantly increased with postnatal day[19]. Another study showed that serotonin is actively involved in pathophysiological processes of visceral hyperalgesia because serotonin concentration is significantly decreased in the spinal cord and but not in the colon; and 5-HT significantly increased in the colons of rats after CRD[13]. It is well known that 5-HT in the gastrointestinal tract is generally believed to be one of the most important mediators and regulators of bowel sensation and motility[9,25]. The current study found that 5-HT concentrations in the colons of the NMS vehicle group were significantly higher than that in the NH vehicle group supporting the concept that 5-HT is an important mediator involved in NMS-induced visceral hyperalgesia.

Our data also showed that JCM-16021 not only significantly reduces the 5-HT concentration but also significantly increases the 5-HIAA concentration. Serotonin, as an important gastrointestinal signaling molecule, is synthesized from the amino acid tryptophan *via* a short metabolic pathway consisting of two enzymes: tryptophan hydroxylase and amino acid decarbo-carboxylase. Recent studies have demonstrated that distinct changes in intestinal EC cell number and 5-HT content have significant relationships with symptoms in IBS

patients[26,27]. Therefore, the serotonin signaling pathway has been proposed as a therapeutic target to improve the symptoms of IBS[11]. Our current study found that NMS not only increases 5-HT concentration but also decreases the levels of its metabolite 5-HIAA in the colon of rats. After JCM-16021 treatment, the 5-HT concentration was decreased while 5-HIAA concentration increased. 5-HIAA is a major product of 5-HT breakdown, which is excreted in the urine. The increase in 5-HIAA indicates that more 5-HT was broken down after JCM-16021 treatment. Therefore, the data indicate that JCM-16021 affected 5-HT action and its metabolism in the colon.

It is well-known that a large proportion of 5-HT in the body is found in the gastrointestinal tract, and is primarily contained within EC cells[26]. This study found that EC cell number in NMS control rats is higher than that in NH control rats. It is possible that NMS induces hyperplasia of EC cells, thus NMS rats have higher concentrations of 5-HT in their colons compared to NH rats. Our results also showed that JCM-16021 cannot significantly change EC cell number in the colon of rats. This suggests that the hyperplasia of EC cells may be a permanent change similar to the elevated activation of the cingulate cortex and sensitization of the ascending pathway involving the spinal cord and the thalamo-cortico-amygdala pathway[24]. Therefore, EC cell number was not changed with treatment.

SERT is necessary for termination of serotonergic action in the colon. After the release by EC cells, serotonin is taken up again from the mucosa into the nerve fibers[11]. Altered SERT expression and function could contribute to the abdominal hypersensitivity and abnormal colonic motility associated with IBS and IBD[27]. A previous report showed 5-HT and mucosal SERT are both decreased in ulcerative colitis, diarrhea-predominant IBS and constipation-predominant IBS[28]. Our study showed that NMS rats with vehicle have significantly increased SERT expression in the colon with increased concentration of 5-HT, compared with NH rats with vehicle. The increase in SERT expression could be due to an adaptive response to improve disturbed gut function and ameliorate symptoms; thus increased 5-HT could be terminated quickly under different stimulations. After treatment with JCM-16021, SERT expressions were decreased along with 5-HT content. Such decreases could result in the inhibition of SERT function. It is reported that inhibition of SERT function leads to decreased transiency in the gut and lower sensitivity[27,29,30]. Our data showed that JCM-16021 reduced SERT expression in the colon of rats, indicating that JCM-16021 inhibits SERT function so as to induce lower sensitivity.

In summary, the present findings provide evidence for the analgesic effect of JCM-16021 on visceral hyperalgesia in rats. This effect may be mediated through changes in the synthesis and metabolism of 5-HT in the colons of rats.

COMMENTS

Background

Increasing numbers of irritable bowel syndrome (IBS) sufferers are seeking help from complementary and alternative medicines because conventional therapies have not been proven to be more effective than placebo in providing overall relief of symptoms in randomized, controlled clinical trials. The 5-HT signaling pathway represents a promising target for IBS treatment. JCM-16021 improved the symptoms of IBS patients but the mechanism is unknown.

Research frontiers

A neonatal maternal separation (NMS)-induced visceral hyperalgesia rat model is often used to study the mechanism of IBS and to evaluate the pharmacological effects of potential IBS therapies. This study aimed to investigate the analgesic effect of JCM-16021 on NMS-induced visceral hyperalgesia in rats, and its potential underlying mechanism.

Innovations and breakthroughs

Recent studies have highlighted the role of serotonin (5-HT) in the generation of IBS symptoms and in other gastrointestinal functional disorders. Furthermore, novel serotonergic agents, such as the 5-HT_3 antagonist alosetron and the 5-HT_4 agonist tegaserod, have significant impacts on IBS symptoms through their visceral analgesic properties and diverse effects on motor functions in the lower gastrointestinal tract. This is the first study to report that the analgesic effect of JCM-16021, a Chinese herbal formula, on visceral hyperalgesia in rats may be mediated through changes in the synthesis and metabolism of 5-HT in the colons of rats.

Applications

This study provides direct evidence for the analgesic effect of JCM-16021 on visceral hyperalgesia in rats. JCM-16021 might become a reliable therapy to relieve the symptoms of IBS patients.

Peer review

This is an interesting paper showing the analgesic effect of a Chinese Medicine herb, JCM-16021 on maternal separation stress induced visceral hypersensitivity in male rats. Together with their previous data in IBS patients, this additional preclinical work suggests that JCM-16021 may be of therapeutic interest for IBS.

REFERENCES

1 **Somers SC**, Lembo A. Irritable bowel syndrome: evaluation and treatment. *Gastroenterol Clin North Am* 2003; **32**: 507-529
2 **Tillisch K**, Chang L. Diagnosis and treatment of irritable bowel syndrome: state of the art. *Curr Gastroenterol Rep* 2005; **7**: 249-256
3 **Camilleri M**. Management of the irritable bowel syndrome. *Gastroenterology* 2001; **120**: 652-668
4 **Farthing MJ**. Treatment of irritable bowel syndrome. *BMJ* 2005; **330**: 429-430
5 **Sung JJY**, Bian ZX, Wu JCY, Ziea ET, Suen BY, Leung WK. Herbal medicine versus hyosine in the treatment of irritable bowel syndrome: A double-blinded randomized study. *Gut* 2007; **56** (Suppl III): A338
6 **Gershon MD**. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment Pharmacol Ther* 1999; **13** Suppl 2: 15-30
7 **Spiller RC**, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; **47**: 804-811
8 **Miwa J**, Echizen H, Matsueda K, Umeda N. Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. *Digestion* 2001; **63**: 188-194
9 **Crowell MD**, Shetzline MA, Moses PL, Mawe GM, Talley NJ. Enterochromaffin cells and 5-HT signaling in the pathophysiology of disorders of gastrointestinal function. *Curr Opin Investig Drugs* 2004; **5**: 55-60
10 **Mawe GM**, Coates MD, Moses PL. Review article: intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; **23**: 1067-1076
11 **Camilleri M**. Serotonergic modulation of visceral sensation: lower gut. *Gut* 2002; **51** Suppl 1: i81-i86

12 **Coutinho SV**, Plotsky PM, Sablad M, Miller JC, Zhou H, Bayati AI, McRoberts JA, Mayer EA. Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G307-G316

13 **Ren TH**, Wu J, Yew D, Ziea E, Lao L, Leung WK, Berman B, Hu PJ, Sung JJ. Effects of neonatal maternal separation on neurochemical and sensory response to colonic distension in a rat model of irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G849-G856

14 **Zhang XJ**, Li Z, Leung WM, Liu L, Xu HX, Bian ZX. The analgesic effect of paeoniflorin on neonatal maternal separation-induced visceral hyperalgesia in rats. *J Pain* 2008; **9**: 497-505

15 **Bian ZX**, Zhang M, Xu HX, Sung JJY. Chinese medicine formula JCM-16021 attenuates visceral pain of neonatal maternal separation rats via serotonergic pathway. *Gut* 2008; **57** (SupplII): A153

16 Pharmacopoeia Commission of the Ministry of Public Health of PRC. Chinese Pharmacopoeia: Chemical Industry Press, 2005

17 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285

18 **Li Z**, Zhang XJ, Xu HX, Sung JJ, Bian ZX. Intracolonical administration of protease-activated receptor-2 agonists produced visceral hyperalgesia by up-regulating serotonin in the colon of rats. *Eur J Pharmacol* 2009; **606**: 199-204

19 **Tian XY**, Bian ZX, Hu XG, Zhang XJ, Liu L, Zhang H. Electro-acupuncture attenuates stress-induced defecation in rats with chronic visceral hypersensitivity via serotonergic pathway. *Brain Res* 2006; **1088**: 101-108

20 **Sjölund K**, Sandén G, Håkanson R, Sundler F. Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterology* 1983; **85**: 1120-1130

21 **Azpiroz F**, Bouin M, Camilleri M, Mayer EA, Poitras P, Serra J, Spiller RC. Mechanisms of hypersensitivity in IBS and functional disorders. *Neurogastroenterol Motil* 2007; **19**: 62-88

22 **Bradesi S**, Schwetz I, Ennes HS, Lamy CM, Ohning G, Fanselow M, Pothoulakis C, McRoberts JA, Mayer EA. Repeated exposure to water avoidance stress in rats: a new model for sustained visceral hyperalgesia. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G42-G53

23 **Ladd CO**, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res* 2000; **122**: 81-103

24 **Chung EK**, Zhang X, Li Z, Zhang H, Xu H, Bian Z. Neonatal maternal separation enhances central sensitivity to noxious colorectal distention in rat. *Brain Res* 2007; **1153**: 68-77

25 **Gershon MD**, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; **132**: 397-414

26 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659

27 **Coates MD**, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 2004; **126**: 1657-1664

28 **Coates MD**, Johnson AC, Greenwood-Van Meerveld B, Mawe GM. Effects of serotonin transporter inhibition on gastrointestinal motility and colonic sensitivity in the mouse. *Neurogastroenterol Motil* 2006; **18**: 464-471

29 **Wang SH**, Dong L, Luo JY, Gong J, Li L, Lu XL, Han SP. Decreased expression of serotonin in the jejunum and increased numbers of mast cells in the terminal ileum in patients with irritable bowel syndrome. *World J Gastroenterol* 2007; **13**: 6041-6047

30 **O'Mahony S**, Chua AS, Quigley EM, Clarke G, Shanahan F, Keeling PW, Dinan TG. Evidence of an enhanced central 5HT response in irritable bowel syndrome and in the rat maternal separation model. *Neurogastroenterol Motil* 2008; **20**: 680-688

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ORIGINAL ARTICLE

Celecoxib inhibits *Helicobacter pylori* colonization-related factors

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Abstract

AIM: To investigate the effect of celecoxib, a selective COX-2 inhibitor, on *Helicobacter pylori* (*H. pylori*) colonization-related factors and its mechanism.

METHODS: After co-incubation with celecoxib, morphology of *H. pylori* strain 26695 was observed under a transmission electron microscope. Flagella motility was assessed by stab agar motility test. Adherence of *H. pylori* to AGS cells was determined by enzyme linked immunosorbent assay. Levels of mRNA expression in flagellar genes (*flaA*, *flaB*), urease genes (*ureA*, *ureB*) and adhesin genes (*babA*, *sabA*, *alpA*, *alpB*, *hpaA*, *hopZ*) were measured by real-time polymerase chain reaction.

RESULTS: Separation and non-integrity of bacterial cell wall, rarefaction and asymmetry of cytoplasm, and even lysis of *H. pylori* were observed in the presence of celecoxib. When *H. pylori* strains were incubated in the presence of celecoxib, their flagellar motility and adherence to AGS cells were inhibited. The expression of *ureA*, *ureB*, *babA*, *sabA*, *alpA*, *alpB*, *hpaA*, *hopZ* was up-regulated while the expression of *flaA*, *flaB* was down-regulated in the presence of celecoxib.

CONCLUSION: Celecoxib inhibits flagellar motility and adherence of *H. pylori* to AGS cells, and destructs their normal structure *in vitro*.

Key words: *Helicobacter pylori*; Celecoxib; Colonization; Ultrastructure

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INTRODUCTION

About 30% of the population in developed countries and up to 90% of the population in developing countries are chronically infected with *Helicobacter pylori* (*H. pylori*)[1,2]. Non-steroidal anti-inflammatory drugs (NSAID) are the most commonly used drugs, on a world-wide scale, which are used by at least 30 million people[3]. NSAID and *H. pylori* infection are two major factors for gastric injuries. Subjects taking NSAID are often infected with *H. pylori*. However, whether these two factors exert synergistic or antagonistic actions on gastric mucosa is still controversial[4,5]. Data from a meta-analysis review have shown that the risk of peptic

ulcer is approximately 60-fold higher in *H. pylori* positive subjects taking NSAID than in *H. pylori* negative subjects not taking NSAID[6]. Since both *H. pylori* and NSAID are responsible for mucosal damage, they can increase the risk of developing uncomplicated and complicated peptic ulcer. However, data from several studies do not always confirm such an assumption[5]. A large clinical trial demonstrated that eradication of *H. pylori* delays the healing of gastric ulcers in NSAID users after treatment with omeprazole[7], implying that *H. pylori* may protect individuals against NSAID-induced ulcer, possibly by stimulating mucosal prostaglandins and other protective factors.

Recent studies *in vitro* also suggested that aspirin and celecoxib, a selective COX-2 inhibitor, inhibit the growth of *H. pylori* and decrease the activity of urease and vacuolating cytotoxin in a dose-dependent manner[8-12], indicating that NSAID may antagonize injuries of gastric mucosa caused by *H. pylori* infection. Colonization of *H. pylori* in gastric mucosa is a prerequisite for pathogenicity and needs to have at least 4 basic characteristics: integrate helical shape, motility of flagella, specific binding to adhesin and its receptors, and urease activity that provides an appropriate microenvironment[13]. We hypothesize that NSAID and celecoxib may influence the pathogenicity of *H. pylori* in gastric mucosa injury by altering the colonization. Therefore, the aim of the present study was to investigate the effect of celecoxib on *H. pylori* colonization- related factors and its mechanism *in vitro*.

MATERIALS AND METHODS

Bacterial culture

H. pylori 26695 strain was cultured at 37℃ in a microaerobic atmosphere containing 5% O_2, 85% N_2, and 10% CO_2 for 48 h on Colombia agar medium supplemented with 8% (v/v) defibrinated goat blood containing 0.02 mmol/L celecoxib or vehicle control (1/1000 DMSO).

Stab agar motility test

H. pylori strains were grown on Colombia agar medium for 48 h and then harvested into a brain heart infusion (37 g/L). After the concentration of bacteria was adjusted to 10^8 CFU/mL, 10 μL was inoculated into a 0.3% agar Brucella broth medium containing 8% defibrinated goat blood using a sterile picker. Five days after incubation under microaerobic condition at 37℃, the halo diameter was measured.

Ultrastructural analysis

Forty-eight hours after exposure to 0.02 mmol/L celecoxib, *H. pylori* cells were collected and rinsed three times with 0.01 mol/L PBS, fixed in phosphate-buffer solution containing 2.5% glutaraldehyde at 4℃ for 2 h. After centrifugation, pellets were embedded in 2% agar, fixed in 1% osmium tetraoxide (OsO4) at 4℃, and rinsed three times with 0.01 mol/L PBS. After dehydrated in a series of graded acetone at 4℃, specimens were embedded in Epon 812 (Emicron). The sample was cut into 90 nm-thick sections which were stained with uranyl acetate and lead citrate, and observed under a JEM1230 transmission electron microscope.

Adhesion of H. pylori to AGS cells

AGS cells (10^4/well) were seeded in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum in a 96-well plate containing 5% CO_2 at 37℃ for 20 h. *H. pylori* (10^7 CFU/well) were pretreated with celecoxib at the concentrations of 0.01, 0.02 and 0.03 mmol/L. The plate was agitated at 60 r/min for 30 min at 37℃. Cultures were fixed with 1% paraformaldehyde. After washed with PBS, *H. pylori* cells were blocked with 5% bovine serum albumin (BSA) for 30 min, and incubated for 24 h with mouse monoclonal anti-*H. pylori* antibody (Santa cruz). After washed three times with PBS, goat anti-mouse IgG-HRP (Santa cruz) was added for 1 h. Binding was visualized by incubating with 100 μL TMB substrate for 30 min. Absorbance was read at 450 nm after 2 mol/L of sulphuric acid was added to terminate the reaction. Adherence of *H. pylori* to AGS cells was calculated according to the formula: [(*A* AGS cells with *H. pylori* -*A* AGS cells without *H. pylori*)/(*A* positive control -*A* negative control)] × 100. For positive control, only bacteria were added and allowed to adhere to the well. Wells containing neither AGS cells nor *H. pylori* were prepared as a negative control.

H. pylori RNA isolation and reverse transcription

Forty-eight hours after pretreatment with 0.02 mmol/L celecoxib, strains of *H. pylori* were rinsed with Tris-HCl and cleared with 1 mL of TRIzol. After 200 μL of chloroform was added, the sample was vigorously shaken and centrifuged. RNA in aqueous phase was precipitated with 0.5 mL of isopropanol. The pellet was washed with ethanol and dried. The RNA was resuspended in sterile water and quantified by UV absorbance. Total RNA (4 μg) treated with RO1 RNase-free DNase (Promega) to remove DNA was used for reverse transcription reaction. In brief, 1.5 μL of random primers was added, the samples were heated to 70℃ for 5 min. Then, 10 μL of 5 × RT buffer, 2.5 μL of dNTPs, and 2 μL of M-MLV were added. cDNA synthesis reaction was performed for 60 min at 37℃ and then at 70℃ for 10 min. Aliquots of cDNA were stored at -70℃.

Real-time polymerase chain reaction (RT-PCR)

mRNA levels of flagellar genes (*flaA, flaB*), urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) were measured by real-time PCR using the ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems, Foster City, Calif). Specific primers and house-keeping gene 16SrRNA were designed with the aid of Primer Express 3.0 software (Applied Biosystem Perkin-Elmer) (Table 1). Real-time PCR was performed in a 25 μL reaction volume containing 2.5 μL

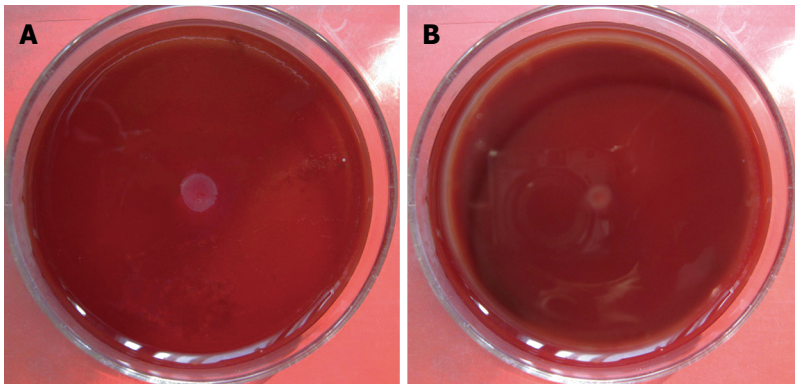

Figure 1 Stab agar motility tests showing the *H. pylori* motility. A: DMSO control (1/1000); B: Celecoxib (0.02 mmol/L).

Table 1 Primers and probes used in real-time quantitative PCR

Gene	Primer (5′-3′)
flaA-F	ATTGGCGTGTTAGCAGAAGTGA
flaA-R	TGACTGGACCGCCACATC
flaB-F	ACATCATTGTGAGCGGTGTGA
flaB-R	GCCCCTAACCGCTCTCAAAT
ureA-F	GCTGGTGCGATTGGCTTTA
ureA-R	GGATAGCGACTTGCACATCGT
ureB-F	TCCTGATGGGACAAAACTCGTA
ureB-R	ACGGCTTTTTTGCCTTCGT
babA-F	TGCTCAGGGCAAGGGAATAA
babA-R	ATCGTGGTGGTTACGCTTTTG
sabA-F	GGTGTGCTGCAACAGACTCAA
sabA-R	CATAAGCTGTTGCGCCAAATT
alpA-F	GCACGATCGGTAGCCAGACT
alpA-R	ACACATTCCCCGCATTCAAG
alpB-F	ACGCTAAGAAACAGCCCTCAAC
alpB-R	TCATGCGTAACCCCACATCA
hpaA-F	GAGCGTGGTGGCTTTGTTAGT
hpaA-R	TCGCTAGCTGGATGGTAATTCA
hopZ -F	GCGCCGTTACTAGCATGATCA
hopZ-R	GAAATCTTTCGGCGCGTTT
16SrRNA-F	CCGCCTACGCGCTCTTTAC
16SrRNA-R	CTAACGAATAAGCACCGGCTAAC

PCR: Polymerase chain reaction.

of cDNA, 12.5 μL of SYBR green real time PCR master mix (Toyobo), 1 μL of sense and antisense primers (5 pmol/L), and 9 μL of DEPC water. PCR was carried out at 95℃ for 10 min, followed by 40 cycles at 95℃ for 15 s, at 61℃ for 1 min. A further melting curve step analyzing the purity of PCR products was performed at 95℃ for 15 s, at 61℃ for 30 s, and at 96℃ for 15 s. A standard curve was plotted using 10-fold serial dilution of each cDNA. mRNA level was expressed as the ratio of detected mRNA to 16S rRNA mRNA [detected mRNA (U/mL)/16S rRNA mRNA (U/mL) × 100000]. PCR was carried out in quintuple using samples prepared at the same time.

Statistical analysis

All experiments were performed at least in triplicate. Data were presented as mean ± SD. Statistical analysis between sample and control was conducted by Student's *t*-test using SPSS 11.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of celecoxib on H. pylori motility

The halo diameter for the growth of *H. pylori* in the presence of celecoxib was 5.92 ± 1.20 mm after 5-d incubation, which was significantly smaller than that (8.21 ± 1.63 mm) of DMSO control ($P < 0.05$, Figure 1), indicating that the motility of *H. pylori* is decreased in the presence of celecoxib.

Ultrastructural effects of celecoxib on H. pylori

Transmission electron microscopy demonstrated that both cytoplasmic and outer membranes of *H. pylori* were intact, the cytoplasm was well-distributed and the electron density was moderate in DMSO control. When incubated with 0.02 mmol/L of celecoxib, V- and U-shaped *H. pylori* were observed. The cell wall of *H. pylori* was attenuated with abscission, or even perforation but no integrity. Separation of the outer membrane from the cytoplasmic membrane (cell wall breakaway) and even cell lysis were observed. Rarefaction and asymmetry were observed in cytoplasm of *H. pylori* and the components of *H. pylori* cells disappeared and distributed abnormally (Figure 2).

Effects of celecoxib on H. pylori adherence to AGS cells

Compared to the DMSO control (1/1000), celecoxib significantly inhibited the adherence of *H. pylori* to AGS cells in a dose-dependent manner ($P < 0.05$) (Figure 3).

Effects of celecoxib on H. pylori flagellin, urease and adhesin gene expression

The mRNA expression levels in flagellar genes (*flaA, flaB*), urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) were measured by real-time PCR. After treatment with 0.02 mmol/L celecoxib, the mRNA expression levels in *flaA* and *flaB* were lower than those in DMSO control ($P < 0.05$). However, the mRNA expression levels were higher in urease genes (*ureA, ureB*) and adhesin genes (*babA, sabA, alpA, alpB, hpaA, hopZ*) than in DMSO control ($P < 0.05$). The

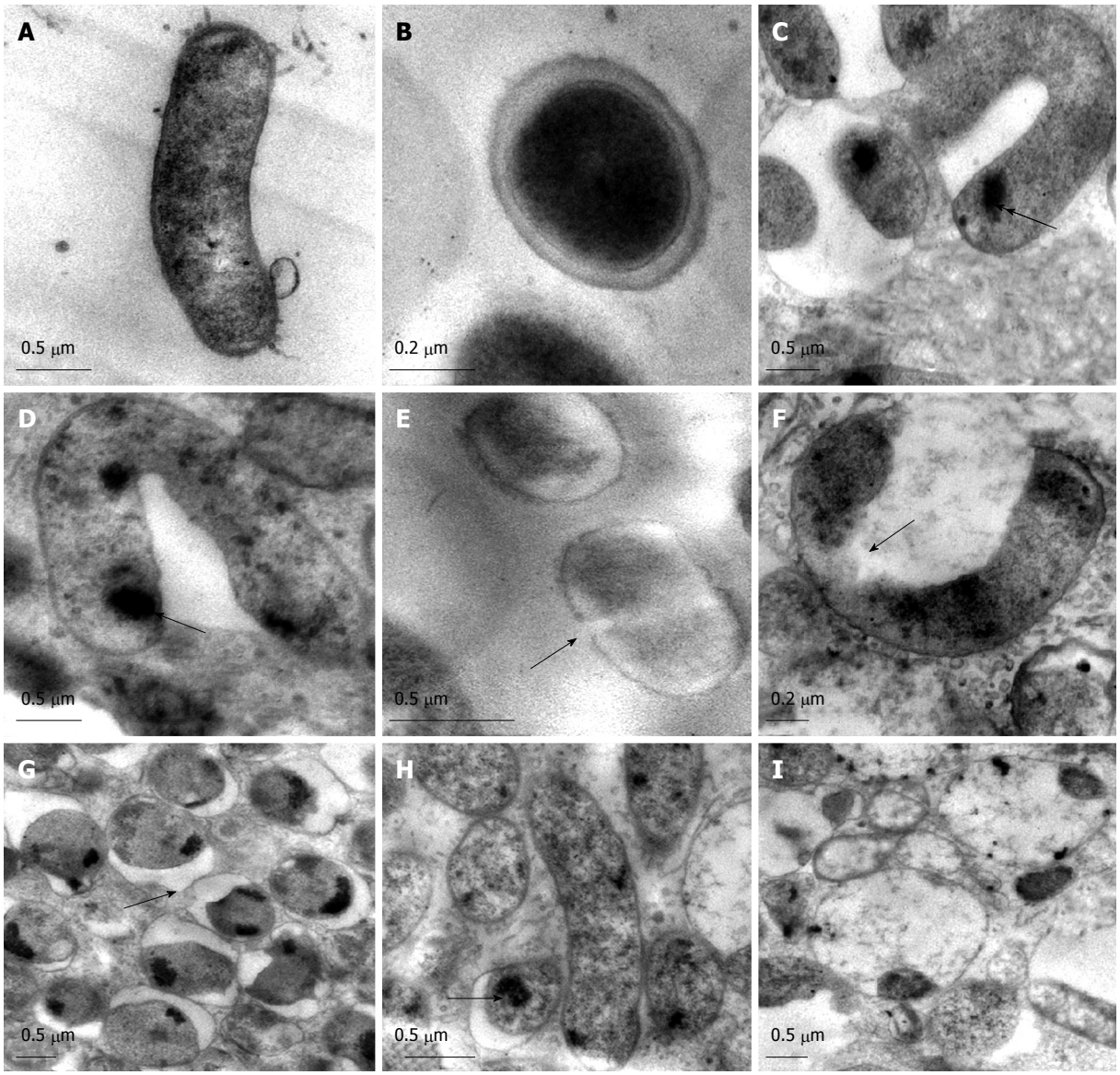

Figure 2 Transmission electron microscopy (TEM). TEM showing rod-shaped *H. pylori* (A), well-distributed cytoplasm and moderate electron density (B), U-shaped (C, arrow) and V-shaped (D, arrow) *H. pylori*, non-integrity (E, arrow) and abscission (F, arrow) of *H. pylori* cell wall, outer membrane separated from the cytoplasmic membrane (G, arrow), decreased electron density in cytoplasm (H, arrow), and cell lysis (I) after treatment with celecoxib.

Table 2 mRNA levels in *H. pylori* flagellin, urease and adhesin genes meausred by real-time quantitative PCR (mean ± SD)

Gene	Celecoxib (0.02 mmol/L)	DMSO control (1/1000)
flaA	23.08 ± 1.70[a]	51.08 ± 6.91
flaB	16.01 ± 0.04[a]	34.80 ± 7.13
ureA	19.61 ± 1.78[a]	7.65 ± 0.38
ureB	29.59 ± 5.31[a]	13.80 ± 1.63
babA	16.78 ± 0.91[a]	12.38 ± 0.38
sabA	49.00 ± 4.10[a]	22.55 ± 2.26
alpA	15.55 ± 0.78[a]	7.34 ± 0.20
alpB	14.07 ± 0.23[a]	8.95 ± 0.38
hpaA	123.98 ± 11.82[a]	57.15 ± 2.56
hopZ	100.25 ± 4.37[a]	45.54 ± 11.64

[a]$P < 0.05$ *vs* DMSO control.

mRNA expression levels in the above genes increased or decreased 1.5-2.5 folds in the presence of celecoxib (Table 2).

DISCUSSION

NSAID and *H. pylori* infection are the two main etiological factors for peptic ulcers. However, their role in the pathogenesis of gastric mucosal damage is still controversial[6]. It has been demonstrated that eradication of *H. pylori* can decrease the recurrence rate of peptic ulcer and its complications in chronic NSAID users[14], while their co-existence aggravating gastric mucosal damage has not been confirmed[4,5]. It was reported that the prostaglandin synthesis level in mucosa is significantly higher in *H. pylori* positive patients than in *H. pylori* negative patients[15,16], demonstrating that colonization of *H. pylori* reduces the inhibitory effect of NSAID

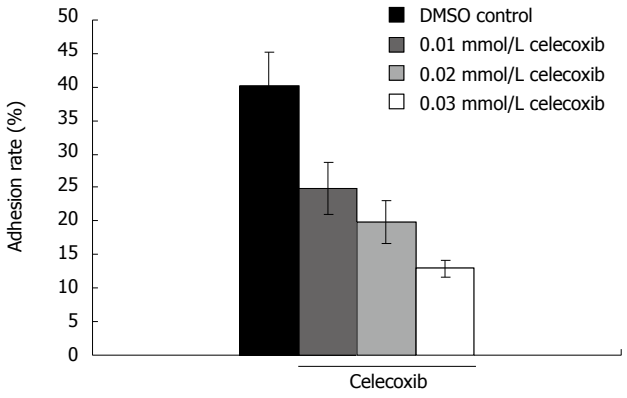

Figure 3 Adhesion of *H. pylori* to AGS cells after treatment with celecoxib at different concentrations.

on prostaglandin synthesis. *In vitro* studies further revealed that NSAID can inhibit the growth of *H. pylori*, and decrease the activity of urease and vacuolating cytotoxin[8-12], suggesting that NSAID may alter the pathogenicity of *H. pylori* in gastric mucosa injury when the two factors are co-existed in gastric mucosa.

H. pylori infection may persist for many years in the host and *H. pylori* colonization-related factors include its spiral shape, flagellar motility, urease and adhesin. Urease neutralizes the pH around *H. pylori* during exposure to the acidic lumen of stomach. The flagella and the spiral shape of *H. pylori* enable *H. pylori* strains to move and penetrate the mucin layer where they come into contact with gastric epithelial cells. Adherence of *H. pylori* to AGS cells is a crucial initial step in colonization[17,18], as non-adhering *H. pylori* strains would be washed away during peristalsis-mediated flushing of stomach. NSAID and celecoxib do not increase the colonization of *H. pylori* in gastric mucosa[19-23]. On the contrary, the incidence of *H. pylori* infection in patients taking NSAID is low[24,25], which may be partially explained by the fact that celecoxib can destruct the normal structure of *H. pylori,* and inhibit the motility of flagella, and the adherence of *H. pylori* to AGS cells and the activity of urease[12], which is consistent with the findings in our study.

Adhesin, exposed on the surface of *H. pylori* cells, facilitates interaction with host cellular receptors. The particularly more important adhesins of *H. pylori* are BabA, SabA, AlpA, AlpB, HpaA, HopZ[26-30]. Their content and expression under different environmental conditions are variable. In our *in vitro* study, celecoxib inhibited the adherence of *H. pylori* to AGS cells, but increased the mRNA expression levels in *babA, sabA, alpA, alpB, hpaA, hopZ*. Whether the increased mRNA expression in such genes is accompanied with an increased competent protein or just a compensatory increase in mRNA expression for the inhibition of *H. pylori* growth and adherence activity remains to be further studied. On the other hand, variable expression of cell receptors in a single host and genetic variability of receptor expression in different hosts make the adherence system very complex. Host receptor expression is up-regulated following *H. pylori* adherence[31]. In this study, the impaired adherence of *H. pylori* to AGS cells in the presence of celecoxib down-regulated the host receptor expression. In this condition, although the expression of *H. pylori* adhesins increases, the adherence of *H. pylori* to AGS cells may decrease.

Urease in *H. pylori* accounts for approximately 10% of the total bacterial protein pool[32]. Urease hydrolyzes urea and releases ammonia, which neutralizes acid, thus enabling survival and initial colonization. It has been shown that urease activity is essential for the initial bacterial colonization[33-35]. Anti-ulcer drug, ecabet, interferes with *H. pylori* colonization by inhibiting urease activity[36]. In the present study, celecoxib inhibited the urease activity in a dose-dependent manner, suggesting that it may further influence *H. pylori* colonization.

Urease is composed of two structural subunits, UreA and UreB. Urease gene clusters include *ure A, B, C, D, E, F, G, H, I,* with *ureA* and *ureB* being the structural genes. *ureC* and *ureD* are located before the structural genes. *ureI, ureE, ureF, urgG* and *ureH* are auxiliary genes. These genes and the structural genes are necessary for urease activity[37]. Urease is a metal enzyme possessing nickel and its activity depends on the two Ni^{2+} inserted into its 6 active sites. The insertion process is accomplished by proteins encoded by auxiliary genes in a urease gene cluster. At present, a variety of identified proteins can regulate the activity of urease by influencing nickel ions. Besides proteins, different ion concentrations also accommodate urease activity[38]. Urease inhibitors can be generally classified into active site-directed (substrate-like) and mechanism-directed inhibitors. Since active site-directed inhibitors bridge the two paramagnetic nickel ions in the active site of urease, the octahedral nickel ions and the amino acid residues in the active site-directed inhibitors are in an orientation similar to those of the urease substrate, the mechanism-directed inhibitors are designed to interfere with the urease's catalysis mechanism leading to enzyme inactivation. In the present study, celecoxib inhibited the urease activity in *H. pylori*, but increased the mRNA expression levels in *ureA* and *ureB*. The mechanism still remains unclear. Further studies are needed to determine whether alterations occur at protein translation or modification level or some other mechanisms are involved.

The motility of *H. pylori* is considered another colonization factor. Less motile strains are less able to colonize or survive in the host than fully motile strains. It has been demonstrated that the degree of the motility of *H. pylori* strains is correlated with the degree of infectivity in gnotobiotic piglets. The most motile strains have a 100% infection rate, while the least motile strains have an infection rate of only 17%[39]. Strains without flagella or flagellar mutant strains cannot colonize the gastric mucosa, thus losing their pathogenicity. The flagella consist mainly of the flagellins, FlaA and FlaB.

Both genes coding for these flagellins are necessary for the full motility of *H. pylori*. Elimination of *flaB* yields normal-looking flagella that retain some functions and propel about 60% of the bacteria[40,41]. Elimination of *flaA* yields truncated flagella that only slightly move the bacteria. Elimination of both flagellins results in aflagellated immobile bacteria[41]. It was reported that NSAID inhibit the movement of *Proteus vulgaris, Proteus mirablis, Providencia rettgeri, Providencia stuartiii and Burkholderia cepacia* in a dose- dependent manner[42], and prevent emergence of *Escherichia coli* flagella by inhibiting flagellin synthesis[43]. In this study, celecoxib inhibited the motility of *H. pylori* and decreased the mRNA expression in *flaA* and *flaB*.

The relation between the degree of *H. pylori* motility, cytokine response levels and the severity of disease has been extensively studied[44,45]. The *H. pylori* motility levels are correlated with IL-8 induction[44]. Kurihara[45] also found that the degree of *H. pylori* motility is low in strains isolated from remnant gastritis, which is distinct from chronic gastritis, peptic ulceration or gastric cancer, indicating that the type and phase of *H. pylori*-related diseases dictate the selective pressure for maintenance of high *H. pylori* motility levels. Further study is needed to demonstrate whether celecoxib prevents the progress of *H. pylori*-related diseases by inhibiting *H. pylori* motility.

Besides the flagella, the shape of *H. pylori* strains makes them possible to penetrate the mucin layer where they come into contact with the gastric epithelial cells. In the present study, transmission electron microscopy showed that celecoxib could impair the formation of *H. pylori*, break the bacterial outer membrane, and destruct its structure. Since the spiral shape of *H. pylori* is one of the important virulence factors, celecoxib-related morphological changes may have an impact on the progress of *H. pylori*-induced diseases.

Gastric carcinoma is the forth most common cancer and the second leading cause of cancer-related deaths worldwide. The high mortality is largely attributed to the huge number of at-risk individuals. Chemoprevention appears to be the most promising approach in reducing the incidence and mortality of *H. pylori*-related gastric cancer. WHO defined *H. pylori* as a risk factor for gastric carcinoma and classified *H. pylori* strains as group I carcinogen in 1994[46]. The prevalence of *H. pylori* infection increases with age[47], and 50% of NSAID users are over 60-year old. NSAID contribute to the chemoprevention of gastric cancer and prevention of lymphatic metastasis by inhibiting angiogenesis and inducing apoptosis of epithelial cells through the COX-dependent and independent pathway. It has been shown that long-term intake of NSAID and aspirin can significantly reduce the incidence of non-cardial gastric cancer in a dose-dependent manner[48]. The results of our study further suggest that celecoxib can reduce *H. pylori* colonization, thus attenuating the pathogenesis in gastric mucosa. Although regular use of aspirin can prevent gastric cancer, it may be disadvantageous for populations with a lower risk of gastric cancer. Those with a high risk of gastric cancer can use celecoxib, a selective COX-2 inhibitor with few gastrointestinal side-effects.

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COMMENTS

Background

Use of non-steroidal anti-inflammatory drugs (NSAID) and *Helicobacter pylori* (*H. pylori*) infection are the two main etiological factors for gastric injuries. Subjects taking NSAID are often co-infected with *H. pylori*, but the interaction between NSAID taking and infection with *H. pylori* remains unclear. Data from clinical and epidemiological studies are still controversial.

Research frontiers

The relation between NSAID and *H. pylori* in the pathogenesis of gastric mucosal damage is still controversial. A number of studies have shown that it is not simply additive, synergistic or antagonistic. There may be complex interactions between them which affect the pathogenicity of each other.

Innovations and breakthroughs

NSAID, as a harmful factor for gastric mucosal barrier, may be expected to increase the colonization of *H. pylori* in gastric mucosa. However, evidence from epidemiological studies indicates a lower prevalence of *H. pylori* infection in patients taking NSAID, which may partially be explained by the fact that celecoxib destructs the normal structure of *H. pylori*, and inhibits the flagellar motility, the adherence of *H. pylori* to AGS cells and the urease activity, as observed in this study.

Applications

Colonization of *H. pylori* is a crucial initial step in the pathogenesis of *H. pylori* in gastric mucosa. The present study suggested that celecoxib could reduce the colonization of *H. pylori*, thus attenuating the pathogenicity in gastric mucosa.

Terminology

SYBR green teal-time polymerase chain reaction (PCR): a quantitative PCR method for determination of the cope number of PCR templates such as DNA or cDNA in a PCR reaction. SYBR green: A dye that binds to the minor groove of double stranded DNA. When SYBR green dye binds to double stranded DNA, the intensity of fluorescent emissions increases. As more double stranded amplicons are produced, SYBR green dye signals increase.

Peer review

The study described the effect of celecoxib on *H. pylori*. The study is well-designed. The experimental data are sufficient to support its conclusion.

REFERENCES

1 **Telford JL**, Covacci A, Rappuoli R, Chiara P. Immunobiology of Helicobacter pylori infection. *Curr Opin Immunol* 1997; **9**: 498-503
2 **Walker MM**, Crabtree JE. Helicobacter pylori infection and the pathogenesis of duodenal ulceration. *Ann N Y Acad Sci* 1998; **859**: 96-111
3 **Antonov KI**, Isacson DG. Prescription and nonprescription analgesic use in Sweden. *Ann Pharmacother* 1998; **32**: 485-494
4 **Loeb DS**, Talley NJ, Ahlquist DA, Carpenter HA, Zinsmeister AR. Long-term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: the role of Helicobacter pylori. *Gastroenterology* 1992; **102**: 1899-1905
5 **Stack WA**, Atherton JC, Hawkey GM, Logan RF, Hawkey CJ. Interactions between Helicobacter pylori and other risk factors for peptic ulcer bleeding. *Aliment Pharmacol Ther* 2002; **16**: 497-506

6 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22
7 **Hawkey CJ**, Tulassay Z, Szczepanski L, van Rensburg CJ, Filipowicz-Sosnowska A, Lanas A, Wason CM, Peacock RA, Gillon KR. Randomised controlled trial of Helicobacter pylori eradication in patients on non-steroidal anti-inflammatory drugs: HELP NSAIDs study. Helicobacter Eradication for Lesion Prevention. *Lancet* 1998; **352**: 1016-1021
8 **Gu Q**, Xia HH, Wang WH, Wang JD, Wong WM, Chan AO, Yuen MF, Lam SK, Cheung HK, Liu XG, Wong BC. Effect of cyclo-oxygenase inhibitors on Helicobacter pylori susceptibility to metronidazole and clarithromycin. *Aliment Pharmacol Ther* 2004; **20**: 675-681
9 **Wang WH**, Wong WM, Dailidiene D, Berg DE, Gu Q, Lai KC, Lam SK, Wong BC. Aspirin inhibits the growth of Helicobacter pylori and enhances its susceptibility to antimicrobial agents. *Gut* 2003; **52**: 490-495
10 **Wang WH**, Hu FL, Wong BCY, Berg DE, Lam SK. Inhibitory effects of aspirin and indometacin on the growth of Helicobacter pylori in vitro. *Chin J Dig Dis* 2002; **3**: 172-177
11 **Shirin H**, Moss SF, Kancherla S, Kancherla K, Holt PR, Weinstein IB, Sordillo EM. Non-steroidal anti-inflammatory drugs have bacteriostatic and bactericidal activity against Helicobacter pylori. *J Gastroenterol Hepatol* 2006; **21**: 1388-1393
12 **Ma HX**, Wang WH, Hu FL, Li J. Effect of aspirin and celecoxib on Helicobacter pylori in vitro. *Shijie Huaren Xiaohua Zazhi* 2006; **14**: 2747-2752
13 **Andersen LP**. Colonization and infection by Helicobacter pylori in humans. *Helicobacter* 2007; **12** Suppl 2: 12-15
14 **Chan FK**, To KF, Wu JC, Yung MY, Leung WK, Kwok T, Hui Y, Chan HL, Chan CS, Hui E, Woo J, Sung JJ. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. *Lancet* 2002; **359**: 9-13
15 **Bhang CS**, Lee HS, Kim SS, Song HJ, Sung YJ, Kim JI, Chung IS, Sun HS, Park DH, Lee YS. Effects of selective cyclooxygenase-2 inhibitor and non-selective NSAIDs on Helicobacter pylori-induced gastritis in Mongolian gerbils. *Helicobacter* 2002; **7**: 14-21
16 **Hudson N**, Balsitis M, Filipowicz F, Hawkey CJ. Effect of Helicobacter pylori colonisation on gastric mucosal eicosanoid synthesis in patients taking non-steroidal anti-inflammatory drugs. *Gut* 1993; **34**: 748-751
17 **Mobley HL**. Helicobacter pylori factors associated with disease development. *Gastroenterology* 1997; **113**: S21-S28
18 **Chow J**, Ma L, Ch C. The role of adhesion molecules in gastric ulcer healing. *World J Gastroenterol* 1998; **4**: 467-468
19 **Caselli M**, Pazzi P, LaCorte R, Aleotti A, Trevisani L, Stab ellini G. Campylobacter-like organisms, nonsteroidal anti-inflammatory drugs and gastric lesions in patients with rheumatoid arthritis. *Digestion* 1989; **44**: 101-104
20 **Shallcross TM**, Rathbone BJ, Wyatt JI, Heatley RV. Helicobacter pylori associated chronic gastritis and peptic ulceration in patients taking non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther* 1990; **4**: 515-522
21 **Graham DY**, Lidsky MD, Cox AM, Evans DJ Jr, Evans DG, Alpert L, Klein PD, Sessoms SL, Michaletz PA, Saeed ZA. Long-term nonsteroidal antiinflammatory drug use and Helicobacter pylori infection. *Gastroenterology* 1991; **100**: 1653-1657
22 **Loeb DS**, Talley NJ, Ahlquist DA, Carpenter HA, Zinsmeister AR. Long-term nonsteroidal anti-inflammatory drug use and gastroduodenal injury: the role of Helicobacter pylori. *Gastroenterology* 1992; **102**: 1899-1905
23 **Santucci L**, Fiorucci S, Patoia L, Di Matteo FM, Brunori PM, Morelli A. Severe gastric mucosal damage induced by NSAIDs in healthy subjects is associated with Helicobacter pylori infection and high levels of serum pepsinogens. *Dig Dis Sci* 1995; **40**: 2074-2080
24 **Taha AS**, Nakshabendi I, Lee FD, Sturrock RD, Russell RI. Chemical gastritis and Helicobacter pylori related gastritis in patients receiving non-steroidal anti-inflammatory drugs: comparison and correlation with peptic ulceration. *J Clin Pathol* 1992; **45**: 135-139
25 **Laine L**, Marin-Sorensen M, Weinstein WM. Nonsteroidal antiinflammatory drug-associated gastric ulcers do not require Helicobacter pylori for their development. *Am J Gastroenterol* 1992; **87**: 1398-1402
26 **Borén T**, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895
27 **Mahdavi J**, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, Altraja S, Wadström T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarström L, Borén T. Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. *Science* 2002; **297**: 573-578
28 **Odenbreit S**, Till M, Hofreuter D, Faller G, Haas R. Genetic and functional characterization of the alpAB gene locus essential for the adhesion of Helicobacter pylori to human gastric tissue. *Mol Microbiol* 1999; **31**: 1537-1548
29 **Evans DG**, Karjalainen TK, Evans DJ Jr, Graham DY, Lee CH. Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of Helicobacter pylori. *J Bacteriol* 1993; **175**: 674-683
30 **Evans DG**, Evans DJ Jr, Lampert HC, Graham DY. Restriction fragment length polymorphism in the adhesin gene hpaA of Helicobacter pylori. *Am J Gastroenterol* 1995; **90**: 1282-1288
31 **Bode G**, Malfertheiner P, Ditschuneit H. Pathogenetic implications of ultrastructural findings in Campylobacter pylori related gastroduodenal disease. *Scand J Gastroenterol Suppl* 1988; **142**: 25-39
32 **Bauerfeind P**, Garner R, Dunn BE, Mobley HL. Synthesis and activity of Helicobacter pylori urease and catalase at low pH. *Gut* 1997; **40**: 25-30
33 **Eaton KA**, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. *Infect Immun* 1991; **59**: 2470-2475
34 **Skouloubris S**, Thiberge JM, Labigne A, De Reuse H. The Helicobacter pylori UreI protein is not involved in urease activity but is essential for bacterial survival in vivo. *Infect Immun* 1998; **66**: 4517-4521
35 **Eaton KA**, Gilbert JV, Joyce EA, Wanken AE, Thevenot T, Baker P, Plaut A, Wright A. In vivo complementation of ureB restores the ability of Helicobacter pylori to colonize. *Infect Immun* 2002; **70**: 771-778
36 **Ito Y**, Shibata K, Hongo A, Kinoshita M. Ecabet sodium, a locally acting antiulcer drug, inhibits urease activity of Helicobacter pylori. *Eur J Pharmacol* 1998; **345**: 193-198
37 **Mobley HL**, Island MD, Hausinger RP. Molecular biology of microbial ureases. *Microbiol Rev* 1995; **59**: 451-480
38 **Pérez-Pérez GI**, Gower CB, Blaser MJ. Effects of cations on Helicobacter pylori urease activity, release, and stability. *Infect Immun* 1994; **62**: 299-302
39 **O'Toole PW**, Lane MC, Porwollik S. Helicobacter pylori motility. *Microbes Infect* 2000; **2**: 1207-1214
40 **Josenhans C**, Labigne A, Suerbaum S. Comparative ultrastructural and functional studies of Helicobacter pylori and Helicobacter mustelae flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in Helicobacter species. *J Bacteriol* 1995; **177**: 3010-3020
41 **Suerbaum S**, Josenhans C, Labigne A. Cloning and genetic characterization of the Helicobacter pylori and Helicobacter mustelae flaB flagellin genes and construction of H. pylori

flaA- and flaB-negative mutants by electroporation-mediated allelic exchange. *J Bacteriol* 1993; **175**: 3278-3288
42 **Kunin CM**, Hua TH, Bakaletz LO. Effect of salicylate on expression of flagella by Escherichia coli and Proteus, Providencia, and Pseudomonas spp. *Infect Immun* 1995; **63**: 1796-1799
43 **Farber BF**, Wolff AG. The use of nonsteroidal antiinflammatory drugs to prevent adherence of Staphylococcus epidermidis to medical polymers. *J Infect Dis* 1992; **166**: 861-865
44 **Watanabe S**, Takagi A, Tada U, Kabir AM, Koga Y, Kamiya S, Osaki T, Miwa T. Cytotoxicity and motility of Helicobacter pylori. *J Clin Gastroenterol* 1997; **25** Suppl 1: S169-S171
45 **Kurihara N**, Kamiya S, Yamaguchi H, Osaki T, Shinohara H, Kitahora T, Ishida H, Ozawa A, Otani Y, Kubota T, Kumai K, Kitajima M. Characteristics of Helicobacter pylori strains isolated from patients with different gastric diseases. *J Gastroenterol* 1998; **33** Suppl 10: 10-13
46 **Logan RP**. Helicobacter pylori and gastric cancer. *Lancet* 1994; **344**: 1078-1079
47 **Dooley CP**, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989; **321**: 1562-1566
48 **Wang WH**, Huang JQ, Zheng GF, Lam SK, Karlberg J, Wong BC. Non-steroidal anti-inflammatory drug use and the risk of gastric cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2003; **95**: 1784-1791

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ORIGINAL ARTICLE

MicroRNA155 is induced in activated CD4$^+$ T cells of TNBS-induced colitis in mice

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Abstract

AIM: To investigate the expression of microRNA155 (miRNA155) in trinitrobenzene sulphonic acid (TNBS)-induced colitis and the relationship between miRNA155 and tumor necrosis factor (TNF) expressions.

METHODS: In TNBS colitis mice, miRNA155 and TNF mRNA expressions were measured in colons and CD4$^+$ T cells of draining lymph nodes (LNs). CD4$^+$ T cells were cultured *in vitro* with or without anti-CD3/CD28 antibody, and the expressions of miRNA155 and TNF mRNA in cells and TNF concentration in culture media were examined.

RESULTS: miRNA155 and TNF mRNA expressions in colons and in cells of LNs were significantly increased in TNBS colitis compared with controls. In TNBS colitis, miRNA155 and TNF mRNA expressions in CD4$^+$ T cells of LNs and TNF concentration in CD4$^+$ T cells culture media increased compared with controls. When cultured with anti-CD3/CD28 antibody, miRNA155 and TNF mRNA expressions in CD4$^+$ T cells and TNF concentration in the CD4$^+$ T cells culture media were significantly higher than those cultured without anti-CD3/CD28 antibody. Following analysis using the Pearson's correlation coefficient, miRNA155 expression had a significant positive correlation with either TNF mRNA expression in CD4$^+$ T cells (r = 0.860, P < 0.05) or TNF concentration in CD4$^+$ T cells culture media (r = 0.892, P < 0.05).

CONCLUSION: miRNA155 is induced in colons and activated CD4$^+$ T cells in TNBS colitis, and the levels of miRNA155 and TNF expressions have a significant positive correlation.

Key words: Colitis; Crohn's disease; Lymph nodes; microRNA; Tumor necrosis factor

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INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory

disorder of the gastrointestinal tract. CD is thought to be a multifactorial, polygenic disease, however, the exact pathogenesis of CD is still unclear[1-3]. Some studies have suggested that CD is mediated by T helper type 1 (Th1) cells producing interferon-γ (IFN-γ), tumor necrosis factor (TNF) and interleukin (IL)-12[4-8]. TNF, which is a proinflammatory cytokine secreted by monocytes/macrophages, T cells, B cells, NK cells and mast cells, is necessary for both the initiation and persistence of the Th1 response and contributes to intestinal inflammation in CD patients[9-11]. Today, many animal models of CD are available, and each model has reflected one aspect of human CD. Hapten-induced colitis, in which trinitrobenzene sulfonic acid (TNBS) is delivered intrarectally to rodents, displays Th1 activity of local $CD4^+$ T cells and is considered to closely resemble CD[12-14].

MicroRNAs (miRNAs) are a group of small non-coding RNAs which posttranscriptionally regulate gene expression[15,16]. More than 700 miRNAs have been identified in mammals and are involved in a wide variety of biological processes[15,17]. They are transcribed as primary transcripts by RNA polymerase Ⅱ, cleaved into a precursor miRNA by the Drosha nuclease, and exported from the nucleus by exportin 5. In the cytoplasm, the precursor miRNAs are further processed by the Dicer nuclease and are incorporated into the RNA-induced silencing complexes. The miRNAs guide the RNA-induced silencing complexes binding to the mRNAs 3'-untranslated regions (UTRs), resulting in either the mRNAs degradation or translational inhibition[18-22].

Although the exact functions of most miRNAs have yet to be elucidated, many studies have suggested that miRNAs have been implicated in many aspects of innate and acquired immunity, such as differentiation, survival and functions of immune cells, and the intracellular signaling pathways[23-25]. miRNA155, which was reported to be involved in the production of TNF and regulation of immunity[26,27], is processed from an exon of the noncoding RNA known as bic[27,28]. In this study, we mainly evaluated the expression of miRNA155 in TNBS colitis, and speculated on the relationship between miRNA155 and TNF expressions.

MATERIALS AND METHODS

Mice

Female 6- to 8-wk-old BALB/C mice weighing 18-22 g were obtained from Shanghai Experimental Animals Centre of Chinese Academy of Sciences. The mice were maintained under specific pathogen free conditions in a room at 23 ± 2℃ with a 12 h light-dark cycle, and had free access to food and water during the study. They were allowed to acclimate to these conditions for at least seven days before inclusion in an experiment. All procedures were approved by the Investigation and Ethics Committee of Shanghai Jiaotong University School of Medicine.

Establishment of TNBS-induced colitis

Colitis was induced in BALB/C mice as described previously with some modification[13,29]. For sensitization, a 2 cm × 2 cm field of the abdominal skin was shaved and 150 μL of 2.5% TNBS (Sigma Chemical Co., St. Louis, MO, USA) in 50% ethanol was applied. Five days after sensitization, mice were anesthetized slightly with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (50 mg/kg), and then intrarectally administered 100 μL solution of 1% TNBS dissolved in 45% ethanol *via* a 3.5-French catheter equipped with a 1 mL syringe. The tip of the catheter was inserted 4 cm proximal to the anal verge. Mice were held in a vertical position for 1 min after the intrarectal injection. Control mice were given 100 μL 45% ethanol solution without TNBS using the same technique.

Clinical observations and histologic assessments of colitis

Daily body weight, stool consistency, and occult blood (measured by the guaiac reaction, hemoccult) were assessed. Three days after intrarectal injection, mice were killed by cervical dislocation after being anesthetized with diethyl ether and entire colons were removed from the cecum to the anus, and flushed with saline. Colon specimens located 2 cm above the anal verge were achieved. One section of the specimen was fixed overnight in 4% paraformaldehyde and embedded in paraffin, and then sections stained with hematoxylin and eosin were examined. The other sections of the colon were immediately frozen in liquid nitrogen after dissection and used for quantification of miRNA155, IL-1β, IL-6, TNF and IFN-γ mRNA.

Cell preparation

Three days after intrarectal injection, colon draining lymph nodes (LNs) were aseptically removed. Single-cell suspensions were prepared by pressing LNs through a 40 μm cell strainer using the plunger of a 1 mL syringe. $CD4^+$ T cells were isolated from the cell suspensions with magnetic beads labeled with anti-CD4 (L3T4) monoclonal antibodies (Miltenyi Biotec Inc, Bergisch Gladbach, Germany). Cells were incubated in media (RPMI 1640 supplemented with 100 U/mL penicillin/streptomycin, 2 mmol/L L-glutamine, 50 mol/L 2-mercaptoethanol, and 10% fetal calf serum) at 8×10^4 cells in 150 μL media per well in 96-well plates for 48 h in the absence or presence of dynabeads CD3/CD28 T cells activator (Invitrogen, Carlsbad, CA, USA) at a concentration of 2 μL/well.

Enzyme-linked immunosorbent assay (ELISA)

After incubation for 48 h, the supernatants of the culture media were harvested and assayed for TNF concentration by ELISA using an ELISA kit (R&D Systems, Minneapolis, MN, USA).

Table 1 Primers used for RT or PCR of mRNA or miRNA

Gene name		Primer sequences (5′-3′)
IL-1β	Sense	GCAACTGTTCCTGAACTCAACT
	Antisense	ATCTTTTGGGGTCCGTCAACT
IL-6	Sense	CCACTTCACAAGTCGGAGGCTTA
	Antisense	GCAAGTGCATCATCGTTGTTCATAC
IFN-γ	Sense	TCAAGTGGCATAGATGTGGAAGAA
	Antisense	TGGCTCTGCAGGATTTTCATG
TNF	Sense	CCACCACGCTCTTCTGTCTAC
	Antisense	TGGGCTACAGGCTTGTCACT
β-actin	Sense	CTAGGCACCAGGGTGTGAT
	Antisense	TGCCAGATCTTCTCCATGTC
miRNA155	Stem-loop primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCCT
	Sense	GCCCGCTTAATGCTAATTGTGAT
	Antisense	GTGCAGGGTCCGAGGT
U6	Sense	CTCGCTTCGGCAGCACA
	Antisense	AACGCTTCACGAATTTGCGT

RT: Reverse transcription; PCR: Polymerase chain reaction.

Quantitative real-time polymerase chain reaction (qPCR) analysis of mRNA detection

Total RNA from cells and colon samples were extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentrations were determined with a spectrophotometer (Eppendorf, Hamburg, Germany). 0.2-0.5 μg of total RNA was reverse transcribed, and RNA expression levels were quantified by sybergreen-based qPCR using a sequence detection system (Prism 7500; Applied Biosystems Inc., Foster City, USA). β-actin served as the endogenous control. Gene-specific primers for the reported genes are indicated in Table 1. To evaluate the relative expression of each target gene, the comparative threshold (Ct) cycle method was used according to the manufacturer's manual. The threshold cycle (Ct) for each gene was determined as the cycle number at which the reaction crossed an arbitrarily placed threshold, and the relative amount of each mRNA to β-actin was described using the formula $2^{-\Delta Ct}$ where $\Delta Ct = (Ct_{mRNA} - Ct_{\beta\text{-actin}})$.

qPCR analysis of miRNA detection

Total RNA from cells and colon samples were isolated using the TRIzol reagent. Real time quantitative analyses for miRNAs were performed using stem-loop RT-PCR[30,31]. 0.2-0.5 μg of total RNA was reverse transcribed to cDNA using a target-specific stem-loop primer indicated in Table 1. qPCR was performed on a sequence detection system (Prism 7500; Applied Biosystems Inc., Foster City, USA). In brief, cDNA in water was added to 5 μL of the 2 × SYBR green master mix (Applied Biosystems Inc., Foster City, USA), 400 nmol/L of gene-specific primer and water to 10 μL. The reactions were amplified at 95℃ for 10 min followed by 40 cycles at 95℃ for 15 s and 60℃ for 60 s. U6 small nuclear RNA (U6) served as the endogenous control. At the end of the qPCR, the thermal denaturation protocol was run to determine the number of products that were present in the reaction. The relative amount of miRNA to U6 was calculated using the Ct cycle method. The relative amount of each miRNA to U6 was described using the formula $2^{-\Delta Ct}$ where $\Delta Ct = (Ct_{miRNA} - Ct_{U6})$[30,31].

Statistical analysis

Each group contained 5-8 mice, and results were expressed as the mean ± SD. A comparison between the two groups was made using the Student's *t*-test. The relationship between the two targets was tested with Pearson's correlation coefficient. Differences were considered significant at $P < 0.05$.

RESULTS

Successful establishment of experimental colitis

Administration of TNBS to presensitized mice resulted in a severe illness characterized by bloody diarrhea, rectal prolapse accompanied by sustained weight loss. At day 3-4, the disease reached a peak. Histologic examination of the colons showed severe depletion of mucin-producing goblet and epithelial cells, large areas of ulceration, a marked increase in the thickness of the muscular layer, and transmural inflammation involving all colon wall layers with infiltration of lymphocytes, macrophages and neutrophils extending from the mucosa into the muscular and serosal layers (Figure 1).

Increased expressions of miRNA155, IL-1β, IL-6, TNF, and IFN-γ mRNA in the colons of TNBS-induced colitis mice

To investigate miRNA155 and cytokine expressions in colons, we assessed miRNA155, IL-1β, IL-6, TNF and IFN-γ mRNA in colon homogenates by qPCR. We found that miRNA155 expression in colon homogenates was significantly increased in TNBS-induced colitis, which was 3.10-fold higher than in control mice. IL-1β, IL-6, TNF, and IFN-γ mRNA expressions in colon homogenates were also significantly increased in TNBS-induced colitis,

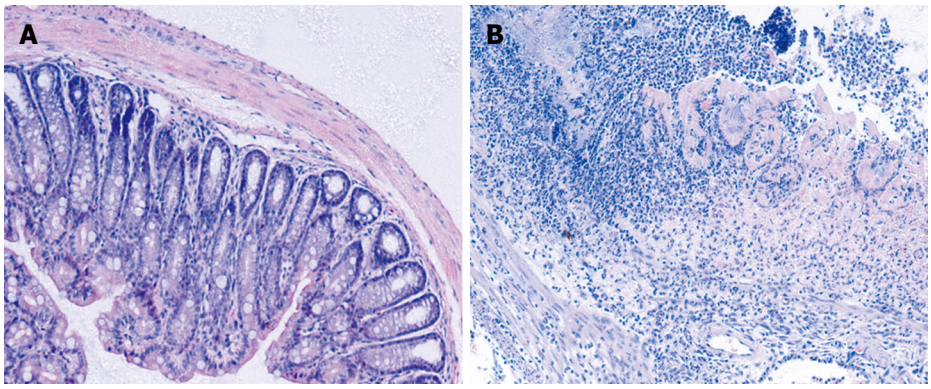

Figure 1 Representative microphotographs of the colon sections stained with hematoxylin and eosin (original magnification, × 100). A: Control colon; B: Colon was obtained on day 3 after intrarectal TNBS.

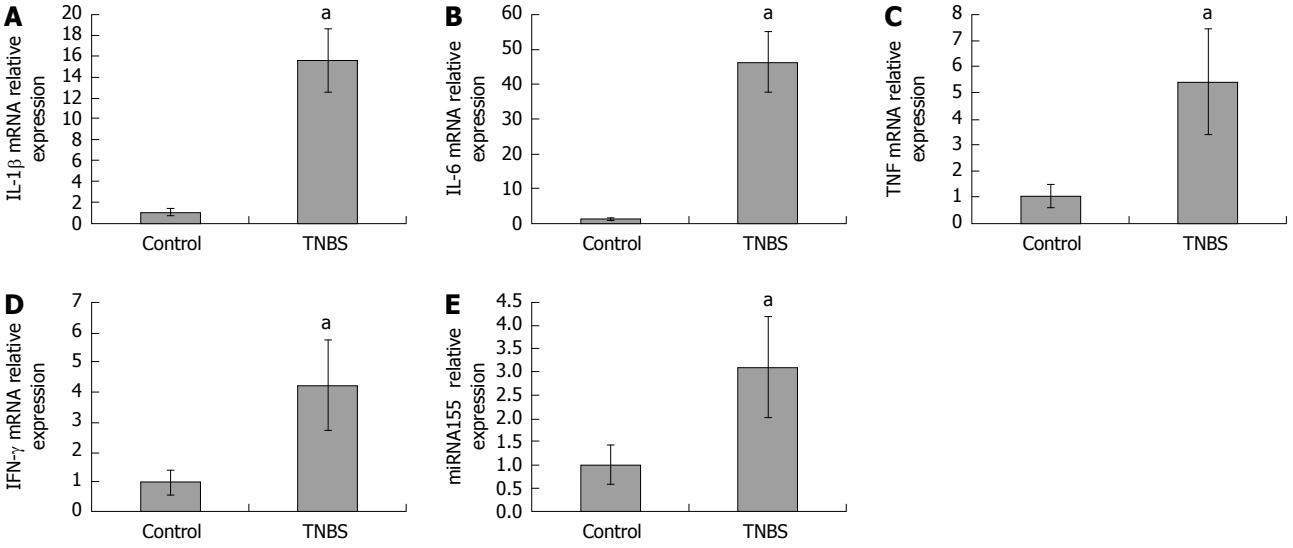

Figure 2 IL-1β (A), IL-6 (B), TNF (C), IFN-γ (D) mRNA and miRNA155 (E) expressions in colon homogenates of control and TNBS colitis groups. qPCR-derived IL-1β, IL-6, TNF, IFN-γ, and miRNA155 expressions in colon homogenates were significantly increased in TNBS-induced colitis. $^{a}P < 0.05$ *vs* control.

and were 15.58-, 46.34-, 5.43-, and 4.23-fold higher than in control mice, respectively (Figure 2).

miRNA155 and TNF mRNA expressions in the CD4$^+$ T cells of colon draining LNs and TNF concentration in CD4$^+$ T cells culture media

As CD4$^+$ T cells play a central role in Th1 response, we evaluated the levels of miRNA155 and TNF mRNA expressions in LNs and CD4$^+$ T cells from LNs. In TNBS colitis, miRNA155 in draining LNs or CD4$^+$ T cells from LNs increased and were 3.74- and 3.07-fold higher than in controls, respectively (Figure 3). TNF mRNA in draining LNs or CD4$^+$ T cells from LNs of TNBS colitis were 3.34- and 2.06-fold higher than in controls (Figure 3). In the CD4$^+$ T cells culture media, the concentration of TNF protein increased in TNBS colitis and was 4.19-fold higher than in controls (128.04 ± 38.71 pg/mL *vs* 30.55 ± 8.37 pg/mL, $P < 0.05$, Figure 4).

Anti-CD3/CD28 antibody promoted the miRNA155 and TNF mRNA expressions in the CD4$^+$ T cells and the TNF concentration in the supernatants of CD4$^+$ T cells culture media

To study whether the T cell receptor (TCR) and the costimulatory receptor were involved in miRNA155 expression, and to study the relationship between miRNA155 and TNF production, we used anti-CD3/CD28 antibody to stimulate CD4$^+$ T cells. When cultured with anti-CD3/CD28 antibody, the CD4$^+$ T cells became larger and displayed an activated appearance. In control and TNBS colitis, the miRNA155 expressions in CD4$^+$ T cells cultured with anti-CD3/CD28 antibody were 4.72- and 3.61-fold higher than cells cultured without anti-CD3/CD28 antibody, and the TNF mRNA expression in CD4$^+$ T cells cultured with anti-CD3/CD28 antibody were 3.42- and 3.03-fold higher than cells cultured without anti-CD3/CD28 antibody, respectively (Figure 4). The TNF concentration in the supernatants of culture media which contained anti-CD3/CD28 antibody increased both in control and TNBS colitis mice, and were 4.28- and 6.87-fold higher than in media cultured without anti-CD3/CD28 antibody (135.66 ± 32.11 pg/mL *vs* 30.55 ± 8.37 pg/mL, $P < 0.05$; 850.94 ± 219.49 pg/mL *vs* 128.04 ± 38.71 pg/mL, $P < 0.05$, Figure 4).

Relationship between miRNA155 and TNF gene expressions

Since TNF plays an important role in the pathogenesis

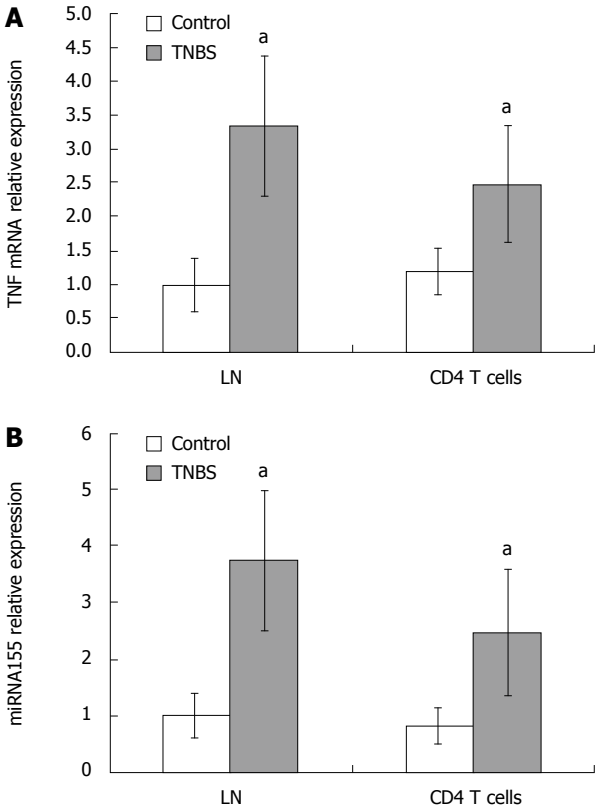

Figure 3 TNF mRNA (A) and miRNA155 (B) expressions in LNs and CD4$^+$ T cells in control and TNBS colitis groups. TNF mRNA and miRNA155 expressions in LNs and CD4$^+$ T cells were significantly increased in TNBS-induced colitis. $^aP < 0.05$ *vs* control.

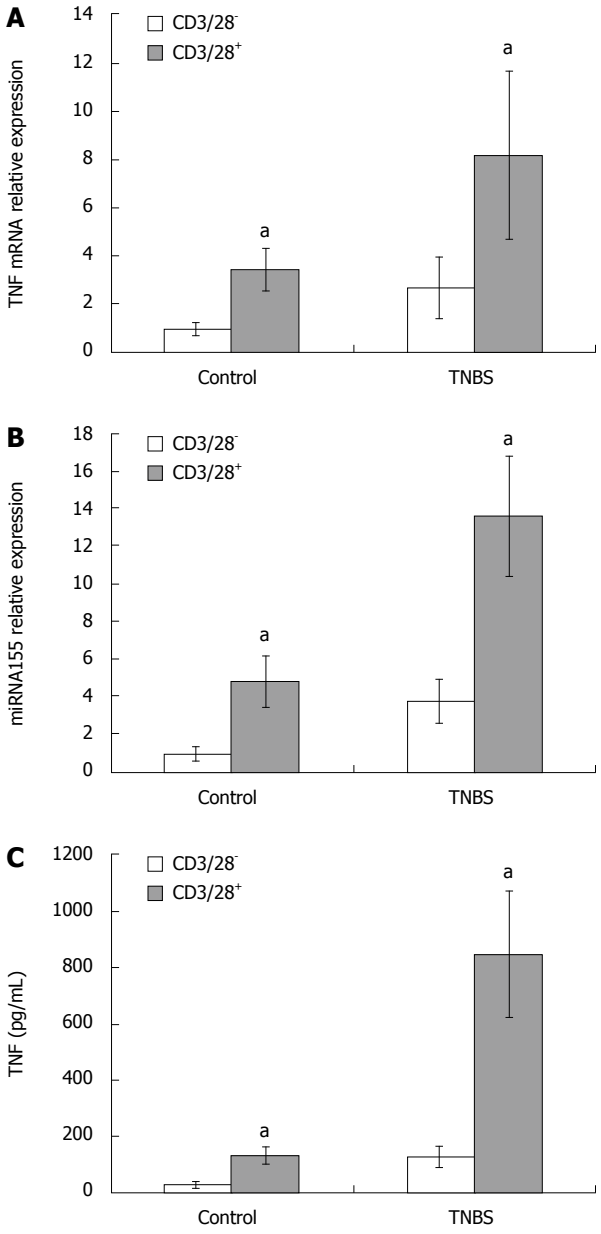

Figure 4 TNF mRNA (A) and miRNA155 (B) expressions in CD4$^+$ T cells cultured with or without anti-CD3/CD28 antibody, and the TNF protein expression (C) in the supernatants of CD4$^+$ T cells culture media with or without anti-CD3/CD28 antibody. TNF mRNA and miRNA155 expressions in CD4$^+$ T cells cultured with anti-CD3/CD28 antibody, and the TNF concentration in the supernatants of culture media containing anti-CD3/CD28 antibody increased in comparison to those in cells or media cultured without anti-CD3/CD28 antibody. $^aP < 0.05$ vs control.

of CD, we evaluated the potential correlation between miRNA155 and TNF gene expressions. Our data indicated that there was a significant positive correlation between miRNA155 and TNF mRNA expressions in CD4$^+$ T cells ($r = 0.860$, $P < 0.05$, Figure 5), and miRNA155 expression in CD4$^+$ T cells and TNF protein concentration in CD4$^+$ T cells culture media ($r = 0.892$, $P < 0.05$, Figure 5).

DISCUSSION

As the exact etiology of CD is still unclear, TNBS-induced colitis was used to study many important aspects of the pathogenesis in CD. TNBS colitis is thought to resemble CD because of the mucosal inflammation mediated by excessive IFN-γ, TNF and other proinflammatory cytokine production[12-14]. In agreement with previous results, our data also showed that TNBS colitis is a Th1 model with elevated IFN-γ and TNF expressions in colon. In this study, we found that miRNA155 was increased in colons and in CD4$^+$ T cells of LNs in TNBS colitis and the levels of miRNA155 and TNF expressions had a significant positive correlation.

MiRNAs are a group of small noncoding RNAs which are thought to posttranscriptionally regulate gene expressions. Dysregulation of miRNAs has been associated with several autoimmune diseases[32,33]. miRNA155 is processed from an exon of the noncoding RNA. Some studies have shown that miRNA155 is required for normal innate and acquired immunity[26,27,34,35].

In macrophages, miRNA155 was reported to enhance the production of TNF, but may target transcripts encoding for several proteins, such as IκBε kinase and Fas-associated death domain protein whose ultimate function results in the activation of the lipopolysaccharide (LPS)/TNF pathway[26]. Therefore, miRNA155 may exert both positive and negative effects on the activation of innate immunity. In acquired immunity, miRNA155 was reported to affect lymphoid cell development[27,34,36]. In

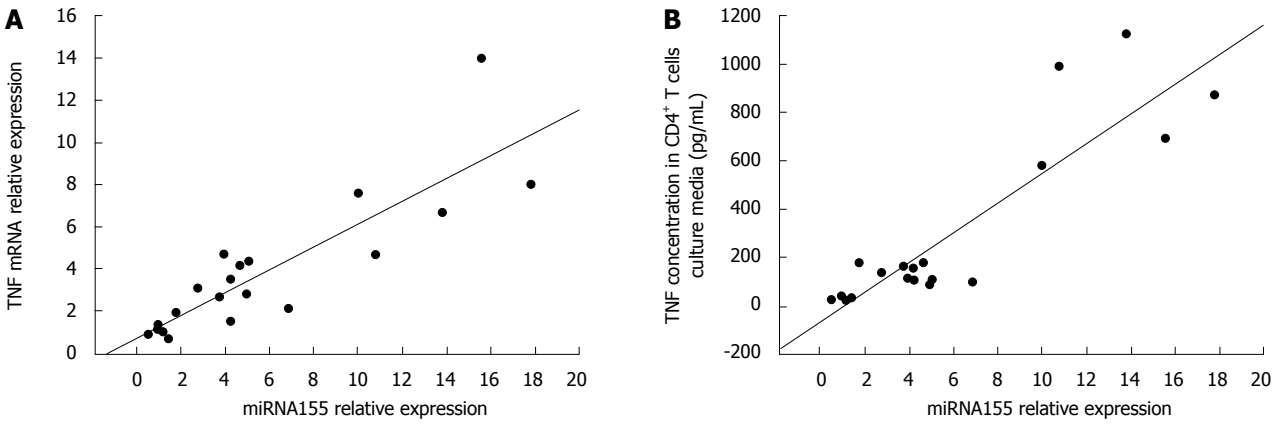

Figure 5 Relationships between miRNA155 expression in CD4$^+$ T cells and TNF mRNA expression in CD4$^+$ T cells (A) or TNF concentration in CD4$^+$ T cells culture media (B). Data analyzed by Pearson's correlation coefficient.

miRNA155$^{-/-}$ mice, CD4$^+$ T cells are intrinsically biased toward Th2 differentiation. miRNA155 modulates the level of a transcription factor c-Maf in T cells and is likely to induce the attenuation of Th2 cell responses *in vivo*[34]. With regard to B cells, several studies have proved that B cells require miRNA155 for normal production of isotype-switched, high-affinity antibodies and for a memory response[27,36]. However, the expression of miRNA155 and its relationship with CD remains unclear. In this paper, we reported that miRNA155 expression is increased in the colon and it's draining LNs in TNBS colitis. This result hints that miRNA155 may have a role in the pathogenesis of TNBS colitis. As CD4$^+$ T cells play a central role in Th1 response, we determined the level of miRNA155 expression in the CD4$^+$ T cells from LNs in TNBS colitis and found that miRNA155 was increased in CD4$^+$ T cells in TNBS colitis.

Considering TNF plays an important role in the pathogenesis of CD, we evaluated the relationship between miRNA155 and TNF expression in CD4$^+$ T cells. Our data indicated that the gene expression of miRNA155 in CD4$^+$ T cells of draining LNs in TNBS colitis has a significant positive correlation with either TNF mRNA in CD4$^+$ T cells or the concentration of TNF protein in the culture media. Some reports have shown that miRNA155 may be involved in TNF production[26,27]. Eu-miR-155 transgenic mice which specifically overexpress miRNA155 in B cells produced more TNF when challenged with LPS, and were hypersensitive to LPS/D-galactosamine-induced septic shock[26]. In addition, miRNA155$^{-/-}$ B cells produce less TNF when activated *in vitro* by BCR cross-linking[27]. Scientists have found that the miRNA155 effects on TNF production are at both the transcriptional and posttranscriptional level[26,27]. More TNF transcripts were observed in wild-type mice compared with miRNA155$^{-/-}$ mice[27]. At the posttranscriptional level, miRNA155 may target the 3'-UTR of TNF transcripts to increase the stability of transcripts and enhance translation[26]. Our results showed that the TNF protein concentration in the CD4$^+$ T cells culture media increased more than the TNF mRNA in CD4$^+$ T cells, which is in agreement with the proposition that miRNA155 regulated TNF production at both the transcriptional and posttranscriptional levels. These findings suggested that miRNA155 may prompt the production of TNF in some types of immune cells. From our results, miRNA155 increased and had a positive relationship with TNF expression in CD4$^+$ T cells, therefore we suppose that miRNA155 may influence TNF expression in CD4$^+$ T cells and is possibly involved in the pathogenesis of TNBS colitis.

The exact mechanism of miRNA155 expression regulation in CD4$^+$ T cells remains unclear. To study the role of TCR in miRNA155 expression and the relationship between miRNA155 expression and the activation of CD4$^+$ T cells, we used anti-CD3/CD28 antibody to stimulate CD4$^+$ T cells, and found that the miRNA155 expression level was elevated in CD4$^+$ T cells when cultured with the antibody. Stimulation of the TCR/CD3 complex and costimulatory receptor CD28 in CD4$^+$ T cells by anti-CD3/CD28 antibody can lead to activation of multiple transcription factors, including NF-AT and NF-κB, which ultimately control transcription of cytokines and T-cell proliferation[37]. miRNA155 level was reported to have a link with the NF-κB pathway. NF-κB activity is required for the change in miRNA155 levels following TNF stimulation in macrophages, however, this relationship remains elusive[26]. The precise mechanism of the regulation of miRNA155 expression may be a new issue for future research.

In conclusion, miRNA155 expression was found to increase in colons and activated CD4$^+$ T cells in TNBS colitis. A significant positive correlation was observed between miRNA155 expression in CD4$^+$ T cells and the expression of TNF mRNA in CD4$^+$ T cells and the TNF protein concentration in CD4$^+$ T cells culture media. miRNA155 may be involved in the activation and TNF production of CD4$^+$ T cells in TNBS colitis.

COMMENTS

Background

MicroRNAs (miRNAs) are a group of small noncoding RNAs which post-

transcriptionally regulate gene expression. miRNA155 is reported to be involved in the production of TNF and the regulation of immunity. Tumor necrosis factor (TNF) plays an important role in the pathogenesis of Crohn's disease (CD). However, the expression of miRNA155 and its relationship with TNF production in CD remains unclear.

Research frontiers

The exact etiology of CD is still unclear. MiRNA research may represent a new way to explore the pathogenesis of CD. The expressions and functions of most miRNAs in CD remain a mystery.

Innovations and breakthroughs

The study found that miRNA155 is increased in colons and draining lymph nodes. In $CD4^+$ T cells which play a central role in Th1 response, miRNA155 expression is higher in TNBS colitis than in controls. As $CD4^+$ T cells were activated by anti-CD3/CD28 antibody, the miRNA155 expression was higher than that when cultured without anti-CD3/CD28 antibody. In addition, the levels of miRNA155 and TNF expressions had a significant positive correlation.

Applications

The results of this study may enhance our understanding of the pathogenesis of CD. By investigating the exact function of miRNA155 in the pathogenesis of CD, the findings may contribute to improvements in future drug therapies.

Terminology

MiRNA: MicroRNAs (miRNAs) are a group of small noncoding RNAs which post-transcriptionally regulate gene expressions. More than 700 miRNAs have been identified in mammals and are involved in a wide variety of biological processes. Stem-loop RT-PCR: RNA is reverse transcribed to cDNA using a gene-specific stem-loop RT primer, and then the RT products are quantified using conventional real time PCR.

Peer review

This paper reports a set of relatively new data about the pathogenic mechanism of IBD that may be referred by those investigators working on IBD.

REFERENCES

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
2 **Kucharzik T**, Maaser C, Lügering A, Kagnoff M, Mayer L, Targan S, Domschke W. Recent understanding of IBD patho genesis: implications for future therapies. *Inflamm Bowel Dis* 2006; **12**: 1068-1083
3 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434
4 **Strober W**, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002; **20**: 495-549
5 **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521
6 **Mizoguchi A**, Mizoguchi E, Bhan AK. Immune networks in animal models of inflammatory bowel disease. *Inflamm Bowel Dis* 2003; **9**: 246-259
7 **Elson CO**, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005; **206**: 260-276
8 **Neurath MF**, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. *Nat Med* 2002; **8**: 567-573
9 **Braegger CP**, MacDonald TT. Immune mechanisms in chronic inflammatory bowel disease. *Ann Allergy* 1994; **72**: 135-141
10 **Neurath MF**, Fuss I, Pasparakis M, Alexopoulou L, Haralambous S, Meyer zum Büschenfelde KH, Strober W, Kollias G. Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice. *Eur J Immunol* 1997; **27**: 1743-1750
11 **Strober W**, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002; **20**: 495-549
12 **Morris GP**, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989; **96**: 795-803
13 **Neurath MF**, Fuss I, Kelsall BL, Stüber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med* 1995; **182**: 1281-1290
14 **Neurath MF**, Pettersson S, Meyer zum Büschenfelde KH, Strober W. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. *Nat Med* 1996; **2**: 998-1004
15 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297
16 **Engels BM**, Hutvagner G. Principles and effects of microRNA-mediated post-transcriptional gene regulation. *Oncogene* 2006; **25**: 6163-6169
17 **Griffiths-Jones S**, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006; **34**: D140-D144
18 **Cai X**, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* 2004; **10**: 1957-1966
19 **Han J**, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho Y, Zhang BT, Kim VN. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* 2006; **125**: 887-901
20 **Yi R**, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003; **17**: 3011-3016
21 **Chendrimada TP**, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, Pasquinelli AE, Shiekhattar R. MicroRNA silencing through RISC recruitment of eIF6. *Nature* 2007; **447**: 823-828
22 **Chendrimada TP**, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 2005; **436**: 740-744
23 **Taganov KD**, Boldin MP, Baltimore D. MicroRNAs and immunity: tiny players in a big field. *Immunity* 2007; **26**: 133-137
24 **Baltimore D**, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: new regulators of immune cell development and function. *Nat Immunol* 2008; **9**: 839-845
25 **Bi Y**, Liu G, Yang R. MicroRNAs: novel regulators during the immune response. *J Cell Physiol* 2009; **218**: 467-472
26 **Tili E**, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, Fabbri M, Alder H, Liu CG, Calin GA, Croce CM. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol* 2007; **179**: 5082-5089
27 **Thai TH**, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL, Schmidt-Supprian M, Rajewsky N, Yancopoulos G, Rao A, Rajewsky K. Regulation of the germinal center response by microRNA-155. *Science* 2007; **316**: 604-608
28 **Tam W**. Identification and characterization of human BIC, a gene on chromosome 21 that encodes a noncoding RNA. *Gene* 2001; **274**: 157-167
29 **te Velde AA**, Verstege MI, Hommes DW. Critical appraisal of the current practice in murine TNBS-induced colitis. *Inflamm Bowel Dis* 2006; **12**: 995-999
30 **Chen C**, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ. Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res* 2005; **33**: e179
31 **Schmittgen TD**, Lee EJ, Jiang J, Sarkar A, Yang L, Elton TS, Chen C. Real-time PCR quantification of precursor and mature microRNA. *Methods* 2008; **44**: 31-38

32 **Tang Y**, Luo X, Cui H, Ni X, Yuan M, Guo Y, Huang X, Zhou H, de Vries N, Tak PP, Chen S, Shen N. MicroRNA-146A contributes to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins. *Arthritis Rheum* 2009; **60**: 1065-1075
33 **Wu F**, Zikusoka M, Trindade A, Dassopoulos T, Harris ML, Bayless TM, Brant SR, Chakravarti S, Kwon JH. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. *Gastroenterology* 2008; **135**: 1624-1635.e24
34 **Rodriguez A**, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. *Science* 2007; **316**: 608-611
35 **O'Connell RM**, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA* 2007; **104**: 1604-1609
36 **Vigorito E**, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KG, Rada C, Enright AJ, Toellner KM, Maclennan IC, Turner M. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity* 2007; **27**: 847-859
37 **Wang D**, You Y, Case SM, McAllister-Lucas LM, Wang L, DiStefano PS, Nuñez G, Bertin J, Lin X. A requirement for CARMA1 in TCR-induced NF-kappa B activation. *Nat Immunol* 2002; **3**: 830-835

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BRIEF ARTICLE

Colorectal cancer prognosis twenty years later

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Author contributions: Bujanda L and Hijona L participated in the experimental design and writing of the manuscript; Sarasqueta C performed data analysis and interpretation of results; Hijona L, Cosme A, Gil I, Elorza JL, Asensio JI, Larburu S, Enríquez-Navascués JM, Jover R, Balaguer F, Llor X, Bessa X, Andreu M, Paya A and Castells A collected and supplied the clinical data of the patients; Study organization and investigators from the Gastrointestinal Oncology Group of the Spanish Gastroenterological Association participated in the study. All authors have critically reviewed the manuscript and approved the final version.
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Abstract

AIM: To evaluate changes in colorectal cancer (CRC) survival over the last 20 years.

METHODS: We compared two groups of consecutive CRC patients that were prospectively recruited: Group Ⅰ included 1990 patients diagnosed between 1980 and 1994. Group Ⅱ included 871 patients diagnosed in 2001.

RESULTS: The average follow up time was 21 mo (1-229) for Group Ⅰ and 50 mo (1-73.4) for Group Ⅱ. Overall median survival was significantly longer in Group Ⅱ than in Group Ⅰ (73 mo *vs* 25 mo, $P < 0.001$) and the difference was significant for all tumor stages. Post surgical mortality was 8% for Group Ⅰ and 2% for Group Ⅱ ($P < 0.001$). Only 17% of Group Ⅰ patients received chemotherapy compared with 50% of Group Ⅱ patients ($P < 0.001$).

CONCLUSION: Survival in colorectal cancer patients has doubled over the past 20 years. This increase seems to be partly due to the generalization in the administration of chemotherapy and to the decrease of post surgical mortality.

Key words: Colon cancer; Prognosis; Survival; Chemotherapy; Surgery

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INTRODUCTION

Colorectal cancer (CRC) is the second most common form of cancer and the second leading cause of cancer death in both men and women in most developed countries including Spain. Mortality has increased by an annual average of 2.6% for men and 0.8% for women since 1975, without variations[1]. It is estimated that CRC caused 11 900 deaths in Spain in 2000, which represents 11% of the total deaths from cancer in men and 15% of those in women[1].

The primary treatment for this condition is surgical resection. Despite resection of all macroscopic tumors, patients whose primary tumor has penetrated the serosa or that have regional lymph node metastases at the time of surgery have high recurrence rates. An effective adjuvant program to eradicate microscopic tumor foci is clearly needed for such high-risk patients[2]. A major advance in adjuvant treatment of CRC came with trials that explored the combination of 5-fluorouracil and levamisole or leucovorin (FL). Therapy with FL reduced the overall death rate by 33% relative to surgery alone in patients with stage Ⅲ disease. During the last few years, sequential advances in chemotherapy after surgical resection (adjuvant chemotherapy) have had an irrefutable and substantial benefit[3].

The aim of the present study was to evaluate CRC survival over the last 20 years.

MATERIALS AND METHODS

Study population

A total of 1990 patients patients diagnosed with CRC between 1980 and 1994 and 2001 were included in a prospective and consecutive manner. Patients were enrolled in two time periods: Group Ⅰ included 1119 patients recruited between 1980 and 1994. Group Ⅱ included 871 patients recruited during 2001. Patients were recruited as part of the EPICOLON project[4,5]. Age, sex, tumor features (location, TNM stage, differentiation) and type of chemotherapy administered, if any, was collected for all patients. Stage was defined according to the 4th Edition of the TNM classification[6].

EPICOLON was a prospective, multicenter, nationwide study that was set up to record consecutive cases of CRC in 25 hospitals in Spain over one year. The initial aim of the study was to determine the incidence and characteristics of familial forms of CRC in Spain.

Patients went through periodical follow up in medical consultations or by telephone. Patients with familial adenomatous polyposis or inflammatory bowel disease were excluded.

In order to evaluate survival trends, Group Ⅰ was divided in three subgroups: patients diagnosed between 1980 and 1985 (343); patients diagnosed between 1986 and 1990 (392); and patients diagnosed between 1991 and 1994 (384).

Death during the first 30 d post surgery was considered postoperative mortality.

All patients provided written informed consent before enrollment in the study. The study was approved by the Institutional Review Board or Ethics Committee at each center and complied with the provisions of the Good Clinical Practice guidelines.

Statistical analysis

Continuous variables are defined with (mean ± SD). Discrete variables are defined by absolute and relative frequencies. The function of survival is calculated by the Kaplan Meier estimator. The accumulated probability of survival is presented at 3, 5 and 10 years of monitoring. The Log-Rank test is used to evaluate the differences in survival between the different categories of independent variables. Multivariable models are constructed using Cox regression analysis and the independent effect of each variable on mortality is estimated using Relative Risks (Hazard Ratio) and the respective confidence intervals of 95%. The SPSS v.15 program was used for analysis.

RESULTS

Tumor features, follow up and treatment

The average follow up time was 21 mo for Group Ⅰ and 50 mo for Group Ⅱ. Average follow up for deceased patients was 12 mo for Group Ⅰ and 16 mo for Group Ⅱ. For patients still alive, follow up was 67 mo for Group Ⅰ and 60 mo for Group Ⅱ.

The characteristics of the patients are described in Table 1. Both groups were similar regarding tumor stages at diagnose and tumor location. Significant differences were observed in sex and age: the average age of Group Ⅰ was 66.7 years compared to 69 years in Group Ⅱ; the percentage of men was 61% and 57%, respectively. Slight differences were also observed in tumor differentiation: Group Ⅰ had 4% of poorly differentiated tumors and Group Ⅱ 8%. The greatest differences were found in the proportion of patients that received chemotherapy: 17% in Group Ⅰ and 47% in Group Ⅱ.

Mortality

The probability of survival at 3 and 5 years was approximately 20% higher in Group Ⅱ than in Group Ⅰ. Three year survival was 44% for Group Ⅰ and 65% for Group Ⅱ. Five year survival was 35% for Group Ⅰ and 57% for Group Ⅱ.

Overall median survival was significantly longer in Group Ⅱ than in Group Ⅰ (73 mo *vs* 25 mo, $P < 0.001$).

Table 1 Characteristics of the patients *n* (%)

	Group Ⅰ (*n* = 1119)	Group Ⅱ (*n* = 871)	*P* value
Age mean (SD)	66.7 (12.4)	69.1 (11.5)	0.000
Sex			0.027
Males	638 (57)	531 (61)	
Females	481 (43)	340 (39)	
Tumor localization			
Distal to splenic flexure	831 (75)	644 (74)	
Proximal to splenic flexure	272 (25)	227 (26)	
TNM			0.11
Ⅰ	190 (17)	122 (14)	
Ⅱ	403 (36)	348 (40)	
Ⅲ	302 (27)	244 (28)	
Ⅳ	224 (20)	157 (18)	
Tumor differentiation			0.000
Poor	45 (4)	70 (8)	
Well-moderate	1074 (96)	801 (92)	
Receiving chemotherapy	190 (17)	409 (46.9)	0.000

Table 2 Mortality variation in relation to 4 independent risk factors

	Hazard ratio (95% CI)
Stage	
Ⅱ *vs* Ⅰ	1.6 (1.2–2.1)
Ⅲ *vs* Ⅰ	3.5 (2.7–4.6)
Ⅳ *vs* Ⅰ	13.7 (10.4-18.0)
Group	
Ⅰ *vs* Ⅱ	2.0 (1.7–2.4)
Chemotherapy	
No *vs* Yes	1.6 (1.3–1.9)
Grade	
Ⅰ, Ⅱ *vs* Ⅲ	1.5 (1.1–1.9)

Three years after cancer diagnosis, 21% more patients were alive in Group Ⅱ than in Group Ⅰ.

Multivariate analysis showed 4 independent factors associated with a higher mortality risk (Table 2).

Survival in Group Ⅰ: In Group I survival increased over the years in parallel to chemotherapy use (Figure 1). Ten percent of patients (35 patients) received chemotherapy between 1980 and 1985, 16% (63) in the period between 1986 and 1990 and 24% (92) in the period between 1991 and 1994. The median survival was 17 mo for the period between 1980 and 1985, 28 mo for the period between 1986 and 1990 and 34 mo for the period between 1991 and 1994 (Figure 1).

Survival and stages

Survival was greater for group II than for group I for all tumor stages (Figure 2). Postoperative mortality was 8% for Group I and 2% for Group Ⅱ (Table 3). However, when patients in Group I who had died within 30 d of emergency surgery were excluded the postoperative mortality rate was equal to that in Group Ⅱ. Postoperative mortality in patients operated electively in Group Ⅰ was 3% (5 patients) for stage Ⅰ, 2% (10 patients) for stage Ⅱ, 3% (10 patients) for stage Ⅲ and 4% (10 patients) for stage Ⅳ.

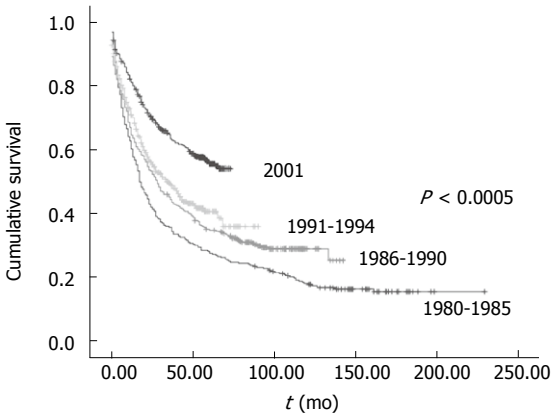

Figure 1 Kaplan-Meier analysis of overall survival in the different groups.

These data were similar to Group Ⅱ. Twenty six percent more patients received chemotherapy in Group Ⅱ than in Group Ⅰ for stage Ⅲ CRC For the other tumor stages the percentage of patients that received chemotherapy was also greater in Group Ⅱ, but to a smaller degree than stage Ⅲ: 3% for stage Ⅰ, 14% for stage Ⅱ and 11% for stage Ⅳ.

DISCUSSION

The last few years have seen great advances in treatment of CRC. The survival has increased substantially. In our study survival rate almost doubled over the last 20 years, from 35% to 57% at 5 years. Determining factors in this increased survival have been the generalization of chemotherapeutic agents and surgical advances, especially pertaining to rectal cancer.

The first chemotherapeutical agent used in the treatment of CRC was fluorouracil (FL) in 1958[7]. Since then and up to the last decade, FL has been the only drug used in CRC treatment. In 1988 a meta-analysis on the effect of FL showed a 10% reduction in the risk of death and an increase of 2.3% in survival after 5 years. In a subgroup of patients the risk of death was reduced by 17% and global survival after 5 years improved up to 34%[7]. Based on the results of this and other studies, the National Cancer Institute and the American Society of Clinical Oncology recommended FL-based post surgical treatment in their annual conference in 1997. In stage Ⅲ disease, FL increases overall five year survival from about 51% to 64%[8]. The use of adjuvant FL in patients with stage Ⅱ disease is controversial. FL in advanced CRC prolongs median survival by approximately 5 mo (6 mo without treatment to 11 mo with FL)[9].

The US Food and Drug Administration (FDA) has approved a host of new chemotherapeutic agents for advanced colon cancer and for relapsing disease. Since the year 2000, irinotecan was approved as a first line treatment in metastatic CRC. In 2002, oxaliplatin was approved for use along with other drugs as adjuvant treatment of relapsing CRC. Sequential exposure to various combinations of FL, irinotecan and oxaliplatin extends median overall survival by approximately 20 mo[10]. The

Table 3 Comparison of age, rectal localization, post-surgery mortality and administration of chemo and/or radiotherapy *n* (%)

	Stage Ⅰ		Stage Ⅱ		Stage Ⅲ		Stage Ⅳ	
	Group Ⅰ (*n* = 190)	Group Ⅱ (*n* = 122)	Group Ⅰ (*n* = 403)	Group Ⅱ (*n* = 348)	Group Ⅰ (*n* = 302)	Group Ⅱ (*n* = 244)	Group Ⅰ (*n* = 224)	Group Ⅱ (*n* = 157)
Age	66.7	70	66.7	70	66.7	70	67.5	70
Rectal localization	93 (49)	65 (44)	121 (30)	115 (33)	103 (34)	81 (33)	67 (30)	49 (31)
Postoperative mortality	17 (9)	2 (2)	20 (5)	7 (2)	21 (7)	5 (2)	29 (13)	6 (4)
Chemotherapy and/or radiotherapy	10 (5)	10 (8)	48 (12)	90 (26)	91 (30)	137 (56)	54 (24)	55 (35)

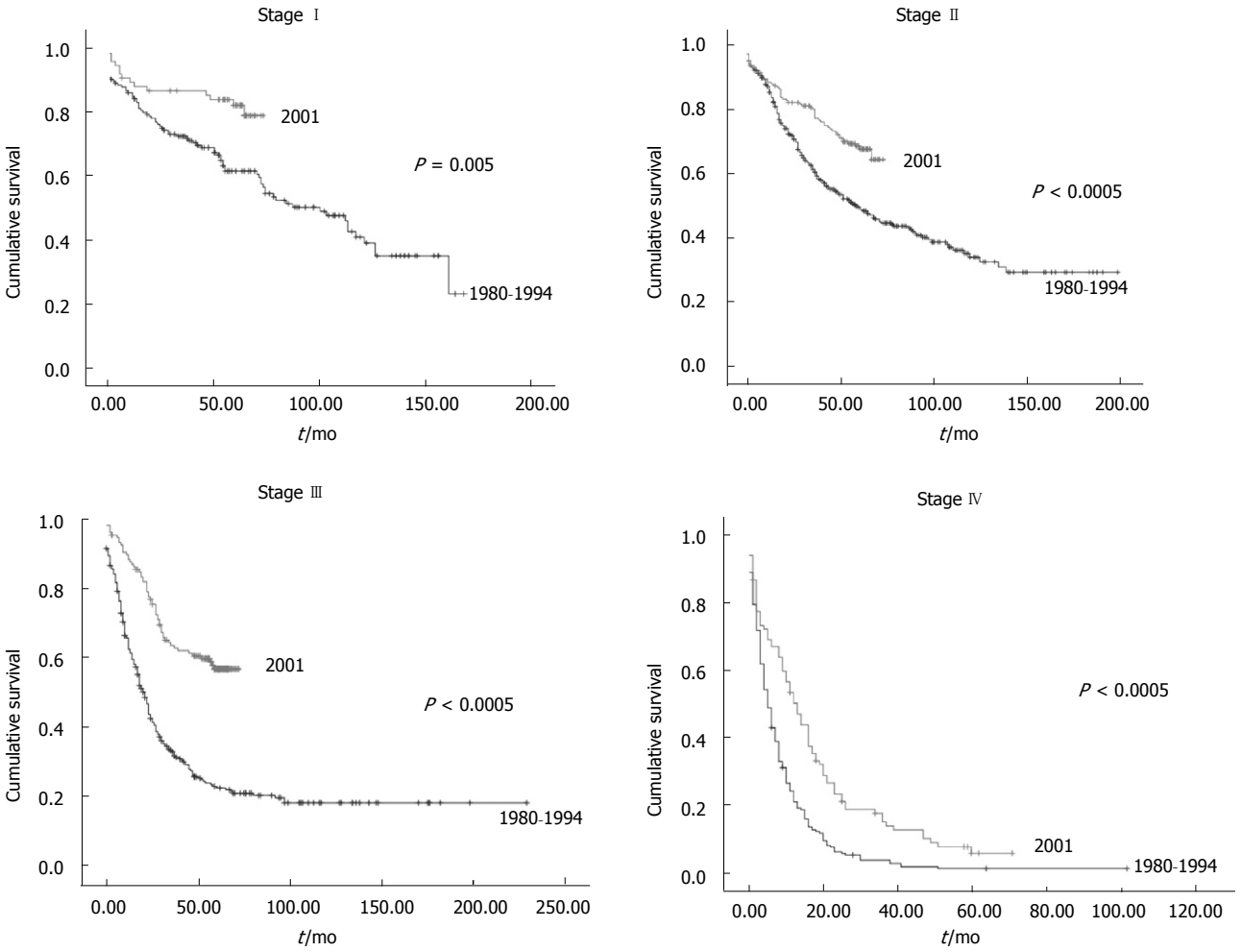

Figure 2 Kaplan-Meier analysis of overall survival and the stage of colorectal cancer according to the group of patients.

FDA approved capecitabine in 2005. Bevacizumab, a monoclonal humanized antibody united with the vascular endothelial growth factor (VEGF) was approved by the FDA for metastatic colon cancer in 2005. The availability of these new agents has resulted in a sustained increase in the use of chemotherapy for CRC patients from 17% in the period of 1980 to 1994 to 50% in 2001. This has been key to the increase of survival, fundamentally in stage Ⅲ and slightly less in the other CRC stages.

The other important factor that has contributed to an increased survival has been surgical improvements, specifically in the rectal area. Surgery-related mortality has consistently decreased over the years. For example, a Belgian study showed a decrease in surgical mortality from 20.1% between 1973 and 1979 to 7.8% between 1980 and 1986[11]. In our hospital, mortality decreased from 9.1% in 1981 to 8% in 1991[12]. In the last decade, the postoperative mortality for CRC varied between 1% and 9.9%[12-18]. Emergency surgery for CRC is associated with a high postoperative morbidity and mortality[14,18,19]. In rectal cancer, the number of abdomino-perineal resections has decreased from 26.3% in 1981 to 17.2% in 1991[12]. Between 1987 and 1992, the mortality in the Istituto di Patología Speciale Chirurgica of the University of Bologna was 7.6% in anterior resections and 14.2% in abdomino-perineal resections[20]. Our study

also observed a significant decrease in the percentage of abdomino-perineal resections, from 30% in 1980 to 18% in 1994. The use of mechanical sutures in rectal cancer operations has allowed a higher rate of sphincter preservation after low anterior resection[21]. On the other hand, the number of anterior rectal resections has increased due to the use of mechanical sutures in the middle third of the rectum.

The availability since 1994 of self-expanding stents for obstructive colorectal cancer[22] has resulted in a dramatic decrease in the number of urgent palliative surgeries in patients with metastases and a subsequent decrease in the associated postoperative mortality in our study and others[23].

Other factors that we have not analyzed and may also have played a significant role in the increased survival include the use of new antibiotics, improvements in the pre- and post-surgical care of patients, the improved application of radiotherapy and chemotherapy in rectal cancer, the surgical treatment of metastases and the improvement in bowel cleansing. Another limitation of our study is that the Group Ⅱ cohort was not entirely similar to that of Group Ⅰ, however, it was a cohort suitable for comparing with Group Ⅰ (20 years earlier) and showing the differences in different variables such as survival, postoperative mortality or the application of chemotherapy-radiotherapy. In both groups of patients data were collected consecutively and protocols followed strictly.

In conclusion, we observed the survival has increased steadily over the years and is now almost double that 20 years ago. This increase has been in parallel with an increase in the administration of adjuvant chemotherapy and a decline in postoperative mortality.

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COMMENTS

Background

Colorectal cancer (CRC) is the second leading cause of cancer mortality. In the last two decades major advances have occurred in treatment. Adjuvant chemotherapy has extended its indications and incorporated new drugs. Surgery outcomes have also significantly improved, with less emergency operations, lower postoperative mortality and a decrease in the number of abdomino-perineal resections. There are few studies geared at analyzing these factors and their influence on CRC survival looking at a 20 year time span.

Research frontiers

Numerous clinical trials have shown that chemotherapy and surgical advances have dramatically improved the prognosis in patients with CRC. Few studies outside the mentioned clinical trials have examined the impact of these advances on clinical outcome over the past two decades.

Innovations and breakthroughs

This study has quantified the effect of new adjuvant chemotherapy and improved surgical techniques on the prognosis of patients with CRC.

Applications

These data show that the increased use of chemotherapy for CRC and better surgical techniques are directly linked to improved survival.

Peer review

This article compares survival differences between two cohorts of patients operated by colorectal cancer from 1980 to 1994 and 2001. It emphasizes the

importance of the highest percentage of patients receiving chemotherapy and better postsurgical mortality in the last group doubled over the last 20 years.

REFERENCES

1 Department of Health, Government of Spain. La situación del cáncer en España. Madrid. 2005
2 **Bresalier RS.** Malignant neoplasms of the large intestine. In: Feldman M, Friedman LS, editors. Sleisenger & Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management. Sleisenger. 7 th ed. Philadelphia, Pennsylvania: Saunders, 2002: 2215-2261
3 **Allegra C**, Sargent DJ. Adjuvant therapy for colon cancer--the pace quickens. *N Engl J Med* 2005; **352**: 2746-2748
4 **Piñol V**, Castells A, Andreu M, Castellví-Bel S, Alenda C, Llor X, Xicola RM, Rodríguez-Moranta F, Payá A, Jover R, Bessa X; Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. *JAMA* 2005; **293**: 1986-1994
5 **Rodríguez-Moranta F**, Castells A, Andreu M, Piñol V, Castellví-Bel S, Alenda C, Llor X, Xicola RM, Jover R, Payá A, Bessa X, Balaguer F, Cubiella J, Argüello L, Morillas JD, Bujanda L; Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. Clinical performance of original and revised Bethesda guidelines for the identification of MSH2/MLH1 gene carriers in patients with newly diagnosed colorectal cancer: proposal of a new and simpler set of recommendations. *Am J Gastroenterol* 2006; **101**: 1104-1111
6 TNM Atlas. Ilustrated Guide to the TNM/pTNM Classification of Malignant Tumors, 4.ª ed. Berlín: Springer Verlag, 1997
7 **Curreri AR,** Asnsfield FJ, McIver FA, Waisman HA, Heidelberger C. Clinical studies with 5-fluorouracil. *Cancer Res* 1958; **18**: 478-484
8 **Buyse M**, Zeleniuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer. Why we still don't know. *JAMA* 1988; **259**: 3571-3578
9 **Gill S**, Loprinzi CL, Sargent DJ, Thomé SD, Alberts SR, Haller DG, Benedetti J, Francini G, Shepherd LE, Francois Seitz J, Labianca R, Chen W, Cha SS, Heldebrant MP, Goldberg RM. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004; **22**: 1797-1806
10 **Mayer RJ**. Two steps forward in the treatment of colorectal cancer. *N Engl J Med* 2004; **350**: 2406-2408
11 **Canivet JL**, Damas P, Desaive C, Lamy M. Operative mortality following surgery for colorectal cancer. *Br J Surg* 1989; **76**: 745-747
12 **Ruiz Montesinos I**, Elorza Orúe JL, Alcón Caracena A, Olaizola Ayerdi A, Irazusta Goena M, Álvarez Caperochipi FJ. Evolución del carcinoma colorrectal a lo largo de una década. *Cir Esp* 1997; **61**: 273-277
13 **Semmens JB**, Platell C, Threlfall TJ, Holman CD. A population-based study of the incidence, mortality and outcomes in patients following surgery for colorectal cancer in Western Australia. *Aust N Z J Surg* 2000; **70**: 11-18
14 **Nickelsen TN**, Jørgensen T, Kronborg O. Thirty-day mortality after surgery for colorectal cancer in Denmark. *Colorectal Dis* 2005; **7**: 500-506
15 **Glen P**, Simpson MF, Donnelly L, Leonard S, Macdonald A. Thirty-day mortality from colorectal cancer surgery within a deprived population. *Colorectal Dis* 2005; **7**: 193-195
16 **Alves A**, Panis Y, Mathieu P, Mantion G, Kwiatkowski F, Slim K. Postoperative mortality and morbidity in French patients undergoing colorectal surgery: results of a prospective multicenter study. *Arch Surg* 2005; **140**: 278-283, discussion 284
17 **Ong ES**, Alassas M, Dunn KB, Rajput A. Colorectal cancer surgery in the elderly: acceptable morbidity? *Am J Surg* 2008; **195**: 344-348; discussion 348
18 **Kelz RR**, Gimotty PA, Polsky D, Norman S, Fraker D, DeMichele A. Morbidity and mortality of colorectal carcinoma surgery differs by insurance status. *Cancer* 2004; **101**: 2187-2194
19 **Itani KM**, Denwood R, Schifftner T, Joehl RJ, Wright C, Henderson WG, DePalma RG. Causes of high mortality in colorectal surgery: a review of episodes of care in Veterans Affairs hospitals. *Am J Surg* 2007; **194**: 639-645
20 **Boschi L**, Lecce F, Bazzocchi F, Del Gaudio A. [Surgical treatment of rectal cancer: comparison of anterior resection and abdominoperineal excision] *Minerva Chir* 1995; **50**: 831-834
21 **Crucitti F**, Sofo L, Doglietto GB, Bellantone R, Perri V, Zucchetti F. [Mechanical sutures in surgery of cancer of the rectum (a personal contribution)] *Chir Ital* 1983; **35**: 823-830
22 **Tejero E**, Mainar A, Fernández L, Tobío R, De Gregorio MA. New procedure for the treatment of colorectal neoplastic obstructions. *Dis Colon Rectum* 1994; **37**: 1158-1159
23 **Tilney HS**, Lovegrove RE, Purkayastha S, Sains PS, Weston-Petrides GK, Darzi AW, Tekkis PP, Heriot AG. Comparison of colonic stenting and open surgery for malignant large bowel obstruction. *Surg Endosc* 2007; **21**: 225-233

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BRIEF ARTICLE

Surgical outcome after docetaxel-based neoadjuvant chemotherapy in locally-advanced gastric cancer

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Abstract

AIM: To investigate feasibility, morbidity and surgical mortality of a docetaxel-based chemotherapy regimen randomly administered before or after gastrectomy in patients suffering from locally-advanced resectable gastric cancer.

METHODS: Patients suffering from locally-advanced (T3-4 any N M0 or any T N1-3 M0) gastric carcinoma, staged with endoscopic ultrasound, bone scan, computed tomography, and laparoscopy, were assigned to receive four 21 d/cycles of TCF (docetaxel 75 mg/m^2 day 1, cisplatin 75 mg/m^2 day 1, and fluorouracil 300 mg/m^2 per day for days 1-14), either before (Arm A) or after (Arm B) gastrectomy. Operative morbidity, overall mortality, and severe adverse events were compared by intention-to-treat analysis.

RESULTS: From November 1999 to November 2005, 70 patients were treated. After preoperative TCF (Arm A), thirty-two (94%) resections were performed, 85% of which were R0. Pathological response was complete in 4 patients (11.7%), and partial in 18 (55%). No surgical mortality and 28.5% morbidity rate were observed, similar to those of immediate surgery arm (P = 0.86). Serious chemotherapy adverse events tended to be more frequent in arm B (23% *vs* 11%, P = 0.07), with a single death per arm.

CONCLUSION: Surgery following docetaxel-based chemotherapy was safe and with similar morbidity to immediate surgery in patients with locally-advanced resectable gastric carcinoma.

Key words: Gastric cancer; Docetaxel; Neoadjuvant chemotherapy; Laparoscopy; Endoscopic ultrasonography; Morbidity

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INTRODUCTION

In spite of a declining incidence in the Western world, gastric cancer is still a major malignant disease in many populations, and the second leading cause for cancer mortality worldwide[1]. While localized disease, limited to the submucosa, can be best treated surgically, with a long-term survival of 70%-95%, the prognosis of locally-advanced tumor is poorer, due to a high unresectability rate at presentation, and a much higher relapse rate after radical surgery[2], thus demanding further studies regarding adjuvant and neoadjuvant treatment. Docetaxel (Taxotere®; Sanofi-Aventis, Paris, France) has been approved for treatment of metastatic gastric cancer, when combined with cisplatin and infused fluorouracil (TCF regimen), showing superiority in survival, time to progression, and response rate (RR) *vs* cisplatin/fluorouracil (CF) in a randomized phase Ⅲ trial[3]. A better RR for docetaxel/cisplatin (TC) *vs* epirubicin/cisplatin/protracted venous infusion fluorouracil (ECF) has been documented in a randomized phase Ⅱ trial[4]. These data suggested investigational use TCF in a preoperative neoadjuvant setting. This analysis aimed to test the hypothesis that preoperative chemotherapy with TCF does not influence negatively the results of subsequent surgery, when compared to immediate surgery. Primary endpoints of this study were operative morbidity and mortality rates; secondary endpoints were surgical and pathological assessments of downstaging and assessment by the surgeon as to whether the surgery was curative or not.

MATERIALS AND METHODS

Patient selection and treatment

Patients with histologically-proven locally-advanced resectable gastric carcinoma (T3-4 any N M0 or any T N1-3 M0 as defined in the 1997 TNM classification) were screened for eligibility. Other inclusion criteria were: World Health Organization (WHO) performance status ≤ 2; age 18-75 years; adequate blood counts (white blood cell count ≥ 4000/mm^3, platelets ≥ 100 000/mm^3); normal renal (calculated creatinine clearance ≥ 60 mL/min) and liver function. Patients suffering from Siewert type I cardia location adenocarcinoma (extended mostly into the lower esophagus) were excluded. All patients underwent chest X-ray, gastric endoscopic ultrasound (EUS), spiral thoraco-abdominal computerized tomography (CT) scan, bone scintigraphy and staging laparoscopy to define nodal status and rule out distant deposits and/or peritoneal seeding.

The trial was approved in all centers by relevant ethics committees. All patients gave written informed consent for participation in the trial.

Patients were stratified by center, tumor size, tumor location (cardia adenocarcinoma Siewert Ⅱ and Ⅲ *vs* tumors of the rest of the stomach) and nodal status (N+ *vs* N-). Patients received four 21-d/cycles of TCF (docetaxel 75 mg/m^2, 1-h Ⅳ infusion, day 1; cisplatin 75 mg/m^2 4-h Ⅳ infusion, day 1; and 5-fluorouracil (5-FU) 300 mg/m^2 per day continuous Ⅳ infusion, days 1 to 14), either preoperatively (Arm A) or postoperatively (Arm B). Just before starting chemotherapy all patients underwent placement of a totally implantable central venous port. In Arm A, a re-evaluation was performed after 2 cycles. If local progression had occurred, then the patient immediately underwent surgery. Otherwise two more TCF cycles were administered and surgery was performed within 3-5 wk after day 1 of the last cycle. In Arm B, surgery was scheduled to take place within 1 wk after randomization. Postoperative TCF was to be initiated 3 to 6 wk after surgery.

Perioperative complications

Data about postoperative course and complications were reported on hospital cards by surgical teams. In addition, an epidemiology nurse was in charge of regularly collecting microbiology data with respect to nosocomial infections (surgical site, pulmonary, urinary, and/or intravascular catheter infections). Data on hospital infections were regularly submitted to the infection central committees on a 3-mo basis.

As conclusive assessments of surgical procedures remain difficult, and there is a lack of consensus on how to define complications and to stratify them by severity, we decided to apply a single classification, proposed by Dindo *et al*[5], which is based on the evaluation of a cohort of 6336 patients and the results of a survey. In this classification, the therapy used to correct a specific complication is the cornerstone in ranking a complication. For example, life-threatening complications requiring an intermediate or intensive care_management (IC/ICU) have to be differentiated from complications treated on the ward; as such complications are associated with a high mortality, stress for the patients, and substantial resource consumption. Therefore, registered complications in both groups were analyzed accordingly, with the exception of those classified as grade I (deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic or radiological interventions). This grade also included wound infections treated at bedside.

Surgery

A careful intraoperative staging of disease was first performed, in order to rule out peritoneal seeding, ovarian involvement, "drop" metastasis in the pelvis, or periaortic gross adenopathy. Attention was directed to the liver, greater omentum and root of the mesentery below the transverse colon. The stomach was always inspected and gently

Table 1 Extent of lymphadenectomy D1 *vs* D2 according to the JRSGC[5,6]

	D1	D2
Upper third	1-2-3-4	D1+5-6-7-8-9-10-11-110
Middle third	1-3-4-5-6	D1+2-7-8 -9 -10 -11
Lower third	3-4-5-6	D1+1-7-8-9

JRSGC: Japanese Research Society for the Study of Gastric Cancer.

palpated to assess the location and the extent of the tumor and to exclude direct invasion of adjacent structures. Frozen sections of every suspect tissue were obtained (e.g. preaortic, infracolic nodes); intraoperative, histology-proven recognition of metastatic spread was considered an exclusion criterion. The extent of gastrectomy depended on the proximal distance of the tumor from the cardia; therefore, total gastrectomy was performed in all patients with cardia locations and in those having antral and body tumors in whom a 6-cm gross proximal margin could not be obtained. Subtotal distal gastrectomy was performed in the others (almost exclusively small-size body or antral locations). Proximal gastric resection was never carried out, as total gastrectomy with 2-3 cm extent to abdominal esophagus was chosen for all cardia locations.

Lymphadenectomy included excision of all N1 and most N2 stations (stations 7, 8, 9 and station 11), according to the classification of the Japanese Research Society for the Study of Gastric Cancer (JRSGC)[6,7] (Table 1). Hepatoduodenal ligament nodes (station 12) were also dissected, limiting the lymphadenectomy to the 12a station (left side of the hepatic artery), and leaving undissected the parts b and p of the station (right side of the ligament and just posteriorly to the portal vein, respectively). Lymph nodes of the surgical specimen were routinely dissected by experienced pathologists, using standard techniques; in some cases, depending on the pathologist's judgement, clearing fixatives were used prior to the dissection. Splenectomy was only carried out in cases of direct invasion of the spleen by the tumor, or gross appearance of metastatic nodes at station 10 (splenic hilum). Caudal pancreas was always preserved, according to the Maruyama's technique, even when splenectomy was performed, unless tumor direct involvement was clinically evident.

Surgeons were asked to document the extent of node dissection and to state whether the procedure was likely to be curative as follows: (1) Absolutely curative: absence of hepatic and/or peritoneal metastasis; serosa not involved; no infiltration within 10 mm of the proximal resection line; (2) Relatively curative: as above, but serosa involved, and/or cancer infiltrates within 10 mm of the proximal resection line, and/or nodal involvement (N stage) equals D number; and (3) Non-radical: resection line involvement; any residual disease after resection.

Reconstruction technique (Roux-en-Y, Braun or others) was entirely left to the discretion of the surgeon.

Pathology

Pathological response to chemotherapy was centrally evaluated and classified as follows: (1) Complete response (pCR): No residual tumor could be found after *in toto* examination of the potential tumor site. Acellular mucus or acellular necrosis in the gastric wall or in lymph nodes was not considered as residual tumor and therefore was not taken into consideration for staging (neither for T, nor for N); (2) Partial microscopic response: Microscopic residual tumor (persistence of microscopic islands of tumor cells); (3) Partial macroscopic response: Macroscopic residual tumor, but overt necrosis or calcification, or downstaging of the tumor; and (4) No response: Only minor necrosis and no downstaging of the tumor.

Statistical analysis

Initially a target sample size at 240 patients was set, assuming a 3-year event-free survival rate of 20% in arm B and 35% in arm A (+ 15%). Trial was prematurely stopped at 70 randomized patients, due to insufficient accrual. Only two centers out of nine showed a good accrual rate, whereas most participating groups were not ready to be involved in such a multi-disciplinary approach. Moreover, some patients refused to participate to this kind of trial because they wanted to be operated on as soon as possible. For this reason, these study results are underpowered to detect any possibly significant differences in the experimental groups. Nevertheless, results were descriptively compared between the two arms on an intention-to-treat basis, using Mann-Whitney *U*-test and Kruskal-Wallis test to compare means and medians, respectively. χ^2 or Fisher's exact test were used to compare proportions. All tests were two-sided. A P value less than 0.05 was assumed significant. Intention-to-treat principle was adopted.

RESULTS

This trial was activated in November 1999 and closed in November 2005 due to insufficient accrual. From December 1999 to August 2005 a total of 70 patients were enrolled from 9 Institutions in three countries. Eighty-five percent of included patients were from two Institutions; from Milan (IEO-European Institute of Oncology) and Geneva (University Hospitals of Geneva). One patient withdrew consent, did not receive any chemotherapy, and was excluded from the analysis. Of the remaining 69 patients, 34 were randomized to Arm A (TCF followed by surgery) and 35 to Arm B (surgery followed by TCF). One patient in Arm A did not receive any chemotherapy because he died before starting. This patient was included in the analysis, in agreement with the intention-to-treat principle. A trial profile, conforming to the Consolidated Standards of Reporting Trials (CONSORT) is shown in Figure 1. The two groups of patients were similar with respect to various characteristics (Table 2).

Table 3 shows details about surgical procedures performed and pathology reports. Thirty-two patients in Arm A (94%) underwent laparotomy: 29 (85%) had an R0 resection, and two a non-radical resection; one had no resection due to unsuspected peritoneal carcinomatosis. All 35 patients in Arm B underwent laparotomy; 32

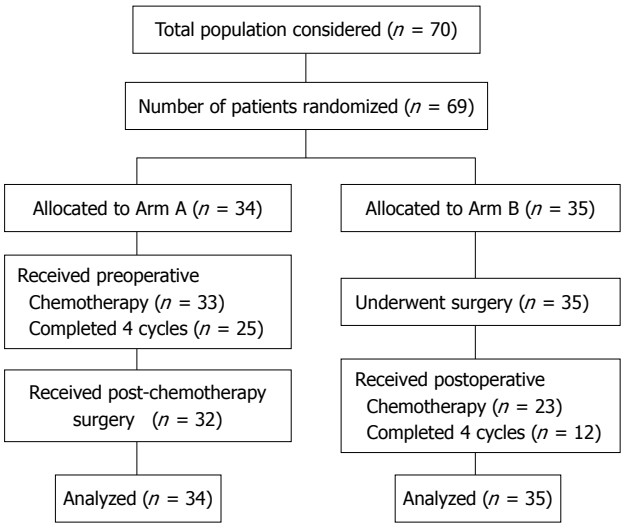

Figure 1 Trial profile conforming to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Table 2 Patient characteristics

	Arm A (*n* = 34)	Arm B (*n* = 35)
Age (yr): median (range)	57 (25-75)	59 (39-76)
Male (%)	68	71
PS 0/1/2 (%)	91/6/3	86/14/0
Tumor site (%)		
Cardia	21	20
Fundus/body	38	40
Antrum/pylorus	41	40
Stage (by EUS + CT scan)		
I B	2	1
II	14	13
III	18	21

EUS: Endoscopic ultrasound; CT: Computerized tomography.

(91%) had an R0 resection, two a non-radical resection and one no resection due to peritoneal carcinomatosis. In Arm A, pathological response was complete in 4 patients (11.7%), and partial (macro- or microscopic) in 18 (55%). The respective proportions of total *vs* subtotal gastrectomies, D ⩾ 2 *vs* D1 lymph node dissections, median number of excised lymph nodes and of metastatic nodes were very similar in the two arms of the study, and all differences were not statistically significant.

Postoperative mortality and morbidity events are detailed in Table 4; they are stratified by severity, applying the classification proposed by Dindo *et al*[5]. In Arm A these included four septic intraabdominal complications (one anastomotic leak, two abdominal abscesses and one infected fluid peritoneal collection), one gastrojejunal anastomosis bleeding, one pneumonia requiring ICU admission, one pulmonary embolism, one urinary infection, and one fever of unknown origin. Morbidity events in Arm B included three septic intraabdominal complications (one anastomotic leak, one abdominal abscess, and one infected fluid peritoneal collection), and six extra abdominal infections (one infected mediastinal collection, three pneumonias requiring ICU admission,

Table 3 Details of surgical procedures and pathology

	Arm A (*n* = 32)	Arm B (*n* = 35)
Complete resection R0 (%)	29 (85)	32 (91)
Non-radical resection (%)	2 (5.8)	2 (5.7)
No resection pM1 (peritoneum) (%)	1 (2.9)	1 (2.8)
pCR *n* (%)	4 (11.7)	NA
pPR *n* (%)	18 (55)	NA
Total gastrectomy	20	24
Subtotal gastrectomy	11	10
D-2 lymphadenectomy	29	31
D-3 lymphadenectomy	-	2
Excised lymph nodes median (range)	20 (9-39)	26 (13-76)
Metastatic lymph nodes median (range)	1 (0-23)	5 (0-50)

pCR: Pathological complete response; pPR: Pathological partial response; NA: Not available.

Table 4 Postoperative morbidity and mortality, ranked according to Dindo *et al*[5]

Type of complication	Arm A (*n* = 32)	Arm B (*n* = 35)
Anastomotic leak	1-IVb	1-IVb
Abdominal abscess	2-IIIa	1-IIIa
Infected peritoneal collection	1-IIIa	1-IIIa
Anastomotic bleeding	1-IIIb (re-operation)	-
Pneumonia requiring ICU	1-IVa	3-IVa-IVa, V (re-operation, death)
Pulmonary embolism	1-II	-
Urinary infection	1-II	-
Fever of unknown origin	1-II	-
Mediastinal infected collection + MOF	-	1-V (re-operation, death)
Central venous catheter-related blood stream infection	-	2-II
Total[a]	9 (28.5%), 1 re-operation	9 (25.7%), 2 re-operations, 1 death

[a]*P* = 0.86. ICU: Intensive care unit; MOF: Multiple organ failure.

and two central venous catheter-related blood stream infections). Three re-operations were performed, one in Arm A due to anastomotic hemorrhage, and two in Arm B, due to infected mediastinal collection and pleural empyema complicating severe pneumonia, respectively. Overall, 9 morbidity events occurred in each arm (28.5% in Arm A and 25.7% in Arm B). Two postoperative deaths occurred, in Arm B, as a consequence of multiple organ failure (MOF) complicating mediastinal infected fluid collection, in spite of re-operation. All these differences were not statistically significant ($P = 0.86$).

A total of 189 TCF cycles were administered; 118 in Arm A and 71 in Arm B (Table 5). In Arm A, 25 patients (74%) received all 4 cycles, two patients 3 cycles, and six patients 2 cycles. In Arm B, only 12 patients received all 4 cycles (34%), five patients 3 cycles, two patients 2 cycles, four patients 1 cycle and 12 patients received no cycle. A 64-year-old female patient who had received one cycle of preoperative TCF died after severe worsening of performance status and dyspnoea. Excluding this case, serious adverse events (SAEs) oc-

Table 5 Treatment administration and SAEs

	Arm A (n = 33)	Arm B (n = 23)
Total number of cycles	118	71
Causes of treatment failure		
Progression of disease	1	0
G4 toxicity	2	6
Death	1	1
Patient refusal	1	1
Investigator's decision	2	3
Other	1	1
Total	8	12
Severe adverse events (% of cycles)[a]	13 (11)	16 (23)
No. of patients involved (% pts. treated)[b]	10 (30)	14 (60)

[a]P = 0.07, [b]P = 0.15. SAE: Severe adverse event.

curred more frequently in Arm B. In Arm A, 13 SAEs in 10 patients were observed (13 SAEs out of 118 cycles = 11%), 7 of them infectious (3 febrile neutropenia). In Arm B, 16 SAEs occurred in 14 patients (16 SAEs out of 71 cycles = 23%). All these differences were not statistically significant (P = 0.07 and 0.15, respectively).

A 58-year-old male patient in Arm B died suddenly 38 d after gastrectomy from severe arrhythmia and pulmonary infection. Table 5 details reasons for cessation of therapy in the two arms of the study.

DISCUSSION

Prognosis of locally-advanced gastric cancer is generally poor in Western surgical and population-based series, with 5-year overall survival rates of 25% or less[8], in spite of complete excision of the gastric and nodal components of the disease[2]. This is the consequence of a high relapse rate after radical surgery, and has prompted many studies in the last decade, aimed at improving these results by means of adjuvant and neoadjuvant treatments[9-12]. Both these approaches remain controversial and are under current investigation. A large randomized trial [Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC)][13], demonstrated a survival benefit with the use of perioperative chemotherapy (i.e. pre- and postoperatively delivered) as compared with surgery alone. Similarly, the FFCD 9703 trial[14] showed an improvement in both disease free survival (DFS) and overall survival (OS) with the use of perioperative chemotherapy (FP regimen: 5-FU continuous infusion + cisplatin) as compared to surgery. To date, no trial has so far investigated the effects of the same chemotherapy regimen given either pre- or postoperatively.

Although our study is underpowered to detect any possible significant difference in short-term postoperative outcome, it gives some preliminary answers to questions not yet available in the medical literature that could be interesting for future studies. The first relevant information provided by the present study is the safety of surgery following a preoperative docetaxel-based chemotherapy regimen. In fact, we did not register any mortality and we had a 28.5% morbidity rate, without any significant difference between pre- and postoperative administration of chemotherapy. Although our patient population was slightly different from that of the MAGIC and FFCD trials, since Type I Siewert adenocarcinomas of the lower third of the esophagus were excluded, results of our study compare favorably with those of the MAGIC trial, where a 45% morbidity rate and 5% mortality rate were observed. In the FFCD trial, postoperative morbidity was 21% in the surgical arm, and 28% in the perioperative chemotherapy arm, whereas surgical mortality was 5% for both groups. A possible favorable factor was that 85% of patients in our series were operated on in two high-volume institutions where D2 gastrectomy is routinely carried out as standard treatment of gastric cancer. Our results support the conclusions that D2 gastrectomy can be considered a safe treatment of gastric cancer in Western patients, at least when performed in experienced centers[15], and that gastric cancer resection should probably be added to the growing list of procedures which are safer when performed in high-volume institutions[16,17]. This could explain why reports from single large volume institutions continue to demonstrate low operative mortality after D2 radical gastrectomy, while randomized trials show no survival benefit and severely increased surgical mortality after this procedure[18,19].

In addition, our data indicate that chemotherapy-related SAEs tended to be more frequent in Arm B (adjuvant) than in Arm A (neoadjuvant), suggesting that lower patient tolerance to treatment is a key factor in determining higher toxicity. This could be explained by an increased risk of gastrointestinal toxicity after surgery, when patients are already deeply affected in their eating capacity by the gastrectomy. For instance, it was shown that, in a population of 23 patients followed for dietary intake and nutritional status after total gastrectomy, no patient reached recommended dietary allowances by first monthly follow up[20].

Our trial confirms the difficulties in administering intensive adjuvant chemotherapy in gastric cancer. In the MAGIC trial, 34% of patients who completed preoperative chemotherapy and surgery did not start postoperative chemotherapy, mostly owing to early progression, patient refusal and/or surgical complication. In the weekly-PELF trial[21], only 14% of experimental arm patients completed the scheduled adjuvant treatment without time and/or dose modifications. Even without preoperative chemotherapy, 12 patients in this series did not receive any adjuvant chemotherapy. The most frequent reasons were patient refusal and medical decision. However, even when adjuvant chemotherapy was started, only 34% of the patients received all the four cycles. In the ACT-GC Group Trial (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer), evaluating an oral fluoropyrimidine as adjuvant agent, enrollment was stopped after 1 year as a consequence of the higher rate of overall survival in the S-1 treated group than in controls who had only surgery[22]. Nevertheless, among the 517 patients who received S-1, 71 refused to continue treatment because of adverse events, and in 72 the

decision of the investigators was to terminate treatment because of adverse events or complications (143/571, 25.04%). The dose of S-1 was reduced in 219 of the 517 treated patients (42.4%).

Laparoscopy has been reported to improve clinical staging when compared to conventional methods, identifying unexpected peritoneal or liver metastases in up to 20% of operable patients[23,24]; consequently, a significant proportion of patients can avoid unnecessary laparotomy[25]. Although in our study staging laparoscopy has been confirmed as an effective tool to demonstrate peritoneal deposits even when missed by preoperative CT scan, minimal peritoneal deposits were found and biopsied at laparotomy in 3 patients previously judged peritoneal seeding-free at laparoscopy. These false-negative results of laparoscopy occurred within the omentum and/or the lesser sac, emphasizing the limits of staging laparoscopy to demonstrate a minimal peritoneal spread in these difficult locations. Similarly, in a recent report, the sensitivity for detecting peritoneal carcinomatosis was 85% for laparoscopy[26,27]. The possible role of the preoperative PET scan in reducing the rate of false negative results of staging laparoscopy is currently under investigation, with conflicting preliminary evidence[28,29]; it seems a priori highly improbable that such small-volume disease could be detected by this imaging technique.

Finally, our data confirm the huge difficulties in performing this kind of study, which requires a high level of cooperation between different disciplines. Principal investigators analyzed the reasons for the slow accrual of patients for their neoadjuvant study with FAMTX[30] for operable gastric cancer, and observed that around half of the participating centers were not ready for such a multi-disciplinary approach, not believing in the potential efficacy of the neoadjuvant treatment. Moreover, several patients refused to participate in this kind of trial because they wanted to be operated on as soon as possible.

In conclusion, our study does not provide information on efficacy of preoperatively-delivered TCF, due to early discontinuation for slow accrual. It is also underpowered to detect any possible significant differences in short-term postoperative outcome. Nevertheless, data regarding TCF efficacy and feasibility in the preoperative setting and TCF feasibility in the adjuvant setting could be interesting for future studies. In particular, neoadjuvant TCF achieved promising results with a 12% pCR rate. This evidence prompts further studies, since patients achieving a pCR tend to have a much better outcome, as underlined in a recent phase Ⅱ trial of preoperative chemo-radiation therapy for resectable gastric cancer[31]. Surgery was safe after TCF preoperative chemotherapy, while toxicity (especially gastrointestinal) made adjuvant postoperative TCF more difficult to administer fully compared to the neoadjuvant setting. These data are consistent with the results of the recent FFCD trial[14], where postoperative chemotherapy was completed in less than 50% of the patients. This should be carefully considered when an intensive adjuvant chemotherapy regimen is planned.

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COMMENTS

Background

In spite of a declining incidence in the Western world, gastric cancer is still a major malignant disease in many populations, and the second leading cause for cancer mortality worldwide. While localized disease, limited to the submucosa, can be best treated surgically, with a long-term survival rate of 70%-95%, the prognosis of locally-advanced tumor is poorer, due to a high unresectability rate at presentation, and a much higher relapse rate after radical surgery. Docetaxel (Taxotere®; Sanofi-Aventis, Paris, France) has been approved for treatment of metastatic gastric cancer, when combined with cisplatin and infused fluorouracil (TCF regimen), showing superiority in survival, time to progression, and response rate (RR) *vs* cisplatin/fluorouracil (CF) in a randomized phase Ⅲ trial.

Research frontiers

The above mentioned results obtained in metastatic disease suggested the investigational use of the TCF regimen in a preoperative neoadjuvant setting. The present trial aimed to test the hypothesis that preoperative chemotherapy with TCF does not influence negatively the results of subsequent surgery, when compared to immediate surgery.

Innovations and breakthroughs

This trial proved that surgery is safe after TCF preoperative chemotherapy, while toxicity (especially gastrointestinal) makes adjuvant postoperative TCF more difficult to administer fully compared to the neoadjuvant setting. Moreover, neoadjuvant TCF achieved promising results with a 12% pCR (pathological complete response) rate.

Applications

Obtained data regarding TCF efficacy and feasibility in the preoperative setting and TCF feasibility in the adjuvant setting could be interesting for future studies. In fact, patients achieving a pCR tend to have a much better oncology outcome. Finally, data here presented are consistent with the results of the recent FFCD trial, where postoperative chemotherapy was completed in less than 50% of the patients. This should be carefully considered when an intensive adjuvant chemotherapy regimen is planned.

Peer review

This is an interesting report of the efficacy of neoadjuvant chemotherapy for advanced gastric cancer.

REFERENCES

1 **Forman D**, Burley VJ. Gastric cancer: global pattern of the disease and an overview of environmental risk factors. *Best Pract Res Clin Gastroenterol* 2006; **20**: 633-649

2 **Briasoulis E**, Liakakos T, Dova L, Fatouros M, Tsekeris P, Roukos DH, Kappas AM. Selecting a specific pre- or postoperative adjuvant therapy for individual patients with operable gastric cancer. *Expert Rev Anticancer Ther* 2006; **6**: 931-939

3 **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

4 **Roth AD**, Fazio N, Stupp R, Falk S, Bernhard J, Saletti P, Köberle D, Borner MM, Rufibach K, Maibach R, Wernli M, Leslie M, Glynne-Jones R, Widmer L, Seymour M, de Braud F. Docetaxel, cisplatin, and fluorouracil; docetaxel and cisplatin; and epirubicin, cisplatin, and fluorouracil as systemic treatment for advanced gastric carcinoma: a randomized phase II trial of the Swiss Group for Clinical Cancer Research. *J Clin Oncol* 2007; **25**: 3217-3223

5 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213

6 **Japanese Research Society for Gastric Cancer**. Japanese classification of gastric carcinoma. First English Edition. Tokio: Kanehara & Co. Ltd, 1995

7 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma-2nd English Edition-*Gastric Cancer* 1998; **1**: 10-24

8 **Siewert JR**, Böttcher K, Roder JD, Busch R, Hermanek P, Meyer HJ. Prognostic relevance of systematic lymph node dissection in gastric carcinoma. German Gastric Carcinoma Study Group. *Br J Surg* 1993; **80**: 1015-1018

9 **Hermans J**, Bonenkamp JJ, Boon MC, Bunt AM, Ohyama S, Sasako M, Van de Velde CJ. Adjuvant therapy after curative resection for gastric cancer: meta-analysis of randomized trials. *J Clin Oncol* 1993; **11**: 1441-1447

10 **Earle CC**, Maroun JA. Adjuvant chemotherapy after curative resection for gastric cancer in non-Asian patients: revisiting a meta-analysis of randomised trials. *Eur J Cancer* 1999; **35**: 1059-1064

11 **Janunger KG**, Hafström L, Glimelius B. Chemotherapy in gastric cancer: a review and updated meta-analysis. *Eur J Surg* 2002; **168**: 597-608

12 **Lim L**, Michael M, Mann GB, Leong T. Adjuvant therapy in gastric cancer. *J Clin Oncol* 2005; **23**: 6220-6232

13 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20

14 **Boige V**, Pignon J, Saint-Aubert B, Lasser P, Conroy T, Bouché O, Segol P, Bedenne L, Rougier P, Ychou M. Final results of a randomized trial comparing preoperative 5-fluorouracil (F)/cisplatin (P) to surgery alone in adenocarcinoma of stomach and lower esophagus (ASLE): FNLCC ACCORD 07-FFCD 9703 trial [Abstract]. *Proc Am Soc Clin Oncol* 2007; **25** (18S): 4510

15 **Biffi R**, Chiappa A, Luca F, Pozzi S, Lo Faso F, Cenciarelli S, Andreoni B. Extended lymph node dissection without routine spleno-pancreatectomy for treatment of gastric cancer: low morbidity and mortality rates in a single center series of 250 patients. *J Surg Oncol* 2006; **93**: 394-400

16 **Degiuli M**, Sasako M, Calgaro M, Garino M, Rebecchi F, Mineccia M, Scaglione D, Andreone D, Ponti A, Calvo F. Morbidity and mortality after D1 and D2 gastrectomy for cancer: interim analysis of the Italian Gastric Cancer Study Group (IGCSG) randomised surgical trial. *Eur J Surg Oncol* 2004; **30**: 303-308

17 **Sierra A**, Regueira FM, Hernández-Lizoáin JL, Pardo F, Martínez-Gonzalez MA, A-Cienfuegos J. Role of the extended lymphadenectomy in gastric cancer surgery: experience in a single institution. *Ann Surg Oncol* 2003; **10**: 219-226

18 **Cuschieri A**, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, Sydes M, Fayers P. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. *Br J Cancer* 1999; **79**: 1522-1530

19 **Hartgrink HH**, van de Velde CJ, Putter H, Bonenkamp JJ, Klein Kranenbarg E, Songun I, Welvaart K, van Krieken JH, Meijer S, Plukker JT, van Elk PJ, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H, Sasako M. Extended lymph node dissection for gastric cancer: who may benefit? Final results of the randomized Dutch gastric cancer group trial. *J Clin Oncol* 2004; **22**: 2069-2077

20 **Braga M**, Zuliani W, Foppa L, Di Carlo V, Cristallo M. Food intake and nutritional status after total gastrectomy: results of a nutritional follow-up. *Br J Surg* 1988; **75**: 477-480

21 **Cascinu S**, Labianca R, Barone C, Santoro A, Carnaghi C, Cassano A, Beretta GD, Catalano V, Bertetto O, Barni S, Frontini L, Aitini E, Rota S, Torri V, Floriani I, Pozzo C, Rimassa L, Mosconi S, Giordani P, Ardizzoia A, Foa P, Rabbi C, Chiara S, Gasparini G, Nardi M, Mansutti M, Arnoldi E, Piazza E, Cortesi E, Pucci F, Silva RR, Sobrero A, Ravaioli A. Adjuvant treatment of high-risk, radically resected gastric cancer patients with 5-fluorouracil, leucovorin, cisplatin, and epidoxorubicin in a randomized controlled trial. *J Natl Cancer Inst* 2007; **99**: 601-607

22 **Sakuramoto S**, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, Furukawa H, Nakajima T, Ohashi Y, Imamura H, Higashino M, Yamamura Y, Kurita A, Arai K. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. *N Engl J Med* 2007; **357**: 1810-1820

23 **D'Ugo DM**, Coppola R, Persiani R, Ronconi P, Caracciolo F, Picciocchi A. Immediately preoperative laparoscopic staging for gastric cancer. *Surg Endosc* 1996; **10**: 996-999

24 **Lehnert T**, Rudek B, Kienle P, Buhl K, Herfarth C. Impact of diagnostic laparoscopy on the management of gastric cancer: prospective study of 120 consecutive patients with primary gastric adenocarcinoma. *Br J Surg* 2002; **89**: 471-475

25 **Hünerbein M**, Rau B, Hohenberger P, Schlag PM. The role of staging laparoscopy for multimodal therapy of gastrointestinal cancer. *Surg Endosc* 1998; **12**: 921-925

26 **Nakagawa S**, Nashimoto A, Yabusaki H. Role of staging laparoscopy with peritoneal lavage cytology in the treatment of locally advanced gastric cancer. *Gastric Cancer* 2007; **10**: 29-34

27 **Gretschel S**, Siegel R, Estévez-Schwarz L, Hünerbein M, Schneider U, Schlag PM. Surgical strategies for gastric cancer with synchronous peritoneal carcinomatosis. *Br J Surg* 2006; **93**: 1530-1535

28 **Chen J**, Cheong JH, Yun MJ, Kim J, Lim JS, Hyung WJ, Noh SH. Improvement in preoperative staging of gastric adenocarcinoma with positron emission tomography. *Cancer* 2005; **103**: 2383-2390

29 **Lim JS**, Kim MJ, Yun MJ, Oh YT, Kim JH, Hwang HS, Park MS, Cha SW, Lee JD, Noh SH, Yoo HS, Kim KW. Comparison of CT and 18F-FDG pet for detecting peritoneal metastasis on the preoperative evaluation for gastric carcinoma. *Korean J Radiol* 2006; **7**: 249-256

30 **Hartgrink HH**, van de Velde CJ, Putter H, Songun I, Tesselaar ME, Kranenbarg EK, de Vries JE, Wils JA, van der Bijl J, van Krieken JH. Neo-adjuvant chemotherapy for operable gastric cancer: long term results of the Dutch randomised FAMTX trial. *Eur J Surg Oncol* 2004; **30**: 643-649

31 **Ajani JA**, Winter K, Okawara GS, Donohue JH, Pisters PW, Crane CH, Greskovich JF, Anne PR, Bradley JD, Willett C, Rich TA. Phase II trial of preoperative chemoradiation in patients with localized gastric adenocarcinoma (RTOG 9904): quality of combined modality therapy and pathologic response. *J Clin Oncol* 2006; **24**: 3953-3958

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BRIEF ARTICLE

A novel cleansing score system for capsule endoscopy

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Abstract

AIM: To suggest a new cleansing score system for small bowel preparation and to evaluate its clinical efficacy.

METHODS: Twenty capsule endoscopy cases were reviewed and small bowel preparation was assessed with the new scoring system. For the assessment, two visual parameters were used: proportion of visualized mucosa and degree of obscuration. Representative frames from small bowel images were serially selected and scored at 5-min intervals. Intraclass correlation coefficient (ICC) was obtained to assess the reliability of the new scoring system. For efficacy evaluation and validation, scores of our new scoring system were compared with another previously reported cleansing grading system.

RESULTS: Concordance with the previous system, inter-observer agreement, and intra-patient agreement were excellent with ICC values of 0.82, 0.80, and 0.76, respectively. The intra-observer agreements at four-week intervals were also excellent. The cut-off value of adequate image quality was found to be 2.25.

CONCLUSION: Our new scoring system is simple, efficient, and can be considered to be applicable in clinical practice and research.

Key words: Capsule endoscopy; Cleansing score system

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INTRODUCTION

Capsule endoscopy (CE) was introduced as a method for investigating the full length of the small intestine[1-3]. However, there are limitations such as impaired visualization by air bubbles, food residue, bile, and blood clots. Unfortunately, current CE does not have functions which allow suctioning of fluid or washing the small bowel mucosa during the examination. Therefore, since some of the lesions can be overlooked and missed, adequate bowel cleansing is mandatory for a successful CE[4]. There have been many studies on the necessity and methods of bowel preparation for CE[1,5-8]. Howev-

er, the benefits of bowel preparation prior to CE remain controversial and it is unclear which method is best.

One of the reasons for this controversy is that the current grading systems have not been standardized and therefore, a generally accepted grading system is not available. The presence of numerous grading systems has caused difficulties in comparing the results of studies on small bowel preparation prior to CE[4,9]. In addition, most of the previously reported grading systems are time-consuming, complicated, and difficult to apply in the clinical setting. The aim of this study is to suggest a new cleansing score system for small bowel preparation and to evaluate its clinical efficacy.

MATERIALS AND METHODS

Twenty CE cases were reviewed according to the protocol by three examiners who had experience in interpreting more than 50 cases of CE. These examiners separately assessed small bowel cleanliness by using the grading system described below. The software program Rapid Reader 4 (Given Imaging Ltd, Yoqneam, Israel) was used to review and score the images.

Scoring system

Two visual parameters were used in our scoring system. The first parameter was the proportion of visualized mucosa. This was scored using a 4-step scale ranging from 0 to 3: score 3, greater than 75%; score 2, 50% to 75%; score 1, 25% to 50%; score 0, less than 25% (Table 1, Figure 1). The second parameter was the degree of obscuration by bubbles, debris, and bile *etc*. This was also scored using a 4-step scale ranging from 0 to 3: score 3, less than 5%, no obscuration; score 2, mild (5% to 25%) obscuration; score 1, moderate (25% to 50%) obscuration; score 0, severe (greater than 50%) obscuration (Table 1, Figure 1). Images from the entire small bowel were serially selected at 5-min intervals (1 frame/5 min) by manual mode using the RAPID system. If the capsule got stuck or remained in the same place for more than 5 min, the frames were scored only once and not repeatedly.

Mean scores of each parameter were obtained by summing the scores of all selected images and dividing them by the number of examined frames. The representative values for each parameter were then calculated by the overall average of two mean scores.

Efficacy evaluation

First, 20 CE cases were reviewed and frames were selected twice: once according to our scoring system and once according to one of the previously reported systems which evaluated CE images for 2 min in every 5-min period (e.g. 240 frames/5 min or 40% of the small bowel images)[6]. The images from selected frames were then graded using the aforementioned parameters. The concordance of scores between the two grading systems was analyzed.

Second, the reliability of our grading system was evaluated by assessing the inter-observer, intra-patient, and intra-observer agreement. For the assessment on inter-observer agreement, three examiners each scored the same selected frames separately at 5-min intervals which were then compared. For the evaluation of intra-patient agreement, each examiner reviewed the same case after choosing their own starting frame within the first 5 min of the capsule's entrance into the duodenum, from where the ensuing frames were picked up at 5-min intervals and scored accordingly. For the analysis on intra-observer agreement, the same frames from the same cases were scored once again after four weeks and scores were compared with the previous results.

Third, in order to determine the cut-off value for adequate cleansing, our scoring system was compared with another grading system, which examined all of the images obtained from the entire small bowel. This grading system defined small bowel mucosa as "clean" if less than 25% of the mucosal surface was covered by intestinal contents or food debris, and cleanliness was graded as "adequate" if the time the mucosa appeared clean was greater than 90% of the total examination time[5]. Overall adequacy was compared with that of our scoring system and the results were analyzed using the receiver operating characteristic (ROC) curve.

Table 1 CE image scoring system

The proportion of visualized mucosa	
Score 3	≥ 75%
Score 2	50%-75%
Score 1	25%-50%
Score 0	< 25%
The degree of bubbles, debris and bile	
Score 3	< 5%, no obscuration
Score 2	5%-25%, mild obscuration
Score 1	25%-50%, moderate obscuration
Score 0	≥ 50%, severe obscuration

CE: Capsule endoscopy.

Statistical analysis

Concordance was determined by using the intraclass correlation coefficient (ICC). An ICC value less than 0.40 was considered poor, between 0.40 and 0.75 was considered fair to good, and greater than 0.75 was considered excellent[6,10,11]. The cut-off value of the cleansing scores according to our scoring system, with optimal sensitivity and specificity, was determined using the ROC curve. Area under the curve (AUC) was used for assessing the overall accuracy of our scoring system which employed the ROC curve. All statistical analyses were performed with SPSS version 12.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Twenty cases were selected from previously diagnosed patients. Mean age of the subjects was 48.5 (21-80) years and 80% (18/20) were male. Their indications for CE

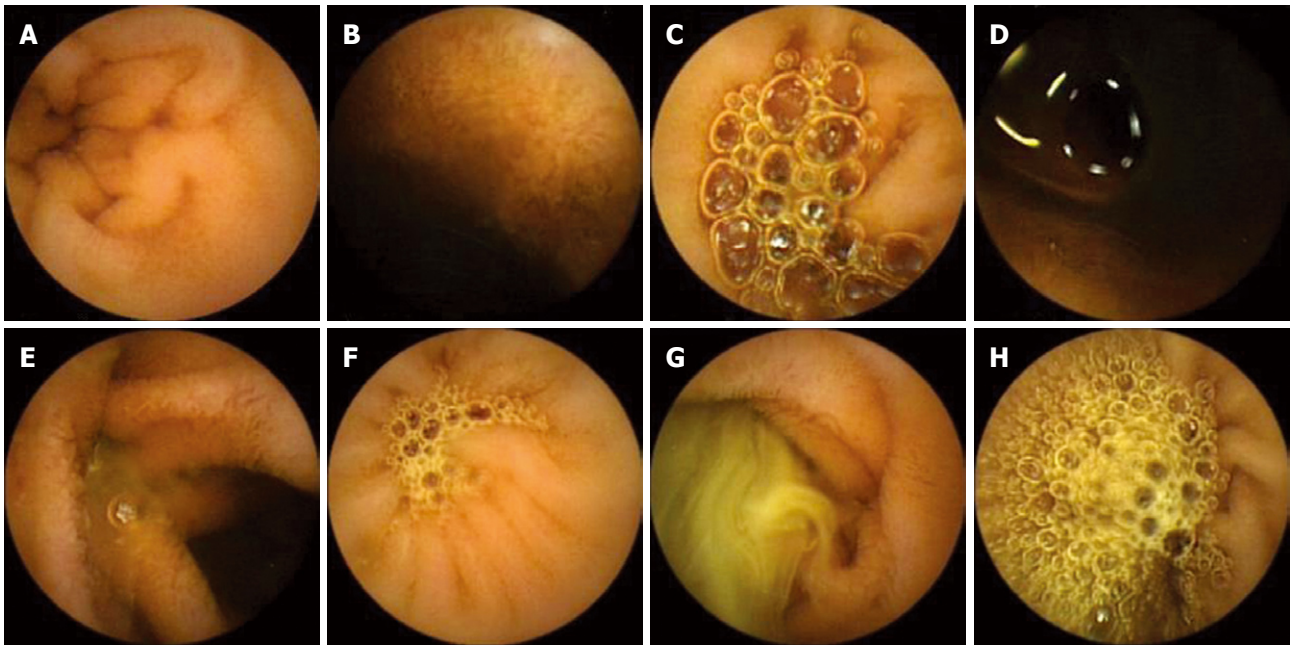

Figure 1 Images of scores according to the proportion of the visualized mucosa (A-D) and the degree of obscuration (E-H). A: Score 3; B: Score 2; C: Score 1; D: Score 0; E: Score 3; F: Score 2; G: Score 1; H: Score 0.

Table 2 Agreement between our scoring system A and previous scoring system B

Parameter	System	Score (median and IQR)	Inter-system ICC (95% CI)
Visualized mucosa	A	2.44 (2.20-2.62)	0.72 (0.27, 0.89)
	B	2.24 (2.11-2.48)	
Degree of obscuration	A	2.13 (1.92-2.35)	0.86 (0.53, 0.95)
	B	1.98 (1.87-2.22)	
Overall average	A	2.30 (2.09-2.50)	0.82 (0.33, 0.94)
	B	2.12 (1.97-2.36)	

A: 1 frame per 5 min; B: 2 min per 5 min. IQR: Interquartile range; ICC: Intraclass correlation coefficient.

Table 3 Inter-observer agreement of our scoring system among three examiners

Parameter	Observer	Score (median and IQR)	Inter-observer ICC (95% CI)
Visualized mucosa	A	2.61 (2.40-2.81)	0.88 (0.47, 0.99)
	B	2.62 (2.42-2.82)	
	C	2.44 (2.20-2.62)	
Degree of obscuration	A	2.34 (2.17-2.55)	0.71 (0.39, 0.87)
	B	2.28 (2.12-2.56)	
	C	2.13 (1.92-2.35)	
Overall average	A	2.47 (2.32-2.63)	0.80 (0.35, 0.93)
	B	2.47 (2.26-2.62)	
	C	2.30 (2.09-2.50)	

were gastrointestinal bleeding (12/20, 60%), iron deficiency anemia (4/20, 20%), abdominal pain (3/20, 15%), and diarrhea (1/20, 5%). Cases were prepared with 4 L of polyethylene glycol (PEG) four hours before examination without prokinetic agent or simethicone. The concordance between our cleansing score system and a previously reported system, which selected the frames and evaluated them for 2 min in every 5-min period, was excellent with an ICC value of 0.82 [95% confidence interval (CI), 0.33-0.94, Table 2).

As for the assessment on reliability, inter-observer and intra-patient agreement were excellent with ICC values of 0.80 (95% CI: 0.35-0.93, Table 3) and 0.76 (95% CI: 0.41-0.93, Table 4), respectively. The data regarding the assessment on intra-observer agreement was available from three examiners and the results were also excellent with ICC values of 0.80, 0.82, and 0.92, respectively (Figure 2).

To assess the overall adequacy of small bowel cleansing, the ROC curve was generated to determine the cut-off value of image quality. The cut-off value, estimated by the ROC curve at an optimal level of sensitivity and specificity, was 2.25 with 85% sensitivity and 87% specificity. The AUC of the scoring system was 0.925 (95% CI: 0.859-0.990, Figure 3).

DISCUSSION

CE has provided a new perspective for diagnosing, treating, and monitoring small bowel diseases, such as obscure GI bleeding, Crohn's disease, celiac sprue, polyposis syndromes, and small-bowel tumors.

However, CE has several limitations, one of which is image quality. Although 12 h-fasting or PEG ingestion is used for small bowel preparation in CE, air bubbles, intestinal secretions, bile or food residue occasionally cover the small bowel mucosa and obscure the view. The capsule endoscope is not equipped with functions to allow suctioning, inflating, and washing the lumen of the small intestine. Therefore, some parts of the lumen will not be

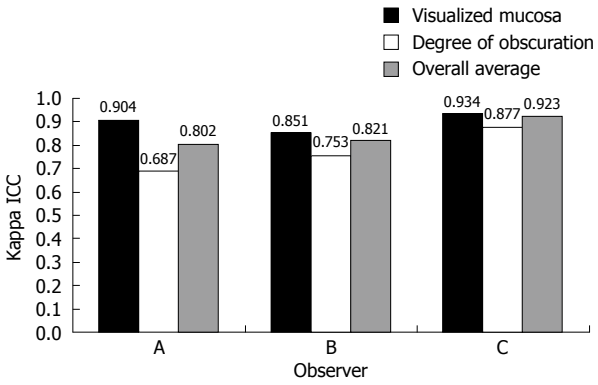

Figure 2 Intra-observer agreements between the scores at 4-wk intervals for two parameters and the overall average.

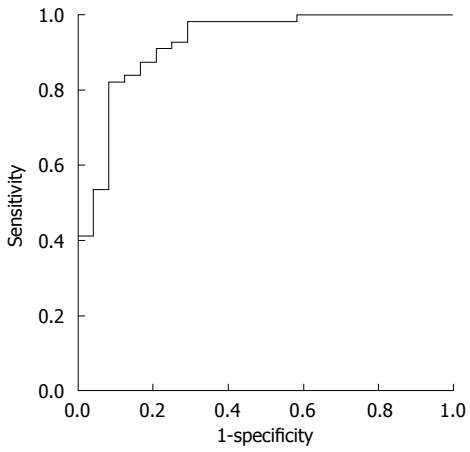

Figure 3 The area under the curve (AUC) was 0.925 (95% confidence interval, 0.859-0.990) in receiver operating characteristic (ROC) curve of image quality scores for grading small bowel cleansing.

Table 4 Intra-patient agreement of our scoring system among different starting frames

Parameter	Frame	Score (median and IQR)	Intra-patient ICC (95% CI)
Visualized mucosa	A	2.33 (1.93-2.59)	0.95 (0.81, 0.98)
	B	2.39 (1.88-2.66)	
	C	2.31 (1.90-2.44)	
Degree of obscuration	A	2.13 (1.97-2.61)	0.72 (0.39, 0.87)
	B	2.01 (1.52-2.34)	
	C	2.01 (1.57-2.20)	
Overall average	A	2.28 (2.08-2.61)	0.76 (0.41, 0.93)
	B	2.45 (1.70-2.50)	
	C	2.16 (2.09-2.50)	

visualized and examiners are unable to observe the entire small bowel mucosa thoroughly. This means that small bowel preparation plays an important role because it can improve image quality by cleansing the small bowel. There have been many studies on the preparation for CE, however, there is no standardized procedure for small bowel preparation for CE. In addition, preparation type, doses, and time of administration differ among centers.

To assess the effect of bowel preparation on the cleansing of the small bowel mucosa objectively, scoring systems for grading small bowel cleanliness have been introduced[1,5,6,12-14]. Prior studies on preparation, formulated and used their own scoring systems to grade small bowel cleanliness, thus making it difficult to compare the effect of preparation among studies[4,9]. For example, the grading system by Viazis *et al*[5] simply graded the small bowel cleanliness as "adequate" or "inadequate" and the time, during which the small bowel mucosa appeared unclean, was recorded using a timer; the concordance among the investigators was excellent (92.5%). However, thousands of frames per case had to be reviewed with this system. Therefore, grading the cleanliness is time-consuming and laborious. In the studies by Niv *et al*[1,7] the capsule images were graded according to the proportion of the small bowel transit time during which the intraluminal fluid interfered with visualization and interpretation. Although concordance on the quality of bowel preparation was excellent (kappa statistic = 0.91, $P < 0.001$), this grading system, in which the entire small bowel could be examined, is also time-consuming.

The study by Dai *et al*[13] assessed the visibility of the small bowel as a percentage of the visualized intestinal wall during 10-min video segments at 1-h intervals. In the study by Shiotani *et al*[6] individual frames were examined for 2 min of every 5-min period and scored using four visual parameters: circumference, bubbles, debris, and lightning. In recent studies, five video segments (each 5 or 10 min long) were selected from all the videos[15-17]. However, no standard guidelines for grading small bowel cleanliness are currently available. Most of the reported grading systems are time-consuming, complicated, and difficult to apply clinically. In addition, the reliability and efficacy of these grading systems have rarely been evaluated. Therefore, we designed a novel, simple, and time-sparing grading system of small bowel preparation for CE. In our scoring system, one frame was selected every 5 min (1 frame/5 min) to reduce the duration of grading small bowel cleansing. As a result, the duration of grading was greatly shortened compared with prior systems, which evaluated the entire small bowel mucosa. Our scoring system was compared with a previous method, which analyzed the frames for 2 min of every 5-min period; this method is a grading system that has recently been reported, but is time-consuming in that a substantial number of frames have to be analyzed. The concordance between this grading system and our scoring system was excellent.

Validity and reliability are two minimum qualities required for a grading system. Without reliability, even a valid scale can differ among study groups[18]. In a recent study, the quantitative index (QI) was better than the qualitative evaluation (QE) for intra-observer and inter-observer reliability[19]. To make the scoring system more reliable, we selected two parameters: the proportion of visualized mucosa and the degree of obscuration by bubbles, debris, bile, *etc*. For each parameter, small bowel cleanliness can be graded objectively by scoring the im-

ages according to the percent of the area. As a result, although the agreement on the degree of obscuration tended to be lower than that of the visualized mucosa in some cases, inter-observer, intra-patient, and intra-observer agreements were excellent. If the examiners in our study had received a calibration exercise before grading cleanliness, the concordance may have been better.

To determine whether our grading system was reasonable for assessing the adequacy of small bowel preparation, we compared our system with the grading system by Viazis *et al*[5], and the AUC of the ROC curve was 0.925. This means that our grading system had good discriminative power. From the ROC curve, we found that the cut-off value of image quality score on adequate bowel cleansing was 2.25, which was considered to have optimal sensitivity and specificity. Therefore, this value may be used as a criterion for determining the overall adequacy of small bowel preparation for CE.

In conclusion, our novel scoring system for CE was simple, objective, and efficient. It showed excellent inter-observer, intra-patient, and intra-observer agreement. In addition, a cut-off value for the adequacy of small bowel preparation was also proposed. Therefore, our scoring system may be useful in clinical practice and in studies determining the optimal small bowel cleansing method.

COMMENTS

Background

For successful capsule endoscopy (CE), adequate bowel cleansing is mandatory. However, the benefits of bowel preparation prior to CE remain controversial and it is unclear which method is best. One of the reasons for this controversy is that the current grading systems have not been standardized and a generally accepted grading system is not available.

Research frontiers

Most of the reported grading systems are time-consuming, complicated, and difficult to apply clinically. In addition, the reliability and efficacy of these grading systems have rarely been evaluated. Therefore, the authors designed a novel, simple, and time-sparing grading system of small bowel preparation for CE. For assessment, two visual parameters were used: the proportion of visualized mucosa and degree of obscuration. Representative frames from small bowel images were serially selected and scored at 5-min intervals. Intraclass correlation coefficient (ICC) was obtained to assess the reliability of the new scoring system. For efficacy evaluation and validation, scores of this new scoring system were compared with those of another previously reported cleansing grading system. Concordance with the previous system, inter-observer agreement, and intra-patient agreement were excellent with ICC values of 0.82, 0.80, and 0.76, respectively. The intra-observer agreements at four-week intervals were also excellent. The cut-off value for adequate image quality was shown to be 2.25.

Innovations and breakthroughs

In this scoring system, one frame was selected every 5 min (1 frame/5 min) to reduce the duration of grading small bowel cleansing. As a result, the duration of grading was greatly shortened compared with prior systems, which evaluated the entire small bowel mucosa. From the receiver operating characteristic (ROC) curve, the found that the cut-off value of image quality score on adequate bowel cleansing was 2.25, which was considered to have optimal sensitivity and specificity. Therefore, this value may be used as a criterion to determine the overall adequacy of small bowel preparation for capsule endoscopy.

Applications

The new scoring system is simple, efficient, and may be useful in clinical practice and in studies to determine the optimal small bowel cleansing method.

Terminology

ICC: A descriptive statistic that can be used when quantitative measurements are made on units that are organized into groups. It describes how strongly units in the same group resemble each other. Its major application is in the assessment of consistency or reproducibility of quantitative measurements made by different observers measuring the same quantity.

Peer review

In this manuscript, authors have reported a novel cleansing score system for capsule endoscopy. This new scoring system is simple, and efficient. Furthermore, it showed good inter-observer, intra-patient, and intra-observer agreement.

REFERENCES

1 **Niv Y**, Niv G, Wiser K, Demarco DC. Capsule endoscopy - comparison of two strategies of bowel preparation. *Aliment Pharmacol Ther* 2005; **22**: 957-962

2 **Ell C**, Remke S, May A, Helou L, Henrich R, Mayer G. The first prospective controlled trial comparing wireless capsule endoscopy with push enteroscopy in chronic gastrointestinal bleeding. *Endoscopy* 2002; **34**: 685-689

3 **Chong AK**, Taylor A, Miller A, Hennessy O, Connell W, Desmond P. Capsule endoscopy vs. push enteroscopy and enteroclysis in suspected small-bowel Crohn's disease. *Gastrointest Endosc* 2005; **61**: 255-261

4 **Villa F**, Signorelli C, Rondonotti E, de Franchis R. Preparations and prokinetics. *Gastrointest Endosc Clin N Am* 2006; **16**: 211-220

5 **Viazis N**, Sgouros S, Papaxoinis K, Vlachogiannakos J, Bergele C, Sklavos P, Panani A, Avgerinos A. Bowel preparation increases the diagnostic yield of capsule endoscopy: a prospective, randomized, controlled study. *Gastrointest Endosc* 2004; **60**: 534-538

6 **Shiotani A**, Opekun AR, Graham DY. Visualization of the small intestine using capsule endoscopy in healthy subjects. *Dig Dis Sci* 2007; **52**: 1019-1025

7 **Niv Y**, Niv G. Capsule endoscopy: role of bowel preparation in successful visualization. *Scand J Gastroenterol* 2004; **39**: 1005-1009

8 **Fireman Z**, Paz D, Kopelman Y. Capsule endoscopy: improving transit time and image view. *World J Gastroenterol* 2005; **11**: 5863-5866

9 **Ben-Soussan E**, Savoye G, Antonietti M, Ramirez S, Ducrotté P, Lerebours E. Is a 2-liter PEG preparation useful before capsule endoscopy? *J Clin Gastroenterol* 2005; **39**: 381-384

10 **Szklo M**, Nieto FJ. Epidemiology: beyond the basics. Gaithersburg, MD: Aspen Publishers, 2000

11 **Landis JR**, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; **33**: 159-174

12 **Ge ZZ**, Chen HY, Gao YJ, Hu YB, Xiao SD. The role of simeticone in small-bowel preparation for capsule endoscopy. *Endoscopy* 2006; **38**: 836-840

13 **Dai N**, Gubler C, Hengstler P, Meyenberger C, Bauerfeind P. Improved capsule endoscopy after bowel preparation. *Gastrointest Endosc* 2005; **61**: 28-31

14 **Albert J**, Göbel CM, Lesske J, Lotterer E, Nietsch H, Fleig WE. Simethicone for small bowel preparation for capsule endoscopy: a systematic, single-blinded, controlled study. *Gastrointest Endosc* 2004; **59**: 487-491

15 **van Tuyl SA**, den Ouden H, Stolk MF, Kuipers EJ. Optimal preparation for video capsule endoscopy: a prospective, randomized, single-blind study. *Endoscopy* 2007; **39**: 1037-1040

16 **Lapalus MG**, Ben Soussan E, Saurin JC, Favre O, D'Halluin PN, Coumaros D, Gaudric M, Fumex F, Antonietti M, Gaudin JL, Jacob P, Heresbach D, Pilichos C, Fan R, Mozer M, Heyries L, Dumortier J, Ponchon T. Capsule endoscopy and bowel preparation with oral sodium phosphate: a prospective randomized controlled trial. *Gastrointest Endosc* 2008; **67**:

1091-1096
17 **Endo H**, Kondo Y, Inamori M, Ohya TR, Yanagawa T, Asayama M, Hisatomi K, Teratani T, Yoneda M, Nakajima A, Matsuhashi N. Ingesting 500 ml of polyethylene glycol solution during capsule endoscopy improves the image quality and completion rate to the cecum. *Dig Dis Sci* 2008; **53**: 3201-3205
18 **Rostom A**, Jolicoeur E. Validation of a new scale for the assessment of bowel preparation quality. *Gastrointest Endosc* 2004; **59**: 482-486
19 **Brotz C**, Nandi N, Conn M, Daskalakis C, DiMarino M, Infantolino A, Katz LC, Schroeder T, Kastenberg D. A validation study of 3 grading systems to evaluate small-bowel cleansing for wireless capsule endoscopy: a quantitative index, a qualitative evaluation, and an overall adequacy assessment. *Gastrointest Endosc* 2009; **69**: 262-270, 270.e1

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BRIEF ARTICLE

Etiological and clinicopathologic characteristics of intrahepatic cholangiocarcinoma in young patients

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Abstract

AIM: To investigate the prevalence, risk factors, and clinicopathologic characteristics of intrahepatic cholangiocarcinoma (ICC) in young patients.

METHODS: A retrospective analysis was performed in ICC patients referred to the Eastern Hepatobiliary Surgery Hospital in Shanghai, China. Among 317 consecutively enrolled patients, 40 patients were aged ≤ 40 years (12.61%). We compared the risk factors and clinicopathologic characteristics of these patients (group Ⅰ: n = 40) with those aged > 40 years (group Ⅱ: n = 277).

RESULTS: Group I had distinct features compared with group Ⅱ, including a low frequency of hepatolithiasis (P = 0.000); a high positive rate of serum hepatitis B surface antigen (P = 0.000) and hepatitis B virus (HBV)-associated cirrhosis (P = 0.038); a high frequency of α-fetoprotein (> 400 μg/L) (P = 0.011); a low frequency of carbohydrate antigen 19-9 (> 37 U/mL) (P = 0.017); and a high frequency of liver histological inflammation (P = 0.002). Although there was no significant difference between the two groups in regards to hepatic schistosomiasis, alcohol-associated cirrhosis and cirrhosis due to other causes (P > 0.05), they only occurred in the elderly group.

CONCLUSION: The risk factors are significantly different between young and elderly ICC patients. HBV and HBV-associated cirrhosis are the most important risk factors for young ICC patients.

Key words: Intrahepatic cholangiocarcinoma; Young patients; Clinicopathologic features; Hepatitis B virus; Risk factor

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is a fatal cancer of the biliary epithelium, arising within the intrahepatic bile ducts. Globally, ICC is the most common primary hepatic malignancy after hepatocellular carcinoma (HCC). The incidence rates of ICC vary greatly among different areas of the world; this variation is related to the distribution of risk factors. Viral infection [hepatitis B virus (HBV) or hepatitis C virus (HCV)][1], primary sclerosingcholangitis (PSC)[2], liver fluke infestation (particularly the endemic *Opisthorchis viverrini*)[3,4], and hepatolithiasis are known as the risk factors for ICC[5,6]. ICC, similar to HCC, affects

predominantly those individuals aged > 40 years[7]; however, younger patients have recently been diagnosed with ICC. The risk factors and clinicopathologic features of young ICC patients have not yet been studied.

The aim of the present study was to investigate the prevalence, risk factors, and the clinicopathologic characteristics of ICC in young patients, and to compare these findings with the characteristics and risk factors associated with elderly patients with ICC.

MATERIALS AND METHODS

We performed a retrospective analysis using medical records of the patients initially diagnosed with ICC in the Eastern Hepatobiliary Surgery Hospital of the Second Military Medical University, Shanghai, China from January 2003 to December 2006. The diagnosis of ICC was confirmed pathologically. We evaluated the age and sex distribution of the patients, and compared the risk factors and clinicopathologic characteristics of patients aged ≤ 40 years (group Ⅰ) with those of patients aged > 40 years (group Ⅱ).

To clarify a difference in the risk factors between the two groups, we analyzed HBV or HCV infection, liver cirrhosis, hepatolithiasis, and hepatic schistosomiasis (our previous study verified that these are the main risk factors for ICC patients from China). The presence of a seropositive HBsAg and/or anti-HCV (Abbott Laboratories, North Chicago, IL, USA) and HCV RNA (real time PCR; Abbott, IL, USA) was interpreted as an indication of chronic hepatitis infection. The diagnosis of ICC was confirmed by pathology. Liver function and serum tumor marker (carbohydrate antigen 19-9 and α-fetoprotein) concentrations were evaluated in all the patients.

To determine the pathological characteristics, the diameter of the largest tumor was measured directly in the surgical specimens from patients who had undergone hepatic resection. The WHO tumor classification was used for pathological grading of the tumor (well, moderate or poor differentiation). When histological diversity was observed in a tumor, the higher grade, according to the classification system, was taken as the overall grade. The tumor mass was estimated by computed tomography (CT), and enlarged lymph nodes were defined as lymph nodes 0.1 cm in diameter at the portal, celiac, retrocrural or retroperitoneal lymph node stations. Ultrasound, CT scan, or surgery was used to diagnose portal vein thrombosis.

Statistical analysis

Statistical analysis was performed using the χ^2 test to compare discrete variables, and analysis of variance (ANOVA) was made to compare continuous variables. SPSS for Windows (version 16.0) was used. $P < 0.05$ was considered as significant difference.

RESULTS

Age and sex distribution

A total of 317 patients (223 men and 94 women, a male to female ratio, 2.37:1) initially diagnosed with ICC were enrolled in the present study. The mean age was 53.05 ± 10.53 years (range 21-78). Most of ICC developed during the 4th-7th decades, with a peak at 54 years of age. Forty (12.61%) patients were aged ≤ 40 years, including 30 men and 10 women (Figure 1).

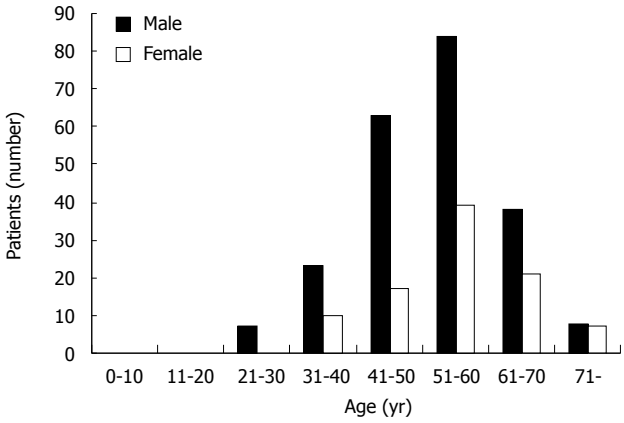

Figure 1 Age and sex distribution of intrahepatic cellular carcinoma (ICC) between 2003 and 2006 at the Eastern Hepatobiliary Surgery Hospital in Shanghai, China.

Table 1 Risk factors for intrahepatic cholangiocarcinoma *n* (%)

Risk factors	Group Ⅰ (*n* = 40)	Group Ⅱ (*n* = 277)	*P* value
HBV infection	34 (85.0)	120 (43.3)	0.000
HCV infection	0 (0.0)	1 (0.4)	0.703
Liver cirrhosis			0.038
Related to HBV	16 (40.0)	68 (24.5)	0.347
Related to alcohol	0 (0.0)	6 (2.2)	0.392
Other causes	0 (0.0)	5 (1.8)	
Total	16 (40.0)	79 (28.52)	
Hepatolithiasis	0 (0.0)	25 (9.0)	0.000
Liver schistosomiasis	0 (0.0)	16 (5.8)	0.119

HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Risk factor distribution

The risk factors for ICC are listed in Table 1. Of the 40 young ICC patients, 34 (85.0%) were seropositive for HBsAg, 16 (40.0%) had liver cirrhosis related to HBV, and none had intrahepatic-duct stones (IHD stones), liver schistosomiasis, or HCV (Figure 2A). In group Ⅱ, 120 (43.3%) patients were seropositive for HBsAg, 1 (0.4%) was seropositive for anti-HCV and HCV RNA, 79 (28.5%) had liver cirrhosis, including 68 (24.5%) whose cirrhosis was related to HBV, 6 (2.2%) with liver cirrhosis related to alcohol, 5 (1.8%)with liver cirrhosis due to other causes (1 with liver cirrhosis related to HCV, 1 with nonalcoholic liver cirrhosis, and 3 with occult liver cirrhosis), 25 (9.0%) had IHD stones, and 16 (5.8%) had liver schistosomiasis (Figure 2B). It is worth mentioning that the HCV that is prevalent in Japan and some Western countries has been found to be the significant cause of ICC. In our series, only one case of ICC had HCV.

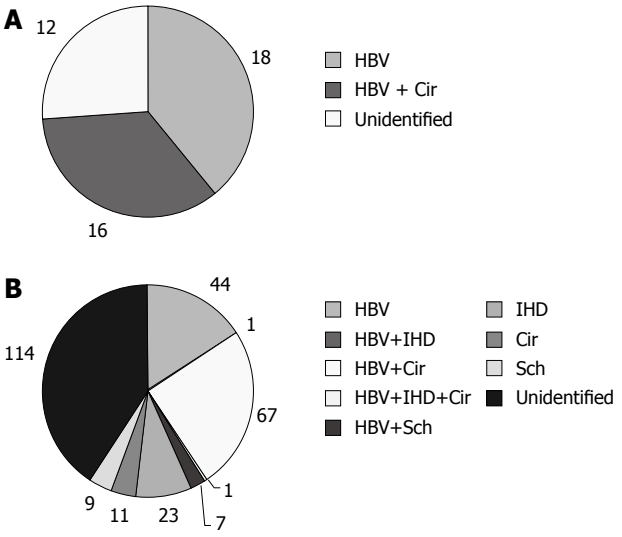

Figure 2 Distribution of potential risk factors for ICC among young ICC patients (A) and elderly ICC patients (B). HBV = Seropositivity for HBsAg; Cir = Liver cirrhosis; IHD = Intrahepatic duct stone (Hepatolithiasis); Sch = Liver schistosomiasis.

Clinical features

For further comparison between the two groups, the following clinical variables were investigated: gender, total bilirubin (TBIL) (> 20 μmol/L *vs* ≤ 20 μmol/L), albumin, alanine aminotransferase (ALT) (> 42 U/L *vs* ≤ 42 U/L), aspartate aminotransferase (AST) (> 37 U/L *vs* ≤ 37 U/L), r-glutamyl-transferase (r-GT) (> 64 U/L *vs* ≤ 64 U/L), alkaline phosphatase (ALP) (> 119 U/L *vs* ≤ 119 U/L), α-fetoprotein (AFP) (> 400 μg/L *vs* ≤ 400 μg/L), and carbohydrate antigen 19-9 (CA19-9, > 37 ng/mL *vs* ≤ 37 ng/mL). AFP (> 400 μg/L) and CA19-9 (> 37 U/mL) were significantly different between group Ⅰ and group Ⅱ by univariate analysis (Table 2).

Pathological characteristics

Table 3 reveals the pathological characteristics in the two groups. There was significantly more histological inflammation (present *vs* no hepatitis) in the younger patient group ($P = 0.002$). No difference was detected between the two groups with regard to the tumor number (< 2 *vs* ≥ 2), location (right lobe, left lobe, or both lobes), tumor size (main tumor or the largest one), capsule (present *vs* absent), tumor differentiation (well, moderate or poor), portal vein invasion (invasion *vs* no invasion), microscopic satellite lesion (tiny nodule present around the main tumor *vs* no satellite), and immunohistochemical examination (cytokeratin 18 and cytokeratin 19).

DISCUSSION

Although several risk factors have been associated with ICC, such as viral infection (HBV and/or HCV), liver cirrhosis, IHD stones, and liver parasite infestation, the distribution characteristics of these risk factors in young and elderly ICC patients and the mechanism responsible for ICC remain unknown. Here, we not only demonstrated different etiological characteristics of ICC between young and elderly patients, but also the clinicopathologic features of young patients.

Our study focused on patients with ICC who were ≤ 40 years of age, and the "young patients" were defined as those ≤ 40 years of age at diagnosis. We chose the cut-off age of 40 years based on the recommended age for starting regular HCC screening for men in Asia[8] and according to Lam *et al*[9]. The age distribution of patients with ICC in China is described by a triangular-shaped curve (Figure 1). Only 12.61% of patients were ≤ 40 years of age.

HBV or HCV infection was strongly associated

Table 2 Comparison of clinical features of ICC between the two groups *n* (%)

	Group Ⅰ (*n* = 40)	Group Ⅱ (*n* = 277)	*P* value
Gender (M/F)	30/10	193/84	0.491
ALT (> 42 U/L)	15 (60.00)	89 (32.13)	0.499
AST (> 37 U/L)	12 (30.00)	103 (37.18)	0.377
TBIL (> 20 μmol/L)	7 (17.50)	62 (22.38)	0.484
Albumin (g/L)	43.82 ± 4.27	42.07 ± 5.12	0.236
GGT (> 64 U/L)	19 (47.50)	176 (63.54)	0.051
ALP (> 119 U/L)	15 (11.19)	134 (48.38)	0.198
AFP (> 400 μg/L)	7 (17.50)	17 (6.14)	0.011
CA19-9 (> 37 U/mL)	14 (35.00)	153 (55.23)	0.017

M: Male; F: Female; TBIL: Total bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AFP: α-fetoprotein; ALP: Alkaline phosphatase; r-GT: r-glutamyl-transferase; CA 19-9: Carbohydrate antigen 19-9. Group I: ICC patients aged ≤ 40 years; Group II: ICC patients aged > 40 years.

Table 3 Comparison of pathological features of ICC between the two groups *n* (%)

	Group Ⅰ (*n* = 40)	Group Ⅱ (*n* = 277)	*P* value
Tumor location			0.827
Right lobe	25 (6.25)	160 (57.76)	
Left lobe	14 (35.00)	111 (40.07)	
Both lobes	1 (2.50)	6 (15.00)	
Tumor size (cm)	6.80 ± 3.92	6.67 ± 3.38	0.892
Tumor number			0.474
1	36 (90.00)	258 (93.14)	
≥ 2	4 (10.00)	19 (6.86)	
Histological inflammation	15 (37.50)	47 (16.97)	0.002
Capsule	5 (12.50)	35 (12.64)	0.981
Tumor differentiation			0.506
Well	0 (0.00)	9 (3.25)	
Moderate	29 (72.50)	191 (68.95)	
Poor	11 (27.50)	71 (25.63)	
Microvascular invasion	10 (25.00)	41 (14.80)	0.101
Perineural infiltration	0 (0.00)	4 (1.44)	0.444
Portal vein invasion	3 (7.50)	30 (10.83)	0.519
Lymphatic metastasis	8 (20.00)	46 (16.61)	0.594
Microscopic satellite lesion	7 (17.50)	69 (24.91)	0.305
Immunohistochemical examinations			
Ck18 positive	38 (95.00)	269 (97.11)	0.475
Ck19 positive	40 (100.00)	267 (93.39)	0.222

Ck: Cytokeratin.

with ICC risk. Korean investigators performed a case-control study comparing 41 cases of ICC with 406 controls without cancer and found that 13.8% of cases and 3.5% of controls were anti-HCV positive and 12.5% of cases and 2.3% of controls were HBsAg positive[10]. In a Japanese hospital-based study, investigators found that 36% of 50 patients with ICC but only 3% of 205 controls (surgical patients who did not have primary liver cancer) were HCV seropositive [OR (odds ratio) = 16.87; 95% CI (confidence interval): 5.69-50.00][11]. Through a population based cohort study including 146 394 HCV-infected and 572 293 HCV-uninfected patients, El-Serag *et al*[12] showed a strong association of ICC with HCV infection (hazard ratio = 2.55, 95% CI: 1.31-4.95), but the association was not observed in extrahepatic cholangiocarcinoma. Our previous study also found that the incidence of HBV infection in ICC patients was significantly higher than that in non-cancer individuals (48.6% *vs* 6.6%), indicating that chronic HBV infection was independently the most important risk factor for ICC in Chinese population (OR = 9.669, 95% CI: 6.329-14.770). Our findings were consistent with that reported by Zhou *et al*[13] recently. In our study, 85.0% of young ICC patients had chronic HBV infection, which is significantly higher than that of elderly ICC patients (43.3%). It is interesting to note that HBV infection was also the only risk factor identified in young ICC patients. It is worth mentioning that the HCV that is prevalent in Japan and some Western countries has been proven to be the significant cause of ICC. In our series, only one case of ICC had HCV.

Cirrhosis, of any cause, has also been associated with intrahepatic cholangiocarcinoma[5]. A cohort study of over 11 000 patients with cirrhosis, followed up over 6 years, showed a 10-fold risk compared with the general population[14]. A prospective controlled study from Japan reported the risk of developing cholangiocarcinoma in patients with cirrhosis related to HCV as 3.5% at 10 years, 1000 times higher than in the general population[15]. The data obtained from our previous study also showed that cirrhosis, particularly HBV-associated cirrhosis, was an important risk factor for ICC in Chinese population. In the present study, the etiological distribution of cirrhosis was significantly different between young ICC patients and elderly ICC patients. HBV-associated cirrhosis had a higher incidence in young ICC patients than in the elderly group, while alcoholic cirrhosis and cirrhosis due to other causes only occurred in elderly ICC patients.

Hepatolithiasis is rare in Western countries, but relatively common in some parts of Asia, and is associated particularly with peripheral intrahepatic cholangiocarcinoma[4]. In Taiwan, up to 70% of patients with intrahepatic cholangiocarcinoma undergoing resection reportedly have intrahepatic biliary stones, and in Japan this figure is 6%-18%[16]. Biliary stones are thought to cause bile stasis, predisposing to recurrent bacterial infections and subsequent inflammation, a potential cofactor for cholangiocarcinogenesis. In our cohort, hepatolithiasis only occurred in elderly ICC patients. The result indicates hepatolithiasis may be not a risk factor of ICC development for young patients.

A large body of experimental and epidemiological data suggests a pathogenic association between liver parasite infection, especially *Opisthorchis viverrini* (and less definitively *Clonorchis sinensis*) and intrahepatic cholangiocarcinoma[1]. Our previous study showed that hepatic schistosomiasis had a higher incidence in ICC patients than in no-cancer individuals. The data suggests that hepatic schistosomiasis was a risk factor for ICC development (OR = 11.06, 95% CI: 3.368-36.337). Although there was no significant difference between the young ICC patents and elderly ICC patients in regards to hepatic schistosomiasis ($P > 0.05$), similar to alcohol-cirrhosis and cirrhosis due to other causes, hepatic schistosomiasis also only occurred in the elderly group.

α-fetoprotein (AFP), a 70-kDa glycoprotein, is normally produced during fetal development by the liver and yolk sac. The protein levels drop off rapidly after birth, and by the second year of life only trace amounts are detectable in the serum. AFP is increased in the majority of patients with HCC and is useful in the diagnosis and follow-up of cases. Studies suggest that, in patients with suspected HCC clinically, AFP levels > 400 ng/mL should strongly confirm the presence of HCC *via* a tissue diagnosis[17,18]. Some cancers may originate from cancer stem cells, which may form *via* the carcinogenesis of normal stem cells[19-21]. It has been suggested that hepatocytes and cholangiocytes arise from the same pool of hepatic precursor cells, also called oval cells. Carcinogenesis of such hepatic precursor cells may cause ICC[22]. Hepatic progenitor cells were also shown to strongly express AFP mRNA and produce AFP during differentiation[23]. Compared with the elderly ICC patients, young ICC patients exhibited a higher incidence of AFP > 400 μg/L (17.50% *vs* 6.14%). Our data indicated that the neoplastic transformation of oval cells may be one of the mechanisms for ICC development and that the oval cell precursor retains its ability to produce AFPin the process of malignant transformation.

In a recent prospective study, serum CA19-9 was found to be useful in diagnosing cholangiocarcinoma, in deciding whether the tumor had been radically resected and in monitoring the effect of treatment. Serum CA19-9 concentrations were significantly elevated in patients with cholangiocarcinoma compared with patients with HCC, benign biliary disease or healthy individuals. After curative resection, serum CA19-9 decreasesd to a preoperative level[24]. In the present study, young ICC patientsexhibited a lower serum CA19-9 level, similar to HCC.

In conclusion, the risk factors for ICC are different between young and elderly patients. HBV infection and HBV-associated cirrhosis may be the main risk factors for young ICC patients. Young ICC patients share etiological and many clinicopathologic similarities with HCC patients. These results indicated that ICC in young patients and HCC have a common process of

carcinogenesis (through a similar long-term inflammatory carcinogenic process) and that both may arise from hepatic progenitor cells.

COMMENTS

Background

Although several risk factors have been associated with the development of Intrahepatic cholangiocarcinoma (ICC), such as hepatitis B virus (HBV), hepatitis C virus (HCV) or cirrhosis, the risk factors and clinicopathologic features of young ICC patients have not been fully studied.

Applications

ICC in young patients and hepatocellular carcinoma (HCC) shared a common process of carcinogenesis (through a similar long-term inflammatory carcinogenic process) and that both may arise from hepatic progenitor cells. However, this presumption awaits verification by more studies.

Peer review

The authors clearly demonstrated that the risk factors for ICC differed significantly between young patients and elderly patients in a Chinese population. They also showed that HBV infection was closely associated with the development of ICC in young patients.

REFERENCES

1 **Shaib YH**, El-Serag HB, Nooka AK, Thomas M, Brown TD, Patt YZ, Hassan MM. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *Am J Gastroenterol* 2007; **102**: 1016-1021

2 **Chalasani N**, Baluyut A, Ismail A, Zaman A, Sood G, Ghalib R, McCashland TM, Reddy KR, Zervos X, Anbari MA, Hoen H. Cholangiocarcinoma in patients with primary sclerosing cholangitis: a multicenter case-control study. *Hepatology* 2000; **31**: 7-11

3 **Watanapa P**, Watanapa WB. Liver fluke-associated cholangiocarcinoma. *Br J Surg* 2002; **89**: 962-970

4 **Hughes NR**, Pairojkul C, Royce SG, Clouston A, Bhathal PS. Liver fluke-associated and sporadic cholangiocarcinoma: an immunohistochemical study of bile duct, peribiliary gland and tumour cell phenotypes. *J Clin Pathol* 2006; **59**: 1073-1078

5 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125

6 **Shaib YH**, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005; **128**: 620-626

7 **Kim JH**, Choi MS, Lee H, Kim do Y, Lee JH, Koh KC, Yoo BC, Paik SW, Rhee JC. Clinical features and prognosis of hepatocellular carcinoma in young patients from a hepatitis B-endemic area. *J Gastroenterol Hepatol* 2006; **21**: 588-594

8 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236

9 **Lam CM**, Chan AO, Ho P, Ng IO, Lo CM, Liu CL, Poon RT, Fan ST. Different presentation of hepatitis B-related hepatocellular carcinoma in a cohort of 1863 young and old patients - implications for screening. *Aliment Pharmacol Ther* 2004; **19**: 771-777

10 **Shin HR**, Lee CU, Park HJ, Seol SY, Chung JM, Choi HC, Ahn YO, Shigemastu T. Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 1996; **25**: 933-940

11 **Yamamoto S**, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, Takemura S, Tanaka H, Yamazaki O, Hirohashi K, Tanaka T. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. *Cancer Sci* 2004; **95**: 592-595

12 **El-Serag HB**, Engels EA, Landgren O, Chiao E, Henderson L, Amaratunge HC, Giordano TP. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: A population-based study of U.S. veterans. *Hepatology* 2009; **49**: 116-123

13 **Zhou YM**, Yin ZF, Yang JM, Li B, Shao WY, Xu F, Wang YL, Li DQ. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China. *World J Gastroenterol* 2008; **14**: 632-635

14 **Sorensen HT**, Friis S, Olsen JH, Thulstrup AM, Mellemkjaer L, Linet M, Trichopoulos D, Vilstrup H, Olsen J. Risk of liver and other types of cancer in patients with cirrhosis: a nationwide cohort study in Denmark. *Hepatology* 1998; **28**: 921-925

15 **Kobayashi M**, Ikeda K, Saitoh S, Suzuki F, Tsubota A, Suzuki Y, Arase Y, Murashima N, Chayama K, Kumada H. Incidence of primary cholangiocellular carcinoma of the liver in japanese patients with hepatitis C virus-related cirrhosis. *Cancer* 2000; **88**: 2471-2477

16 **Okuda K**, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: recent progress. Part 1: epidemiology and etiology. *J Gastroenterol Hepatol* 2002; **17**: 1049-1055

17 **Bruce MG**, Bruden D, McMahon BJ, Christensen C, Homan C, Sullivan D, Deubner H, Williams J, Livingston SE, Gretch D. Clinical significance of elevated alpha-fetoprotein in Alaskan Native patients with chronic hepatitis C. *J Viral Hepat* 2008; **15**: 179-187

18 **Murugavel KG**, Mathews S, Jayanthi V, Shankar EM, Hari R, Surendran R, Vengatesan A, Raghuram K, Rajasambandam P, Murali A, Srinivas U, Palaniswamy KR, Pugazhendhi T, Thyagarajan SP. Alpha-fetoprotein as a tumor marker in hepatocellular carcinoma: investigations in south Indian subjects with hepatotropic virus and aflatoxin etiologies. *Int J Infect Dis* 2008; **12**: e71-e76

19 **Al-Hajj M**, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; **100**: 3983-3988

20 **Singh SK**, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004; **432**: 396-401

21 **Singh SK**, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003; **63**: 5821-5828

22 **Wilkens L**, Bredt M, Flemming P, Klempnauer J, Heinrich Kreipe H. Differentiation of multicentric origin from intra-organ metastatic spread of hepatocellular carcinomas by comparative genomic hybridization. *J Pathol* 2000; **192**: 43-51

23 **Ishikawa K**, Sasaki A, Haraguchi N, Yoshikawa Y, Mori M. A case of an alpha-fetoprotein-producing intrahepatic cholangiocarcinoma suggests probable cancer stem cell origin. *Oncologist* 2007; **12**: 320-324

24 **Qin XL**, Wang ZR, Shi JS, Lu M, Wang L, He QR. Utility of serum CA19-9 in diagnosis of cholangiocarcinoma: in comparison with CEA. *World J Gastroenterol* 2004; **10**: 427-432

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BRIEF ARTICLE

Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis

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Abstract

AIM: To perform a meta-analysis of observational studies and randomized controlled trials (RCTs) on the association between *Helicobacter pylori* (*H. pylori*) and iron deficiency anemia (IDA).

METHODS: A defined search strategy was used to search Medline, Embase, the Cochrane Library, Clinical Trials, Cochrane Central Register of Controlled Trials, Premedline and Healthstar. Odds ratio (OR) was used to evaluate observational epidemiology studies, and weighted mean difference (WMD) was used to demonstrate the difference between control and intervention groups.

RESULTS: Fifteen observational studies and 5 RCTs were identified and used for calculation. The pooled OR for observational studies was 2.22 (95% CI: 1.52-3.24, $P < 0.0001$). The WMD for hemoglobin (HB) was 4.06 g/L (95% CI: -2.57-10.69, $P = 0.01$), and the WMD for serum ferritin (SF) was 9.47 μg/L (95% CI: -0.50-19.43, $P < 0.0001$). Results were heterogeneous for all comparisons.

CONCLUSION: This meta-analysis on observational studies suggests an association between *H. pylori* and IDA. In RCTs, eradication of *H. pylori* can improve HB and SF levels but not significantly.

Key words: *Helicobacter pylori*; Iron-deficiency anemia; Meta-analysis; Hemoglobins; Odds ratio

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Qu XH, Huang XL, Xiong P, Zhu CY, Huang YL, Lu LG, Sun X, Rong L, Zhong L, Sun DY, Lin H, Cai MC, Chen ZW, Hu B, Wu LM, Jiang YB, Yan WL. Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis. *World*

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INTRODUCTION

Anemia, defined as a hemoglobin concentration below established cut-off levels, is a widespread public health problem with major consequences for human health as well as social and economic development[1]. The World Health Organization (WHO) estimates that about 2 billion people in the world are suffering from this disease, and that approximately 50% of all anemia cases are diagnosed as iron deficiency anemia (IDA)[2,3]. IDA affects the work capacity of patients and may contribute to mortality, thus limiting economic development. The overall death rate from IDA has been underestimated in most surveys from many developing and developed countries[4,5]. WHO suggested that researchers and clinical doctors should investigate the etiology of IDA and develop therapeutic strategies because timely treatment restores personal health and increases national productivity by 20%[6,7].

It is known that a variety of causes such as inadequate iron intake, chronic blood loss, chronic disease, malabsorption, hemolysis, or a combination of these, can induce IDA[5,8-10]. Among possible causes, the involvement of *Helicobacter pylori* (*H. pylori*) infection remains controversial[11-13]. *H. pylori* is a highly prevalent microbial infection. Over 50% people in the world are infected by *H. pylori*. In Africa, Mexico, South America and Central America, *H. pylori* infection reaches 70%-90% of the population[14,15]. *H. pylori* has been considered as a major cause for the development of peptic ulcer disease, gastric malignancy and dyspeptic symptoms[16-19]. Recent studies have shown that *H. pylori* can also cause other extragastric diseases[20-22]. However, knowledge regarding any relation between *H. pylori* infection and IDA is limited. Moreover, studies regarding the role of *H. pylori* infection in IDA and the effectiveness of the eradication of *H. pylori* in the treatment of IDA are controversial.

This clinical research question is addressed by this meta-analysis. The aim of the study was to evaluate the association between *H. pylori* infection and IDA and examine the effect of *H. pylori* eradication on serum hemoglobin (HB) and serum ferritin (SF) levels. Observational epidemiological studies have demonstrated an association between *H. pylori* and IDA by comparing IDA risk between *H. pylori*-infected and non-infected participants. Randomized controlled trials (RCTs) have established a cause and effect relationship between *H. pylori* and IDA. In this meta-analysis, we hypothesized that there is a significant difference in IDA risk between *H. pylori*-infected and non-infected participants, and that *H. pylori* eradication therapy can significantly increase HB and SF concentration, thus alleviating IDA. We tested our hypothesis by pooling the results of studies on *H. pylori* and IDA.

MATERIALS AND METHODS

Search strategy and identification of studies

We searched, without language restrictions, for all publications on *H. pylori* and IDA between January 1966, and June 2009. Searches were performed on Medline, Embase, Clinical Trials, Database of Abstracts of Reviews of Effects (DARE), Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews, Premedline, Healthstar, by using the MeSH heading: "*Helicobacter pylori*", "iron-deficiency anemia", "anemia", "iron" and "hemoglobin" and the non-MeSH terms "sideropenic refractory anemia" and "serum ferritin". The reference lists of major textbooks, review articles, and of all the included articles identified by the search were then individually searched to find other potentially eligible studies. Information about unpublished and ongoing RCTs was sought from authors of the included RCTs, and experts in the field.

Selection criteria and validity assessment

The present meta-analysis followed the Quality of Reports of Meta-Analyses of RCTs (QUOROM) guideline for RCTs and observational studies in epidemiology [Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Methodological Index for Non-Randomized Studies (MINORS)] guideline in observational studies[23-25]. To avoid selection bias, selection criteria were established before searching. Two reviewers (Qu XH and Huang XL) identified articles eligible for further review by performing an initial screen of the abstracts or titles of the search results. The second screening was based on a full-text review according to the selection criteria. The observed agreement between reviewers for eligibility of articles was 96.3%, corresponding to modest agreement (κ = 0.40). Discrepancies were resolved by discussion and consultation with other reviewers (Xiong P and Zhu CY).

Observational epidemiology studies: Observational epidemiology studies (cross-sectional, case-control, or cohort) investigating the prevalence of IDA in *H. pylori*-positive patients and negative controls were included in this meta-analysis. Duplicate publications and those studies in which patients had other underlying common IDA causes (e.g. aspirin/NSAID use, colonic carcinoma, gastric carcinoma, angiodysplasia) were excluded.

Randomized controlled trials: In order for an RCT to be included, its participants must have had both *H. pylori* infection and IDA/iron deficiency (ID). At least 2 authors independently assessed the methodological quality of included RCTs by Jadad scores[26]. In addition, for a study

to be eligible for inclusion, the use of therapy to eradicate *H. pylori* in intervention groups and administration of oral ferrous sulfate to both intervention and control groups were required. Discrepancies in data extraction were resolved by discussion among authors (Qu XH and Huang XL).

Data abstraction

For observational epidemiology studies, we collected information on the year of publication, location of the study, age groups, number of cases and controls, country and region, number of IDA positive and negative patients, test method for *H. pylori*.

For RCTs, we collected information on references, year of publication, sample size, age group, treatment therapies, *H. pylori* testing methods and changes in mean ± SD of HB and SF in both the intervention and control groups.

Statistical analysis

For observational epidemiology studies, we recorded the prevalence of IDA in *H. pylori*-positive patients and controls for each study as an odds ratio (OR) and 95% CI and the weight of the studies. We used the heterogeneity χ^2 (Cochran Q) statistic to formally analyze heterogeneity across included studies. Meta-analysis was performed using Review Manager Version 5 (Cochrane Collaboration and Update Software) for observational studies[27].

For RCTs, we collected changes in HB and SF concentration after *H. pylori* eradication and evaluated them by using weighted mean difference (WMD) with 95% CI. A χ^2 test was used to assess heterogeneity of the studies. If the studies were found to be heterogeneous, (i.e. χ^2 > df), we utilized the DerSimonian and Laird random-effects model[28] rather than a fixed effects model to reassess the pooled estimates. The source of heterogeneity was investigated as described below. Publican bias was performed by both Review Manager Version 5 and STATA version 10. We also performed the Duval and Tweedie nonparametric "trim and fill" procedure[29] to further assess the possible effect of publication bias in our meta-analysis.

Subgroup analysis

Subgroup analysis was performed to assess the factors that might impact the pooled estimates and to investigate the source of heterogeneity. Sensitivity analysis was also conducted to test whether the analysis was robust by changing statistical methods, reanalyzing the data, and comparing the 2 results by the *t* test.

Publication bias

Funnel plots and Begg's test[30] are thought to detect the existence of publication bias of pooled ORs within observational studies. Small studies are scattered widely at the bottom of the graph, while the spread narrows for larger studies. When a funnel plot seemed to be asymmetrical, we used Duval and Tweedie's nonparametric "trim and filled" method as a sensitivity analysis to reassess the pooled estimates[29]. This method considers the possibility of hypothetical "missing" studies that might exist and recalculates the results with the imputed missing studies.

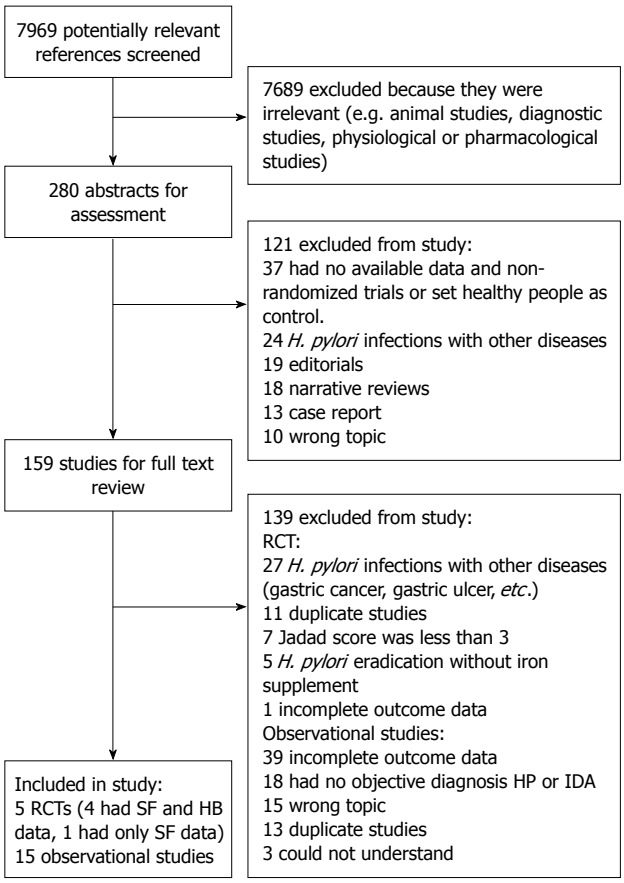

Figure 1 Flow chart showing the trial flow for selection of RCTs and observational studies to be included. RCT: Randomized controlled trial; HP: *H. pylori*; IDA: Iron deficiency anemia; HB: Hemoglobin; SF: Serum ferritin.

RESULTS

Search results

The search strategy retrieved 7969 potentially relevant references. Of these, 7689 were not relevant, e.g. animal studies, physiological or pharmacological studies. The remaining 280 references were assessed by screening their abstracts, and we excluded any references that were editorials or narrative reviews. One hundred and fifty nine studies were subjected to a full text review and excluded according to the selection criteria as described earlier. Supplementary studies were identified that had been published only as abstracts from conference proceedings of scientific meetings. We then excluded all RCTs with a Jadad score under 3 to ensure the quality of eligible trials. Fifteen observational studies[31-45] and 5 RCTs[46-49] (4 of which[46-49] provided both HB and SF data, and one study[40] provided only SF data) meeting our criteria were included in our meta-analysis (Figure 1).

Table 1 Summary characteristics of studies and participants

Ref.	Participants	Age group (yr)	Male (%)	Prevalence of *H. pylori* infection (%)		*H. pylori* test methods
Observational studies					Sampling mode	
Milman *et al*[31]	2264	30-60	1153 (51)	32.0	Hospital/health examination	Serum IgG
Choe *et al*[32]	375	10-15	205 (55)	16.8	High school/questionnaires	Serum IgG
Cuoco *et al*[33]	362	16-58	115 (32)	21.0	Hospital diagnosed patients	Histological examination
Choe *et al*[34]	660	15-17	376 (57)	29.5	High school/physical examination	Serum IgG
Nahon *et al*[35]	210	57.4 (21.4) (SD)	80 (38)	NA	Hospital diagnosed patients	Histological examination
Choe *et al*[36]	937	10-18	475 (51)	20.8	-/physical examination	Serum IgG
Choi[37]	674	9-12	344 (51)	13.6	Middle-class families/physical examination	Serum IgG
Ciacci *et al*[38]	55	> 17	22 (40)	60.0	Hospital diagnosed patients	Histological examination/rapid urease test
Hershko *et al*[39]	210	16-77	NA	NA	Hospital diagnosed patients	UBT/Serum IgG
Gessner *et al*[40]	690	7-11	NA	87.0	8 most populous villages/physical examination	UBT
Baggett *et al*[41]	668	7-11	354 (53)	86.5	10 predominantly villages/physical examination	UBT
Cardenas *et al*[42]	7462	⩾ 3	NA	27.1	NHANES/questionnaire, laboratory, examination data	Serum IgG
Süoglu *et al*[43]	70	4-16	NA	50.0	Hospital diagnosed patients	Histological examination
Haghi-Ashtiani *et al*[44]	209	2-14	111 (45)	47.8	Hospital diagnosed patients	Histological examination
Mulayim *et al*[45]	117	NA	0 (0)	61.5	Hospital diagnosed patients	UBT
Randomized controlled trials					Treatment therapies	
Choe *et al*[46]	13	10-17	NA	19.7	B+A+M	Histology
Gessner *et al*[40]	201	7-11	NA	87.0	L+A+C	UBT
Chen *et al*[47]	86	18-76	50 (58)	NA	B+A+M	UBT
Vijayan *et al*[48]	22	> 13	NA	NA	L+T+C	Rapid urease test/histology
Sarker *et al*[49]	99	2-5	46 (46)	NA	O+A+C	UBT

UBT: Urea breath test; IgG: Immunoglobulin G; NHANES: National Health and Nutrition Examination Survey; B: Bismuth; A: Amoxicillin; M: Metronidazole; L: Lansoprazole; C: Clarithromycin; T: Tinidazole; O: Omeprazole.

Study characteristics and quality

In total, 15 183 patients from 20 studies (15 observational and 5 RCTs) were included in the meta-analysis, and the characteristics of the sample are summarized in Table 1.

Of the observational studies, 4 studies with participants over 18 years old[31,35,38,39] and one study with patients aged 16-58 years old[33] were classified as adult groups. Three studies with patients aged 10-18 years old were classified as adolescent groups[32,34,36]. Two studies with patients younger than 11 years old were classified as child groups[41,49]. For the presence of *H. pylori*, 6 studies utilized serum immunoglobulin G (IgG)[31,32,34,36,37,42], 5 utilized histological examination[33,35,38,43,44] and 3 used the urea breath test (UBT)[40,41,45]. UBT and serum IgG were utilized by Hershko *et al*[39] in their studies. Of the RCTs, 2 studies with participants aged 2-11 years old were classified as child groups[40,49] and 3 were classified as adolescent and adult groups[46-48]. All the RCTs used eradication triple therapy for *H. pylori* as intervention.

Study methodological quality is shown in Table 2 (observational studies) and Table 3 (RCTs). In observational studies, the MINORS quality score ranged from 6 to 14 points. Only 8 (53%) of the articles included employment outcomes as part of the main study aim. Five studies satisfied the criteria for inclusion. Most data were collected according to a protocol established before the beginning of the study, and most studies have no exclusion or details about the reasons for exclusion. In RCTs, qualities of all the studies were evaluated by Jadad sore. All of the studies included had a score greater than 3. Only one study fulfilled all of the evaluated quality criteria. All studies were randomized, and for 4 of them, the generation of allocation sequence was judged adequate. Only 2 studies were designed as double-blind, but placebo was offered in only one study. Three of the 5 RCTs had no exclusions, and one of the 5 RCTs gave details on the reasons for exclusion.

Summary estimates

Risk of IDA for *H. pylori*-positive *versus H. pylori*-negative patients: We tested the heterogeneity of the 15 observational studies that provided information about prevalence OR, and the heterogeneity χ^2 statistic was 51.29 ($P < 0.00001$). Therefore, the pooled estimates were evaluated under a random effects model instead of a fixed effects model. The pooled OR was 2.22 and the 95% CI was 1.52-3.24 ($P < 0.0001$) suggesting that IDA is associated with *H. pylori* (Figure 2).

***H. pylori* eradication effect in IDA patients:** Four RCTs[46,48-49] reported blood parameter (HB levels and SF concentrations) differences and one RCT[40] reported

Table 2 Methodological quality assessment based on MINORS[1]

Source	Aim[2]	Rate[3]	Data[4]	Measure[5]	Bias[6]	Time[7]	Loss[8]	Size[9]	Total[10]
Milman *et al*[31]	2	2	2	2	2	1	1	0	12
Choe *et al*[32]	2	1	1	1	2	1	0	0	8
Cuoco *et al*[33]	1	1	1	2	2	0	0	1	8
Choe *et al*[34]	2	1	1	2	2	1	0	0	9
Nahon *et al*[35]	1	2	2	1	2	1	1	0	10
Choe *et al*[36]	2	2	1	1	0	0	0	0	6
Choi[37]	2	1	1	2	2	2	1	0	11
Ciacci *et al*[38]	1	1	2	1	2	1	0	0	8
Hershko *et al*[39]	1	1	1	1	2	1	1	0	8
Gessner *et al*[40]	2	2	2	1	2	2	2	1	14
Baggett *et al*[41]	2	1	2	1	2	1	1	0	10
Cardenas *et al*[42]	2	2	2	1	2	1	1	1	12
Süoglu *et al*[43]	1	1	1	1	2	1	0	0	7

[1]Assessed with the adapted Methodological Index for Non-Randomized Studies (MINORS)[25]; [2]Clearly stated aim (0,1,2 points); [3]Inclusion of consecutive patients and response rate (0,1,2); [4]Prospective collection of data (0,1,2); [5]Inclusion of employment measure (0,1,2); [6]Unbiased assessment of study end points (0 or 2); [7]Follow-up time appropriate (0,1,2); [8]Loss to follow-up (0,1,2); [9]Prospective calculation of the study size (0 or 1); [10]Total: minimum equals 0; maximum equals 15 points.

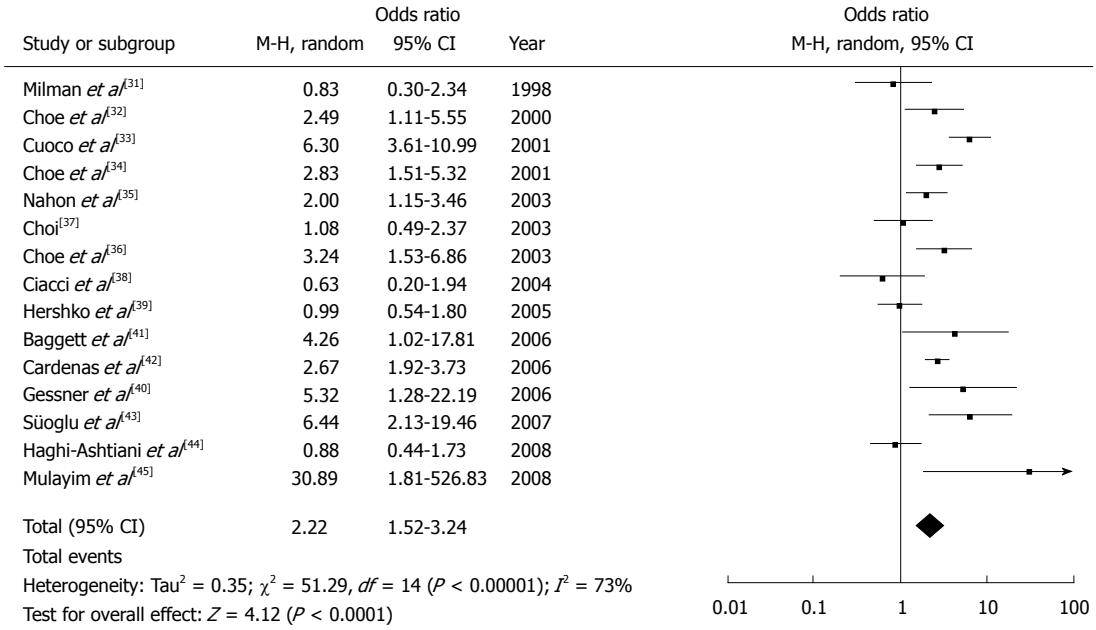

Figure 2 Forest plot of the observational studies. M-H, Random: Mantel-Haenszel heterogeneity random effects model. Horizontal lines = 95% CI. The rectangles represent the point estimates of the study and the size of the rectangle represents the weight given to each study in the meta-analysis. The diamond represents the summary estimate; the size of the diamond represents the CIs of the summary estimate.

Table 3 Quality evaluation of the included studies

RCT	Randomization	Blindness	Withdraw and dropout	Total
Choe *et al*[46]	2	2	0	4
Gessner *et al*[40]	2	1	1	4
Chen *et al*[47]	1	1	1	3
Vijayan *et al*[48]	2	2	1	5
Sarker *et al*[49]	2	0	1	3

SF concentration differences between the intervention and control groups after *H. pylori* eradication therapy. We pooled summary estimates to demonstrate the treatment effect and underlying connection between *H. pylori* and IDA. The results showed that *H. pylori* eradication therapy can improve IDA. Four RCTs compared the increase in HB levels and 5 in SF concentrations achieved with *H. pylori* eradication (plus iron) treatment and with iron administration alone in patients with IDA, and found a greater effect in the eradication group (WMD of HB: 4.06 g/L, 95% CI: -2.57-10.69, $P = 0.01$; WMD of SF: 9.47 μg/L; 95% CI: -0.50-19.43, $P < 0.0001$, Figure 3).

Subgroup analysis

Subgroup analysis was performed to investigate the source of heterogeneity and detect the influential factors that could impact the summary estimates. The methodological

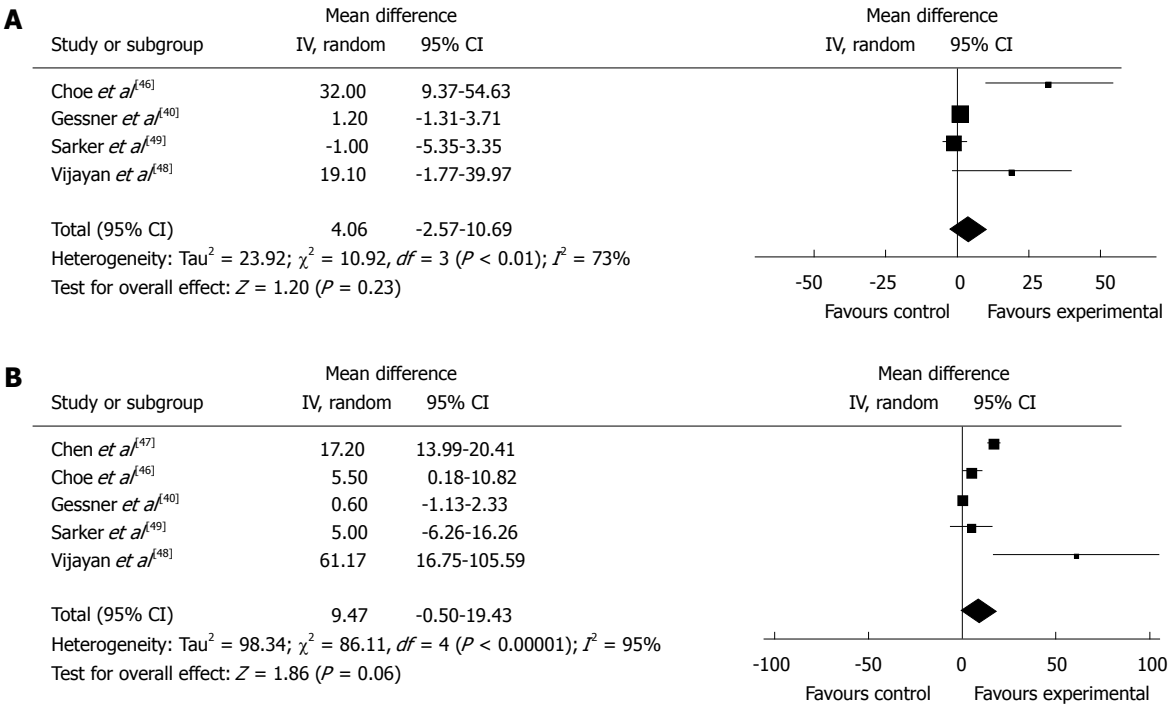

Figure 3 The treatment effect and underlying connection between *H. pylori* and IDA. A: Weighted mean difference (WMD) forest plots of HB (g/L) involved in the meta-analysis; B: Forest plots of studies estimate changes in SF (μg/L) level. IV, Random: Inverse variance heterogeneity random effects model. Horizontal lines = 95% CI. The size of the data marker corresponds to the weight of that study. The diamond represents the summary estimate. The result favors experimental groups.

and biological heterogeneity of the studies made it possible to explore the summary estimates in many different subgroups.

In observational studies, differences in the sensitivity of the *H. pylori* test methods could partly result in different pooled ORs. The 15 observational studies utilized 3 methods, enzyme-linked immunosorbent assay (ELISA) serum IgG, histological biopsy and UBT, to test for the presence of *H. pylori*. The pooled OR of ELISA serum IgG was lowest (OR = 2.16, 95% CI: 1.49-3.14, $P = 0.10$) and the pooled OR of UBT was highest (OR = 5.88, 95% CI: 2.27-15.23, $P = 0.47$). The pooled ORs were consistent with the sensitivity of these methods.

Subgroup analysis for different age groups revealed a significant difference between children, adolescents and adults in the association between *H. pylori* and IDA. The pooled OR of children younger than 11 years was 4.76 (95% CI: 1.73-13.08, $P = 0.83$). Adolescents yielded a pooled OR of 2.85 (95% CI: 1.68-4.31, $P = 0.89$), while adults had a pooled OR of 1.55 (95% CI: 0.67-3.62, $P < 0.0001$).

In RCTs, factors included in the subgroup analysis were age and therapy of each study. The summary estimate from children was 0.65 g/L (95% CI: -1.52-2.82, $P = 0.39$) for HB changes, significantly different from the pooled estimate of 25.03 g/L (95% CI: 9.69-40.37, $P = 0.41$) from adolescent and adult groups, indicating that adult IDA patients react more strongly to *H. pylori* eradication therapy. The WMD for SF changes was 0.70 μg/L (95% CI: -1.01-2.41, $P = 0.45$) in children while the WMD was 14.79 μg/L (95% CI: 2.53-27.05, $P = 0.0001$) in adolescent and adult patients.

To examine the method of therapy, we separated the studies into a bismuth triple therapy group and a proton pump inhibitor (PPI) triple therapy group. Bismuth triple therapy showed an obvious advantage (WMD of SF = 11.55 μg/L, 95% CI: 0.09- 23.01, $P = 0.0002$) over PPI triple therapy (WMD of SF = 7.15 μg/L, 95% CI: -6.45-20.75, $P = 0.002$), particularly when used together with oral ferrous sulfate for *H. pylori* patients with IDA.

We analyzed the association between *H. pylori* and IDA in developed areas and less developed areas, large sample subgroups and small sample subgroups, and did not find any significant difference between those parameters. Table 4 shows the summary estimates of subgroups of both RCTs and observational studies.

Sensitivity analysis

We chose to change the weights of every observational study involved so as to detect the stability of this meta-analysis. We then reanalyzed the data using different statistical methods. The pooled OR using a fixed effects model was 2.34 (95% CI: 1.97-2.78), which is not a significant change from the original random effects model ($P = 0.74$).

Publication bias

Visual inspection of the Begg's funnel plot revealed asymmetry ($P < 0.01$). This raises the possibility of publication bias, so we undertook a sensitivity analysis using the trim and fill method. This method conservatively imputes hypothetical negative unpublished studies

Table 4 Summary of subgroup analyses of both observational studies and experimental studies

	Subjects (*n*)		95% CI	Heterogeneity (χ^2)	*P*[1]
Observational studies		Odds ratio (OR)			
H. pylori test methods					
ELISA serum IgG	12372	2.16	1.49-3.14	9.27	0.10
Histological biopsy	906	2.17	0.90-5.26	28.85	< 0.0001
UBT	1475	5.88	2.27-15.23	1.53	0.47
Age					
Child	1358	4.76	1.73-13.08	0.05	0.83
Adolescent	1972	2.85	1.68-4.31	0.22	0.89
Adult	3101	1.55	0.67-3.62	28.41	< 0.0001
Randomized controlled trials[2]		Weighted mean difference (WMD)			
Hemoglobin (g/L)					
Age					
Child	300	0.65	-1.52-2.82	0.74	0.39
Adolescent and adult	35	25.03	9.69-40.37	0.67	0.41
Therapy					
PPI	322	0.95	-2.98-4.87	3.71	0.16
Bismuth	13	32.00	9.37-54.63	-	-
H. pylori test methods					
Histological biopsy	223	7.03	-9.41-23.47	2.79	0.10
UBT	35	25.03	9.69-40.37	0.67	0.41
Serum ferritin (μg/L)					
Age					
Child	300	0.70	-1.01-2.41	0.57	0.45
Adolescent and adult	121	14.79	2.53-27.05	17.91	0.0001
Therapy					
PPI	322	7.15	-6.45-20.75	7.68	0.002
Bismuth	99	11.55	0.09-23.01	13.61	0.0002

[1]*P*-value tested for heterogeneity of subgroups; [2]Data were derived from hemoglobin changes and serum ferritin changes.

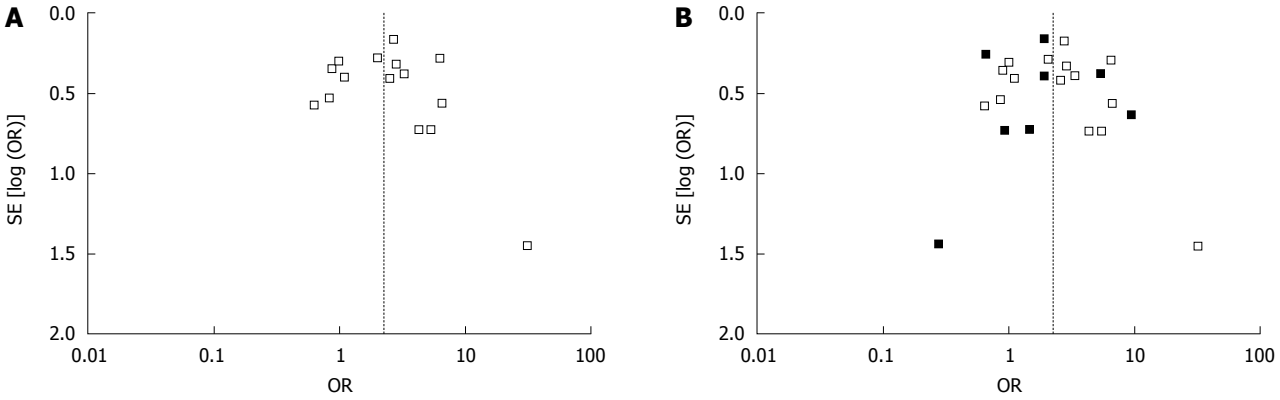

Figure 4 Funnel plots without (A) and with (B) trim and fill. The pseudo 95% CI is computed as part of the analysis that produces the funnel plot, and corresponds to the expected 95% CI for a given standard error (SE). OR: Odds ratio.

to mirror the positive studies that cause funnel plot asymmetry. The adjusted summary OR is based on the eventually filled funnel plot (4.32, 95% CI: 3.00-5.66, $P < 0.001$), which continued to show a statistically significant association between *H. pylori* and IDA (Figure 4).

DISCUSSION

Our results from a meta-analysis of 15 observational epidemiological studies revealed a correlation between *H. pylori* and IDA (OR, 2.22; 95% CI: 1.52-3.24, $P < 0.0001$), although some studies reported only a slight association. In addition, In RCTs, eradication of *H. pylori* can improve HB and SF levels but not significantly (WMD of HB: 4.06 g/L, 95% CI: -2.57-10.69, $P = 0.01$; WMD of SF: 9.47 μg/L, 95% CI: -0.50-19.43, $P < 0.0001$).

Dufour *et al*[50] first reported that *H. pylori* eradication had a positive effect on sideropenic refractory anemia, indicating a possible underlying association between *H. pylori* and IDA. A large population-based study from the USA reported that *H. pylori* infection was an independent risk factor for IDA in 7462 children, adolescents, and adults[42]. This research reported that *H. pylori* infection was associated with an increased risk of IDA (OR, 2.6; 95% CI: 1.5-4.6). Compared to former studies, our meta-analysis was a detailed and comprehensive investigation

procedure. It included RCTs of highly detailed power and large-scale observational epidemiology studies.

It is reported that *H. pylori* infection is observed in over 50% people in the world with peaks of 70%-90% for some countries. Moreover, IDA affects 2 billion people in the world. When 2 diseases have such a high prevalence in the population they may appear to be associated with each other. Recent studies regarding the role of *H. pylori* infection in IDA are controversial. However, whether eradication of *H. pylori* prevents IDA has been widely debated. This meta-analysis was performed to clarify this issue: whether iron deficiency could specifically be related to *H. pylori* infection. The observational studies in our meta-analysis prove the association between *H. pylori* and IDA. In fact, in the RCTs of our meta-analysis, after iron replacement, HB and SF were not different between the groups with or without *H. pylori* eradication. However, these data should be interpreted with caution because of the marked heterogeneity among studies. We carefully performed subgroup analysis, and found age and therapies had an impact on the increase in the levels of HB and SF. These results should be investigated further in the future. Larger scale RCTs should be recommended to test the results of our meta-analysis.

As with all meta-analyses, the results we obtained could be impacted by 3 factors: heterogeneity within the studies involved, bias (including selection bias[51,52] and detection bias[53,54]), and publication bias[55-57]. The generation of heterogeneity could occur by virtue of the methodological and biological heterogeneity of the studies analyzed, such as differences in diagnostic methods, the population under study, the sample size, and language of publication. Each of the subgroups described above contributed partly to the heterogeneity of the observational studies.

We assessed the included studies with caution. For observational studies, the MINORS quality score ranged from 6 to 14 points. In RCTs, quality of all the studies were evaluated by the Jadad sore. All of the studies included had a score greater than 3. Our test for heterogeneity was significant, and hence we utilized a random effects model that accounted for inter-study variation. Compared with the fixed effects model, the random effects model evenly distributes weight among studies, minimizing the impact of heterogeneity[58].

We used age as one subset determinant for the pooled estimates. A significant association between *H. pylori* and IDA was found in children younger than 11 years. The common causes of IDA, such as colonic carcinoma, gastrectomy or menstruation, were usually absent in children. Thus, *H. pylori* infection can be treated as the only indicator for refractory IDA in children, and *H. pylori* infection should be considered first in children with IDA[59-61]. The same scenario occurred in adolescents, as adolescents are particularly susceptible to ID. Because of the requirement for a large amount of iron to sustain their growth, dietary deficiency, and menstrual blood loss, girls should be more strongly affected by *H. pylori* infection[10,34,62]. In adults, no such strong association was found. The possible explanation for this phenomenon is that *H. pylori* plays a smaller part in the etiology of IDA in adults[1,5,63]. In RCT subgroup analysis, eradication therapy for *H. pylori* did not demonstrate the same curative effect on IDA in children as in adults. This may arise from the special characteristics of children (quickly growing blood volume and large requirement)[59-61].

Different diagnostic methods for *H. pylori* contribute to the variation of the pooled estimates because of their different sensitivities. The pooled OR value increased with the sensitivity of the diagnostic method. Therefore, UBT, which has the highest sensitivity of the 3 tests used[64-66], obtained the highest pooled OR, while ELISA serum IgG tests yielded the lowest pooled OR. Recent guidelines have indicated that UBT is regarded as a gold standard diagnostic method and the most reliable nonendoscopic test for the existence of *H. pylori*[67].

The way in which RCTs chose to eradicate *H. pylori* can make a difference in pooled analyses. Bismuth-based triple therapy indicated a much better response to iron intake than PPI-based triple therapy. The work done by McColl and Hutchinson may explain this phenomenon[68,69]. It was reported that PPI therapy lowers the concentration of vitamin C in gastric juice and reduces the bioavailability of ingested vitamin C thus resulting in low absorption of nonheme iron. It may also retard the clinical response to iron supplementation. Vitamin C, as an essential factor in alimentary iron absorption, not only converts ferric iron to the ferrous form, which maintains solubility at the alkaline pH of the duodenum, but also chelates with ferric chloride which is also stable at a pH > 3. PPI can also reduce the absorption of vitamin B_{12}, a significant factor in iron absorption, probably by inhibiting intragastric proteolysis.

Publication bias was tested by Begg's test and illustrated by funnel plots. The results indicated the existence of publication bias. The funnel plot showed that there were some missing small sample studies. Therefore, meta-analysis would underestimate the association between IDA and *H. pylori*. The "trim and fill" method helped to resolve this problem by imputing the hypothetical studies symmetrically and reassessing the pooled estimates as a sensitivity analysis. The filled funnel plot showed a strong association between IDA and *H. pylori*. Possible sources of asymmetry in funnel plots were explored: variations in sample size, etc, could contribute to publication bias.

Sensitivity analysis was performed in several ways to test concordance of the results by changing the statistical methods used. The sensitivity analyses that were performed did not materially change the results, increasing the confidence that can be placed in these results when applying the conclusion in practice.

Our study has limitations. We have excluded trials that studied the relationship between *H. pylori* and iron deficiency. However, these studies have been reviewed elsewhere[70]. Furthermore, in the experimental studies,

eradication treatment without ferrous sulfate but with placebo groups were excluded because the number of studies was not adequate. This kind of design can illustrate the role that *H. pylori* plays in IDA in a better way, and we expect further investigations will take that design into consideration. Lastly, results were markedly heterogeneous for all comparisons. These data should be interpreted with caution because of the marked heterogeneity among studies.

In conclusion, our meta-analysis of 15 observational studies demonstrated an association between *H. pylori* and IDA. In addition, the meta-analysis of RCTs showed that eradication of *H. pylori* can improve HB and SF levels, though not significantly. From the analysis, we also concluded that IDA could not specifically be related to *H. pylori* infection. We do not recommend a strategy of population-based screening and treatment for *H. pylori* infection to prevent IDA. This concept should be discussed in the future. UBT is the most reliable nonendoscopic test for the existence of. Bismuth-based triple therapy has a better response to increase HB and SF levels than PPI-based triple therapy. There are no significant differences between less developed areas and developed areas in the association between *H. pylori* infection and IDA.

COMMENTS

Background

Both *Helicobacter pylori* (*H. pylori*) and iron deficiency anemia (IDA) have high prevalence worldwide. The relationship between these 2 remains controversial. Recent guidelines for *H. pylori* and IDA both focus on the role *H. pylori* plays in the process of IDA.

Research frontiers

The interaction between *H. pylori* and IDA is a current 'hot topic'. *H. pylori* infection impairs iron absorption causing a considerable decrease in the concentration of gastric juice ascorbic acid (vitamin C) that is the best promoter of nonheme iron absorption. It thus causes the decline of hemoglobin in red blood cells and directly leads to anemia. The gastric colonization by *H. pylori* increases lactoferrin uptake from neutrophils and increases iron demand.

Innovations and breakthroughs

To the best of the authors' knowledge, this is the first published meta-analysis assessing the association between *H. pylori* infection and IDA (evaluating children, adolescents and adults) and assessing the effect of *H. pylori* eradication on hemoglobin (HB) and serum ferritin (SF) levels.

Applications

The research showed an association between *H. pylori* and IDA. Eradication of *H. pylori* can improve HB and SF levels but not significantly. The authors do not recommend a strategy of population-based screening and treatment for *H. pylori* infection to prevent IDA.

Peer review

This is a good and interesting meta-analysis.

REFERENCES

1 World Health Organization, The United Nations Children's Fund, United Nations University. Iron deficiency anaemia: assessment, prevention, and control. Available from: URL: http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf, accessed 27 July, 2004
2 **Zimmermann MB**, Hurrell RF. Nutritional iron deficiency. *Lancet* 2007; **370**: 511-520
3 **Clark SF**. Iron deficiency anemia: diagnosis and management. *Curr Opin Gastroenterol* 2009; **25**: 122-128
4 **Jolobe O**. Guidelines for the management of iron deficiency anaemia. *Gut* 2001; **49**: 158
5 World Health Organization, United Nations University, The United Nations Children's Fund. Iron deficiency anaemia. Assessment, prevention and control:A guide for programme managers. Available from: URL: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.3.pdf, accessed 2001
6 **Ramakrishnan U**. Prevalence of micronutrient malnutrition worldwide. *Nutr Rev* 2002; **60**: S46-S52
7 World Health Organization. Micronutrient deficiencies—Iron deficiency anaemia. Available from: URL: http://www.who.int/nutrition/topics/ida/en/index.html, accessed 2001
8 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023
9 **Annibale B**, Marignani M, Monarca B, Antonelli G, Marcheggiano A, Martino G, Mandelli F, Caprilli R, Delle Fave G. Reversal of iron deficiency anemia after Helicobacter pylori eradication in patients with asymptomatic gastritis. *Ann Intern Med* 1999; **131**: 668-672
10 **Gasche C**, Lomer MC, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. *Gut* 2004; **53**: 1190-1197
11 **DuBois S**, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. *Am J Gastroenterol* 2005; **100**: 453-459
12 **Bini EJ**. Helicobacter pylori and iron deficiency anemia: guilty as charged? *Am J Med* 2001; **111**: 495-497
13 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
14 **Howden CW**, Hunt RH. Guidelines for the management of Helicobacter pylori infection. Ad Hoc Committee on Practice Parameters of the American College of Gastroenterology. *Am J Gastroenterol* 1998; **93**: 2330-2338
15 World Gastroenterology Organisation. WGO Practice Guideline: Helicobacter pylori in Developing Countries. Available from: URL: http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/11_helicobacter_pylori_developing_countries_en.pdf, accessed 2005
16 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186
17 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22
18 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194
19 **Jaakkimainen RL**, Boyle E, Tudiver F. Is Helicobacter pylori associated with non-ulcer dyspepsia and will eradication improve symptoms? A meta-analysis. *BMJ* 1999; **319**: 1040-1044
20 **Gasbarrini A**, Franceschi F, Armuzzi A, Ojetti V, Candelli M, Torre ES, De Lorenzo A, Anti M, Pretolani S, Gasbarrini G. Extradigestive manifestations of Helicobacter pylori gastric infection. *Gut* 1999; **45** Suppl 1: I9-I12
21 **Peterson WL**, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease: guidelines for testing and treatment. *Arch Intern Med* 2000; **160**: 1285-1291
22 **Leontiadis GI**, Sharma VK, Howden CW. Non-gastrointestinal tract associations of Helicobacter pylori infection. *Arch Intern Med* 1999; **159**: 925-940
23 **Moher D**, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet* 1999; **354**: 1896-1900

24 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012

25 **Slim K**, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg* 2003; **73**: 712-716

26 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12

27 The Cochrane Collaboration. Cochrane Handbook for Systematic Reviews of Interventions Version 5.0.0[updated September 2008]. Available from: URL: http://www.cochrane-handbook.org/. accessed 7 November, 2008

28 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188

29 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558

30 **Begg C**, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, Rennie D, Schulz KF, Simel D, Stroup DF. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. *JAMA* 1996; **276**: 637-639

31 **Milman N**, Rosenstock S, Andersen L, Jørgensen T, Bonnevie O. Serum ferritin, hemoglobin, and Helicobacter pylori infection: a seroepidemiologic survey comprising 2794 Danish adults. *Gastroenterology* 1998; **115**: 268-274

32 **Choe YH**, Kim SK, Hong YC. Helicobacter pylori infection with iron deficiency anaemia and subnormal growth at puberty. *Arch Dis Child* 2000; **82**: 136-140

33 **Cuoco L**, Cammarota G, Jorizzo RA, Santarelli L, Cianci R, Montalto M, Gasbarrini A, Gasbarrini G. Link between Helicobacter pylori infection and iron-deficiency anaemia in patients with coeliac disease. *Scand J Gastroenterol* 2001; **36**: 1284-1288

34 **Choe YH**, Kwon YS, Jung MK, Kang SK, Hwang TS, Hong YC. Helicobacter pylori-associated iron-deficiency anemia in adolescent female athletes. *J Pediatr* 2001; **139**: 100-104

35 **Nahon S**, Lahmek P, Massard J, Lesgourgues B, Mariaud de Serre N, Traissac L, Bodiguel V, Adotti F, Delas N. Helicobacter pylori-associated chronic gastritis and unexplained iron deficiency anemia: a reliable association? *Helicobacter* 2003; **8**: 573-577

36 **Choe YH**, Kim SK, Hong YC. The relationship between Helicobacter pylori infection and iron deficiency: seroprevalence study in 937 pubescent children. *Arch Dis Child* 2003; **88**: 178

37 **Choi JW**. Does Helicobacter pylori infection relate to iron deficiency anaemia in prepubescent children under 12 years of age? *Acta Paediatr* 2003; **92**: 970-972

38 **Ciacci C**, Sabbatini F, Cavallaro R, Castiglione F, Di Bella S, Iovino P, Palumbo A, Tortora R, Amoruso D, Mazzacca G. Helicobacter pylori impairs iron absorption in infected individuals. *Dig Liver Dis* 2004; **36**: 455-460

39 **Hershko C**, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, Lahad A. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica* 2005; **90**: 585-595

40 **Gessner BD**, Baggett HC, Muth PT, Dunaway E, Gold BD, Feng Z, Parkinson AJ. A controlled, household-randomized, open-label trial of the effect that treatment of Helicobacter pylori infection has on iron deficiency in children in rural Alaska. *J Infect Dis* 2006; **193**: 537-546

41 **Baggett HC**, Parkinson AJ, Muth PT, Gold BD, Gessner BD. Endemic iron deficiency associated with Helicobacter pylori infection among school-aged children in Alaska. *Pediatrics* 2006; **117**: e396-e404

42 **Cardenas VM**, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and Helicobacter pylori infection in the United States. *Am J Epidemiol* 2006; **163**: 127-134

43 **Süoglu OD**, Gökçe S, Saglam AT, Sökücü S, Saner G. Association of Helicobacter pylori infection with gastroduodenal disease, epidemiologic factors and iron-deficiency anemia in Turkish children undergoing endoscopy, and impact on growth. *Pediatr Int* 2007; **49**: 858-863

44 **Haghi-Ashtiani MT**, Monajemzadeh M, Motamed F, Mahjoub F, Sharifan M, Shahsiah R, Kashef N. Anemia in children with and without Helicobacter pylori infection. *Arch Med Res* 2008; **39**: 536-540

45 **Mulayim B**, Celik NY, Yanik FF. Helicobacter pylori infection detected by 14C-urea breath test is associated with iron deficiency anemia in pregnant women. *J Obstet Gynaecol Res* 2008; **34**: 980-985

46 **Choe YH**, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of Helicobacter pylori eradication for iron-deficiency anemia in preadolescent children and adolescents. *Helicobacter* 1999; **4**: 135-139

47 **Chen LH**, Luo HS. Effects of H. pylori therapy on erythrocytic and iron parameters in iron deficiency anemia patients with H. pylori-positive chronic gastristis. *World J Gastroenterol* 2007; **13**: 5380-5383

48 **Vijayan G**, Sundaram RC, Bobby Z, Hamide A, Selvaraj N, Dasse NR. Increased plasma malondialdehyde and fructosamine in anemic H. pylori infected patients: effect of treatment. *World J Gastroenterol* 2007; **13**: 796-800

49 **Sarker SA**, Mahmud H, Davidsson L, Alam NH, Ahmed T, Alam N, Salam MA, Beglinger C, Gyr N, Fuchs GJ. Causal relationship of Helicobacter pylori with iron-deficiency anemia or failure of iron supplementation in children. *Gastroenterology* 2008; **135**: 1534-1542

50 **Dufour C**, Brisigotti M, Fabretti G, Luxardo P, Mori PG, Barabino A. Helicobacter pylori gastric infection and sideropenic refractory anemia. *J Pediatr Gastroenterol Nutr* 1993; **17**: 225-227

51 **Kunz R**, Vist G, Oxman AD. Randomisation to protect against selection bias in healthcare trials. *Cochrane Database Syst Rev* 2007; MR000012

52 **Guyatt GH**, Oxman AD, Kunz R, Vist GE, Falck-Ytter Y, Schünemann HJ. What is "quality of evidence" and why is it important to clinicians? *BMJ* 2008; **336**: 995-998

53 **Schulz KF**, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA* 1995; **273**: 408-412

54 **Karlowski TR**, Chalmers TC, Frenkel LD, Kapikian AZ, Lewis TL, Lynch JM. Ascorbic acid for the common cold. A prophylactic and therapeutic trial. *JAMA* 1975; **231**: 1038-1042

55 **Easterbrook PJ**, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991; **337**: 867-872

56 **Dickersin K**. The existence of publication bias and risk factors for its occurrence. *JAMA* 1990; **263**: 1385-1389

57 **Sutton AJ**, Duval SJ, Tweedie RL, Abrams KR, Jones DR. Empirical assessment of effect of publication bias on meta-analyses. *BMJ* 2000; **320**: 1574-1577

58 **Poole C**, Greenland S. Random-effects meta-analyses are not always conservative. *Am J Epidemiol* 1999; **150**: 469-475

59 **Oski FA**. Iron deficiency in infancy and childhood. *N Engl J Med* 1993; **329**: 190-193

60 **Kurekci AE**, Atay AA, Sarici SU, Yesilkaya E, Senses Z, Okutan V, Ozcan O. Is there a relationship between childhood Helicobacter pylori infection and iron deficiency anemia? *J Trop Pediatr* 2005; **51**: 166-169

61 **Russo-Mancuso G**, Branciforte F, Licciardello M, La Spina M. Iron deficiency anemia as the only sign of infection with Helicobacter pylori: a report of 9 pediatric cases. *Int J Hematol*

2003; **78**: 429-431
62 **Hallberg L**, Hultén L, Lindstedt G, Lundberg PA, Mark A, Purens J, Svanberg B, Swolin B. Prevalence of iron deficiency in Swedish adolescents. *Pediatr Res* 1993; **34**: 680-687
63 **Yip R**, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *Am J Clin Nutr* 1984; **39**: 427-436
64 **Thijs JC**, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, Luijt DS, Meyer BC, Kleibeuker JH. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. *Am J Gastroenterol* 1996; **91**: 2125-2129
65 **Atherton JC**, Spiller RC. The urea breath test for Helicobacter pylori. *Gut* 1994; **35**: 723-725
66 **Logan RP**, Polson RJ, Misiewicz JJ, Rao G, Karim NQ, Newell D, Johnson P, Wadsworth J, Walker MM, Baron JH. Simplified single sample 13Carbon urea breath test for Helicobacter pylori: comparison with histology, culture, and ELISA serology. *Gut* 1991; **32**: 1461-1464
67 **Chey WD**, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; **102**: 1808-1825
68 **McColl KE**. Effect of proton pump inhibitors on vitamins and iron. *Am J Gastroenterol* 2009; **104** Suppl 2: S5-S9
69 **Hutchinson C**, Geissler CA, Powell JJ, Bomford A. Proton pump inhibitors suppress absorption of dietary non-haem iron in hereditary haemochromatosis. *Gut* 2007; **56**: 1291-1295
70 **Muhsen K**, Cohen D. Helicobacter pylori infection and iron stores: a systematic review and meta-analysis. *Helicobacter* 2008; **13**: 323-340

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BRIEF ARTICLE

Betaine inhibits Toll-like receptor 4 expression in rats with ethanol-induced liver injury

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Abstract

AIM: To test whether ethanol feeding could induce Toll-like receptor 4 (TLR4) responses, assess the hepatoprotective effect of betaine and its inhibitive effect on TLR4 in animal models of alcoholic liver injury.

METHODS: Forty-eight female Sprague-Dawley rats were randomly divided into four groups as control, model, low and high dose betaine groups. Except control group, all rats were fed with high fat-containing diet plus ethanol and fish oil gavages for 8 wk. Betaine was administered intragastrically after exposure of ethanol for 4 wk. The changes of liver histology were examined. The expression of TLR4 mRNA and protein was detected by RT-PCR and Western blotting, respectively. The serum aminotransferase activity [alanine transarninase (ALT), aspartate aminotransferase (AST)], serum endotoxin, and liver inflammatory factors [tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-18 (IL-18)] were also assayed.

RESULTS: Compared with control group, rats of model group developed marked liver injury, accompanied by an increase of ALT (159.41 ± 7.74 U/L *vs* 59.47 ± 2.34 U/L, $P < 0.0001$), AST (248.25 ± 1.40 U/L *vs* 116.89 ± 3.48 U/L, $P < 0.0001$), endotoxin (135.37 ± 30.17 ng/L *vs* 44.15 ± 7.54 ng/L, $P < 0.0001$), TNF-α (20.81 ± 8.58 pg/mL *vs* 9.34 ± 2.57 pg/mL, $P = 0.0003$), IFN-γ (30.18 ± 7.60 pg/mL *vs* 16.86 ± 9.49 pg/mL, $P = 0.0039$) and IL-18 (40.99 ± 8.25 pg/mL *vs* 19.73 ± 9.31 pg/mL, $P = 0.0001$). At the same time, the expression of TLR4 mRNA and protein was markedly induced in the liver after chronic ethanol consumption (1.45 ± 0.07 *vs* 0.44 ± 0.04, $P < 0.0001$; 1.83 ± 0.13 *vs* 0.56 ± 0.08, $P < 0.0001$). Compared with model group, betaine feeding resulted in significant decreases of ALT (64.93 ± 6.06 U/L *vs* 159.41 ± 7.74 U/L, $P < 0.0001$), AST (188.73 ± 1.11 U/L *vs* 248.25 ± 1.40 U/L, $P < 0.0001$), endotoxin (61.80 ± 12.56 ng/L *vs* 135.37 ± 30.17 ng/L, $P < 0.0001$), TNF-α (9.79 ± 1.32 pg/mL *vs* 20.81 ± 8.58 pg/mL, $P = 0.0003$), IFN-γ (18.02 ± 5.96 pg/mL *vs* 30.18 ± 7.60 pg/mL, $P = 0.0008$) and IL-18 (18.23 ± 7.01 pg/mL *vs* 40.99 ± 8.25 pg/mL, $P < 0.0001$). Betaine also improved liver steatosis. The expression levels of TLR4 mRNA or protein in liver tissues were significantly lowered (0.62 ± 0.04 *vs* 1.45 ± 0.07, $P < 0.0001$; and 0.65 ± 0.06 *vs* 1.83 ± 0.13, $P < 0.0001$). There was a statistical difference of TLR4 mRNA and protein expression between high- and low-dose betaine groups (0.62 ± 0.04 *vs* 0.73 ± 0.05, $P < 0.0001$, and 0.65 ± 0.06 *vs* 0.81 ± 0.09, $P < 0.0001$).

CONCLUSION: Betaine can prevent the alcohol-induced liver injury effectively and improve the liver function. The expression of TLR4 increases significantly in ethanol-fed rats and betaine administration can inhibit TLR4 expression.

Key words: Betaine; Toll-like receptor 4; Alcoholic liver injury; Expression

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INTRODUCTION

Previous studies have shown that the chronic ingestion of ethanol can induce functional and structural changes in liver. Bacterial lipopolysaccharide (LPS; endotoxin), an abundant and essential component of the outer membrane of gram negative bacteria, causes liver injury in many experimental models[1,2]. Chronic alcohol administration increases gut-derived endotoxin in the portal circulation, thereby activating Kupffer cells to produce several proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin (IL)-1[3,4].

Toll-like receptor 4 (TLR4), a transmembrane protein with a cytoplasmic domain that bears homology to the IL-1 receptor, is expressed in monocytes and macrophages, including Kupffer cells[5]. Recently, TLR4 has been shown to mediate LPS-induced signal transduction in peripheral blood monocytes[6]. Furthermore, it has been shown that Kupffer cell activation by LPS is dependent on the presence of a functional TLR4[7]. It has been confirmed that TLR4 is involved in the mechanism of early alcohol-induced liver injury[8-11]. Ethanol administration can lead to the synthesis of TLR4 protein and its gene expression in Kupffer cells, indicating that TLR4 may play a major role in the development of alcohol-induced liver injury.

Betaine, also known as trimethyl glycine, is the only methyl donor, which can replace folate or S-ademetionine in the human body[12,13]. Betaine is quaternary ammonium salt soluble alkaloids, which participates in the methionine recycling and phosphatidylcholine synthesis[14,15]. Many studies including our previous study have indicated that betaine can prevent the alcohol-induced liver injury effectively and improve the liver function[16,17]. The hepatoprotective mechanism of betaine is related to the inhibition of inflammatory factor, the decrease of lipid peroxidation, the promotion of endoplasmic reticulum stress and the prevention of apoptosis[18-21].

In the present study, we employed the intragastric ethanol-fed rat model, which reproduces the pathological features of early alcohol-induced liver injury, to observe the changes of TLR4 expression and study the effect of betaine in alcohol-induced liver injury animal models.

MATERIALS AND METHODS

Chemicals and reagents

Betaine hydrochlorides (99% of purity) were kindly presented by Juhua Group Co. (Zhejiang, China). Ferrous sulfate was obtained from Shanghai reagent chemicals Co. Ltd (Shanghai, China). Fish oil and 560 mL/L of alcohol were purchased from supermarkets. Limulus amoebocyte lysate assay kit for serum endotoxin assay was purchased from BioWhittaker Inc. (USA). Enzyme-linked immunosorbent (ELISA) kits for rat TNF-α, IFN-γ, and IL-18 detection were purchased from Shanghai Senxiong Biotech industry Co. Ltd. (Shanghai, China). TRIzol reagent was purchased from Invitrogen Co. (USA). DL1000 DNA ladder marker was purchased from TaKaRa Biotech Co. Ltd. (Japan). M-MLV reverse transcriptase, deoxyribonucleotide (dNTP, 10 mmol/L), oligo (dT)$_{15}$ primer, Taq DNA polymerase, RNasin were purchased from Promega Biotech Co. Ltd. (USA). Polymerase chain reaction (PCR) primers for TLR4 and GAPDH were synthesized by Sai-Bai-Sheng Biocompany (Shanghai, China).

Animal models

Forty-eight female specific pathogen free (SPF) Sprague-Dawley rats, weighing 150 ± 10 g, were purchased from the Experimental Animal Center of Wuhan University. After acclimation for 1 wk, animals were randomly divided into four groups as control, model, low dose and high dose betaine groups. Each group contains 12 rats. Except rats of control group fed with ordinary diet and administrated intragastrically with physiological saline, the rats of the other three groups were fed with fat-rich diet containing common animal feeds, lard and whole milk powder (80:10:10), and were administrated intragastrically with ethanol and 0.5 mL fish oil. The initial dose of ethanol was 6 g/kg per day (solutions maximally containing 560 mL/L alcohol). Within the first week, the dose was increased progressively to a maintenance dose of 8 g/kg per day that was continued for 8 more weeks. After exposure of ethanol for 4 wk, the rats of low dose and high dose groups were administrated intragastrically with betaine 200 and 400 mg/kg per day, respectively. Animals were weighted three times per week. At the end of the experiment, animals were anaesthetized with urethane (20%, 1.0 g/kg) and sacrificed by bleeding from femoral arteries. Blood samples were collected. Immediately after exsanguination, the livers were harvested. Small portions of the livers were kept frozen at -70℃ for reverse transcriptase-polymerase chain reaction (RT-PCR), whereas other portions were separated and immersed in 10% buffered formalin solution for histological examination. All animals were given humane care in compliance with the institutional guidelines.

Liver function assay

Blood samples were allowed to clot, and the sera were isolated by centrifugation at 1000 × *g* for 10 min and kept at -20℃ before determination. Serum alanine transaminase (ALT), aspartate transaminase (AST) and albumin (ALB) were determined by routine laboratory methods using a Hitachi Automatic Analyzer (Hitachi, Inc. Japan).

Determination of serum endotoxin, TNF-α, IFN-γ and IL-18

Serum levels of endotoxin, TNF-α, IFN-γ and IL-18 were measured using commercial kits according to the manufacturer's protocol.

Detection of liver TLR4 mRNA in liver tissues

Total RNA was extracted from approximately 100 mg frozen liver tissue using TRIzol reagent according to the manufacturer's protocol. The concentration of total RNA was assayed by ultraviolet spectrophotometric measurements at wavelength of 260 nm, and its purity was estimated by the ratio of A_{260}/A_{280}. The total RNA was reversely transcribed into single-stranded complementary DNA (cDNA) using the following methods: 2 μg RNA, 0.5 μg oligo(dT)$_{15}$ primer and DEPC (diethylpyrocarbonate)-treated water were added to reach a total volume of 15 μL mixture at 70℃ for 5 min, then rapidly chilled on ice. Finally, 5 μL 5 × reaction buffer, 1.25 μL dNTP (10 mmol/L, each), 25 units of RNasin, 200 units of M-MLV reverse transcriptase and DEPC-treated water were added to reach a total volume of 25 μL mixture and incubated at 42℃ for 60 min, then terminated by placing it on ice after deactivation at 85℃ for 5 min. The cDNA was amplified by PCR. The amplification primers for rat TLR 4 were 5'-ACTCGAGCCAGAATGAGGACT-3' and 5'-ACTGCCATGTCTGAGCAATCT-3', for rat GADPH were 5'-TCCCTCAAGATTGTCAGCAA-3' and 5'-AGATCCACAACGGATACATT-3'. The 50 μL PCR reaction mix contained 10 mmol/L dNTP, 2.5 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 25 pmol/L of sense and antisense primers, and 2U of Taq DNA polymerase. Amplification was performed with 35 cycles with initial incubation at 94℃ for 3 min and final extension at 72℃ for 7 min, each cycle consisted of denaturation for 45 s at 94℃, annealing for 45 s at 55℃, and extension for 1 min at 72℃. The PCR products were 237 bp and 309 bp for TLR 4 and GADPH, respectively. In all experiments, possible contamination with genomic DNA was excluded by PCR amplification in the absence of reverse transcriptase. The PCR products were electrophoresed on 2% agarose gel. Semiquantitative evaluation was performed using the Gel Doc 2000 System (BioRad Laboratories GmbH, München, Germany). GADPH was used as a positive internal control and was positive for each specimen. Its expression was used as a correction factor for TLR 4 mRNA, thus the results were calculated as the ratio of the intensity of bands of TLR 4 cDNA per GADPH cDNA on the gel.

Western blotting assay of TLR4 protein in liver tissues

Liver tissue samples of 100 mg were crushed in a liquid nitrogen-cooled grinding bowl and then were lysed in cold RIPA buffer (25 mmol/L Tris-HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) (Pierce Biotechnology, Inc., USA), supplemented with Halt™ Protease Inhibitor Cocktail (Pierce Biotechnology, Inc., USA). Whole cell lysates were obtained by subsequent centrifugation at 15000 × *g* for 10 min at 4℃. Protein concentrations were determined using Bradford Protein Assay Kit with bovine serum albumin (BSA) as standard (SinoBio Biotech Co., Ltd. Shanghai, China). Fifty μg of protein extracts were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a Protran® nitrocellulose membrane (Schleicher & Schuell BioScience GmbH, Whatman Group, Germany). The membrane was incubated with the rabbit anti-TLR4 polyclonal antibody (Bio-Chain, USA) at 4℃ overnight after being blocked with a 10% BSA solution. The membrane was washed with TBST buffer (20 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 0.1% Tween-20) and incubated with a secondary goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibody (Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 2 h at room temperature, and finally detected by chemiluminescence using Enhanced NuGlo™ Chemiluminescent Substrate Kit (Alpha Diagnostic Intl. Inc., USA) followed by autoradiographic and densitometric analysis. β-actin was used as an internal control.

Histological examinations of liver

The liver specimens were fixed in 10% formaldehyde for 12-24 h, embedded in paraffin, sliced into sections of 5 μm thickness and stained with hematoxylin-eosin (HE). Histological assessment was performed by three pathologists independently. The severity of steatosis was scored as 0 (no hepatocytes), 1 (less than 25% of hepatocytes), 2 (26%-50%), 3 (51%-75%), and 4 (greater than 75% of hepatocytes). The severity of the inflammation was scored as 0 (none), 1 (minimal), 2 (mild), 3 (moderate) and 4 (severe) based on the degree of portal and lobular inflammation and the evidence of piecemeal and spotty necrosis. The degree of necrotic hepatocytes was also scored as 0 to 4 (none, minimal, mild, moderate, and severe, respectively) based on the evidence of piecemeal and spotty necrosis.

Statistical analysis

All data were presented as mean ± SE. Differences among groups were assessed using unpaired Student's *t* test and one-way ANOVA. *P* value less than 0.05 was considered to be statistically significant. Calculations were performed with the SPSS11.0 statistical software package.

RESULTS

General conditions of rats

During the experiment, 4 rats in the model group died because fluids were poured mistakenly into trachea when they were administrated intragastrically. The other 44 rats survived.

Changes of weight and liver index of rats

The changes of the rat weight in models were significantly

Table 1 Changes of weight and liver index in rats

Groups	*n*	Weight change(g)	Liver index (Liver wet weight/rats weight × 100)
Control	12	27.27 ± 1.55[b]	3.65 ± 0.22[b]
Model	8	18.44 ± 1.16	5.49 ± 0.34
Low dose betaine	12	19.38 ± 1.95	3.83 ± 0.14[b]
High dose betaine	12	19.34 ± 1.38	3.75 ± 0.68[b]

[b]$P < 0.01$ compared with model group.

Table 2 Changes of liver function in rats

Groups	*n*	ALT (U/L)	AST (U/L)	ALB (g/L)	ALB/GLB
Control	12	59.47 ± 2.34[b]	116.89 ± 3.48[b]	38.1 ± 0.16	1.40 ± 0.34
Model	8	159.41 ± 7.74	248.25 ± 1.40	36.1 ± 1.22	1.55 ± 0.06
Low dose betaine	12	62.82 ± 7.78[b]	189.25 ± 5.9[b]	37.8 ± 2.36	1.50 ± 0.03
High dose betaine	12	64.93 ± 6.06[b]	188.73 ± 1.11[b]	36.4 ± 3.17	1.46 ± 0.12

[b]$P < 0.01$ compared with model group. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; GLB: Globulin.

Table 3 Changes of rat serum endotoxin, TNF-α, IFN-γ and IL-18

Groups	*n*	Endotoxin (ng/L)	TNF-α (pg/mL)	INF-γ (pg/mL)	IL-18 (pg/mL)
Control	12	44.15 ± 7.54[b]	9.34 ± 2.57[b]	16.86 ± 9.49[b]	19.73 ± 9.31[b]
Model	8	135.37 ± 30.17	20.81 ± 8.58	30.18 ± 7.60	40.99 ± 8.25
Low dose betaine	12	87.36 ± 15.93[b]	12.61 ± 1.70[b]	22.63 ± 4.90[b]	26.51 ± 5.59[b]
High dose betaine	12	61.80 ± 12.56[b,d]	9.79 ± 1.32[b,d]	18.02 ± 5.96[b,d]	18.23 ± 7.01[b,d]

[b]$P < 0.01$ compared with model group; [d]$P < 0.01$ compared with low dose betaine group. TNF-α: tumor necrosis factor-α; IL-10: Interleukin-10; IL-18: Interleukin-18; IFN-γ: Interferon-γ.

lower than that in controls ($P < 0.01$). Compared with the model group, there were no significant differences in betaine intervention groups ($P > 0.05$). Liver index was significantly higher in models than in controls ($P < 0.01$). Compared with the model group, liver index decreased significantly in the betaine intervention groups ($P < 0.01$), and there was no statistical difference between high dose betaine group and low dose betaine group ($P > 0.05$) (Table 1).

Changes of liver function

The changes of the ALT and AST in models were significantly higher than in controls ($P < 0.01$). Compared with the model group, the ALT and AST levels were significantly lowered in betaine intervention groups, indicating that the betaine can greatly improve the alcohol-induced liver injury, and there was no statistical difference between high dose betaine group and low dose betaine group ($P > 0.05$). There were no significant differences in ALB and A/G between model group and betaine intervention groups ($P > 0.05$) (Table 2).

Changes of serum endotoxin, TNF-α, IFN-γ and IL-18

The levels of serum endotoxin, TNF-α, IFN-γ and IL-18 were significantly higher in model group than in control group ($P < 0.01$). Compared with model group, serum endotoxin, TNF-α, IFN-γ and IL-18 significantly decreased in betaine intervention groups ($P < 0.01$). There was a statistical difference between high dose betaine group and low dose betaine group ($P < 0.01$) (Table 3).

Expressions of TLR4 mRNA and protein in liver tissue of rats

The software Quantiscan was used to analyze the absorbance of the products of TLR4 mRNA or protein quantitatively. The expression of TLR4 mRNA and protein was both significantly higher in model group than in normal ($P < 0.01$). Compared with the model group, the expression of TLR4 mRNA and protein was significantly reduced in betaine intervention groups ($P < 0.01$). There was a statistical difference of TLR4 expression between high dose betaine group and low dose betaine group ($P < 0.01$) (Table 4 and Figure 1).

Histopathological changes of liver

The liver structure in control group was normal, and no obvious inflammation and hepatic steatosis were observed (Figure 2A). In model group, the structure of hepatic cord was deranged, and various degrees of diffuse hepatic

Table 4 Changes of TLR4 expression in rat liver tissues

Groups	*n*	TLR4 mRNA	TLR4 protein
Control	12	0.44 ± 0.04[b]	0.56 ± 0.08[b]
Model	8	1.45 ± 0.07	1.83 ± 0.13
Low dose betaine	12	0.73 ± 0.05[b]	0.81 ± 0.09[b]
High dose betaine	12	0.62 ± 0.04[b,d]	0.65 ± 0.06[b,d]

[b]$P < 0.01$ compared with model group; [d]$P < 0.01$ compared with low dose betaine group. TLR 4: Toll-like receptor 4.

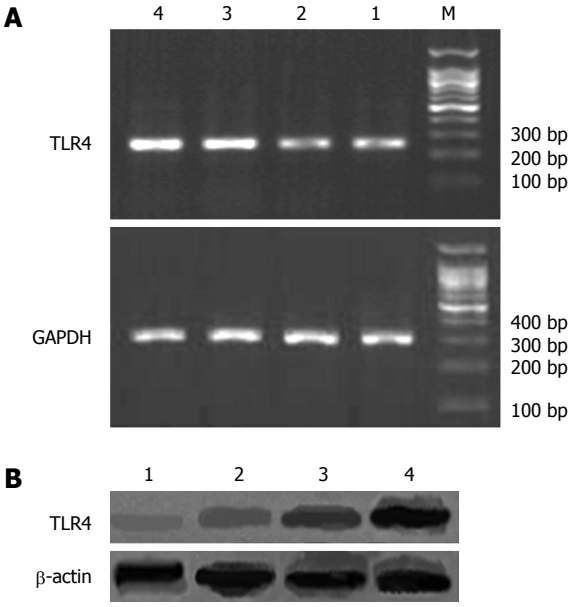

Figure 1 Expression of TLR4 in rat liver tissues. A: RT-PCR assay of TLR4 mRNA; B: Western blotting assay of TLR4 protein. 1: Control; 2: High dose betaine group; 3: Low dose betaine group; 4: Model; M: DNA marker.

steatosis and intralobular inflammation could be found obviously (Figure 2B). Compared with model group, the degree of hepatic steatosis and inflammation was greatly reduced in betaine intervention groups. The improvement of liver histopathology in the high dose betaine group was most significant (Figure 2C and D).

DISCUSSION

In this study, by establishing the intragastric fat-rich and ethanol diet mouse model, we found that the rats in model group had lower weight and higher liver index, obvious liver injury and hepatic steatosis, higher serum endotoxin, TNF-α, IFN-γ and IL-18 levels compared with the rats in control group. Endotoxemia and oxidative stress are two key factors for the progression of alcoholic liver diseases[22]. There are solid data supporting the hypothesis that endotoxin is indeed involved in alcoholic liver injury. First, it has been shown that excessive alcohol intake increases gut permeability of normally nonabsorbed substances[23,24]. Second, intestinal gram-negative bacteria, as well as blood endotoxin levels, are increased both in alcoholic patients and in the Tsukamoto-French enteral alcohol feeding model[25,26]. Third, intestinal sterilization with antibiotics and displacement of gram-negative

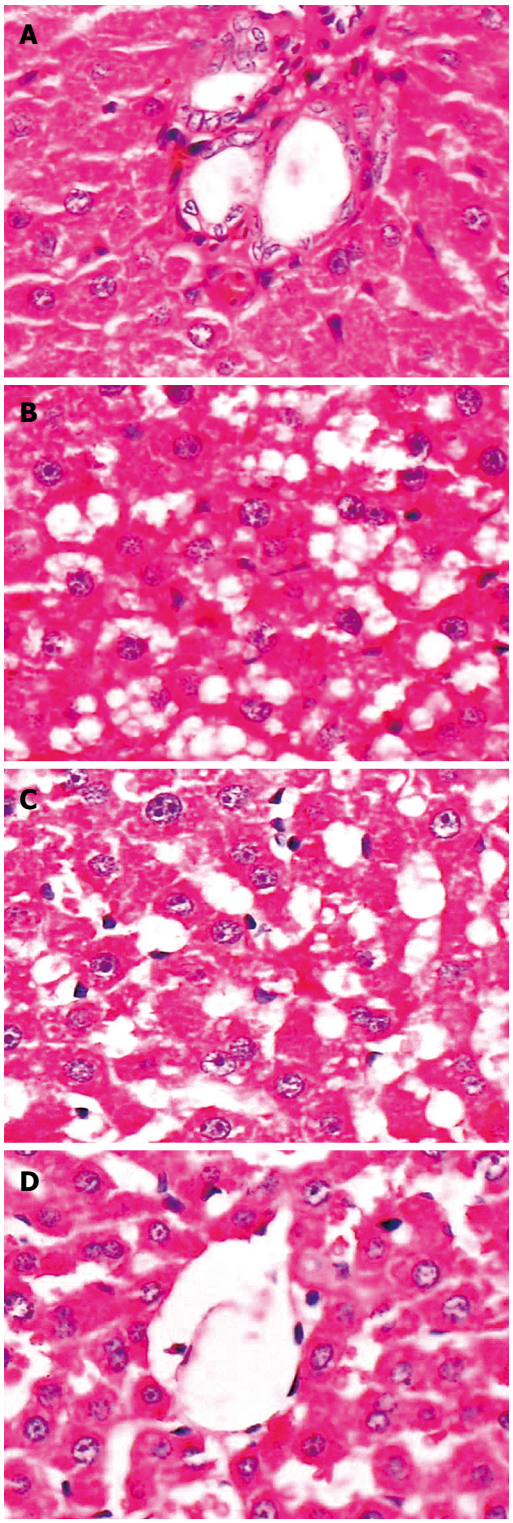

Figure 2 Histopathological changes of rat liver after betaine intervention. A: In control group, liver structure was normal, without obvious inflammation and hepatic steatosis; B: In model group, the structure of hepatic cord was deranged, with various degrees of diffuse hepatic steatosis and intralobular inflammation; C: In low dose betaine group, the degree of hepatic steatosis and inflammation was greatly reduced compared with model group; D: High dose betaine group, showing more significant improvement of hepatic steatosis and inflammation than the low does betaine group. Original magnification × 400.

bacteria with lactobacillus treatment prevents alcohol-induced liver injury[27,28]. Alcohol can increase the levels of circulating endotoxin in the portal blood. Once bound to LPS-binding protein (LBP), this complex is formed with the endotoxin receptor and CD14, activates Kupffer cells *via* TLR4[29]. Kupffer cell activation leads to the up-regulation of key cytokines, including TNF-α. Besides direct toxic effects on hepatocytes, TNF-α can indirectly damage the liver by increasing expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, as well as increasing the production of chemoattractant molecules from inflammatory cells[30].

In this study, we also found that the rats in model group fed with ethanol had a significantly higher expression of TLR4 mRNA and protein than normal rats. Many studies have confirmed that TLR4 is critical for early alcoholic liver injury[8-11]. It was shown that a functional mutation in TLR4 prevents early alcohol-induced liver injury in mice. Specifically, no differences in alcohol levels or plasma endotoxin were observed between the groups fed with ethanol. Moreover, a functional mutation in the TLR4 receptor prevents all downstream events, including increased TNF-α expression, inflammation, and liver injury[11]. These results support the hypothesis that endotoxin and TLR4 play a major role in the development of early alcohol-induced liver injury. CD14, a glycosylphosphatidylinositol-anchored receptor for LPS, is important in mediating the effects of LPS/LPB complexes on peripheral blood monocytes[31], and it is known that ethanol increases expression of CD14 on Kupffer cells[32]. However, CD14 lacks the ability to transduce LPS-induced cytoplasmic signals across a cell membrane, because it is not a transmembrane protein[33]. It had been suggested that LPS-induced inflammatory cell activation *via* CD14 also requires TLR4, which associates with CD14 on the cell surface, mediating LPS-induced signal transduction[34]. The finding that alcoholic liver injury is blocked in both CD14 and TLR4-deficient mice suggests that both of these receptors are necessary to initiate liver damage caused by alcohol[35]. Therefore, pharmacologic manipulation and targeting of the endotoxin-CD14/TLR4 signaling pathways could prove to be useful in alcoholic liver disease.

Previous studies have shown that betaine can prevent the alcohol-induced liver injury effectively and improve the liver function, which is related to the inhibition of inflammatory factor, the decrease of lipid peroxidation, the rivalry of endoplasmic reticulum stress and the prevention of apoptosis[16-21]. Our study indicates that in rats with alcohol-induced liver injury, betaine feeding can decrease the levels of serum ALT, AST, endotoxin, TNF-α, IFN-γ and IL-18, and reduced the expressions of TLR4, and improved the degree of hepatic steatosis and inflammation in liver tissues. It is suggested that betaine can prevent the alcohol-induced liver injury effectively and improve the liver function. The hepatoprotective mechanism of betaine is probably related to the inhibition of endotoxin/TLR4 signaling pathways.

In summary, the results of this study show that the expression of TLR4 increased significantly in ethanol-fed rats. Betaine administration can inhibit TLR4 expression, which may be one of mechanisms of alcoholic liver injury protected by betaine.

COMMENTS

Background

Chronic ethanol ingestion increases gut-derived endotoxin (LPS) in the portal circulation, thereby activating Kupffer cells to produce proinflammatory cytokines and induce liver injury. Toll-like receptor 4 (TLR4), *via* mediating LPS-induced signal transduction, plays a major role in the development of alcohol-induced liver injury. Blocking TLR4 signaling pathways is a therapeutic target of alcoholic liver disease. Many studies reported that betaine can prevent the alcohol-induced liver injury effectively and improve the liver function, but there are few reports about the effects of betaine on TLR4 and endotoxin in alcohol-induced liver injury.

Research frontiers

Betaine is the only methyl donor, which can replace folate or S-ademetionine to participate in methionine recycling and phosphatidylcholine synthesis in the human body. The hepatoprotective effect and mechanism of betaine is a research hotspot in the area of prevention and cure of alcoholic liver disease. Current studies show that the hepatoprotective mechanism of betaine is related to the inhibition of inflammatory factor, the decrease of lipid peroxidation, the promotion of endoplasmic reticulum stress and the prevention of apoptosis. However, whether the inhibition of TLR4 expression and reduction of endotoxin are involved in hepatoprotective effect of betaine in the alcoholic liver injury remains unclear.

Innovations and breakthroughs

In the present study, the authors employed the intragastric ethanol-fed rat model, which reproduces the pathological features of early alcohol-induced liver injury, to observe the changes of TLR4 and endotoxin, and to study the protective effect of betaine on alcohol-induced liver injury. The authors found that the ethanol-fed rats had obvious liver injury and hepatic steatosis, higher serum endotoxin and inflammatory factor (TNF-α, IFN-γ and IL-18) levels, and significantly higher TLR4 expression, whereas betaine feeding can improve the liver function, reduce the expressions of TLR4 and endotoxin levels, and improve the degree of hepatic steatosis and inflammation in liver tissues.

Applications

The study results suggest that betaine can prevent the alcohol-induced liver injury effectively, and one of the hepatoprotective mechanisms of betaine is probably related to the inhibition of endotoxin/TLR4 signaling pathways.

Terminology

Betaine, also known as trimethylglycine, is a chemical compound similar to folic acid and S-ademetionine. These compounds function as "methyl donors" that carry methyl molecules throughout the body, thus helping in the completion of several vital chemical processes. Toll-like receptor 4 (TLR4): TLR4 is a member of the Toll-like receptors (TLRs) family which can recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity, and thereby plays a fundamental role in pathogen recognition and activation of innate immunity.

Peer review

This is a well conducted and well written study. The experiments are described in detail, the results are shown nicely and the figures are impressive. This study for the first time shows that Betaine reduces the expression of TLR4 in rats with ethanol-induced liver injury, and proposes that the hepatoprotective mechanism of betaine is secondary to inhibition of endotoxin/TLR4 signaling pathways.

REFERENCES

1 **Fukui H**. Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. *Alcohol Clin Exp Res* 2005; **29**: 172S-179S

2 **Fang ZH**, Cui JW, Hu YY. [Endotoxin injury in alcoholic liver disease] *Zhonghua Ganzangbing Zazhi* 2005; **13**: 636-638

3 **Xu FL**, You HB, Li XH, Chen XF, Liu ZJ, Gong JP. Glycine attenuates endotoxin-induced liver injury by downregulating TLR4 signaling in Kupffer cells. *Am J Surg* 2008; **196**: 139-148
4 **Enomoto N**, Takei Y, Yamashima S, Ikejima K, Kitamura T, Sato N. Protective effect of pioglitazone against endotoxin-induced liver injury through prevention of Kupffer cell sensitization. *Alcohol Clin Exp Res* 2005; **29**: 216S-219S
5 **Dai Q**, Pruett SB. Ethanol suppresses LPS-induced Toll-like receptor 4 clustering, reorganization of the actin cytoskeleton, and associated TNF-alpha production. *Alcohol Clin Exp Res* 2006; **30**: 1436-1444
6 **Tavener SA**, Kubes P. Is there a role for cardiomyocyte toll-like receptor 4 in endotoxemia? *Trends Cardiovasc Med* 2005; **15**: 153-157
7 **Ferwerda B**, McCall MB, Verheijen K, Kullberg BJ, van der Ven AJ, Van der Meer JW, Netea MG. Functional consequences of toll-like receptor 4 polymorphisms. *Mol Med* 2008; **14**: 346-352
8 **Dolganiuc A**, Bakis G, Kodys K, Mandrekar P, Szabo G. Acute ethanol treatment modulates Toll-like receptor-4 association with lipid rafts. *Alcohol Clin Exp Res* 2006; **30**: 76-85
9 **Pruett SB**, Zheng Q, Fan R, Matthews K, Schwab C. Acute exposure to ethanol affects Toll-like receptor signaling and subsequent responses: an overview of recent studies. *Alcohol* 2004; **33**: 235-239
10 **Zuo G**, Gong J, Liu C, Wu C, Li S, Dai L. Synthesis of Toll-like receptor 4 in Kupffer cells and its role in alcohol-induced liver disease. *Chin Med J* (Engl) 2003; **116**: 297-300
11 **Uesugi T**, Froh M, Arteel GE, Bradford BU, Thurman RG. Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. *Hepatology* 2001; **34**: 101-108
12 **Kim YC**, Jung YS, Kim SK. Effect of betaine supplementation on changes in hepatic metabolism of sulfur-containing amino acids and experimental cholestasis induced by alpha-naphthylisothiocyanate. *Food Chem Toxicol* 2005; **43**: 663-670
13 **Kharbanda KK**, Rogers DD 2nd, Mailliard ME, Siford GL, Barak AJ, Beckenhauer HC, Sorrell MF, Tuma DJ. A comparison of the effects of betaine and S-adenosylmethionine on ethanol-induced changes in methionine metabolism and steatosis in rat hepatocytes. *J Nutr* 2005; **135**: 519-524
14 **Krueger KJ**, McClain CJ, McClave SA, Dryden GW. Nutritional supplements and alternative medicine. *Curr Opin Gastroenterol* 2004; **20**: 130-138
15 **Craig SA**. Betaine in human nutrition. *Am J Clin Nutr* 2004; **80**: 539-549
16 **Zhang P**, Gong ZJ. Effects of betaine on expression of caspase-12 in ethanol-induced liver injury in rats. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 2437-2440
17 **Zhang P**, Gong ZJ, Wang LW, Sun XM, Zhou XR. Effects of Betaine on hyperhomocysteinemia and lipid peroxidation in rats with ethanol-induced liver injury. *Zhongxiyi Jiehe Ganbing Zazhi* 2006; **16**: 30-32
18 **Ji C**, Shinohara M, Vance D, Than TA, Ookhtens M, Chan C, Kaplowitz N. Effect of transgenic extrahepatic expression of betaine-homocysteine methyltransferase on alcohol or homocysteine-induced fatty liver. *Alcohol Clin Exp Res* 2008; **32**: 1049-1058
19 **Samara K**, Liu C, Soldevila-Pico C, Nelson DR, Abdelmalek MF. Betaine resolves severe alcohol-induced hepatitis and steatosis following liver transplantation. *Dig Dis Sci* 2006; **51**: 1226-1229
20 **Kharbanda KK**, Mailliard ME, Baldwin CR, Sorrell MF, Tuma DJ. Accumulation of proteins bearing atypical isoaspartyl residues in livers of alcohol-fed rats is prevented by betaine administration: effects on protein-L-isoaspartyl methyltransferase activity. *J Hepatol* 2007; **46**: 1119-1125
21 **Ji C**, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. *Gastroenterology* 2003; **124**: 1488-1499
22 **Nanji AA**. Role of Kupffer cells in alcoholic hepatitis. *Alcohol* 2002; **27**: 13-15
23 **Tang Y**, Forsyth CB, Farhadi A, Rangan J, Jakate S, Shaikh M, Banan A, Fields JZ, Keshavarzian A. Nitric oxide-mediated intestinal injury is required for alcohol-induced gut leakiness and liver damage. *Alcohol Clin Exp Res* 2009; **33**: 1220-1230
24 **Rajendram R**, Preedy VR. Effect of alcohol consumption on the gut. *Dig Dis* 2005; **23**: 214-221
25 **Parlesak A**, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000; **32**: 742-747
26 **Zuo GQ**, Gong JP, Liu CA, Li SW, Wu XC, Yang K, Li Y. Expression of lipopolysaccharide binding protein and its receptor CD14 in experimental alcoholic liver disease. *World J Gastroenterol* 2001; **7**: 836-840
27 **Zeng MD**, Li YM, Chen CW, Lu LG, Fan JG, Wang BY, Mao YM. Guidelines for the diagnosis and treatment of alcoholic liver disease. *J Dig Dis* 2008; **9**: 113-116
28 **Barve A**, Khan R, Marsano L, Ravindra KV, McClain C. Treatment of alcoholic liver disease. *Ann Hepatol* 2008; **7**: 5-15
29 **Su GL**, Klein RD, Aminlari A, Zhang HY, Steinstraesser L, Alarcon WH, Remick DG, Wang SC. Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. *Hepatology* 2000; **31**: 932-936
30 **Kono H**, Uesugi T, Froh M, Rusyn I, Bradford BU, Thurman RG. ICAM-1 is involved in the mechanism of alcohol-induced liver injury: studies with knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1289-G1295
31 **Triantafilou M**, Triantafilou K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol* 2002; **23**: 301-304
32 **Dai LL**, Gong JP, Zuo GQ, Wu CX, Shi YJ, Li XH, Peng Y, Deng W, Li SW, Liu CA. Synthesis of endotoxin receptor CD14 protein in Kupffer cells and its role in alcohol-induced liver disease. *World J Gastroenterol* 2003; **9**: 622-626
33 **Dobrovolskaia MA**, Vogel SN. Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect* 2002; **4**: 903-914
34 **Muta T**, Takeshige K. Essential roles of CD14 and lipopolysaccharide-binding protein for activation of toll-like receptor (TLR)2 as well as TLR4 Reconstitution of TLR2- and TLR4-activation by distinguishable ligands in LPS preparations. *Eur J Biochem* 2001; **268**: 4580-4589
35 **Miyaso H**, Morimoto Y, Ozaki M, Haga S, Shinoura S, Choda Y, Murata H, Katsuno G, Huda K, Takahashi H, Tanaka N, Iwagaki H. Protective effects of nafamostat mesilate on liver injury induced by lipopolysaccharide in rats: possible involvement of CD14 and TLR-4 downregulation on Kupffer cells. *Dig Dis Sci* 2006; **51**: 2007-2012

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BRIEF ARTICLE

Role of *RECK* methylation in gastric cancer and its clinical significance

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Abstract

AIM: To investigate the relation between *RECK* methylation and clinicopathological characteristics of gastric cancer patients and evaluate the role of *RECK* methylation in peritoneal metastasis of gastric cancer.

METHODS: Methylation of *RECK* gene in 40 paired samples of gastric cancer and its corresponding adjacent normal mucosa, lymph nodes and peritoneal irrigation fluid was detected by methylation-specific polymerase chain reaction.

RESULTS: Aberrant methylation of *RECK* gene was detected in 27.5% (11/40) of the adjacent normal mucosa samples, in 47.5% (19/40) of gastric cancer samples, in 57.1% (12/21) of the lymph node samples, and in 35% (14/40) of peritoneal irrigation fluid samples, respectively, with a significant difference between the adjacent normal mucosa and lymph node samples (P = 0.023). Presence of *RECK* methylation in the primary tumor samples was significantly correlated with tumor invasion (P = 0.023). The accuracy of *RECK* methylation in peritoneal lavage fluid samples for the diagnosis of peritoneal metastasis of gastric cancer was 72.5% (26/40), with a sensitivity of 66.7% (6/9) and a specificity of 74.2% (23/31).

CONCLUSION: Aberrant methylation of *RECK* gene may provide useful information for the early diagnosis and treatment of peritoneal metastasis of gastric cancer.

Key words: *RECK* gene; Hypermethylation; Gastric cancer; Metastasis; Peritoneal lavage fluid

Peer reviewer: Damian Casadesus Rodriguez, MD, PhD, Calixto Garcia University Hospital, J and University, Vedado, Havana City, Cuba

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INTRODUCTION

Gastric cancer seriously threatens the human health worldwide. There is increasing evidence that almost all gastric cancers have epigenetic abnormalities that drive cancer development and progression in collaboration with genetic changes. Aberrant methylation in the promoter CpG island of tumor suppressor genes (*TSG*) where DNA is transcribed into RNA causes its silence. Transcription of DNA is the first major step in decoding DNA into a protein. DNA methylation can inactivate tumor suppressor genes[1]. It has been shown that aberrant methylation and diminished expression of DNA in the promoter CpG island occur in a number of tumor-related genes in gastric cancer[2]. For example, *RASSF1A*, a candidate tumor suppressor gene, is

hypermethylated in gastric cancer[3,4], *TIMP-3,* a silenced tumor suppressor gene, encodes a protease inhibitor that may inhibit tissue invasion[5], and *RECK*, a newly discovered metastasis suppressor gene, is silenced with aberrant CpG island hypermethylation in some common tumors[6-8]. However, the relation between methylation of *RECK* gene and gastric cancer has not been fully studied.

In this study, *RECK* gene methylation was detected in samples of primary tumor tissue and its adjacent normal mucosa, metastatic lymph nodes and peritoneal irrigation fluid by methylation-specific PCR in order to find the relation between *RECK* methylation and clinicopathological characteristics of gastric cancer and the role of *RECK* methylation in diagnosis of peritoneal metastasis of gastric cancer.

MATERIALS AND METHODS

Patients

Forty patients including 28 males and 12 females at the age of 34-78 years underwent resection of their gastric cancer at the First Affiliated Hospital of China Medical University from July 2008 to January 2009. All patients did not receive chemotherapy or radiotherapy before operation.

Samples

Physiological saline (50 mL) was injected into the Douglas cavity at the beginning of operation and aspirated after gentle stirring, and then peritoneal lavage fluid was collected from the cavity before operation. Half of the peritoneal lavage fluid was examined using conventional cytological methods with Papanicolaou's staining and intact cells were harvested from the other half centrifuged at 2000 r/min for 20 min as previously described[9,10] and stored in liquid nitrogen. Samples of primary tumor tissue and its paired adjacent normal mucosa and metastatic lymph nodes were taken immediately after resection of gastric cancer and stored in liquid nitrogen until use. The diagnosis of gastric cancer was made with hematoxylin and eosin (HE) staining. Paired adjacent normal mucosa samples were obtained at least 3 cm from the distal negative surgical margin to confirm the absence of malignancy. Lymph node samples were also stained with HE to confirm the occurrence of metastasis. Differentiation of tumor cells was detected and the tumor was staged following the guidelines of International Union against Cancer (UICC).

DNA extraction and bisulfite treatment

DNA was extracted from the genome with the hydroxybenzene-chloroform extraction method, stored at -70℃, and treated with bisulfite to convert the unmethylated cytosine to uracil.

Methylation-specific PCR

DNA was purified using a Wizard DNA clean-up system (Promega) according to its manufacturer's instructions. A 20 μL reaction volume was consisted of 3 μL DNA, 2 μL 10 × PCR buffer, 0.8 μL dNTP, 0.4 μL primers, 0.15 μL Tap enzyme, and 13.25 μL double-distilled water. PCR conditions were as follows: pre-denaturation at 94℃ for 10 min, followed by 40 cycles at 94℃ for 30 s, at 54℃ for 20 s, at 72℃ for 30 s, and a final extension at 72℃ for 5 min. Methyltransferase Sss I -treated DNA in peripheral blood cells from healthy people was used as a methylation positive control, untreated DNA served as an unmethylation positive control, and double-distilled water served as a negative control[4]. The sequences of primers are as follows: unmethylation primer: UF_*RECK* (5'-GGTTAGTTTTTTTTTTTATTTTAGTGGTTTGA-3') and UR_*RECK* (5'-ATTTCCAAAACCTCCCAAAAACAAAAACA-3'), methylation primer: MF_*RECK* (5'-GTTAGTTTTTTTTTTTTATTTTAGTGGTTCGA-3') and MR_*RECK* (5'-TCCAAAACCTCCCGAAAACGAAAACG-3')[8]. The PCR products (205 bp and 201 bp) were subjected to 2.5% agarose gel electrophoresis at 120 V for 40 min and quantified with the Fluor Chen 2.0 system.

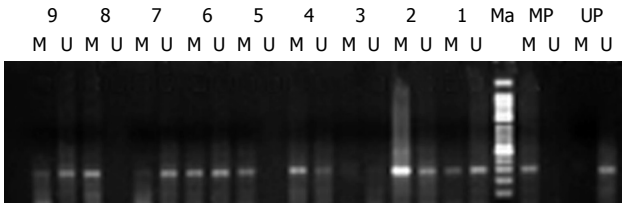

Figure 1 PCR showing methylation of *RECK* in primary tumor and its paired adjacent normal mucosa and metastatic lymphnode samples. M: Methylation; U: Unmethylation; Ma: 50 bp DNA ladder marker; MP: Methylation positive control; UP: Unmethylation positive control; 1-9: Sample number.

Statistical analysis

Statistical analysis was performed using the SPSS13.0 software package. χ^2 test and Fisher's exact test were adopted to verify the difference. $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological characteristics of gastric cancer patients and RECK methylation

The methylation of *RECK* in samples of primary tumor tissue and its paired adjacent normal mucosa and metastatic lymphnode was detected by methylation special-PCR (Figure 1).

Methylation of *RECK* was found in 47.5% (19/40) of primary tumor tissue samples, in 27.5% (11/40) of paired adjacent normal mucosa samples, in 57.1% (12/21) of metastatic lymphnode samples, respectively. A significant relation was found between adjacent normal mucosa and metastatic lymphnode samples. *RECK* methylation was related with tumor invasion ($P = 0.023$) but not with other clinicopathological characteristics of gastric cancer patients such as age,

Table 1 Relation between clinicopathological characteristics and *RECK* methylation in gastric cancer patients

Variable	Patients (*n*)	*RECK* methylation	*P* value
Age (yr)			0.689
≤ 65	32	16	
> 65	8	3	
Tumor size (cm)			0.121
≤ 5	24	9	
> 5	16	10	
Borrmann classification			0.199
1+2	19	7	
3+4	21	12	
Tumor differentiation			0.935
Well	25	12	
Moderate/poor	15	7	
Tumor invasion			0.023
T1+T2	28	10	
T3+T4	12	9	
Nodal status			0.199
N-	19	7	
N+	21	12	

TNM was staged according to the guideline of International Union against Cancer (UICC). N-: Without nodal metastasis; N+: With nodal metastasis.

Table 2 Relation between *RECK* methylation in peritoneal lavage and peritoneal metastasis

PLM	Peritoneal metastasis	
	+	-
+	6	8
-	3	23

PLM: Methylation in peritoneal lavage.

Table 3 Relation between *RECK* methylation in peritoneal lavage and clinicopathological factors

Variable	Patients (*n*)	RECK methylation in peritoneal lavage	*P* value
Tumor invasion			0.193
T1+T2	28	8	
T3+T4	12	6	
Nodal status			0.273
N-	19	5	
N+	21	9	

tumor size, tumor differentiation, and Borrmann classification (Table 1).

Relation between peritoneal metastasis of gastric cancer and RECK methylation in peritoneal lavage fluid

In this study, the promoter of *RECK* gene was hypermethylated in 35% (14/40) of the samples. Among the 14 samples, peritoneal metastasis of gastric cancer was observed in 9. The diagnostic accuracy of *RECK* methylation in peritoneal lavage fluid for peritoneal metastasis of gastric cancer was 72.5%, with a sensitivity of 66.7%, a specificity of 74.2%, a PPV of 47.1%, and a NPV of 95.7% (Table 2).

RECK methylation in peritoneal lavage fluid was found in tumors with lymph node metastasis (42.6%) and without lymph node metastasis (26.3%), although the difference between them was not statistically significant (Table 3).

DISCUSSION

RECK gene was discovered on chromosome region 9p13-p12 by Takahashi *et al*[11] in 1998. It encodes a membrane-anchored glucose protein with a relative molecular mass of 110000. *RECK* protein is an important mediator of tissue remodeling to inhibit *MMP-2, MMP-9* and *MT1-MMP* after transcription[12,13]. *RECK* protein limits tumor invasion and metastasis and angiogenesis through negatively regulated *MMPs*. It has been shown that several common tumors, such as colorectal, breast, and lung carcinomas, are linked to down-regulation of *RECK*[14-16]. In these tumors, *RECK* is down-regulated most likely as a result of inhibition at the *Sp1* promoter site[17]. It was reported that down-regulation of the *RECK* gene is mediated by promoter methylation which causes its silence, just as other tumor suppressor genes[7,8,18]. Epigenetic alteration induced by DNA methyltransferases (DNMT) catalyzing methylation at 5 positions of cytosine ring using S-adenosylmethionine as the donor molecule for the methyl group plays an important role in tumorigenesis and progression[1]. The mechanism underlying *RECK* down-regulation appears to be multifactorial, and more studies are required to define its reasons.

In this study, *RECK* methylation was observed in samples of primary tumor tissue and its paired adjacent normal mucosa and metastatic lymph nodes from gastric cancer patients, indicating that *RECK* methylation in primary tumor tissue samples (47.5%) and in metastatic lymph node samples (57.1%) is much higher than that in paired adjacent normal mucosa samples (27.5%) ($P = 0.023$) and that *RECK* methylation is correlated with tumor invasion ($P = 0.023$). No significant difference was found in other factors, including age, tumor size, tumor differentiation, nodal status, Borrmann classification. However, Song *et al*[19] found that *RECK* expression is negatively related with lymph node metastasis and tumor stage in gastric cancer patients, which may be due to the small sample size, contamination of normal tissues, technical limitations[7], and down-regulation of *RECK*. Cho *et al*[7] showed that *RECK* promoter is methylated in 44% of tumor tissue samples and down-regulation of *RECK* is significantly correlated with promoter methylation ($P < 0.05$), suggesting that *RECK* methylation plays a significant role in inhibiting tumorigenesis and metastasis.

Methylation alteration occurs not only in solid cancer tissues but also in various remote samples from cancer patients. It has been recently reported that DNA methylation can act as a promising biomarker in early diagnosis and prognosis of gastric cancer[20]. In our

study, *RECK* methylation in peritoneal lavage fluid was related with peritoneal metastasis of gastric cancer. Peritoneal metastasis of gastric cancer with cytologically positive peritoneal lavage was found in 9 of 14 patients with promoter hypermethylation. *RECK* promoter hypermethylation in peritoneal lavage showed a higher sensitivity (66.7%) for the diagnosis of peritoneal dissemination of gastric cancer than cytology. The reasons why methylation alteration acts as a biomarker are as follows. First, the methylation signal can act as a marker at a low concentration. Second, the methylation pattern and underlying DNA are more stable than RNA level and molecules[10]. However, methylation alteration in peritoneal lavage has a lower specificity for the diagnosis of peritoneal dissemination of gastric cancer, which can explained as follows. First, most cells in peritoneal lavage are mesothelial cells leading to false positive *RECK* methylation. Second, the discrepancy of methylation profile exists sometimes in peritoneal lavage and cancer tissue. In order to solve these problems, serial test, *RECK* methylation and other examinations, such as carcino-embryonic antigen in peritoneal lavage, can be used in the diagnosis of peritoneal dissemination of gastric cancer. In our study, *RECK* methylation in peritoneal lavage fluid was more frequently found in tumors with lymph node metastasis than in tumors without lymph node metastasis, suggesting that *RECK* methylation in peritoneal lavage can be considered a biomarker for predicting peritoneal metastasis of gastric cancer.

In summary, hypermethylation of *RECK* promoter is a common event in gastric cancer patients. *RECK* methylation in peritoneal lavage fluid acts as a biomarker of peritoneal metastasis of gastric cancer. Promoter hypermethylation of *RECK* gene provides a new tool for the prevention and treatment of gastric cancer. Further study is needed on the mechanism underlying *RECK* hypermethylation in gastric cancer patients.

COMMENTS

Background

Gastric cancer is a common tumor which seriously threatens the human health worldwide. DNA methylation in the promoter CpG island of tumor suppressor genes is one of the reasons for tumorigenesis and progression. It has been shown that DNA methylation, especially in body fluid, can act as a biomarker for predicting tumor metastasis.

Research frontiers

RECK hypermethylation plays an important role in the epigenetic regulation of gene transcription. There is evidence that DNA promoter hypermethylation can cause transcription repression, contributing to tumorigenesis and progression. It has been recently shown that DNA methylation, especially in body fluid, can act as a biomarker for predicting tumorigenesis and prognosis. However, further study is needed on the mechanism underlying *RECK* hypermethylation.

Innovations and breakthroughs

RECK methylation in gastric cancer and peritoneal lavage fluid was detected, showing that *RECK* methylation plays an important role in diagnosing peritoneal metastasis.

Applications

Promoter hypermethylation of *RECK* gene provides a new tool for the prevention and treatment of gastric cancer. In addition, *RECK* methylation, especially in peritoneal lavage fluid, can act as a biomarker for diagnosing peritoneal metastasis.

Peer review

It is a very interested topic for the readers of *WJG*. The results of this study show that promoter hypermethylation of *RECK* gene provides a new tool for the prevention and treatment of gastric cancer and *RECK* methylation, especially in peritoneal lavage fluid, can act as a biomarker for diagnosing peritoneal metastasis, which are of great value for the diagnosis of gastric cancer.

REFERENCES

1 **Herman JG**, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003; **349**: 2042-2054

2 **Wang JF**, Dai DQ. Metastatic suppressor genes inactivated by aberrant methylation in gastric cancer. *World J Gastroenterol* 2007; **13**: 5692-5698

3 **Oliveira C**, Velho S, Domingo E, Preto A, Hofstra RM, Hamelin R, Yamamoto H, Seruca R, Schwartz S Jr. Concomitant RASSF1A hypermethylation and KRAS/BRAF mutations occur preferentially in MSI sporadic colorectal cancer. *Oncogene* 2005; **24**: 7630-7634

4 **Shen WJ**, Dai DQ, Teng Y, Liu HB. Regulation of demethylation and re-expression of RASSF1A gene in gastric cancer cell lines by combined treatment of 5-Aza-CdR and NaB. *World J Gastroenterol* 2008; **14**: 595-600

5 **Kang GH**, Shim YH, Jung HY, Kim WH, Ro JY, Rhyu MG. CpG island methylation in premalignant stages of gastric carcinoma. *Cancer Res* 2001; **61**: 2847-2851

6 **Chang HC**, Cho CY, Hung WC. Downregulation of RECK by promoter methylation correlates with lymph node metastasis in non-small cell lung cancer. *Cancer Sci* 2007; **98**: 169-173

7 **Cho CY**, Wang JH, Chang HC, Chang CK, Hung WC. Epigenetic inactivation of the metastasis suppressor RECK enhances invasion of human colon cancer cells. *J Cell Physiol* 2007; **213**: 65-69

8 **Long NK**, Kato K, Yamashita T, Makita H, Toida M, Hatakeyama D, Hara A, Mori H, Shibata T. Hypermethylation of the RECK gene predicts poor prognosis in oral squamous cell carcinomas. *Oral Oncol* 2008; **44**: 1052-1058

9 **Katsuragi K**, Yashiro M, Sawada T, Osaka H, Ohira M, Hirakawa K. Prognostic impact of PCR-based identification of isolated tumour cells in the peritoneal lavage fluid of gastric cancer patients who underwent a curative R0 resection. *Br J Cancer* 2007; **97**: 550-556

10 **Kamiyama H**, Noda H, Takata O, Suzuki K, Kawamura Y, Konishi F. Promoter hypermethylation of tumor-related genes in peritoneal lavage and the prognosis of patients with colorectal cancer. *J Surg Oncol* 2009; **100**: 69-74

11 **Takahashi C**, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, Ikawa Y, Ratzkin BJ, Arakawa T, Noda M. Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. *Proc Natl Acad Sci USA* 1998; **95**: 13221-13226

12 **Noda M**, Oh J, Takahashi R, Kondo S, Kitayama H, Takahashi C. RECK: a novel suppressor of malignancy linking oncogenic signaling to extracellular matrix remodeling. *Cancer Metastasis Rev* 2003; **22**: 167-175

13 **Clark JC**, Thomas DM, Choong PF, Dass CR. RECK--a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer. *Cancer Metastasis Rev* 2007; **26**: 675-683

14 **Takemoto N**, Tada M, Hida Y, Asano T, Cheng S, Kuramae T, Hamada J, Miyamoto M, Kondo S, Moriuchi T. Low expression of reversion-inducing cysteine-rich protein with Kazal motifs (RECK) indicates a shorter survival after resection in patients with adenocarcinoma of the lung. *Lung Cancer* 2007; **58**: 376-383

15 **Figueira RC**, Gomes LR, Neto JS, Silva FC, Silva ID, Sogayar MC. Correlation between MMPs and their inhibitors in breast cancer tumor tissue specimens and in cell lines with different metastatic potential. *BMC Cancer* 2009; **9**: 20
16 **Oshima T**, Kunisaki C, Yoshihara K, Yamada R, Yamamoto N, Sato T, Makino H, Yamagishi S, Nagano Y, Fujii S, Shiozawa M, Akaike M, Wada N, Rino Y, Masuda M, Tanaka K, Imada T. Clinicopathological significance of the gene expression of matrix metalloproteinases and reversion-inducing cysteine-rich protein with Kazal motifs in patients with colorectal cancer: MMP-2 gene expression is a useful predictor of liver metastasis from colorectal cancer. *Oncol Rep* 2008; **19**: 1285-1291
17 **Sasahara RM**, Takahashi C, Noda M. Involvement of the Sp1 site in ras-mediated downregulation of the RECK metastasis suppressor gene. *Biochem Biophys Res Commun* 1999; **264**: 668-675
18 **Chang HC**, Cho CY, Hung WC. Silencing of the metastasis suppressor RECK by RAS oncogene is mediated by DNA methyltransferase 3b-induced promoter methylation. *Cancer Res* 2006; **66**: 8413-8420
19 **Song SY**, Son HJ, Nam E, Rhee JC, Park C. Expression of reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) as a prognostic indicator in gastric cancer. *Eur J Cancer* 2006; **42**: 101-108
20 **Tost J**. DNA methylation: an introduction to the biology and the disease-associated changes of a promising biomarker. *Methods Mol Biol* 2009; **507**: 3-20

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CASE REPORT

Successful endoscopic procedures for intraductal papillary neoplasm of the bile duct: A case report

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Abstract

Attention has recently been focused on biliary papillary tumors as the novel disease entity intraductal papillary neoplasm of the bile duct (IPNB), which consists of papillary proliferation of dysplastic biliary epithelium. As even benign papillary tumors are considered as premalignant, some investigators recommend aggressive surgical therapy for IPNB, although no guidelines are available to manage this disease. Few reports have described long-term follow-up of patients with benign IPNB without radical resection. If patients with IPNB who are treated only with endoscopic procedures are noted, clinical profiles and alternative therapies other than resection may be recommended. We report the case of a patient who experienced repetitive cholangitis for 10 years and was finally diagnosed with IPNB. Radical resection could not be recommended because of the age of the patient, therefore, endoscopic sphincterotomy was performed. Although an endoscopic retrograde biliary drainage catheter was placed several times for repetitive cholangitis, the patient has done well during follow-up. Our case may offer insights into the natural course and management decisions for the novel disease entity of IPNB.

Key words: Biliary tract neoplasms; Papilloma; Endoscopic sphincterotomy; Endoscopic retrograde biliary drainage

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INTRODUCTION

Recently, the novel disease entity of intraductal papillary neoplasm of the bile duct (IPNB) has been proposed to include biliary papillomatosis, which comprises multiple

biliary papillomas composed of papillary proliferation of the dysplastic biliary epithelium, and papillary cholangiocarcinoma[1]. IPNB is thought to represent the biliary counterpart of pancreatic intraductal papillary mucinous neoplasm (IPMN-P) and is thus considered premalignant[1,2]. The prognosis of patients with IPNB is good when curative surgery is performed, therefore, aggressive resection is recommended as the first choice[3-5]. However, little information is available regarding the prognosis of IPNB without curative surgery, as few patients with IPNB who have been followed for several years without surgery have been reported. Here, we present a patient with IPNB who was followed for 10 years without surgical resection, and has done well during the follow-up period. This case indicates a natural course of the novel entity IPNB and alternative therapies for this disease.

CASE REPORT

A previously healthy 76-year-old woman developed acute cholecystitis caused by gallbladder stones, and cholecystectomy was performed in 1999. Cholangioscopic examination during surgery revealed a bile duct tumor with papillary proliferation protruding into the common bile duct (Figure 1A). The tumor was followed without resection, and a biopsy specimen showed tubular adenoma packed with small glandular or papillary components.

When the patient was admitted for acute cholangitis in 2004, the papillary tumor in the bile duct appeared unchanged. The patient recovered well until she became symptomatic again in September 2007, for which an endoscopic retrograde biliary drainage (ERBD) catheter (FLEXIMA™ Biliary Stent System, Boston Scientific Co., Natick, MA, USA) was placed. In December 2007, the patient developed recurrent cholangitis because of slippage of the ERBD catheter, which was re-inserted into the bile duct. In early January 2008, the ERBD catheter slipped, and the patient was seen for abdominal pain and fever. Laboratory test results included: alkaline phosphatase, 498 U/L (normal: 104-338 U/L); γ-glutamyltranspeptidase, 122 U/L (normal: 18-66 U/L); aspartate aminotransferase, 58 U/L (normal: 10-37 U/L); alanine aminotransferase, 71 U/L (normal: 3-34 U/L); carbohydrate antigen 19-9, 414 U/mL (normal: 0-37 U/mL); white blood cell count, 11 400/mm^3 (normal: 4000-9000/mm^3); and C-reactive protein, 17.3 mg/dL (normal: 0-0.3 mg/dL).

Endoscopic retrograde cholangiography (ERC) revealed extrahepatic bile duct dilatation, which was more marked than that seen in 1999, and the presence of intraductal polypoid and amorphous filling defects (Figure 1B and C). Magnetic resonance cholangiopancreatography (MRCP) showed cystic lesions connected to the intrahepatic bile duct in the left liver. Cystic lesions were enlarged compared with those in 1999 (Figure 1D and E). Abdominal computed tomography (CT) showed both intrahepatic and extrahepatic bile ducts to be dilated and the presence of a 20-mm mass in the distal common bile duct. In addition, the cystic lesion in the left liver was enlarged compared to that in 1999 (Figure 1F-I). Contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) with Sonazoid® (Daiichi-Sankyo, Tokyo, Japan) showed flow signals inside the whole mass, which revealed the papillary structure (Figure 2A and B). CEH-EUS for biliary diseases was approved by the ethical committee of Dokkyo Medical University, and written informed consent was obtained from the patient before the examination. Intraductal ultrasonography (IDUS) showed a 10-mm papillary mass in the middle bile duct (Figure 2C), and multilobular lesions in the distal bile duct (Figure 2D). Duodenoscopy demonstrated a 10-mm discolored mass on the ampulla of Vater. A biopsy specimen from the discolored mass showed adenoma.

As the patient was diagnosed with cholangitis caused by obstruction of the bile duct by intraductal papillary tumor, endoscopic biliary sphincterotomy (EST) was performed (Figure 3). Radical resection was not recommended because of the age of the patient. Through the orifice of the ampulla of Vater, a soft, bead-like mass was extracted by balloon sweep and a net-type catheter.

Pathological diagnosis of the extracted mass was papillary neoplasia without invasive carcinoma (Figure 4A). Immunohistochemical analysis was performed for mucin core proteins and cytokeratins (CKs). Less than 5% of adenoma cells showed positivity for MUC1 in the apical membrane (Figure 4B). Negative results were obtained for MUC2. About 40%-50% of adenoma cells showed positivity for MUC5AC in the cytoplasm (Figure 4C). Both CK7 and CK20 were negative.

Although the patient was readmitted in May 2008 and in June 2009 with signs of cholangitis, for which no special procedures and placement of ERBD catheter were respectively performed, the patient has done well since EST.

DISCUSSION

This case is compatible with the novel disease entity of IPNB[1], and biliary papillomatosis can be diagnosed as discussed below. Biliary papilloma is a rare benign neoplasm that consists of papillary proliferation of atypical biliary epithelium along with delicate fibrovascular stalks[6]. Biliary papillomatosis is defined as the presence of more than three papillomas at different sites of the biliary tree. Some cholangiocarcinomas that show mainly papillary proliferation in the bile duct are designated as papillary cholangiocarcinoma. Zen *et al*[1] have proposed biliary papilloma(tosis) and papillary cholangiocarcinoma with or without mucus hypersecretion as belonging to the novel tumor entity of IPNB. In this case, cholangioscopy and tissue specimens demonstrated that the tumor originated from the biliary epithelium, and various imaging modalities revealed tumors with papillary formation. Two papillary tumors in the extrahepatic bile duct detected by IDUS, and an expanding cystic lesion in the liver contiguous with the intrahepatic bile duct suggested that at least three lesions were present at different sites of the biliary tree. To summarize, this case could be diagnosed as biliary papillomatosis, although whether malignant transformation was present is unknown.

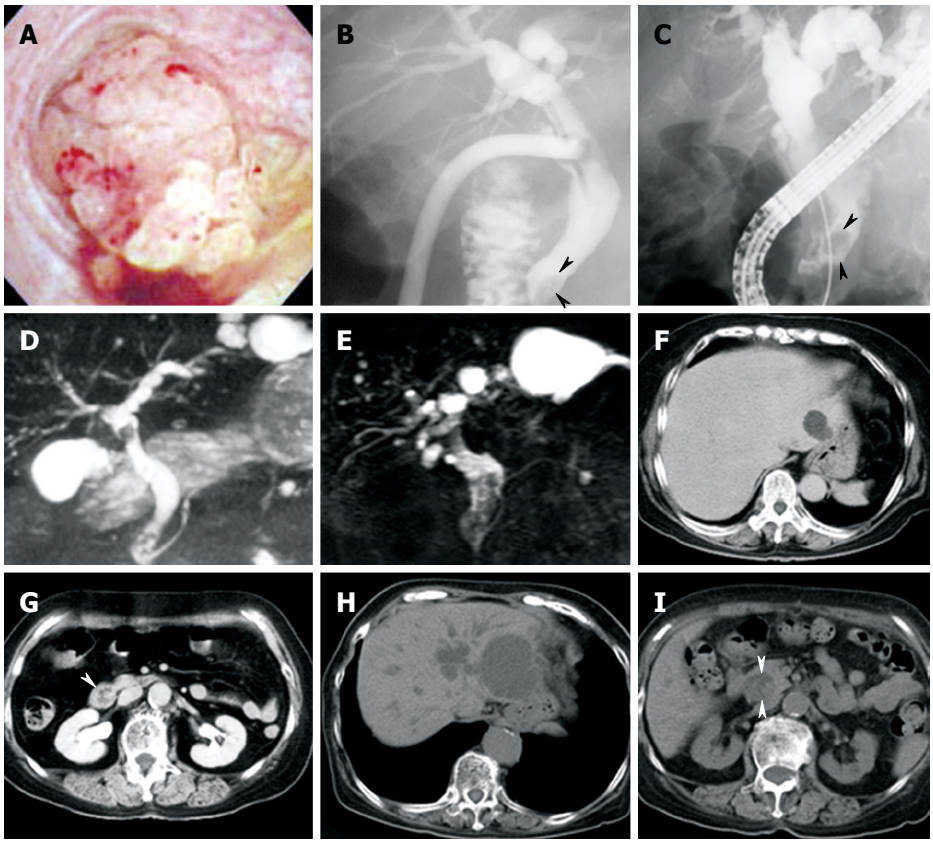

Figure 1 Images of cholangioscopic examination, ERC, MRCP and abdominal CT. A: Cholangioscopic examination performed in 1999. A multilobulated papillary tumor was seen protruding into the common bile duct; B: ERC performed in 1999; C: ERC performed in 2008. The extrahepatic bile duct became more dilated in 2008 than in 1999. A polypoid filling defect (arrow heads) could be detected in the distal bile duct; D: MRCP in 1999; E: MRCP in 2004. Cystic lesions connected to the intrahepatic bile ducts of the left liver became enlarged in 2004; F and G: Abdominal CT in 1999; H and I: Abdominal CT in 2004. Intra- and extrahepatic bile ducts became dilated in 2004. A mass in the distal bile duct (arrow heads) and a cystic lesion in the left liver were enlarged in 2004, compared with those in 1999.

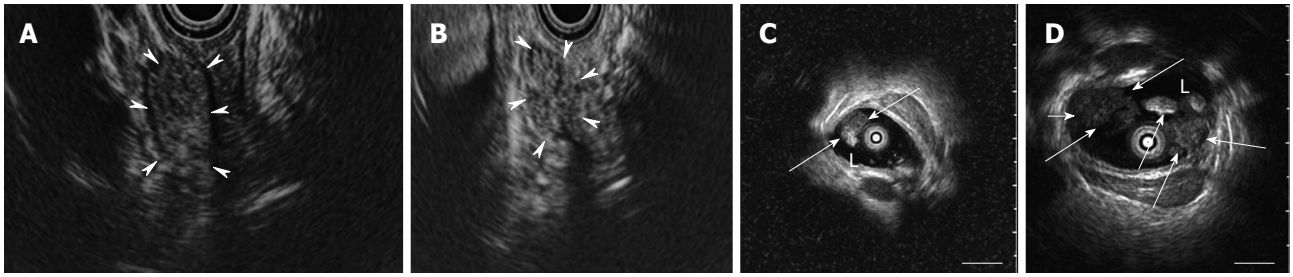

Figure 2 Images of ultrasonographic studies. A: EUS showed a mixed echoic mass in the distal bile duct (arrow heads); B: Papillary structure of the mass could be apparently observed on CEH-EUS with Sonazoid®; C: IDUS showed a 10-mm papillary mass (arrows) in the middle bile duct; D: In the distal bile duct, multilobular and papillary lesions (arrows) were observed. L: Lumen of the bile duct.

Occasional association with mucin hypersecretion is one of the pathological similarities between IPNB and IPMN-P[1]. Zen *et al*[1] have performed immunohistochemical analysis for mucin core proteins in patients with IPNB and compared the results with patients with IPMN-P. They have proposed the typical mucin and cytokeratin expression profile of IPNB as MUC1-negative, MUC2-positive, MUC5AC-positive, CDX2-positive, CK7-positive, CK20-positive. According to Shibahara *et al*[7], patients with MUC1-positive expression show poorer survival than those with MUC1-negative expression in papillary cholangiocarcinoma. In addition, the same group has reported that IPMN-P tends to show MUC1-negative and MUC2-positive expression, in contrast to invasive carcinoma, which shows MUC1-positive and MUC2-negative expression[8]. The mucin and cytokeratin expression profile in the present case was MUC1-positive, MUC2-negative, MUC5AC-positive, CK7-negative, CK20-negative, which differs from the typical profile reported by Zen *et al*[1]. Reasons for this difference might have included: (1) our specimens were taken by biopsy and immunohistochemical analysis was not performed for the entire lesion; (2) even in the original paper by Zen *et al*[1], not all cases showed the typical profile; and (3) MUC1-positivity implies that our case could have malignant potential and needs cautious observation in the future.

Surgical resection is often recommended because of the high malignancy rate, diffuse pattern of disease, and better survival after curative surgery[3-5]. Liver transplantation has been suggested as an alternative[9], while many patients with biliary papillomatosis, which is a disease of the elderly (mean age at time of diagnosis, 63 years)[10], would not be eligible for transplantation.

We consider that curative resection is not necessarily reasonable for every patient with IPNB for the following reasons. First, compared with the prognosis of usual

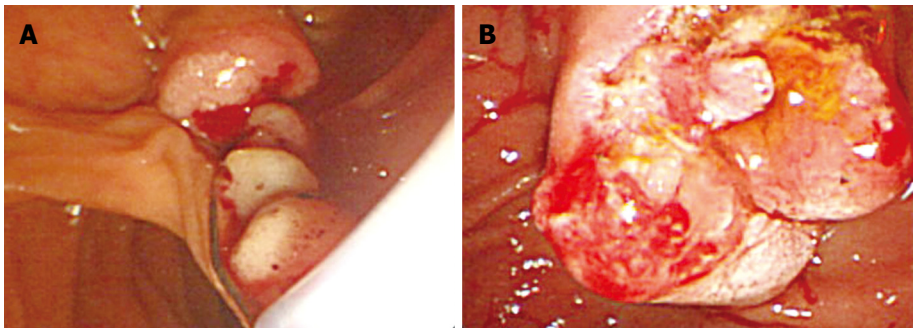

Figure 3 EST was performed. A: A soft, bead-like mass was extracted through the orifice of the ampulla of Vater after EST; B: Image of the ampulla of Vater after extraction of the mass by balloon sweep and a net-type catheter.

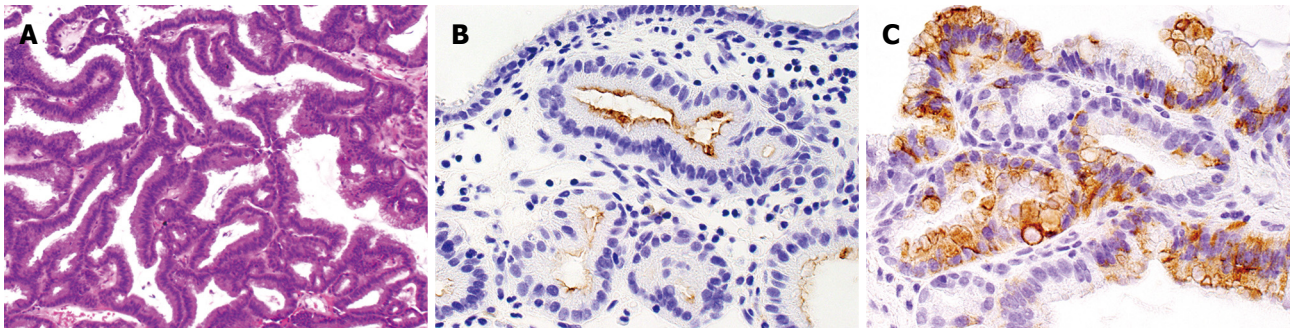

Figure 4 Histopathological findings. A: Hematoxylin-eosin staining; B: Immunohistochemical analysis for MUC1; C: Immunohistochemical analysis for MUC5AC.

Table 1 Patients with IPNB treated by endoscopic procedures reported between 2005 and 2009

Author	Gender	Age (yr)	Follow-up after diagnosis of IPNB	Treatment	Reason surgery was not performed	Outcome
Bechmann *et al*[10]	Male	65	10 years	Whipple, right hepatectomy, PDT	Patient's age	Death without cholestasis
Park *et al*[11]	Female	78	3 wk	EST, EPBD	Unknown	Well for a short time
Brauer *et al*[12]	Male	86	1 mo	APC	Diffuse involvement of biliary system, patient's age and comorbidities	Death due to hepatic encephalopathy
Jazrawi *et al*[13]	Male	37	6 mo	Extrahepatic bile duct resection, APC	Patient's refusal	Being evaluated for liver transplantation due to disease progression
Current case	Female	86	11 years	Cholecystectomy, EST, ERBD	Patient's age	Being well irrespective of slow disease progression

EST: Endoscopic sphincterotomy; EPBD: Endoscopic papillary balloon dilatation; Whipple: Pancreaticoduodenectomy; PDT: Photodynamic therapy; APC: Argon plasma coagulation; ERBD: Endoscopic retrograde biliary drainage.

cholangiocellular carcinoma, that of biliary papillomatosis including benign and malignant cases is much better[2]. This implies that the prognosis of benign biliary papillomatosis, in particular, may be good enough to be observed without radical resection, although precise information on the clinical course of patients with biliary papillomatosis is currently unclear. Second, the Whipple procedure and hemihepatectomy are the therapies of choice depending on the location and extension of the disease. In addition, malignant change is observed in 40%-50% of cases[11], which means that half of the cases remain benign. If radical resection were recommended for all patients, the therapy might be too invasive for the potentially large population of patients with benign disease.

A search of the English-language literature published in the past 5 years was performed using the MEDLINE database with keywords of 'biliary papillomatosis' and 'intraductal papillary neoplasm of the bile duct'. Five patients with pathologically benign IPNB[10-13], including the present case, have been followed up using endoscopic procedures (Table 1). Two patients had been followed for > 10 years. Among these five patients, surgery was not considered a viable option because of the age of the patient in three cases, small range of the disease in one case, and for unknown reasons in the other.

The therapy offered to these patients was EST plus additional endoscopic papillary balloon dilation in two cases, argon plasma coagulation in two cases, and photodynamic therapy (PDT) in one case. Prognosis of these patients was unchanged with occasional cholangitis in two cases, exacerbation in one, death from another disease in one, and unknown in one. Based upon the summaries of

these patients, cases in which malignant transformation cannot be ascertained pathologically could be cautiously followed using endoscopic procedures, and using radical resection as the gold standard is unnecessary. In addition, another patient similar to our own was followed up for 10 years, and treated using endoscopic procedures for recurrent biliary papillomatosis, because there was no surgical option left. As IPNB is considered as the biliary counterpart of IPNM-P[1,7], a follow-up period of over a decade for some patients with IPNB, as in our case, is not necessarily inappropriate.

To conclude, we encountered a patient with IPNB who was treated only with endoscopic procedures for 10 years, which suggests that some patients with benign IPNB could be followed conservatively without radical resection. Moreover, the present case might partly demonstrate the natural course of patients with the novel disease entity of IPNB.

REFERENCES

1 **Zen Y**, Fujii T, Itatsu K, Nakamura K, Minato H, Kasashima S, Kurumaya H, Katayanagi K, Kawashima A, Masuda S, Niwa H, Mitsui T, Asada Y, Miura S, Ohta T, Nakanuma Y. Biliary papillary tumors share pathological features with intraductal papillary mucinous neoplasm of the pancreas. *Hepatology* 2006; **44**: 1333-1343

2 **Lee SS**, Kim MH, Lee SK, Jang SJ, Song MH, Kim KP, Kim HJ, Seo DW, Song DE, Yu E, Lee SG, Min YI. Clinicopathologic review of 58 patients with biliary papillomatosis. *Cancer* 2004; **100**: 783-793

3 **Yeh TS**, Tseng JH, Chiu CT, Liu NJ, Chen TC, Jan YY, Chen MF. Cholangiographic spectrum of intraductal papillary mucinous neoplasm of the bile ducts. *Ann Surg* 2006; **244**: 248-253

4 **Paik KY**, Heo JS, Choi SH, Choi DW. Intraductal papillary neoplasm of the bile ducts: the clinical features and surgical outcome of 25 cases. *J Surg Oncol* 2008; **97**: 508-512

5 **Yeung YP**, AhChong K, Chung CK, Chun AY. Biliary papillomatosis: report of seven cases and review of English literature. *J Hepatobiliary Pancreat Surg* 2003; **10**: 390-395

6 **Adbores-Saavedra J**, Scoazec JC, Wittekind C, Sripa B, Menck HR, Soehendra N, Sriram PVJ. Carcinoma of the gallbladder and extrahepatic bile ducts. In: Hamilton SR, Aaltonen LA, editos. World Health Organization Classification of Tumours. Pathology and genetics of tumours of the digestive system. Lyon: IARC Press, 2000: 206

7 **Shibahara H**, Tamada S, Goto M, Oda K, Nagino M, Nagasaka T, Batra SK, Hollingsworth MA, Imai K, Nimura Y, Yonezawa S. Pathologic features of mucin-producing bile duct tumors: two histopathologic categories as counterparts of pancreatic intraductal papillary-mucinous neoplasms. *Am J Surg Pathol* 2004; **28**: 327-338

8 **Osako M**, Yonezawa S, Siddiki B, Huang J, Ho JJ, Kim YS, Sato E. Immunohistochemical study of mucin carbohydrates and core proteins in human pancreatic tumors. *Cancer* 1993; **71**: 2191-2199

9 **Rambaud S**, Nores JM, Meeus F, Paolaggi JA. Malignant papillomatosis of the bile ducts: a new indication for liver transplantation? *Am J Gastroenterol* 1989; **84**: 448-449

10 **Bechmann LP**, Hilgard P, Frilling A, Schumacher B, Baba HA, Gerken G, Zoepf T. Successful photodynamic therapy for biliary papillomatosis: a case report. *World J Gastroenterol* 2008; **14**: 4234-4237

11 **Park JH**, Park do H, Park SH, Lee SH, Kim SJ, Cho HD. Non-mucin-producing biliary papillomatosis diagnosed by transpapillary endoscopic curettage (with video). *Gastrointest Endosc* 2007; **65**: 519-520, discussion 520

12 **Brauer BC**, Fukami N, Chen YK. Direct cholangioscopy with narrow-band imaging, chromoendoscopy, and argon plasma coagulation of intraductal papillary mucinous neoplasm of the bile duct (with videos). *Gastrointest Endosc* 2008; **67**: 574-576

13 **Jazrawi SF**, Nguyen D, Barnett C, Tang SJ. Novel application of intraductal argon plasma coagulation in biliary papillomatosis (with video). *Gastrointest Endosc* 2009; **69**: 372-374

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Monocyte chemotactic protein-1 gene polymorphism and spontaneous bacterial peritonitis

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Abstract

I read with great interest the article by Gäbele *et al* published in issue 44 of *World J Gastroenterol* 2009. The results of their study indicate that -2518 *Monocyte chemotactic protein-1* (*MCP-1*) genotype AA is a risk factor for spontaneous bacterial peritonitis in patients with alcoholic cirrhosis. However, there are some items that need to be discussed.

Key words: Spontaneous bacterial peritonitis; *Monocyte chemotactic protein-1*; Polymorphism

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TO THE EDITOR

I read with great interest the article by Gäbele *et al*[1] published in issue 44 of *World J Gastroenterol* 2009. The article provides important data. The results of their study indicate that the -2518 *Monocyte chemotactic protein-1* (*MCP-1*) genotype AA is a risk factor for spontaneous bacterial peritonitis (SBP) in patients with alcoholic cirrhosis. The authors suggested that the reduced MCP-1 ascites level may a cause for patients with SBP compared to those with G allele. However, there are some items that need to be discussed. It is debatable to get this conclusion unless ascites MCP-1 levels are measured before and after the treatment of SBP. It has been reported that the MCP-1 level in both sera and ascites is higher in SBP than in non-SBP patients, and decreases after treatment[2]. Infection other than SBP data is also missed in that article. For example, urinary tract infection and even asymptomatic bacteriuria may precede SBP. It is not easy to decide if MCP-1 polymorphism causes urinary tract infection and subsequently SBP, because MCP-1 plays a role even in asymptomatic bacteriuria[3]. Another issue of my concern is the number of SBP episodes. No data in relation with repeated SBP were provided in the article. Did the authors observe repeated SBP episodes in the patients with genotype AA over a 6-year period between 2001-2007? Did the patients respond to the antibiotic therapy well in a similar time interval?

REFERENCES

1 **Gäbele E**, Mühlbauer M, Paulo H, Johann M, Meltzer C, Leidl F, Wodarz N, Wiest R, Schölmerich J, Hellerbrand C. Analysis of monocyte chemotactic protein-1 gene polymorphism in patients with spontaneous bacterial peritonitis. *World J Gastroenterol* 2009; **15**: 5558-5562
2 **Kim JK**, Chon CY, Kim JH, Kim YJ, Cho JH, Bang SM, Ahn SH, Han KH, Moon YM. Changes in serum and ascitic monocyte chemotactic protein-1 (MCP-1) and IL-10 levels in cirrhotic patients with spontaneous bacterial peritonitis. *J Interferon Cytokine Res* 2007; **27**: 227-230
3 **Reyes L**, Reinhard M, Brown MB. Different inflammatory responses are associated with Ureaplasma parvum-induced UTI and urolith formation. *BMC Infect Dis* 2009; **9**: 9

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Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
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, EGHC 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

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

June 20-23
Mannheim, Germany
16th World Congress for Bronchoesophagology-WCBE

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific Sessions

August 28-31
Boston, Massachusetts, United States
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
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases

October 07-09
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 Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

November 13-14
San Francisco, CA, United States
Case-Based Approach to the Management of Inflammatory Bowel Disease

December 02-04
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Format

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1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 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. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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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 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
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