World Journal of
Gastroenterology ®
Volume 17 Number 36
September 28, 2011

Published by Baishideng Publishing Group Co., Limited,
Room 1701, 17/F, Henan Building,
No. 90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

ISSN 1007-9327 CN 14-1219/R Local Post Offices Code No. 82-261

ISSN 1007-9327
9 771007 932045 36>

World Journal of Gastroenterology

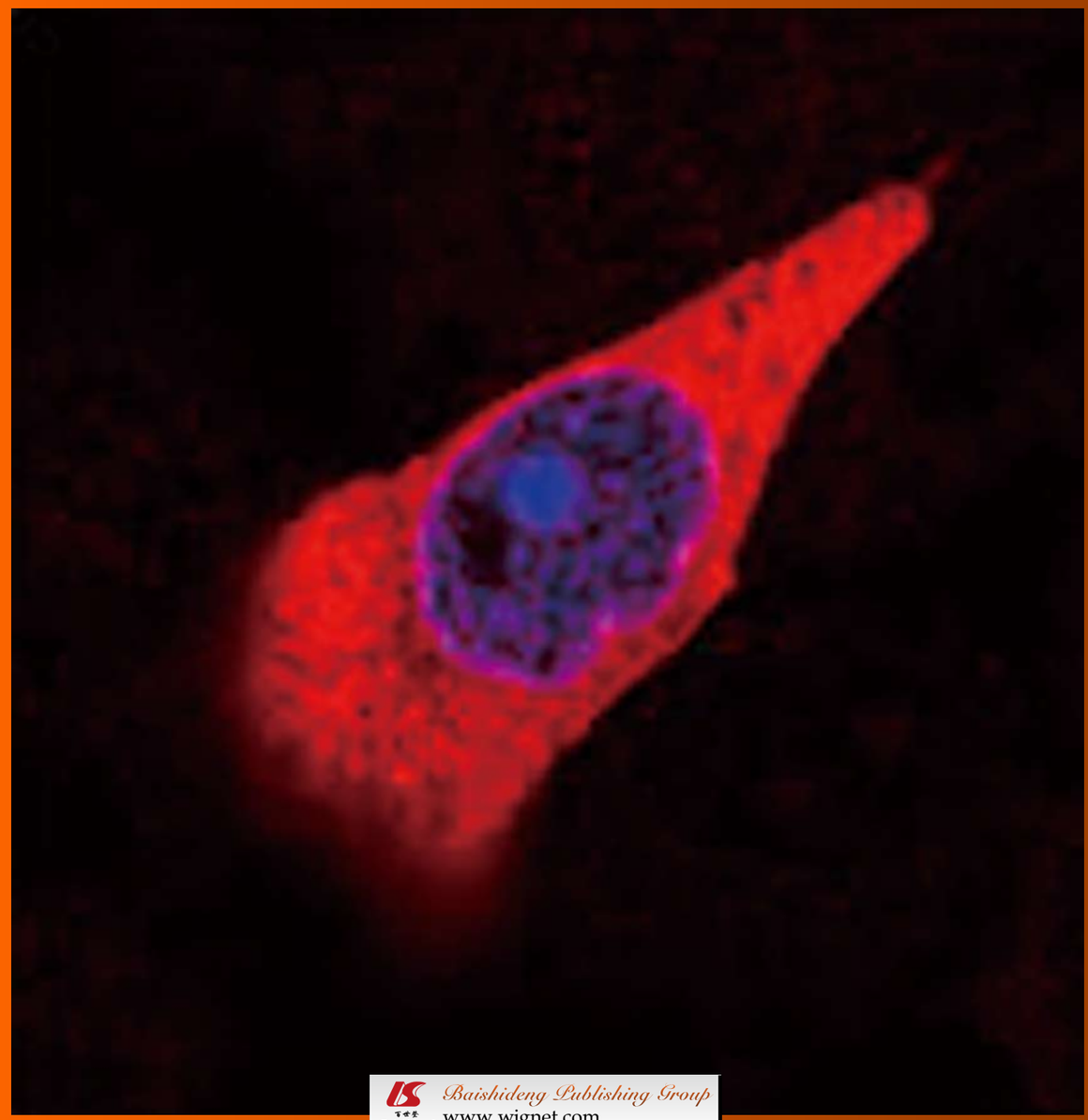
www.wjgnet.com

Volume 17 Number 36 Sep 28 2011

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2011 September 28; 17(36): 4055-4152



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 17 Number 36 September 28, 2011

Contents

World Journal of Gastroenterology
Volume 17 Number 36 September 28, 2011

NAME OF JOURNAL
World Journal of Gastroenterology

LAUNCH DATE
October 1, 1995

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

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

EDITING
Editorial Board of *World Journal of Gastroenterology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

PRINT SUBSCRIPTION
RMB 245 Yuan for each issue, RMB 11760 Yuan for one year.

PUBLICATION DATE
September 28, 2011

ISSN AND EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

EDITORIAL OFFICE
Jian-Xia Cheng, Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/1007-9327office

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4055

World J Gastroenterol 2011 September 28; 17(36): 4055-4062
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis

Metin Basaranoglu, Gökcen Basaranoglu

Metin Basaranoglu, Division of Gastroenterology and Hepatology, Teaching and Consulting, Ankara Yüksek Ihtisas Hospital Gastroenterology Clinic, Sihhiye, Ankara 06100, Turkey
Gökcen Basaranoglu, Department of Anaesthesiology, Bezmialem Vakif University, Fatih, Istanbul 34590, Turkey
Author contributions: Basaranoglu M and Basaranoglu G performed the literature search and designed and wrote the study.
Correspondence to: Metin Basaranoglu, MD, Division of Gastroenterology and Hepatology, Teaching and Consulting, Ankara Yüksek Ihtisas Hospital Gastroenterology Clinic, Sihhiye, Ankara 06100, Turkey. metin_basaranoglu@yahoo.com
Telephone: +90-212-6217580 Fax: +90-212-6217580
Received: March 17, 2011 Revised: May 16, 2011
Accepted: May 23, 2011
Published online: September 28, 2011

Abstract

Chronic hepatitis due to any cause leads to cirrhosis and end-stage liver disease. A growing body of literature has also shown that fatty liver due to overweight or obesity is a leading cause of cirrhosis. Due to the obesity epidemic, fatty liver is now a significant problem in clinical practice. Steatosis has an impact on the acceleration of liver damage in patients with chronic hepatitis due to other causes. An association between hepatitis C virus (HCV) infection, steatosis and the onset of insulin resistance has been reported. Insulin resistance is one of the leading factors for severe fibrosis in chronic HCV infections. Moreover, hyperinsulinemia has a deleterious effect on the management of chronic HCV. Response to therapy is increased by decreasing insulin resistance by weight loss or the use of thiazolidenediones or metformin. The underlying mechanisms of this complex interaction are not fully understood. A direct cytopathic effect of HCV has been suggested. The genomic structure of HCV (suggesting that some viral sequences are involved in the intracellular accumulation of triglycerides), lipid metabolism, the molecular links between the HCV core protein and lipid droplets (the core protein of HCV and its transcriptional regulatory function which induce a triglyceride accumulation in hepatocytes) and increased neolipogenesis and inhibited fatty acid degradation in mitochondria have been investigated.

Key words: Adipocytokines; Fatty acids; Hepatitis B virus; Hepatitis C virus; Inducible nitric oxide synthase; Insulin resistance; Signal transduction and activator of transcription-3; Steatosis; Sterol regulatory element-binding protein-1c; Suppressors of cytokine signaling; Tumor necrosis factor-α

Peer reviewer: Nagarajan Perumal, Dr., Compliance Veterinarian, Center for Life Science, IACUC OFFICE, National University of Singapore, 117456, Singapore

Basaranoglu M, Basaranoglu G. Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis. *World J Gastroenterol* 2011; 17(36): 4055-4062 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4055.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4055

INTRODUCTION

Chronic hepatitis due to any cause leads to cirrhosis and end-stage liver disease. A growing body of literature has also shown that fatty liver due to overweight or obesity is a leading cause of cirrhosis[1-3]. Due to the obesity epidemic, fatty liver is now a significant problem in clinical practice. An association between hepatitis C virus (HCV) infection, steatosis and the onset of insulin resistance has been reported[4-6]. Moreover, steatosis has an impact on the acceleration of liver damage in patients with chronic hepatitis due to other causes. The underlying mechanisms of this complex interaction are not fully understood. A direct cytopathic effect of HCV has been suggested. The genomic structure of HCV (suggesting

that some viral sequences are involved in the intracellular accumulation of triglycerides), lipid metabolism, and the molecular links between the HCV core protein and lipid droplets (the core protein of HCV and its transcriptional regulatory function which induce a triglyceride accumulation in hepatocytes) and increased neolipogenesis and inhibited fatty acid degradation in mitochondria have been investigated (Figure 1).

BACKGROUND OF FATTY LIVER

Excessive accumulation of triglycerides in hepatocytes in the absence of significant alcohol consumption, defined as > 5% fat by weight, occurs in about 20%-30% of adults[1-3]. Excessive fat in the liver predisposes to the development of steatohepatitis which is a significant risk factor for developing cirrhosis and its complications, including hepatocellular carcinoma.

Background of insulin resistance in patients with HCV

The frequency of type 2 diabetes is more common in patients with chronic HCV infection than in hepatitis B infection (21% *vs* 12%, respectively) which is evidence of a link between HCV infection and diabetes mellitus (DM)[4-6]. This relationship is independent of the existence of cirrhosis. A large cross-sectional United States study which included over 9000 individuals showed that the frequency of type 2 DM is 3-fold more common in hepatitis C patients. Both older age and higher body mass index (BMI) are more common among patients with both hepatitis C and type 2 diabetes.

Insulin resistance (IR) is a specific feature of chronic HCV, associated with genotypes 1 and 4 and high serum HCV RNA level[7]. Chronically HCV infected subjects present a 3-fold increased risk of IR and glucose metabolism impairment, with IR occurring in the very early stages of hepatic lesions (fibrosis stage 0 or 1), with a worsening tendency as hepatic fibrosis progresses[8-10]. There is also an association between IR severity and DM, with higher viral load and an improvement in IR after a sustained viral response.

A recently published study which investigated 600 patients [chronic hepatitis C (CHC) in 500 and chronic hepatitis B (CHB) in 100] reported that IR was present in 32.4% of the 462 nondiabetic CHC patients and was associated with the metabolic syndrome, genotypes 1 and 4, significant fibrosis, and severe steatosis[7]. IR was diagnosed in 15% of 145 CHC patients without metabolic syndrome or significant fibrosis, and was associated with genotypes 1 and 4, high serum HCV RNA level, and moderate-to-severe necroinflammation. IR was less frequent in CHB patients than in matched CHC patients (5% *vs* 35%, respectively, $P < 0.001$).

In our clinic, we investigated 76 patients. Of these 76, 12 had hepatitis B, 19 had hepatitis C, 34 had simple steatosis and 11 were control subjects. We found that IR was only significant and associated with severe fibrosis in patients with HCV[11].

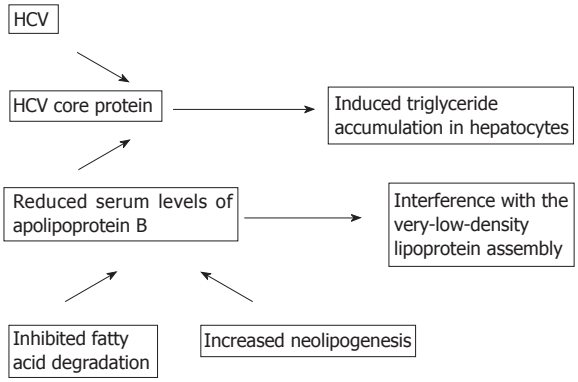


Figure 1 Underlying mechanisms of the complex interaction resulting in steatosis in patients with hepatitis C virus. HCV: Hepatitis C virus.

Whether fat in the liver is an important determinant of IR is debatable. The study showed that insulin secretion assessed by intravenous glucose injection was not impaired in CHC patients compared to the controls[12]. When they studied the IR of 29 people with hepatitis C (14 with genotype 1 and 15 with genotype 3) and confirmed they had high IR, nearly all the IR was found to be in the muscle and hardly any in the liver. Of the 29 patients, 15 had very high levels of fat in the liver and had the same degree of IR as the 14 patients who did not have fatty livers.

IR is one of the leading factors for severe fibrosis in CHC infections independent of steatosis, as compensatory hyperinsulinemia is fibrogenic[12]. Moreover, a relationship between exogenous hyperinsulinemia and hepatocellular carcinoma has been reported. Hyperinsulinemia decreases therapy response and has a deleterious effect on the management of chronic HCV infection. Response to therapy is increased by decreasing insulin resistance by weight loss or the use of thiazolidenediones and metformin. Metformin improved virologic response when added to hepatitis C interferon-ribavirin therapy in those with IR[13].

A relationship between type 1 diabetes and hepatitis C, and type 2 diabetes and hepatitis B has not so far been reported[14].

Background of steatosis in patients with HCV infection

The prevalence of steatosis is 20%-30% in the general population and is 50%-80% in patients with HCV infection. HCV itself has the ability to directly promote steatosis and IR[15-18]. If all steatogenic co-factors are excluded, the prevalence of steatosis remains at 50% resulting in a 2.5-fold increased prevalence as compared with the general population and other forms of chronic liver disease. The prevalence of steatosis is 18% in hepatitis B virus infection[19].

There are 2 types of liver steatosis: metabolic steatosis which is related to high BMI in patients with genotype 1, and viral steatosis which is related to hepatitis C genotype 3. Steatosis is more frequent in association with genotype 3a as compared to other genotypes such

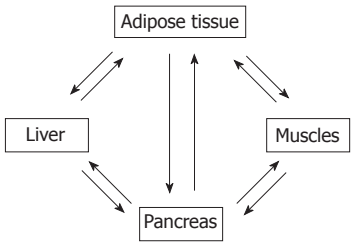


Figure 2 Cross-talk among the insulin sensitive organs.

as genotype 1, 2 and 4 (74% *vs* 50%), which suggests that some sequences of the viral genome may be involved in intracellular lipid accumulation[16]. Additionally, steatosis correlates with viral load and can revert after effective treatment and reoccurs with re-infection in genotype 3 infection.

The localization of steatosis, particularly in genotype 3 infected patients, is predominantly in the periportal zone (acinar 1) and not in the centrilobular zone (acinar 3) and is more typical of metabolic associated steatohepatitis[20-23]. All genotypes are steatogenic, however, genotype 3 is three times more potent. Transgenic mouse models showed that the core protein can induce the appearance of lipid droplets. One possible molecular explanation for the greater steatogenic property of genotype 3, could be a phenylalanine residue at position 164 in core protein domain Ⅱ, instead of tyrosine as in other genotypes, which results in a higher affinity to lipids.

Hyperinsulinism, IR related, directly activates stellate cells and, in association with hyperglycemia, increases connective tissue growth factor, a key cytokine in hepatic fibrogenesis[24,25]. Steatosis also relates to more advanced fibrosis and to accelerated fibrosis progression. Thus, treating HCV infected patients with evidence of hepatic steatosis, even if they only present mild inflammatory activity, has been suggested. How? Steatosis may sensitize the liver to inflammation and apoptosis, and subsequently enhance fibrosis. A recent study showed that hepatic steatosis is associated with higher programmed cell death by apoptosis with stellate cell activation[26].

Background of insulin resistance

A balance exists between energy demand and intake in the human body. Obesity and its consequences such as IR and the metabolic syndrome, is a growing threat to the health of people in developed nations. While insulin receptor defects cause severe IR, most patients with IR have impaired post-receptor intracellular insulin signaling[27].

INSULIN SIGNALING PATHWAY AND GLUCOSE HOMEOSTASIS

There is cross-talk among insulin sensitive tissues such as skeletal muscle, adipose tissue, and liver (Figure 2). Insulin binds α-subunits of its receptor which is a cell surface receptor on insulin sensitive cells such as skeletal muscle, adipocytes, and hepatocytes leading to autophosphorylation of the cytoplasmic domains (β-subunits) of the receptor[25-40]. The insulin receptor has intrinsic tyrosine kinase activity activated by insulin binding and the autophosphorylated receptor activates its substrates that include insulin receptor substrate (IRS)-1, IRS-2, Src homology collagen (Shc), and an adaptor protein with a pleckstrin homology (PH) and Src homology (SH) 2 domain by tyrosine phosphorylation (Figure 3). These phosphorylated docking proteins bind and activate several downstream components of the insulin signaling pathways. Activated IRS-1 associates with phosphatidyl inositol 3-kinase (PI3-K), which then activates Akt. Akt substrate of 160 kDa (AS160), a serine/threonine kinase, was identified in 3T3-L1 adipocytes. In both skeletal muscle and adipose tissue, these insulin-mediated phosphorylation-dephosphorylation signaling cascades induce the translocation of glucose transporters (GLUT), predominantly GLUT4-containing vesicles, from intracellular storage sites to the plasma membrane, increasing glucose uptake to prevent abnormal glucose and insulin elevations in the plasma (insulin-stimulated glucose transport). These events and insulin-dependent inhibition of hepatic glucose output maintain glucose homeostasis. Insulin also affects glucose homeostasis indirectly by its regulatory effect on lipid metabolism. Any interference in this insulin signaling pathway causes glucotoxicity, insulin resistance and, when islet β cells are capable of responding, compensatory hyperinsulinemia. Hepatitis C virus Genotype 1b diminishes IRS-1 levels and causes IR.

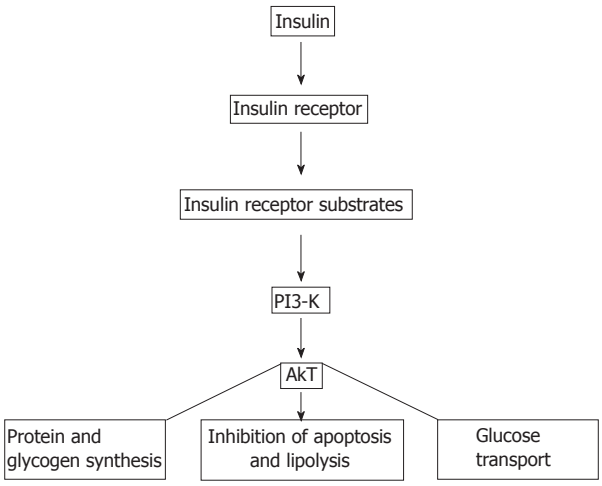


Figure 3 Insulin signaling pathways. PI3-K: Phosphatidyl inositol 3-kinase; AkT: A serine/threonine protein kinase.

Hepatic expression of insulin receptor protein was decreased in chronic hyperinsulinemic states. IRS-1 was more closely linked to glucose homeostasis with the regulation of glucokinase expression, while IRS-2 was more closely linked to lipogenesis with the regulation of lipogenic enzymes sterol regulatory element-binding protein-1c (SREBP-1c) and fatty acid synthase. Moreover, insulin activates synthesis and inhibits catabolism

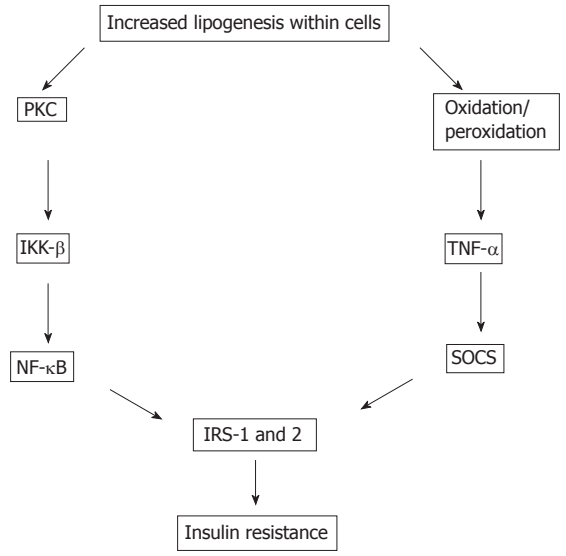


Figure 4 Pathways to insulin resistance. PKC: Protein kinase C; IKK: Inhibitor κB kinase; TNF: Tumor necrosis factor; NF: Nuclear factor; SOCS: Suppressors of cytokine signaling; IRS: Insulin receptor substrate.

of lipids, while shutting off the synthesis of glucose in the liver.

Adipose tissue is one of the major insulin sensitive organs in the human body and the process of differentiation of preadipocytes to adipocytes, induced by insulin, is called adipogenesis. Within the adipose tissue, insulin stimulates triglyceride synthesis (lipogenesis) and inhibits lipolysis by upregulating lipoprotein lipase activity which is the most sensitive pathway in insulin action, facilitating free fatty acid uptake and glucose transport, inhibiting hormone sensitive lipase, and increasing gene expression of lipogenic enzymes. Insulin also induces the degradation of apolipoprotein B100 (apo B100), a key component of very-low-density lipoprotein, in the liver[41].

Insulin resistance

Insulin resistance can be defined as the failure of insulin sensitive cells to respond to insulin normally. It is characterized by elevated plasma glucose and, before attrition of pancreatic β-cells develops, elevated insulin levels. Chronic hyperinsulinemia is a major contributor to glucose and lipid metabolism abnormalities. Insulin resistance also inappropriately activates peripheral lipolysis and stimulates free fatty acid mobilization from adipocytes in the fed state. Increased circulating free fatty acids contribute to fat accumulation in the liver and muscle, further causing these tissues to be insulin resistant by disturbing their downstream insulin signaling cascades.

Mechanisms of insulin resistance (role of tumor necrosis factor-α and plasma free fatty acids)

The most common mechanism of IR is disturbed post-receptor insulin signaling (Figure 4)[42-48]. Whereas most insulin signaling is propagated by tyrosine phosphorylation, serine (Ser) phosphorylation is often inhibitory. Ser phosphorylation of IRS-1 decreases both insulin stimulated tyrosine phosphorylation of IRS-1 (phosphorylated Ser residues of IRS-1 become poor substrates for insulin receptor) and PI3-K activation. This diminishes the downstream insulin signaling and insulin sensitivity of insulin target tissues. IRS-1 has several Ser residues including Ser 307, Ser 612 and Ser 632 which can be phosphorylated. Insulin and tumor necrosis factor-α (TNF-α) can phosphorylate the same Ser residues of IRS-1. IR occurs very early in HCV infection, in parallel with an elevation in TNF-α levels. HCV also directly promotes IR through the proteasomal degradation of IRS-1.

TNF-α and plasma free fatty acids have been shown to be major stimuli of Ser 307 phosphorylation of IRS-1. Inhibition of IRS-1 due to the phosphorylation of its Ser 307 residues also requires the activation of both c-Jun N-terminal kinase (JNK) and inhibitor κB kinase (IKK) β. Both TNF-α and free fatty acids induce JNK and IKK-β activation.

TNF-α stimulates phosphorylation of Ser residues of both IRS-1 and IRS-2 in hepatocytes[48-50]. It was recently reported that monocyte-derived macrophages increasingly accumulated within the adipose tissue of obese patients. In addition to the dysregulated production of adipocytokines by adipocytes, adipose tissue macrophages also produce proinflammatory cytokines such as TNF-α, interleukin-6, and C-reactive protein. Both adipose tissue and its macrophages contribute to the TNF-α burden. Indeed, its circulating concentrations are very low, commonly undetectable even in obese mice or humans.

Elevated free fatty acids in the circulation are also major contributors to IR in both humans and mice by stimulating Ser 307 phosphorylation of IRS-1. Adipose tissue triglycerides are the main source of circulating free fatty acids in obesity. One mechanism of elevated free fatty acid-induced IR in muscle is the impaired activation of protein kinase C lamda (PKCλ) and protein kinase C XI (PKCξ)[50-52]. PKCθ can also activate IKK-β which phosphorylates Ser 307 residues of IRS-1. Additionally, increased acyl CoAs or ceramide which is a derivative of acyl CoAs, promote IR by diminishing Akt1 activation. Increased ceramide activates a phosphatase (protein phosphatase 2A) that reverses tyrosine phosphorylation of Akt/protein kinase B (PKB). Inactivated PKB inhibits the insulin downstream signaling cascade leading to IR in muscles [Le]. Several oxidative stress mediators might also induce IR by affecting insulin downstream signaling. Phosphatases such as phosphatase and tensin homolog, small heterodimer partner 2, and protein tyrosine phosphatase 1B are now recognized to be major mediators involved in IR. Another possible mechanism for IR is defective glucose transport such as down-regulation of GLUT4.

JNK is one of the stress-related kinases and plays an important role in the development of IR[48,52]. The

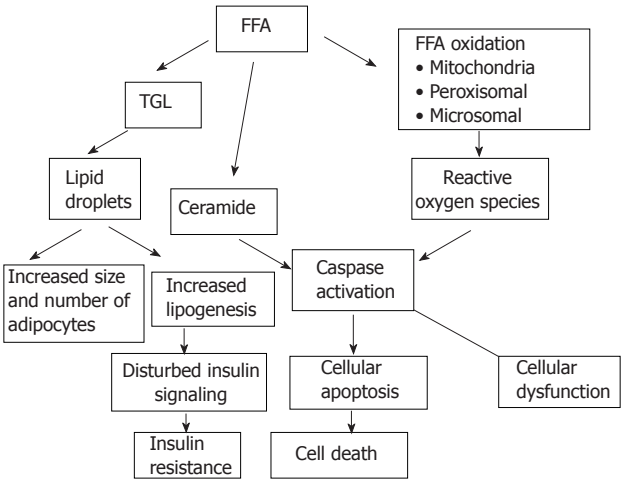


Figure 5 Insulin resistance and cell death. FFA: Free fatty acids; TGL: Tryglycerides.

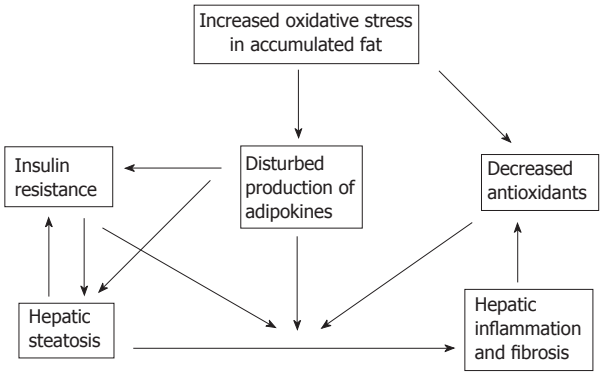


Figure 6 Fate of accumulated fat within hepatocytes.

three members of the JNK group of serine/threonine kinases, namely JNK-1, -2, and -3 are activated by proinflammatory cytokines such as TNF-α as well as free fatty acids and endoplasmic reticulum stress due to metabolic overload, which is an intracellular abnormality found in obesity. Activated JNK induces Ser 307 phosphorylation of IRS-1, disturbs insulin downstream signaling, and subsequently causes insulin resistance. JNK activity has been found to be elevated in liver, muscle, and adipose tissue of experimental obese models. Additionally, the loss of JNK-1 activity such as in JNK-1 knockout mice has been shown to prevent the development of IR in leptin-deficient *ob/ob* mice or mice with high-fat induced dietary obesity.

PKCθ and IKK-β are two proinflammatory kinases involved in insulin downstream signaling. They are activated by lipid metabolites such as high plasma free fatty acid concentrations and there is a positive relationship between the activation of PKCθ and the concentration of intermediate fatty acid products. PKCθ activates both IKK-β and JNK, leading to increased Ser 307 phosphorylation of IRS-1 and IR. IKK-β is a mediator of IR and one of the other stress-related kinases[53,54]. Activation or overexpression of IKK-β diminishes insulin signaling and causes IR, whereas inhibition of IKK-β improves insulin sensitivity. IKK-β phosphorylates the inhibitor of nuclear factor (NF) -κB leading to the activation of NF-κB by the translocation of NF-κB to the nucleus. NF-κB is an inducible transcription factor and promotes specific gene expression in the nucleus. NF-κB has both apoptotic and anti-apoptotic effects. The finding that NF-κB deficient mice were protected from high-fat diet-induced IR suggests that NF-κB directly participates in processes that impair insulin signaling.

Suppressors of cytokine signaling (SOCS) and inducible nitric oxide synthase (iNOS) are two inflammatory mediators recently recognized to play a role in insulin signaling[54-61]. Induction of SOCS proteins [SOCS 1-7 and cytokine-inducible src homology 2 domain-containing protein (CIS)] by proinflammatory cytokines might contribute to the cytokine-mediated IR in obese subjects. SOCS-3 might also regulate central leptin action and play a role in the leptin resistance of obese human subjects. SOCS-1 knockout mice showed low glucose concentrations and increased insulin sensitivity. In animal studies, inactivation of SOCS-3 or SOCS-1 or both in the livers of *db/db* mice partially improved insulin sensitivity and decreased hyperinsulinemia, whereas overexpression of SOCS-1 and SOCS-3 in obese animals caused IR and also increased activation of SREBP-1c[62]. SREBP-1c is one of the key mediators of lipid synthesis from glucose and other precursors (*de novo* lipogenesis) in the liver. Indeed, SOCS proteins markedly induce *de novo* fatty acid synthesis in the liver by both the up-regulation of SREBP-1c and persistent IR with hyperinsulinemia which stimulates SREBP-1c-mediated gene expression.

The molecular mechanism that leads to IRS-1 degradation varies according to genotype in patients with heptitis C virus infection. Genotype 1 promotes the expression of SOCS-3 as genotype 3 promotes SOCS 7 expression, with a mechanism of IRS-1 degradation similar to that induced by SOCS 3; it also inhibits PPAR-γ, further worsening IR. One of the steatogenic mechanisms is the promotion of *de novo* fatty acids synthesis by induced expression of SREBP-1c by HCV infection.

Nitric oxide synthase-2 (NOS2) or iNOS production are also induced by proinflammatory cytokines[63,64]. High-fat diet in rats causes up-regulation of iNOS mRNA expression and increases iNOS protein activity. Increased production of NOS2 might reduce insulin action in both muscle and pancreas and decreased iNOS activity protects muscles from high-fat diet-induced IR.

HCV induces protein phosphatase 2A expression, through an endoplasmic reticulum stress response pathway, which dephosphorylates protein kinase B (PkB)/Akt (a main enzyme in the insulin signaling pathway), and thereby lowers its kinase activity[65].

THE PATHOGENESIS OF HEPATOCELLULAR INJURY IN STEATOSIS

The accumulation of fat within the hepatocytes sensitizes

the liver to injury from a variety of causes and the regenerative capacity of a fatty liver is impaired (Figures 5 and 6)[66-68]. An interacting network of cytokines and adipokines that regulate inflammation is disrupted. p53 is involved in the mechanisms of hepatocellular injury accompanied by steatosis[69].

CONCLUSION

Steatosis is one of the characteristic histopathologic features of HCV caused by chronic liver disease, and is also closely related to IR. Insulin resistance is one of the leading factors for severe fibrosis in chronic HCV infections. Moreover, hyperinsulinemia has a deleterious effect on the management of chronic HCV. The underlying mechanisms of this complex interaction are not fully understood. A direct cytopathic effect of HCV has been suggested. The genomic structure of HCV, lipid metabolism, the molecular links between the HCV core protein and lipid droplets and increased neolipogenesis and inhibited fatty acid degradation in mitochondria have been investigated.

ACKNOWLEDGMENTS

This article is dedicated to my mentors Professor Stephen H Caldwell from the University of Virginia Health System and Professor Brent A Neuschwander-Tetri from the St. Louis University Hospital in gratitude for having guided me into Basic and Clinical Hepatology.

REFERENCES

1 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669
2 **Poonawala A**, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692
3 **Ratziu V**, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 2002; **35**: 1485-1493
4 **Romero-Gómez M**. Insulin resistance and hepatitis C. *World J Gastroenterol* 2006; **12**: 7075-7080
5 **Arao M**, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, Tagaya T, Yamanouchi K, Ichimiya H, Sameshima Y, Kakumu S. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003; **38**: 355-360
6 **White DL**, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 2008; **49**: 831-844
7 **Moucari R**, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, Ripault MP, Sobesky R, Martinot-Peignoux M, Maylin S, Nicolas-Chanoine MH, Paradis V, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. *Gastroenterology* 2008; **134**: 416-423
8 **Lecube A**, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 2004; **27**: 1171-1175
9 **Sougleri M**, Labropoulou-Karatza C, Paraskevopoulou P, Fragopanagou H, Alexandrides T. Chronic hepatitis C virus infection without cirrhosis induces insulin resistance in patients with alpha-thalassaemia major. *Eur J Gastroenterol Hepatol* 2001; **13**: 1195-1199
10 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704
11 **Akdogan M,** Kuran SÖ, Kayhan B, editors. Is there a relationship between insulin resistance and fibrosis in patients with chronic viral hepatitis? Proceedings of the 17th Gastroenterology Conference; 2009 Sep 2-8; Antalya, TR. Ankara: Nobel, 2009: 34
12 **Milner KL**, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, Dore GJ, Zekry A, Weltman M, Fragomeli V, George J, Chisholm DJ. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. *Gastroenterology* 2010; **138**: 932-941.e1-e3
13 **Romero-Gómez M**, Diago M, Andrade RJ, Calleja JL, Salmerón J, Fernández-Rodríguez CM, Solà R, García-Samaniego J, Herrerías JM, De la Mata M, Moreno-Otero R, Nuñez O, Olveira A, Durán S, Planas R. Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702-1708
14 **Chen LK**, Chou YC, Tsai ST, Hwang SJ, Lee SD. Hepatitis C virus infection-related Type 1 diabetes mellitus. *Diabet Med* 2005; **22**: 340-343
15 **Leandro G**, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N, Pazienza V, Giordani MT, Giostra E, Sonzogni A, Ruggiero G, Marcellin P, Powell EE, George J, Negro F. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006; **130**: 1636-1642
16 **Machado MV**, Cortez-Pinto H. Insulin resistance and steatosis in chronic hepatitis C. *Ann Hepatol* 2009; **8** Suppl 1: S67-S75
17 **Cua IH**, Hui JM, Kench JG, George J. Genotype-specific interactions of insulin resistance, steatosis, and fibrosis in chronic hepatitis C. *Hepatology* 2008; **48**: 723-731
18 **Bedossa P**, Moucari R, Chelbi E, Asselah T, Paradis V, Vidaud M, Cazals-Hatem D, Boyer N, Valla D, Marcellin P. Evidence for a role of nonalcoholic steatohepatitis in hepatitis C: a prospective study. *Hepatology* 2007; **46**: 380-387
19 **Thomopoulos KC**, Arvaniti V, Tsamantas AC, Dimitropoulou D, Gogos CA, Siagris D, Theocharis GJ, Labropoulou-Karatza C. Prevalence of liver steatosis in patients with chronic hepatitis B: a study of associated factors and of relationship with fibrosis. *Eur J Gastroenterol Hepatol* 2006; **18**: 233-237
20 **Pazienza V**, Clément S, Pugnale P, Conzelman S, Foti M, Mangia A, Negro F. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. *Hepatology* 2007; **45**: 1164-1171
21 **Abid K**, Pazienza V, de Gottardi A, Rubbia-Brandt L, Conne B, Pugnale P, Rossi C, Mangia A, Negro F. An in vitro model of hepatitis C virus genotype 3a-associated triglycerides accumulation. *J Hepatol* 2005; **42**: 744-751
22 **Moriya K**, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *J Gen Virol* 1997; **78** (Pt 7): 1527-1531
23 **Chang ML**, Chen JC, Yeh CT, Sheen IS, Tai DI, Chang MY, Chiu CT, Lin DY, Bissell DM. Topological and evolutional relationships between HCV core protein and hepatic lipid vesicles: studies in vitro and in conditionally transgenic

mice. *World J Gastroenterol* 2007; **13**: 3472-3477
24 **Paradis V**, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 2001; **34**: 738-744
25 **Paradis V**, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratziu V, Bedossa P. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. *Hepatology* 1999; **30**: 968-976
26 **Walsh MJ**, Vanags DM, Clouston AD, Richardson MM, Purdie DM, Jonsson JR, Powell EE. Steatosis and liver cell apoptosis in chronic hepatitis C: a mechanism for increased liver injury. *Hepatology* 2004; **39**: 1230-1238
27 **Basaranoglu M,** Neuschwander-Tetri BA. Metabolic Aspects of Chronic Liver Disease. Pathophysiology of Nonalcoholic Steatohepatitis. Schattner A, Knobler H, editors. NY: Nova Science, 2008: 1-70
28 **Eckel RH**, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; **375**: 181-183
29 **Dahlman I**, Arner P. Genetics of adipose tissue biology. *Prog Mol Biol Transl Sci* 2010; **94**: 39-74
30 **Le Marchand-Brustel Y**, Gual P, Grémeaux T, Gonzalez T, Barrès R, Tanti JF. Fatty acid-induced insulin resistance: role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling. *Biochem Soc Trans* 2003; **31**: 1152-1156
31 **Okamoto H**, Obici S, Accili D, Rossetti L. Restoration of liver insulin signaling in Insr knockout mice fails to normalize hepatic insulin action. *J Clin Invest* 2005; **115**: 1314-1322
32 **Anai M**, Funaki M, Ogihara T, Terasaki J, Inukai K, Katagiri H, Fukushima Y, Yazaki Y, Kikuchi M, Oka Y, Asano T. Altered expression levels and impaired steps in the pathway to phosphatidylinositol 3-kinase activation via insulin receptor substrates 1 and 2 in Zucker fatty rats. *Diabetes* 1998; **47**: 13-23
33 **Taniguchi CM**, Ueki K, Kahn R. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. *J Clin Invest* 2005; **115**: 718-727
34 **Buettner R**, Straub RH, Ottinger I, Woenckhaus M, Schölmerich J, Bollheimer LC. Efficient analysis of hepatic glucose output and insulin action using a liver slice culture system. *Horm Metab Res* 2005; **37**: 127-132
35 **Fisher SJ**, Kahn CR. Insulin signaling is required for insulin' s direct and indirect action on hepatic glucose production. *J Clin Invest* 2003; **111**: 463-468
36 **Jensen MD**, Caruso M, Heiling V, Miles JM. Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 1989; **38**: 1595-1601
37 **Smith U**, Axelsen M, Carvalho E, Eliasson B, Jansson PA, Wesslau C. Insulin signaling and action in fat cells: associations with insulin resistance and type 2 diabetes. *Ann N Y Acad Sci* 1999; **892**: 119-126
38 **Kahn BB**, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473-481
39 **Summers SA**, Whiteman EL, Birnbaum MJ. Insulin signaling in the adipocyte. *Int J Obes Relat Metab Disord* 2000; **24** Suppl 4: S67-S70
40 **Formiguera X**, Cantón A. Obesity: epidemiology and clinical aspects. *Best Pract Res Clin Gastroenterol* 2004; **18**: 1125-1146
41 **Karlsson HK**, Zierath JR. Insulin signaling and glucose transport in insulin resistant human skeletal muscle. *Cell Biochem Biophys* 2007; **48**: 103-113
42 **Pittas AG**, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab* 2004; **89**: 447-452
43 **Eckel RH**, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; **365**: 1415-1428
44 **Arner P**. The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol Metab* 2003; **14**: 137-145
45 **Nicholls HT**, Kowalski G, Kennedy DJ, Risis S, Zaffino LA, Watson N, Kanellakis P, Watt MJ, Bobik A, Bonen A, Febbraio M, Lancaster GI, Febbraio MA. Hematopoietic cell-restricted deletion of CD36 reduces high-fat diet-induced macrophage infiltration and improves insulin signaling in adipose tissue. *Diabetes* 2011; **60**: 1100-1110
46 **Fritsche L**, Weigert C, Häring HU, Lehmann R. How insulin receptor substrate proteins regulate the metabolic capacity of the liver--implications for health and disease. *Curr Med Chem* 2008; **15**: 1316-1329
47 **Smith U**. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue? *Int J Obes Relat Metab Disord* 2002; **26**: 897-904
48 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336
49 **Pittas AG**, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab* 2004; **89**: 447-452
50 **Kanety H**, Feinstein R, Papa MZ, Hemi R, Karasik A. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. *J Biol Chem* 1995; **270**: 23780-23784
51 **Kim YB**, Shulman GI, Kahn BB. Fatty acid infusion selectively impairs insulin action on Akt1 and protein kinase C lambda /zeta but not on glycogen synthase kinase-3. *J Biol Chem* 2002; **277**: 32915-32922
52 **Lam TK**, Yoshii H, Haber CA, Bogdanovic E, Lam L, Fantus IG, Giacca A. Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am J Physiol Endocrinol Metab* 2002; **283**: E682-E691
53 **Wellen KE**, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; **115**: 1111-1119
54 **Cai D**, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 2005; **11**: 183-190
55 **Perreault M**, Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 2001; **7**: 1138-1143
56 **Emanuelli B**, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol Chem* 2001; **276**: 47944-47949
57 **Ueki K**, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci USA* 2004; **101**: 10422-10427
58 **Rui L**, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* 2002; **277**: 42394-42398
59 **Johnston JA**, O'Shea JJ. Matching SOCS with function. *Nat Immunol* 2003; **4**: 507-509
60 **Farrell GC**. Signalling links in the liver: knitting SOCS with fat and inflammation. *J Hepatol* 2005; **43**: 193-196
61 **Mori H**, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 2004; **10**: 739-743
62 **Horton JD**, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131
63 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage

accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808
64 **Rockey DC**, Shah V. Nitric oxide biology and the liver: report of an AASLD research workshop. *Hepatology* 2004; **39**: 250-257
65 **Shulman GI**. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; **106**: 171-176
66 **Wan G**, Ohnomi S, Kato N. Increased hepatic activity of inducible nitric oxide synthase in rats fed on a high-fat diet. *Biosci Biotechnol Biochem* 2000; **64**: 555-561
67 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562
68 **Yang S**, Lin H, Diehl AM. Fatty liver vulnerability to endotoxin-induced damage despite NF-kappaB induction and inhibited caspase 3 activation. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G382-G392
69 **Yahagi N**, Shimano H, Matsuzaka T, Sekiya M, Najima Y, Okazaki S, Okazaki H, Tamura Y, Iizuka Y, Inoue N, Nakagawa Y, Takeuchi Y, Ohashi K, Harada K, Gotoda T, Nagai R, Kadowaki T, Ishibashi S, Osuga J, Yamada N. p53 involvement in the pathogenesis of fatty liver disease. *J Biol Chem* 2004; **279**: 20571-20575

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4063

World J Gastroenterol 2011 September 28; 17(36): 4063-4066
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Digestive manifestations of parathyroid disorders

Bassam Abboud, Ronald Daher, Joe Boujaoude

Bassam Abboud, Ronald Daher, Department of General Surgery, Hotel Dieu de France Hospital, Faculty of Medicine, Saint-Joseph University, Beirut 166830, Lebanon
Joe Boujaoude, Department of Gastroenterology, Hotel Dieu de France Hospital, Faculty of Medicine, Saint-Joseph University, Beirut 166830, Lebanon
Author contributions: Abboud B designed the research; Daher R, Abboud B and Boujaoude J performed the research; Daher R, Abboud B and Boujaoude J analyzed the data; Daher R and Abboud B wrote the paper.
Correspondence to: Bassam Abboud, MD, Department of General Surgery, Hotel Dieu de France Hospital, Alfred Naccache Street, Beirut 166830, Lebanon. dbabboud@yahoo.fr
Telephone: +961-1-615300 Fax: +961-1-615295
Received: December 29, 2010 Revised: March 25, 2011
Accepted: April 2, 2011
Published online: September 28, 2011

Key words: Dysparathyroidism; Hypoparathyroidism; Hyperparathyroidism; Digestive manifestations; Steatorrhea; Pancreatitis; Peptic ulcer

Peer reviewer: Dan L Dumitrascu, Professor, President, Romanian Society of Neurogastroenterology 2nd Medical Department University of Medicine and Pharmacy Iuliu Hatieganu Cluj, Romania

Abboud B, Daher R, Boujaoude J. Digestive manifestations of parathyroid disorders. *World J Gastroenterol* 2011; 17(36): 4063-4066 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4063.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4063

Abstract

The parathyroid glands are the main regulator of plasma calcium and have a direct influence on the digestive tract. Parathyroid disturbances often result in unknown long-standing symptoms. The main manifestation of hypoparathyroidism is steatorrhea due to a deficit in exocrine pancreas secretion. The association with celiac sprue may contribute to malabsorption. Hyperparathyroidism causes smooth-muscle atony, with upper and lower gastrointestinal symptoms such as nausea, heartburn and constipation. Hyperparathyroidism and peptic ulcer were strongly linked before the advent of proton pump inhibitors. Nowadays, this association remains likely only in the particular context of multiple endocrine neoplasia type 1/Zollinger-Ellison syndrome. In contrast to chronic pancreatitis, acute pancreatitis due to primary hyperparathyroidism is one of the most studied topics. The causative effect of high calcium level is confirmed and the distinction from secondary hyperparathyroidism is mandatory. The digestive manifestations of parathyroid malfunction are often overlooked and serum calcium level must be included in the routine workup for abdominal symptoms.

INTRODUCTION

The parathyroid glands play a major role in calcium homeostasis, and ultimately have an effect on all organs because of the complexity of intracellular calcium physiology. The gut and accessory organs are not spared. However, digestive manifestations of dysparathyroidism are not well known and typically rely on old articles and theories. This paper summarizes the digestive consequences of parathyroid disorders and highlights recent theories based on older studies.

DIGESTIVE MANIFESTATIONS OF HYPOPARATHYROIDISM

Hypoparathyroidism may be transient, genetically inherited, or acquired due to an autoimmune process. It may also be secondary to surgery or neck irradiation[1]. Digestive manifestations of hypoparathyroidism are few and consist mainly of steatorrhea.

Steatorrhea related to hypoparathyroidism is a consequence of bilio-pancreatic exocrine deficit due to insufficient meal-stimulated cholecystokinin secretion by the

duodenal mucosa[2]. The treatment of fat malabsorption in idiopathic hypoparathyroidism comprises: medium-chain triglycerides diet[3], correction of hypoparathyroidism, administration of vitamin D[4], and normalization of hypocalcemia[5]. In contrast, secondary hyperparathyroidism, as a consequence of malabsorption and steatorrhea, is accompanied by normal or sub-normal serum calcium level.

Idiopathic hypoparathyroidism can be associated with other digestive autoimmune diseases that may cause diarrhea. Few reports have been published on the coexistence of primary hypoparathyroidism and celiac disease[6-8]. Kumar *et al*[9] have explored this association in a cross-reactive immunological pathway. If suspected by resistance to vitamin D supplementation[10], the coexistence of celiac sprue must be ruled out by duodenal biopsy. In such cases, gluten-free diet should be included in the treatment regimen[11,12]. Moreover, in the specific context of celiac sprue, Parathyroid hormone (PTH) level might not be elevated because of parathyroid atrophy, and secondary hyperparathyroidism might not appear[13]. Finally, since its description by Reisner *et al*[14] more than 50 years ago, the coexistence of idiopathic hypoparathyroidism and pernicious anemia has not been further reported.

DIGESTIVE MANIFESTATIONS OF HYPERPARATHYROIDISM

The gastrointestinal manifestations of primary hyperparathyroidism (PHPT) have been described many decades ago[15]. Truly asymptomatic hyperparathyroidism is rare when thorough anamnesis looks for subtle symptoms. Most frequent digestive manifestations are constipation, heartburn, nausea and appetite loss that occur in 33%, 30%, 24% and 15% of cases, respectively[16]. Significant reduction in symptom rates is found after parathyroidectomy. Vague abdominal pain can be as frequent as 29%[17]. The exact pathophysiological mechanism is not fully understood. Alterations in gene expression secondary to sustained stimulation of PTH receptors may help explain the symptoms[18]. As a result, gut atony occurs and leads to constipation in the colon and dyspepsia in the stomach[17]. Finally, PHPT has been associated with increased incidence of malignancies, especially of the colon[19].

The association between PHPT and peptic ulcer disease is a yet-to-be-resolved issue. Most studies about this subject date were performed several decades ago[18,20-23], did not include prospective large-scale studies, and led to controversial results. Compared to 30% in adults with hyperparathyroidism[18], peptic ulcer was found in 5% of autopsies in the general population before the advent of the proton pomp inhibitors[20]. Other studies have reported results between these two extremes[21]. On the other hand, among patients with duodenal ulcer, Frame *et al*[22] have shown a 10-fold increase in the incidence of PHPT. As reported in old studies, complete correlation between hyperparathyroidism and increased gastric acid secretion could not be found, and normalization of the latter was not systematic after parathyroidectomy[21,23-28]. Again, the correlation between hypergastrinemia and hyperparathyroidism was not constant throughout previous studies[28,29], although Reeder *et al*[30] have found a direct calcium-to-gastric hypersecretion relationship in hypergastrinemia. The only prospective study conducted by Corleto *et al*[31] failed to confirm these findings. Zollinger-Ellison syndrome (ZES) may coexist with PHPT in the context of multiple endocrine neoplasia type 1. In a prospective study, Norton *et al*[32] reported a significant biochemical improvement of ZES in 20% of patients who underwent resection of more than three parathyroid glands. Finally, pancreatic polypeptide was once correlated with hyperparathyroidism[33].

Acute pancreatitis caused by PHPT was first described by Cope *et al*[34] in 1957. Since that date, the exact relationship between these two entities has been questioned, until PHPT was accepted as an etiology for pancreatitis[35]. Incidence of acute pancreatitis in patients with PHPT has varied from 1%[36] to 12%[37] in retrospective series, with intermediate values[38,39]. Jacob *et al*[40] have shown a 28-fold increased risk of pancreatitis in hyperparathyroid patients compared to the general population. After eliminating all other causes, mean plasma calcium level seems to be the only predictive factor for pancreatitis development[37,40,41]. Its dosage must be included in the etiological work-up, although hyperparathyroidism is found in < 1% of patients who present with acute pancreatitis[42]. Carnaille *et al*[37] have shown that most patients had single adenoma, which suggested that pancreatitis was a consequence (and not the cause) of hyperparathyroidism. Additionally, acute pancreatitis may be the presenting form of PHPT[38,43,44], even in its ectopic localization[45,46]. In contrast, Felderbauer *et al*[39] have stressed that genetic mutations constitute a greater risk factor for pancreatitis than serum calcium.

The pathophysiological mechanism that leads to pancreatitis seems more related to hypercalcemia than to PHPT. It has been shown that hypercalcemia from any cause can lead to pancreatitis[47-49]. As confirmed by experimental studies, calcium ions cause calculus deposition within the pancreatic ductules, with consequent obstruction and inflammation[50]. Moreover, calcium can trigger the pancreatitis cascade by promoting conversion of trypsinogen to trypsin[51,52].

Interrelation between acute pancreatitis and parathyroid function can be summarized as follows: (1) acute pancreatitis results in a tendency to hypocalcemia and secondary hyperparathyroidism[53,54]. Compensation need is correlated to pancreatitis severity as shown by PTH level[55]; (2) severe and/or complicated pancreatitis can lead to overt hypocalcemia through relative deficiency in PTH secretion[54], because exogenous administration of PTH normalizes calcium level[56]; (3) in severe pancreatitis, resistance to PTH action in bones and kidneys may occur because of fluid sequestration and reduction in efficient arterial blood volume[53]; (4) once the diagnosis of PHPT-induced acute pancreatitis is established, parathyroidectomy is mandatory because it prevents recurrence[37,42].

Bhadada *et al*[57] have studied PHPT-induced chronic pancreatitis and compared it to pancreatitis of other causes. PTH and calcium levels are significantly more elevated in PHPT, while in others, elevated PTH level is secondary to maintain normocalcemia. With regard to complications, it seems that chronic pancreatitis secondary to PHPT does not differ from chronic pancreatitis of other causes. This entity needs to be studied by larger studies for further understanding.

In conclusion, serum calcium level must be considered among the usual tests in patients with rare and/or non-specific abdominal symptoms. Hypoparathyroidism mainly manifests in the gut as malabsorptive diarrhea. Laboratory tests are essential for the diagnosis of secondary hypocalcemia when treatment is medical. PHPT causes non-specific digestive symptoms that are consequent to smooth-muscle atony. Association of peptic ulcer with PHPT is not as clear as described by old literature except for ZES in MEN 1. In contrast, PHPT is a confirmed risk factor for acute pancreatitis that can be its presenting form. Finally, PHPT-induced chronic pancreatitis needs further study for confirmation.

REFERENCES

1 **Maeda SS**, Fortes EM, Oliveira UM, Borba VC, Lazaretti-Castro M. Hypoparathyroidism and pseudohypoparathyroidism. *Arq Bras Endocrinol Metabol* 2006; **50**: 664-673
2 **Heubi JE**, Partin JC, Schubert WK. Hypocalcemia and steatorrhea--clues to etiology. *Dig Dis Sci* 1983; **28**: 124-128
3 **Lorenz R**, Burr IM. Idiopathic hypoparathyroidism and steatorrhea: a new aid in management. *J Pediatr* 1974; **85**: 522-525
4 **Clarkson B**, Kowlessar OD, Horwith M, Sleisenger MH. Clinical and metabolic study of a patient with malabsorption and hypoparathyroidism. *Metabolism* 1960; **9**: 1093-1106
5 **Peracchi M**, Bardella MT, Conte D. Late-onset idiopathic hypoparathyroidism as a cause of diarrhoea. *Eur J Gastroenterol Hepatol* 1998; **10**: 163-165
6 **Wortsman J**, Kumar V. Case report: idiopathic hypoparathyroidism co-existing with celiac disease: immunologic studies. *Am J Med Sci* 1994; **307**: 420-427
7 **Frysák Z**, Hrcková Y, Rolinc Z, Hermanová Z, Lukl J. [Idiopathic hypoparathyroidism with celiac disease--diagnostic and therapeutic problem]. *Vnitr Lek* 2000; **46**: 408-412
8 **Gelfand IM**, DiMeglio LA. Hypocalcemia as a presenting feature of celiac disease in a patient with DiGeorge syndrome. *J Pediatr Endocrinol Metab* 2007; **20**: 253-255
9 **Kumar V**, Valeski JE, Wortsman J. Celiac disease and hypoparathyroidism: cross-reaction of endomysial antibodies with parathyroid tissue. *Clin Diagn Lab Immunol* 1996; **3**: 143-146
10 **Marcondes JA**, Seferian Junior P, Mitteldorf CA. Resistance to vitamin D treatment as an indication of celiac disease in a patient with primary hypoparathyroidism. *Clinics* (Sao Paulo) 2009; **64**: 259-261
11 **Isaia GC**, Casalis S, Grosso I, Molinatti PA, Tamone C, Sategna-Guidetti C. Hypoparathyroidism and co-existing celiac disease. *J Endocrinol Invest* 2004; **27**: 778-781
12 **Matsueda K**, Rosenberg IH. Malabsorption with idiopathic hypoparathyroidism responding to treatment for coincident celiac sprue. *Dig Dis Sci* 1982; **27**: 269-273
13 **Jorde R**, Saleh F, Sundsfjord J, Haug E, Skogen B. Coeliac disease in subjects with secondary hyperparathyroidism. *Scand J Gastroenterol* 2005; **40**: 178-182
14 **Reisner DJ**, Ellsworth RM. Coexistent idiopathic hypoparathyroidism and pernicious anemia in a young girl: case report. *Ann Intern Med* 1955; **43**: 1116-1124
15 **St Goar WT**. Gastrointestinal symptoms as a clue to the diagnosis of primary hyperparathyroidism: a review of 45 cases. *Ann Intern Med* 1957; **46**: 102-118
16 **Chan AK**, Duh QY, Katz MH, Siperstein AE, Clark OH. Clinical manifestations of primary hyperparathyroidism before and after parathyroidectomy. A case-control study. *Ann Surg* 1995; **222**: 402-412; discussion 412-414
17 **Gardner EC**, Hersh T. Primary hyperparathyroidism and the gastrointestinal tract. *South Med J* 1981; **74**: 197-199
18 **Ellis C**, Nicoloff DM. Hyperparathyroidism and peptic ulcer disease. *Arch Surg* 1968; **96**: 114-118
19 **Sharma S**, Longo WE, Baniadam B, Vernava AM. Colorectal manifestations of endocrine disease. *Dis Colon Rectum* 1995; **38**: 318-323
20 **Ellison EH**, Abrams JS, Smith DJ. A postmortem analysis of 812 gastroduodenal ulcers found in 20,000 consecutive autopsies, with emphasis on associated endocrine disease. *Am J Surg* 1959; **97**: 17-30
21 **Ostrow JD**, Blanshard G, Gray SJ. Peptic ulcer in primary hyperparathyroidism. *Am J Med* 1960; **29**: 769-779
22 **Frame B**, Haubrich WS. Peptic ulcer and hyperparathyroidism: a survey of 300 ulcer patients. *Arch Intern Med* 1960; **105**: 536-541
23 **Barreras RF**, Donaldson RM. Role of calcium in gastric hypersecretion, parathyroid adenoma and peptic ulcer. *N Engl J Med* 1967; **276**: 1122-1124
24 **McGuigan JE**, Colwell JA, Franklin J. Effect of parathyroidectomy on hypercalcemic hypersecretory peptic ulcer disease. *Gastroenterology* 1974; **66**: 269-272
25 **Ward JT**, Adesola AO, Welbourn RB. The parathyroids, calcium and gastric secretion in man and the dog. *Gut* 1964; **5**: 173-183
26 **Segawa K**, Nakazawa S, Naito Y, Imai K, Yamase H, Yamada K, Yamamoto T, Ichikawa M, Hidano H, Kachi T, Hayashi S, Kawaguchi S, Tsukamoto Y, Kajikawa M, Kimoto E, Ichikawa T. The further investigation on the gastric acid secretion in the primary hyperparathyroidism. *Gastroenterol Jpn* 1977; **12**: 347-351
27 **Patterson M**, Wolma F, Drake A, Ong H. Gastric secretion and chronic hyperparathyroidism. *Arch Surg* 1969; **99**: 9-14
28 **Wilson SD**, Singh RB, Kalkhoff RK. Does hyperparathyroidism cause hypergastrinemia? *Surgery* 1976; **80**: 231-237
29 **Wesdorp RI**, Wang CA, Hirsch H, Fischer JE. Plasma and parathyroid tumor tissue gastrin and hyperparathyroidism. *Am J Surg* 1976; **131**: 60-63
30 **Reeder DD**, Jackson BM, Ban J, Clendinnen BG, Davidson WD, Thompson JC. Influence of hypercalcemia on gastric secretion and serum gastrin concentrations in man. *Ann Surg* 1970; **172**: 540-546
31 **Corleto VD**, Minisola S, Moretti A, Damiani C, Grossi C, Ciardi S, D'Ambra G, Bordi C, Strom R, Spagna G, Delle Fave G, Annibale B. Prevalence and causes of hypergastrinemia in primary hyperparathyroidism: a prospective study. *J Clin Endocrinol Metab* 1999; **84**: 4554-4558
32 **Norton JA**, Venzon DJ, Berna MJ, Alexander HR, Fraker DL, Libutti SK, Marx SJ, Gibril F, Jensen RT. Prospective study of surgery for primary hyperparathyroidism (HPT) in multiple endocrine neoplasia-type 1 and Zollinger-Ellison syndrome: long-term outcome of a more virulent form of HPT. *Ann Surg* 2008; **247**: 501-510
33 **Strodel WE**, Vinik AI, Eckhauser FE, Thompson NW. Hyperparathyroidism and gastroenteropancreatic hormone levels. *Surgery* 1985; **98**: 1101-1106
34 **Cope O**, Culver PJ, Mixter CG, Nardi GL. Pancreatitis, a diagnostic clue to hyperparathyroidism. *Ann Surg* 1957; **145**: 857-863
35 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400
36 **Bess MA**, Edis AJ, van Heerden JA. Hyperparathyroidism

and pancreatitis. Chance or a causal association? *JAMA* 1980; **243**: 246-247
37 **Carnaille B**, Oudar C, Pattou F, Combemale F, Rocha J, Proye C. Pancreatitis and primary hyperparathyroidism: forty cases. *Aust N Z J Surg* 1998; **68**: 117-119
38 **Shepherd JJ**. Hyperparathyroidism presenting as pancreatitis or complicated by postoperative pancreatitis. *Aust N Z J Surg* 1996; **66**: 85-87
39 **Felderbauer P**, Karakas E, Fendrich V, Bulut K, Horn T, Lebert R, Holland-Letz T, Schmitz F, Bartsch D, Schmidt WE. Pancreatitis risk in primary hyperparathyroidism: relation to mutations in the SPINK1 trypsin inhibitor (N34S) and the cystic fibrosis gene. *Am J Gastroenterol* 2008; **103**: 368-374
40 **Jacob JJ**, John M, Thomas N, Chacko A, Cherian R, Selvan B, Nair A, Seshadri M. Does hyperparathyroidism cause pancreatitis? A South Indian experience and a review of published work. *ANZ J Surg* 2006; **76**: 740-744
41 **Curto C**, Caillard C, Desurmont T, Sebag F, Brunaud L, Kraimps JL, Hamy A, Mathonnet M, Bresler L, Henry JF, Mirallié E. [Acute pancreatitis and primary hyperparathyroidism: a multicentric study by the Francophone Association of Endocrine Surgeons]. *J Chir* (Paris) 2009; **146**: 270-274
42 **Prinz RA**, Aranha GV. The association of primary hyperparathyroidism and pancreatitis. *Am Surg* 1985; **51**: 325-329
43 **Lenz JI**, Jacobs JM, Op de Beeck B, Huyghe IA, Pelckmans PA, Moreels TG. Acute necrotizing pancreatitis as first manifestation of primary hyperparathyroidism. *World J Gastroenterol* 2010; **16**: 2959-2962
44 **He JH**, Zhang QB, Li YM, Zhu YQ, Li X, Shi B. Primary hyperparathyroidism presenting as acute gallstone pancreatitis. *Chin Med J* (Engl) 2010; **123**: 1351-1352
45 **Imachi H**, Murao K, Kontani K, Yokomise H, Miyai Y, Yamamoto Y, Kushida Y, Haba R, Ishida T. Ectopic mediastinal parathyroid adenoma: a cause of acute pancreatitis. *Endocrine* 2009; **36**: 194-197
46 **Foroulis CN**, Rousogiannis S, Lioupis C, Koutarelos D, Kassi G, Lioupis A. Ectopic paraesophageal mediastinal parathyroid adenoma, a rare cause of acute pancreatitis. *World J Surg Oncol* 2004; **2**: 41
47 **Brandwein SL**, Sigman KM. Case report: milk-alkali syndrome and pancreatitis. *Am J Med Sci* 1994; **308**: 173-176
48 **Gafter U**, Mandel EM, Har-Zahav L, Weiss S. Acute pancreatitis secondary to hypercalcemia. Occurrence in a patient with breast carcinoma. *JAMA* 1976; **235**: 2004-2005
49 **Hochgelerent EL**, David DS. Acute pancreatitis secondary to calcium infusion in a dialysis patient. *Arch Surg* 1974; **108**: 218-219
50 **Ward JB**, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? *Lancet* 1995; **346**: 1016-1019
51 **Mithöfer K**, Fernández-del Castillo C, Frick TW, Lewandrowski KB, Rattner DW, Warshaw AL. Acute hypercalcemia causes acute pancreatitis and ectopic trypsinogen activation in the rat. *Gastroenterology* 1995; **109**: 239-246
52 **Frick TW**, Fernández-del Castillo C, Bimmler D, Warshaw AL. Elevated calcium and activation of trypsinogen in rat pancreatic acini. *Gut* 1997; **41**: 339-343
53 **Hauser CJ**, Kamrath RO, Sparks J, Shoemaker WC. Calcium homeostasis in patients with acute pancreatitis. *Surgery* 1983; **94**: 830-835
54 **Condon JR**, Ives D, Knight MJ, Day J. The aetiology of hypocalcaemia in acute pancreatitis. *Br J Surg* 1975; **62**: 115-118
55 **McKay C**, Beastall GH, Imrie CW, Baxter JN. Circulating intact parathyroid hormone levels in acute pancreatitis. *Br J Surg* 1994; **81**: 357-360
56 **Robertson GM**, Moore EW, Switz DM, Sizemore GW, Estep HL. Inadequate parathyroid response in acute pancreatitis. *N Engl J Med* 1976; **294**: 512-516
57 **Bhadada SK**, Udawat HP, Bhansali A, Rana SS, Sinha SK, Bhasin DK. Chronic pancreatitis in primary hyperparathyroidism: comparison with alcoholic and idiopathic chronic pancreatitis. *J Gastroenterol Hepatol* 2008; **23**: 959-964

S- Editor Sun H **L- Editor** Kerr C **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4067

World J Gastroenterol 2011 September 28; 17(36): 4067-4075
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Current treatment for colorectal liver metastases

Evangelos P Misiakos, Nikolaos P Karidis, Gregory Kouraklis

Evangelos P Misiakos, Third Department of Surgery, Medical School, University of Athens, Attikon University Hospital, Athens 12462, Greece
Nikolaos P Karidis, Gregory Kouraklis, Second Propedeutic Department of Surgery, Medical School, University of Athens, General Hospital Laiko, Athens 11527, Greece
Author contributions: Misiakos EP organized and prepared the draft of the present review; Karidis NP contributed to reference collection and final preparation of the manuscript; Kouraklis G coordinated and reviewed the manuscript.
Correspondence to: Nikolaos P Karidis, MD, General Surgeon, Second Propedeutic Department of Surgery, University of Athens, General Hospital Laiko, Athens 11527, Greece. npkaridis@gmail.com
Telephone: +30-69-74779016 Fax: +30-21-07791456
Received: October 3, 2010 Revised: November 30, 2010
Accepted: December 7, 2010
Published online: September 28, 2011

Abstract

Surgical resection offers the best opportunity for survival in patients with colorectal cancer metastatic to the liver, with five-year survival rates up to 58% in selected cases. However, only a minority are resectable at the time of diagnosis. Continuous research in this field aims at increasing the percentage of patients eligible for resection, refining the indications and contraindications for surgery, and improving overall survival. The use of surgical innovations, such as staged resection, portal vein embolization, and repeat resection has allowed higher resection rates in patients with bilobar disease. The use of neoadjuvant chemotherapy allows up to 38% of patients previously considered unresectable to be significantly downstaged and eligible for hepatic resection. Ablative techniques have gained wide acceptance as an adjunct to surgical resection and in the management of patients who are not surgical candidates. Current management of colorectal liver metastases requires a multidisciplinary approach, which should be individualized in each case.

Key words: Colorectal liver metastases; Multidisciplinary treatment

Peer reviewer: Ferenc Sipos, MD, PhD, Cell Analysis Laboratory, 2nd Department of Internal Medicine, Semmelweis University, Szentkirályi u. 46, Budapest 1088, Hungary

Misiakos EP, Karidis NP, Kouraklis G. Current treatment for colorectal liver metastases. *World J Gastroenterol* 2011; 17(36): 4067-4075 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4067.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4067

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in the West and the second most common cause of cancer related mortality after lung cancer in Europe and North America[1,2]. More than 50% of patients with CRC will develop liver metastases during their lifespan[2,3]. A quarter of patients with primary CRC are found to have synchronous hepatic secondaries[4]. Almost half of patients undergoing resection for primary CRC eventually develop metachronous liver secondaries[5].Despite improvements in chemotherapies and biological agents, survival is rarely longer than three years[6,7].

Evidence based on numerous retrospective and comparative studies indicates that hepatic resection is the only available treatment that allows long-term survival[8]. Experiences with liver resection is associated with a 25% to 51% 5-year survival[9,10]. By contrast, five-year survivors with chemotherapy alone are anecdotal. Historically, only 5%-10% of patients with colorectal liver metastases were resectable; currently, with the advances in diagnostic methods and new therapies, resectability rates have increased to 20%-25%[11].

Emerging strategies designed to increase the proportion of patients who are candidates for complete surgical resection have been introduced in clinical practice. Neoadjuvant chemotherapy[11], preoperative portal vein embolization[12], and the two-stage resection approach[13]

contribute to this aim. However, even with these new strategies, the majority of patients with colorectal liver metastases are not candidates for a curative resection.

In this review, the current data supporting the use of liver resection in the management of colorectal liver metastases are analyzed. For this purpose, the role of new imaging techniques for the preoperative evaluation and new staging systems to stratify the patients are extensively reported. Moreover, the most recently introduced chemotherapies and biological therapies to prevent recurrence after surgery or to downstage unresectable tumors are analyzed.

NATURAL HISTORY

Liver metastases from colorectal cancer carry a median survival of 5 to 20 mo if left untreated; two-year survival is unusual, and five-year survival is extremely rare[4-14]. Factors associated with a significant disadvantage in the unresected group include extent of liver disease, presence of extrahepatic disease, age of the patient, and carcinoembryonic antigen (CEA) level[14]. Prognosis is closely related to the extent of liver replacement by the tumor[4,15]. Indeed, Wood *et al*[15] in a retrospective study of 113 patients undertaken in the Glasgow Royal Infirmary, reported a one-year survival rate of 5.7% for patients with widespread liver disease, 27% for patients with metastases localized to one hepatic lobe, and 60% for patients with solitary metastases.

Even when hepatic resection is performed with curative intent[16], 60% to 70% of patients will develop local or distant recurrence[17]. Recurrence occurs equally at intrahepatic and extrahepatic sites; 80% of all recurrences occur within two years. The median survival of patients with recurrent disease is 8 to 10 mo without any treatment[14]. Repeat resection is feasible in 10% to 15% of these cases and may achieve a five-year overall survival rate of 15% to 40% in selected patients. Cure is considered after the achievement of 10-year disease-free survival[18].

Chemotherapy alone, whether administered systemically or regionally, has a palliative role and rarely results in prolonged survival. Several retrospective studies have reviewed the clinical outcome of patients with potentially resectable liver metastases treated with chemotherapy alone. An obvious survival advantage for patients undergoing curative resection compared to those treated with chemotherapy was noted[19]. Scheele *et al*[19]compared 183 patients with resected hepatic metastases with 62 patients with resectable lesions who did not undergo surgery and 920 patients with unresectable disease. The median survival for the three groups was 30 mo, 14.2 mo and 6.9 mo, respectively. Although the patients of the second group lived longer than those of the third group, no patient in either group survived more than five years.

These poor results in untreated hepatic metastases from colorectal cancer and the continuous improvements in hepatic surgery provided the rationale for increasingly aggressive hepatic resections for the treatment of this condition[20].

CURRENT CRITERIA OF HEPATIC RESECTION

During the past two decades the five-year survival rates for hepatic colorectal metastases patients have almost doubled, from 30% to 60%[14]. The introduction of new chemotherapeutic agents and the shift in the criteria of surgical resection were the main factors in this progress[21]. Previous absolute or relative contraindications to resection included the presence of extrahepatic disease[8], involvement of hepatic pedicle lymph nodes[22], and an inadequate resection margin of < 1 cm[23]. All above contraindications for hepatic resection have been challenged and have already lost their importance in patient selection for hepatectomy[24,25].

The current criteria focus on what should be left after hepatic resection. Previous criteria for resection, such as the size, location, number of intrahepatic metastases, and the presence of bilobar or extrahepatic disease have been largely abandoned[14,26,27]. Nowadays, the definition of resectability includes a complete resection with tumor-free surgical margins (R0 resection), sparing at least two liver segments having an independent inflow, outflow, and biliary drainage. The amount of the liver remnant after resection should not be less than 20% and 30% of the total liver volume in normal and cirrhotic patients, respectively. This can be accurately predicted by computed tomography (CT) or magnetic resonance imaging (MRI) during preoperative evaluation.

PREOPERATIVE EVALUATION

Preoperative investigations before resection of colorectal liver metastases are focused on: (1) determining the diagnosis; (2) anatomically defining the lesion in the liver parenchyma for surgical planning; and (3) meticulous staging to rule out extrahepatic disease[28].

Preoperative biopsy

Fine needle aspiration (FNA) cytology is a well established approach for diagnosis. The potential benefit of FNA in suspect cases is the cytological confirmation of diagnosis, although this can be effectively obtained by other examinations, together with the patient's history. However, there is a potential for false negative results. Nevertheless, the benefit of this examination may be outweighed by the serious risk of needle tract seeding[29,30]. For these reasons, FNA cytology has been virtually abandoned in the preoperative evaluation of colorectal liver metastases.

Preoperative investigation

Metastatic liver tumors can usually be differentiated by imaging modalities, including ultrasound, CT, MRI and positron emission tomography (PET). CT plays a pivotal role in selecting patients for hepatic resection. The use of multidetector helical CT scans has improved resolution and increased the previously low sensitivity (53%) of detecting colorectal liver metastases to 70%-90%[14,31,32]. Liv-

er metastases can be distinguished as hypodense lesions in the portal phase. A CT scan may provide information regarding the anatomical characteristics of the metastatic lesions and their relation to lobar architecture and major vascular structures. However, a CT scan cannot detect subcentimeter lesions[14]. Colorectal liver metastases usually respect the liver capsule and the intersegmental planes and push these structures away. Even large lesions that appear to involve the inferior vena cava or the diaphragm on a CT scan, often do not do so and such appearances should not preclude surgical exploration[28].

MRI is more useful than CT in detecting small metastatic lesions in a fatty liver, and in defining the relationship of the lesions to the hepatic vasculature and the biliary tree with MR cholangiopancreatography[28]. However, it has a sensitivity of 70% to 80% and it does not offer any significant advantage over a CT scan[14]. Furthermore, MRI angiography and CT angiography have gradually replaced the more invasive direct hepatic angiography.

Ultrasonography is an inexpensive test that may identify small metastatic lesions within the hepatic parenchyma. It can give information regarding the size of the metastatic tumor and the extent of liver involvement. Moreover, Duplex ultrasound can define the relation of the tumor to the hilar structures, the hepatic veins, and the inferior vena cava. Ultrasound may be used as a first-line modality in the diagnostic evaluation of hepatic metastases[28].

A new modality in the diagnosis of colorectal liver metastases is whole body PET. The most common tracer in PET scanning is fluoro-18-deoxyglucose (FDG)-PET, a glucose analog, which can proceed down the glycolytic pathway, and accumulate within the glucose-avid cancer cells. A recent meta-analysis reported a sensitivity and specificity for FDG-PET of 88% and 96%, respectively, for the detection of hepatic metastases, and 90% to 95% for the detection of extrahepatic disease[33]. The combination of CT and FDG-PET increases sensitivity and allows the selection of surgical therapy for patients likely to gain the most benefit[34]. The main limitation of a PET scan is the reduced sensitivity in detecting subcentimeter lesions, mucinous lesions, and lesions that have been treated with neoadjuvant chemotherapy[35].

During the last two decades, laparoscopy has emerged as a new diagnostic modality for patients with liver malignancies. When laparoscopy is employed, unnecessary laparotomy can be avoided in 78% of patients with unresectable disease[35]. In these cases, laparoscopy can decrease the morbidity of surgery, and shorten the delay to systemic therapy[36]. Laparoscopy is indicated in cases in which the results of imaging studies are suspicious, but not diagnostic for extrahepatic tumor, such as enlarged lymph nodes or possible peritoneal dissemination.

PREOPERATIVE TREATMENT

Chemotherapy

Current chemotherapy regimens including oxaliplatin and irinotecan in addition to 5-fluorouracil (5-FU), and leucovorin (LV) have achieved improved response rates in colorectal liver metastases, with significant reduction in disease bulk in almost 50% of patients and a median survival approaching two years[37]. New biological agents, such as those targeting epithelial and vascular endothelial growth factor pathways (bevacizumab, cetuximab) have added a significant survival benefit in these patients[38,39].

The successful use of combination chemotherapy in colorectal liver metastases has led to the concept that these agents could also be used before hepatic resection. In fact, the use of neoadjuvant chemotherapy has the benefit of downstaging the tumor, rendering a previously unresectable tumor resectable. This approach may assess the responsiveness of the tumor to chemotherapy, as the initial response to chemotherapy is strongly predictive of a favorable long-term outcome[40,41]. The development of steatohepatitis is a complication of preoperative chemotherapy, which results in a significantly increased 90-d postoperative mortality[42].

Neoadjuvant chemotherapy

The use of preoperative chemotherapy may exert a downsizing effect on the metastatic tumors, so one may perform surgery as soon as resectability is technically feasible. According to the Paul Brousse experience[43], modern chemotherapeutic regimens allow 12.5% of patients with unresectable colorectal liver metastases to be rescued by hepatic resection. This strategy may offer a possibility of long-term survival (33% at five years and 22% at 10 years) with a low operative risk. It is noteworthy that this strategy involves the wide use of repeat hepatectomies and extrahepatic resections in an effort to eradicate all tumors. Currently most reports suggest that infusional FU/LV with oxaliplatin and/or irinotecan are the most effective protocols for this purpose[31,44]. However, although the response rates are very high when used as first-line therapy, the response rates for second-line therapy are very low[31,45]. Therefore, tumors that progress while on chemotherapy usually have a low likelihood of becoming resectable with second-line chemotherapy.

Neo-adjuvant chemotherapy can also be used *via* hepatic arterial infusion (HAI) with high response rates, as first or second-line therapies[46]. Patients with metastatic lesions confined to the liver, without severe ascites or jaundice, are ideal candidates[47]. Preliminary data from several clinical trials with oxaliplatin or irinotecan *via* HAI have been promising[48]. However, HAI is rarely used outside specialized treatment centers, because of limited expertise, high cost of infusion pumps, and ongoing concerns regarding the considerable morbidity due to catheter-related complications, particularly sclerosing cholangitis[49].

Portal vein embolization

Portal vein embolization (PVE) is another modality used preoperatively for patients where the extent of liver

resection is expected to result in less than the optimal functional liver volume of 25% to 40%, necessary to prevent postoperative liver failure[21,50]. This technique, which induces ipsilateral atrophy and contralateral hypertrophy, is used to expand the number of patients undergoing curative hepatectomy for colorectal liver metastases. The most commonly used agents for embolization include gelatin sponge particles (Gelfoam) with iodized oil (Lipiodol), cyanoacrylate, alcohol, fibrin glue, or gelatin sponge, and they are usually administered percutaneously[14,51]. The amount of liver tissue gained is about 15% of the total liver volume, and the time for maximum regeneration ranges from three to nine weeks[52].

Azoulay *et al*[51] have reported on a group of 30 patients who were deemed ineligible for liver resection because the estimated remnant liver was considered too small. These patients underwent PVE with minimal morbidity and no mortality. PVE substantially increased the remnant liver volume, rendering liver resection feasible in 19 patients (63%), with low morbidity and mortality rates and survival rates similar to the patients who did not undergo PVE. In conclusion, PVE followed by hepatic resection represents a two-stage hepatectomy: progressive atrophy of the embolized area, which triggers compensatory hypertrophy of the future remaining parenchyma, followed by liver resection. Therefore, PVE increases the resectability of colorectal liver metastases with a survival benefit comparable to that obtained with primary liver resection.

Several disadvantages of PVE have emerged as more experience is collected. Thrombosis, and/or migration of the emboli to the contralateral hepatic lobe, hemobilia, hemoperitoneum, and transient liver insufficiency, are complications occurring in 10% of cases and can be easily managed[50]. Another adverse side effect is the possibility that PVE may stimulate the growth of tumors in the contralateral liver lobe, although this has yet to be clarified[53]. A way of counteracting this effect is the administration of concurrent chemotherapy soon after PVE, the so-called "interterm chemotherapy"[14].

LIVER RESECTION

Over the last two to three decades, an aggressive surgical approach has been followed for the treatment of colorectal hepatic metastases, based on the fact that the liver is the first isolated site of metastases for colorectal cancer. This direct treatment of hepatic metastases prevents dissemination of the disease from the liver to other sites[54].

The role of hepatic resection as an effective treatment for colorectal liver metastases was established in 1988 from the registry of hepatic metastases[9]. In a retrospective review on 859 patients with colorectal liver metastases who were surgically treated between 1948 and 1985, the five-year actuarial survival rate and the disease-free survival rate were 33% and 21%, respectively. Along with the gradual improvement in imaging techniques, better understanding of liver anatomy, recent refinements of surgical techniques, and the continuous progress in pre-and postoperative care, the postoperative mortality rate after hepatectomy has been reduced to < 3% and the five-year survival rate after resection of colorectal liver metastases has reached 26%-58%[10,25].

Initially, liver resection was based on the anatomic system described in the early 1950s by Couinaud[55], who defined the intrahepatic divisions of blood vessels and bile ducts. However, there was significant confusion regarding the description of liver anatomy and hepatic resections until the first universally accepted terminology system was introduced. The "Brisbane 2000 terminology of liver anatomy and resections"[56] was based on the internal anatomy and described the several levels of division of the liver segments; today, it has gained wide acceptance among liver specialists.

The main purpose of liver resection is to resect the tumor with a sufficient tumor-free margin, while preserving as much normal parenchyma as possible. Hepatic resections have regularly been along the liver segmental anatomy planes[31]. An alternative approach is a non-anatomical or wedge resection, removing a smaller volume of liver with reduced postoperative morbidity and mortality. However, this carries a higher risk of positive resection margins[41]. However, in a recent series where wedge resections were performed for single rather than multiple lesions, the incidence of positive resection margins was equivalent for both wedge resection and segmental resection (8.3%), and the five-year survival was equivalent in both groups[57].

Intraoperative ultrasound can delineate the interior anatomy of the liver, including intrahepatic vessels, and allows hepatic resection to be performed more safely and anatomically. Moreover, intraoperative ultrasound may identify extrahepatic sites of the disease, such as infiltrated lymph nodes in the celiac axis and the liver hilum, or deposits in the peritoneal cavity[58]. Extrahepatic disease sites in the peritoneal cavity impart a significant disadvantage in prognosis, whereas an excellent five-year survival (20% to 48%) can be achieved with pulmonary metastases with an R0 resection[59].

There is a variety of techniques and devices used for hepatic resection, including the clamp crushing technique, Cavitron Ultrasonic Surgical Aspirator (CUSA, Covidien, Mansfield, MA, United States), Hydrojet (Hydro-Jet, Erbe, Tubingen, Germany), and bipolar sealing devices. Among these, the clamp crushing technique remains the most efficient in terms of reduced operation time, blood loss and total costs[60].

Synchronous disease

Synchronous hepatic metastases occur in about 20%-30% of newly diagnosed colorectal cancers, and they present a challenging problem in the management of these patients[9]. Consensus has not been reached as to the timing of surgical resection of the hepatic secondaries and the primary colorectal tumor. Traditionally, these patients

were managed by a second laparotomy 12-16 wk after the resection of the primary tumors[61]. The advantage of this approach is that it provides less surgical insult to the patient as the incision used in the two operations is different[14]. However, with advances in perioperative care and the continuous improvements regarding the postoperative morbidity and mortality rates after liver resection, most researchers today support simultaneous resection[62,63]. In fact, very few reports in the last decade still strongly oppose the simultaneous procedure.

Today, a simultaneous resection is preferred when there is a right colon primary, or when a single hepatic lesion is contemplated, whereas a staged resection is often done in case of rectal primaries, or multiple liver secondaries[31]. However, no real indications or contraindications exist for simultaneous resection of hepatic metastases, and it seems that the final decision depends on the surgeon's experience and the patient's physical status. In general, the results of simultaneous resection are comparable to staged resection in terms of morbidity, and mortality rates; additionally simultaneous resection offers the advantage of completing the local control of the disease in a single procedure, allowing the use of adjuvant chemotherapy for systemic micrometastases[64].

Locally ablative modalities in combination with liver resection

Locally ablative modalities, such as radiofrequency ablation (RFA)[65], cryotherapy[66], or high intensity focused ultrasound[67], can be used in combination to hepatic resection, to offer curative treatment in patients with unresectable tumors. RFA is the most widely used modality. The goal of the combined approach is to resect the bulk of the metastatic load and to ablate the residual smaller lesions, to achieve a R0 status, preserving at the same time adequate liver parenchyma to avoid postoperative hepatic failure[68]. According to the MD Anderson Cancer Center' s experience[65] in the combined approach for advanced hepatic malignancies (72% were hepatic colorectal metastases), the perioperative mortality and morbidity rates were 2.3% and 19.8%, respectively. In addition, patients with colorectal secondaries had a median actuarial survival of 37.3 mo. The authors point out that the functional residual hepatic volume has to be accurately estimated to avoid fatal hepatic failure postoperatively, which is quite common in this combined approach.

The use of RFA in combination with surgical resection allows the hepatic surgeon to ablate small lesions while removing the large ones. RFA combined with hepatectomy is well tolerated by the patients and adds minimal complexity and morbidity to the operation. However, RFA is inferior for local control of metastatic lesions, systemic spread, and long-term survival. Indeed, there is a higher local recurrence rate associated with RFA than with resection, resulting in inferior disease-free survival rate[21]. Therefore, for the treatment of solitary hepatic metastases, the application of RFA cannot be primarily recommended[69]. On the other hand, RFA can be used as palliative treatment for unresectable metastases, as it achieves better survival than chemotherapy[21]. The only limitations in the use of RFA and other locally ablative modalities are the size of the lesion and its location close to major biliary or vascular structures[31].

Bilobar metastases

The management of bilobar liver metastases demonstrates the advantages of a multidisciplinary approach with a step-by-step strategy and restaging at regular intervals, to achieve a complete resection in most of these patients. The prognostic significance of bilobar distribution of multiple metastases is controversial. Some researchers report bilobar distribution as a poor prognostic factor[9], whereas others support the view that bilobar distribution does not affect overall patient survival[8,10]. In fact, the total tumor volume of liver metastases seems to have a stronger influence on survival than the number or location of metastatic lesions[70].

Surgical resection should be performed only if all the metastatic load of the liver can be removed (R0 resection). In case of involvement of lymph nodes in the hepatic pedicle, with frozen section confirmation, an extensive lymphadenectomy should be performed from the liver hilum to the celiac axis. Moreover, in patients who have more than three poorly differentiated metastatic lesions in segments IV and V, a routine extended lymphadenectomy of the hepatic pedicle seems justified[71,72].

In general, hepatic lymph node involvement is a poor prognostic factor affecting survival of these patients[9], but according to a multi-center study by the Association Francaise de Chirurgie, the five-year survival rate of patients with hepatic pedicle lymph node involvement who underwent lymphadenectomy was 12%, compared to the expected 0% to 2 % without resection[10].

The presence of extrahepatic disease is no longer a contraindication to hepatic resection. Recently, encouraging results have been reported in patients treated for liver metastases and peritoneal carcinomatosis[73]. However, this approach is suitable only for expert teams with experience in liver surgery and intraperitoneal chemotherapy[72].

Two-stage hepatectomy

The aim of this approach is to achieve in two steps a complete resection of the metastases in cases initially considered unresectable. In these cases, a single hepatectomy would have left too small a remnant liver after surgery, with a high risk of liver insufficiency after surgery[72]. In two-stage hepatectomy the highest possible number of tumors are resected first, and the remaining tumors are resected in a second procedure after a period of liver regeneration[13].

The aim of the first hepatectomy is to make the second hepatectomy potentially curative. Mapping permits the surgeon to achieve this by resecting the highest possible number of liver tumors or by clearing the metastatic load from the less invaded hepatic lobe, leaving

the other to be resected after regeneration. Neoadjuvant chemotherapy is given after the first operation, beginning three weeks postoperatively, so it does not interfere with initial liver regeneration. The usual interval between the two stages should be usually around 4 mo, (from 2 to 14 mo), depending on the progress of liver regeneration[13]. Patients with multiple bilobar liver metastases and too small a future remnant liver could be treated with a two-stage procedure with the use of portal vein embolization[72].

This approach can also be used at the time of colectomy when multiple synchronous hepatic lesions preclude a single curative hepatectomy. In such cases, a limited resection of the metastatic load of one hemiliver could be done at the same time as the colectomy, leaving the second major hepatectomy to be done in a second stage[72].

FOLLOW UP AFTER RESECTION

Patients who have undergone hepatic resection of colorectal metastases are monitored to identify early recurrence that may be amenable to repeat resection for cure. Most patients undergo serial physical examination, serum CEA level, chest X-ray, and CT of the upper and lower abdomen every 3 to 4 mo for the first two years and then every 6 mo for the following five years[28]. Most patients surviving after liver resection present with recurrent disease at the liver or lung. The liver is the site of recurrence in 45% to 75% of cases after liver resection[5], and this explains the fact that most chemotherapeutic regimens address mainly the liver.

ADJUVANT CHEMOTHERAPY

Postoperative chemotherapy following complete resection of metastatic disease may lead to improvement in long-term prognosis. The past decade has been marked with significant changes in the options available for this group of patients. In addition to 5-FU, which has been used since 1996, several new drugs have been introduced on the market for the treatment of metastatic colorectal cancer (2006): irinotecan, oxaliplatin, capecitabine, benacizumab, and cetuximab. Therefore the efficacy of treatment regimens has substantially increased[28].

Adjuvant chemotherapy is used to increase survival and decrease the rate of recurrence. Recently, the first randomized clinical trial by Portier *et al*[74], which compared surgery alone to surgery plus adjuvant chemotherapy, provided clear evidence that adjuvant chemotherapy is beneficial for patients with colorectal liver metastases. In this study, 173 patients were randomly assigned to surgery and observation or surgery plus 6 mo of systemic adjuvant chemotherapy. The results showed a significantly improved five-year disease-free survival in the surgery plus chemotherapy group compared to surgery alone (33.5% *vs* 26.7%), with a trend towards improved overall five-year survival.

Adjuvant chemotherapy does not decrease the metastatic recurrence rate in the remnant liver after resection[75]. Indeed, according to another study[76], in patients with complete clinical response to chemotherapy according to CT imaging, in situ recurrence was observed in 78% one year after surgery, because of non-visible but viable tumor cells or microscopic disease.

REPEAT RESECTION

As mentioned in the natural history section, the majority of patients with colorectal liver metastases (55%-60%) will develop recurrent disease in the liver within the first two years after surgery, despite any mode of treatment that they have received[17]. For these patients, the only chance to prolong life would be a repeat resection, usually combined with a locally ablative therapy (RFA). The results of repeat curative resection are comparable to the first one[14].

The only problem with a second or third hepatectomy on the same patient is increased technical difficulty. Repeat resection carries perioperative morbidity and mortality rates of 5%-7% and 20%-39%, respectively[27,77]. Therefore, repeat hepatectomy provides similar long-term survival to primary hepatectomy, without increasing perioperative morbidity and mortality[78]. Indeed, Pessaux *et al*[79] showed that overall five-year survival rates after the first, second and third hepatectomy are similar: 33%, 21% and 36%, respectively.

There are a number of prognostic factors determining patient eligibility and probable success after a third hepatectomy. These factors are: the curative nature of the first two hepatectomies, an interval of more than one year between the two procedures, the number of recurrent tumors, serum carcinoembryonic antigen levels, and the presence of extrahepatic disease[80,81]. The best candidates for repeat resection are patients with a low tumor load, no extrahepatic disease, and removal of all visible metastatic load during the second hepatectomy[69]. However, the role of repeat liver resection in patients with intrahepatic recurrence still remains controversial, because of the disputable survival benefit and the additional risks of repeat surgery.

CONCLUSION

There is an ongoing progress in the diagnostic imaging, chemotherapeutic regimens, and surgical techniques in the management of hepatic colorectal metastases. Hepatic resection has been recognized as the only treatment that could offer long-term survival. Traditional risk factors, indications, and contraindications have been abandoned. The present principle as to resectability is that resection should be performed if all metastases could be removed, while leaving a sufficient remaining liver parenchyma, regardless of their size, number, location and distribution.

Proper use of modern chemotherapy, PVE and/or two-stage hepatectomy and locally ablative modalities

might improve the resectability and prognosis in these patients. This review emphasizes the importance of a multidisciplinary approach for the optimal management of this disease. Moreover, decision making and patient care requires careful assessment of the risks and benefits for each individual, as well as balancing the technical feasibilities and oncological options for each case.

ACKNOWLEDGMENTS

We would like to thank Professor Gregory Kouraklis for his constant support throughout the design and preparation of this review.

REFERENCES

1 **Remontet L**, Estève J, Bouvier AM, Grosclaude P, Launoy G, Menegoz F, Exbrayat C, Tretare B, Carli PM, Guizard AV, Troussard X, Bercelli P, Colonna M, Halna JM, Hedelin G, Macé-Lesec'h J, Peng J, Buemi A, Velten M, Jougla E, Arveux P, Le Bodic L, Michel E, Sauvage M, Schvartz C, Faivre J. Cancer incidence and mortality in France over the period 1978-2000. *Rev Epidemiol Sante Publique* 2003; **51**: 3-30

2 **Jemal A**, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007; **57**: 43-66

3 **Steele G**, Ravikumar TS. Resection of hepatic metastases from colorectal cancer. Biologic perspective. *Ann Surg* 1989; **210**: 127-138

4 **Bengmark S**, Hafström L. The natural history of primary and secondary malignant tumors of the liver. I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy. *Cancer* 1969; **23**: 198-202

5 **Bozzetti F**, Doci R, Bignami P, Morabito A, Gennari L. Patterns of failure following surgical resection of colorectal cancer liver metastases. Rationale for a multimodal approach. *Ann Surg* 1987; **205**: 264-270

6 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345

7 **Saltz LB**, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL, Miller LL. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000; **343**: 905-914

8 **Fong Y**, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg* 1999; **230**: 309-318; discussion 318-321

9 Resection of the liver for colorectal carcinoma metastases: a multi-institutional study of indications for resection. Registry of Hepatic Metastases. *Surgery* 1988; **103**: 278-288

10 **Adam R**, Avisar E, Ariche A, Giachetti S, Azoulay D, Castaing D, Kunstlinger F, Levi F, Bismuth F. Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. *Ann Surg Oncol* 2001; **8**: 347-353

11 **Abdalla EK**, Hicks ME, Vauthey JN. Portal vein embolization: rationale, technique and future prospects. *Br J Surg* 2001; **88**: 165-175

12 **Adam R**, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* 2000; **232**: 777-785

13 **Sharma S**, Camci C, Jabbour N. Management of hepatic metastasis from colorectal cancers: an update. *J Hepatobiliary Pancreat Surg* 2008; **15**: 570-580

14 **Arnaud JP**, Dumont P, Adloff M, Leguillou A, Py JM. Natural history of colorectal carcinoma with untreated liver metastases. *Surg Gastroenterol* 1984; **3**: 37-42

15 **Wood CB**, Gillis CR, Blumgart LH. A retrospective study of the natural history of patients with liver metastases from colorectal cancer. *Clin Oncol* 1976; **2**: 285-288

16 **Steele G**, Bleday R, Mayer RJ, Lindblad A, Petrelli N, Weaver D. A prospective evaluation of hepatic resection for colorectal carcinoma metastases to the liver: Gastrointestinal Tumor Study Group Protocol 6584. *J Clin Oncol* 1991; **9**: 1105-1112

17 **Tomlinson JS**, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, Kemeny N, Brennan MF, Blumgart LH, D'Angelica M. Actual 10-year survival after resection of colorectal liver metastases defines cure. *J Clin Oncol* 2007; **25**: 4575-4580

18 **Adson MA**, van Heerden JA, Adson MH, Wagner JS, Ilstrup DM. Resection of hepatic metastases from colorectal cancer. *Arch Surg* 1984; **119**: 647-651

19 **Scheele J**, Stangl R, Altendorf-Hofmann A. Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. *Br J Surg* 1990; **77**: 1241-1246

20 **Abdalla EK**, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg* 2004; **239**: 818-825; discussion 825-827

21 **Iwatsuki S**, Dvorchik I, Madariaga JR, Marsh JW, Dodson F, Bonham AC, Geller DA, Gayowski TJ, Fung JJ, Starzl TE. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291-299

22 **Cady B**, Jenkins RL, Steele GD, Lewis WD, Stone MD, McDermott WV, Jessup JM, Bothe A, Lalor P, Lovett EJ, Lavin P, Linehan DC. Surgical margin in hepatic resection for colorectal metastasis: a critical and improvable determinant of outcome. *Ann Surg* 1998; **227**: 566-571

23 **Elias D**, Liberale G, Vernerey D, Pocard M, Ducreux M, Boige V, Malka D, Pignon JP, Lasser P. Hepatic and extrahepatic colorectal metastases: when resectable, their localization does not matter, but their total number has a prognostic effect. *Ann Surg Oncol* 2005; **12**: 900-909

24 **Pawlik TM**, Scoggins CR, Zorzi D, Abdalla EK, Andres A, Eng C, Curley SA, Loyer EM, Muratore A, Mentha G, Capussotti L, Vauthey JN. Effect of surgical margin status on survival and site of recurrence after hepatic resection for colorectal metastases. *Ann Surg* 2005; **241**: 715-722, discussion 722-724

25 **Altendorf-Hofmann A**, Scheele J. A critical review of the major indicators of prognosis after resection of hepatic metastases from colorectal carcinoma. *Surg Oncol Clin N Am* 2003; **12**: 165-192, xi

26 **Vauthey JN**, Pawlik TM, Abdalla EK, Arens JF, Nemr RA, Wei SH, Kennamer DL, Ellis LM, Curley SA. Is extended hepatectomy for hepatobiliary malignancy justified? *Ann Surg* 2004; **239**: 722-730; discussion 730-732

27 **Taylor R**, Fong Y. Surgical treatment of hepatic metastases from colorectal cancer. In: Blumgart LH. Surgery of the liver, biliary tract, and pancreas. 4th Ed. Philadelphia, PA: Saunders Elsevier, 2007: 1178-1194

28 **Jones OM**, Rees M, John TG, Bygrave S, Plant G. Biopsy of resectable colorectal liver metastases causes tumour dissemination and adversely affects survival after liver resection. *Br J Surg* 2005; **92**: 1165-1168

29 **Rodgers MS**, Collinson R, Desai S, Stubbs RS, McCall JL. Risk of dissemination with biopsy of colorectal liver metastases. *Dis Colon Rectum* 2003; **46**: 454-458; discussion 458-459

30 **Hao CY**, Ji JF. Surgical treatment of liver metastases of colorectal cancer: Strategies and controversies in 2006. *Eur J Surg Oncol* 2006; **32**: 473-483

31 **Bipat S**, van Leeuwen MS, Comans EF, Pijl ME, Bossuyt

PM, Zwinderman AH, Stoker J. Colorectal liver metastases: CT, MR imaging, and PET for diagnosis--meta-analysis. *Radiology* 2005; **237**: 123-131
32 **Wiering B**, Krabbe PF, Jager GJ, Oyen WJ, Ruers TJ. The impact of fluor-18-deoxyglucose-positron emission tomography in the management of colorectal liver metastases. *Cancer* 2005; **104**: 2658-2670
33 **Selzner M**, Hany TF, Wildbrett P, McCormack L, Kadry Z, Clavien PA. Does the novel PET/CT imaging modality impact on the treatment of patients with metastatic colorectal cancer of the liver? *Ann Surg* 2004; **240**: 1027-1034; discussion 1027-1034
34 **Lubezky N**, Metser U, Geva R, Nakache R, Shmueli E, Klausner JM, Even-Sapir E, Figer A, Ben-Haim M. The role and limitations of 18-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) scan and computerized tomography (CT) in restaging patients with hepatic colorectal metastases following neoadjuvant chemotherapy: comparison with operative and pathological findings. *J Gastrointest Surg* 2007; **11**: 472-478
35 **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
36 **Jarnagin WR**, Conlon K, Bodniewicz J, Dougherty E, DeMatteo RP, Blumgart LH, Fong Y. A clinical scoring system predicts the yield of diagnostic laparoscopy in patients with potentially resectable hepatic colorectal metastases. *Cancer* 2001; **91**: 1121-1128
37 **Goldberg RM**. Advances in the treatment of metastatic colorectal cancer. *Oncologist* 2005; **10** Suppl 3: 40-48
38 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342
39 **Tabernero JM**, Van Cutsem E, Sastre J, Cervantes A, Van Laethem JL, Humblet Y, Soulié P, Corretgé S, Mueser M, De Gramont A. An international phase II study of cetuximab in combination with oxalip-latin/5-fluorouracil (5-FU)/folinic acid (FA) (FORFLOX-4) in the first-line treatment of patients with metastatic colorectal cancer (CRC) expressing Epidermal Growth Factor Receptor (EGFR). Preliminary results. *J Clin Oncol* 2004; **22** Suppl 14: 3512
40 **Adam R**, Pascal G, Castaing D, Azoulay D, Delvart V, Paule B, Levi F, Bismuth H. Tumor progression while on chemotherapy: a contraindication to liver resection for multiple colorectal metastases? *Ann Surg* 2004; **240**: 1052-1061; discussion 1052-1061
41 **Lim E**, Thomson BN, Heinze S, Chao M, Gunawardana D, Gibbs P. Optimizing the approach to patients with potentially resectable liver metastases from colorectal cancer. *ANZ J Surg* 2007; **77**: 941-947
42 **Vauthey JN**, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**: 2065-2072
43 **Adam R**, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, Giacchetti S, Paule B, Kunstlinger F, Ghémard O, Levi F, Bismuth H. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004; **240**: 644-657; discussion 657-658
44 **Tournigand C**, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229-237
45 **Grothey A**, Jordan K, Kellner O, Constantin C, Dietrich G, Kroening H, Mantovani L, Schlichting C, Forstbauer H and Schmoll HJ. Capecitabine/irinotecan (Capiri) and capecitabine/oxaliplatin (CapOx) are active second-line protocols in patients with advanced colorectal cancer (ACRC) after failure of first-line combination therapy: results of a randomized phase II study. *J Clin Oncol* 2004; **22** Suppl 14: 3534
46 Reappraisal of hepatic arterial infusion in the treatment of nonresectable liver metastases from colorectal cancer. Meta-Analysis Group in Cancer. *J Natl Cancer Inst* 1996; **88**: 252-258
47 **Kemeny MM**, Adak S, Gray B, Macdonald JS, Smith T, Lipsitz S, Sigurdson ER, O'Dwyer PJ, Benson AB. Combined-modality treatment for resectable metastatic colorectal carcinoma to the liver: surgical resection of hepatic metastases in combination with continuous infusion of chemotherapy--an intergroup study. *J Clin Oncol* 2002; **20**: 1499-1505
48 **Shimonov M**, Hayat H, Chaitchik S, Brener J, Schachter P, Czerniak A. Combined systemic chronotherapy and hepatic artery infusion for the treatment of metastatic colorectal cancer confined to the liver. *Chemotherapy* 2005; **51**: 111-115
49 **Kelly RJ**, Kemeny NE, Leonard GD. Current strategies using hepatic arterial infusion chemotherapy for the treatment of colorectal cancer. *Clin Colorectal Cancer* 2005; **5**: 166-174
50 **Di Stefano DR**, de Baere T, Denys A, Hakime A, Gorin G, Gillet M, Saric J, Trillaud H, Petit P, Bartoli JM, Elias D, Delpero JR. Preoperative percutaneous portal vein embolization: evaluation of adverse events in 188 patients. *Radiology* 2005; **234**: 625-630
51 **Azoulay D**, Castaing D, Smail A, Adam R, Cailliez V, Laurent A, Lemoine A, Bismuth H. Resection of nonresectable liver metastases from colorectal cancer after percutaneous portal vein embolization. *Ann Surg* 2000; **231**: 480-486
52 **Kokudo N**, Tada K, Seki M, Ohta H, Azekura K, Ueno M, Ohta K, Yamaguchi T, Matsubara T, Takahashi T, Nakajima T, Muto T, Ikari T, Yanagisawa A, Kato Y. Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology* 2001; **34**: 267-272
53 **Elias D**, De Baere T, Roche A, Mducreux J, Lasser P. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. *Br J Surg* 1999; **86**: 784-788
54 **Weiss L**, Grundmann E, Torhorst J, Hartveit F, Moberg I, Eder M, Fenoglio-Preiser CM, Napier J, Horne CH, Lopez MJ. Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. *J Pathol* 1986; **150**: 195-203
55 **Couinaud C**. Le foie; Etudes anatomiques et chirurgicales. Paris: Masson, 1957: 284-289
56 **Pang YY**. The Brisbane 2000 terminology of liver anatomy and resections. HPB 2000; 2: 333-39. *HPB* (Oxford) 2002; **4**: 99-100
57 **Zorzi D**, Mullen JT, Abdalla EK, Pawlik TM, Andres A, Muratore A, Curley SA, Mentha G, Capussotti L, Vauthey JN. Comparison between hepatic wedge resection and anatomic resection for colorectal liver metastases. *J Gastrointest Surg* 2006; **10**: 86-94
58 **Machi J**, Isomoto H, Kurohiji T, Yamashita Y, Shirouzu K, Kakegawa T, Sigel B, Zaren HA, Sariego J. Accuracy of intraoperative ultrasonography in diagnosing liver metastasis from colorectal cancer: evaluation with postoperative follow-up results. *World J Surg* 1991; **15**: 551-556; discussion 557
59 **Sakamoto T**, Tsubota N, Iwanaga K, Yuki T, Matsuoka H, Yoshimura M. Pulmonary resection for metastases from colorectal cancer. *Chest* 2001; **119**: 1069-1072
60 **Lesurtel M**, Selzner M, Petrowsky H, McCormack L, Clavien PA. How should transection of the liver be performed?: a prospective randomized study in 100 consecutive patients: comparing four different transection strategies. *Ann Surg*

2005; **242**: 814-822, discussion 822-823
61 **Fujita S**, Akasu T, Moriya Y. Resection of synchronous liver metastases from colorectal cancer. *Jpn J Clin Oncol* 2000; **30**: 7-11
62 **Tanaka K**, Shimada H, Matsuo K, Nagano Y, Endo I, Sekido H, Togo S. Outcome after simultaneous colorectal and hepatic resection for colorectal cancer with synchronous metastases. *Surgery* 2004; **136**: 650-659
63 **Martin R**, Paty P, Fong Y, Grace A, Cohen A, DeMatteo R, Jarnagin W, Blumgart L. Simultaneous liver and colorectal resections are safe for synchronous colorectal liver metastasis. *J Am Coll Surg* 2003; **197**: 233-241; discussion 241-242
64 **Chua HK**, Sondenaa K, Tsiotos GG, Larson DR, Wolff BG, Nagorney DM. Concurrent vs. staged colectomy and hepatectomy for primary colorectal cancer with synchronous hepatic metastases. *Dis Colon Rectum* 2004; **47**: 1310-1316
65 **Curley SA**, Izzo F, Delrio P, Ellis LM, Granchi J, Vallone P, Fiore F, Pignata S, Daniele B, Cremona F. Radiofrequency ablation of unresectable primary and metastatic hepatic malignancies: results in 123 patients. *Ann Surg* 1999; **230**: 1-8
66 **Brooks AJ**, Wang F, Alfredson M, Yan TD, Morris DL. Synchronous liver resection and cryotherapy for colorectal metastases: survival analysis. *Surgeon* 2005; **3**: 265-268
67 **Kennedy JE**, Wu F, ter Haar GR, Gleeson FV, Phillips RR, Middleton MR, Cranston D. High-intensity focused ultrasound for the treatment of liver tumours. *Ultrasonics* 2004; **42**: 931-935
68 **Khatri VP**, Chee KG, Petrelli NJ. Modern multimodality approach to hepatic colorectal metastases: solutions and controversies. *Surg Oncol* 2007; **16**: 71-83
69 **Small R**, Lubezky N, Ben-Haim M. Current controversies in the surgical management of colorectal cancer metastases to the liver. *Isr Med Assoc J* 2007; **9**: 742-747
70 **Ercolani G**, Grazi GL, Ravaioli M, Cescon M, Gardini A, Varotti G, Del Gaudio M, Nardo B, Cavallari A. Liver resection for multiple colorectal metastases: influence of parenchymal involvement and total tumor volume, vs number or location, on long-term survival. *Arch Surg* 2002; **137**: 1187-1192
71 **Jaeck D**, Nakano H, Bachellier P, Inoue K, Weber JC, Oussoultzoglou E, Wolf P, Chenard-Neu MP. Significance of hepatic pedicle lymph node involvement in patients with colorectal liver metastases: a prospective study. *Ann Surg Oncol* 2002; **9**: 430-438
72 **Jaeck D**, Pessaux P. Bilobar colorectal liver metastases: treatment options. *Surg Oncol Clin N Am* 2008; **17**: 553-568, ix
73 **Elias D**, Benizri E, Pocard M, Ducreux M, Boige V, Lasser P. Treatment of synchronous peritoneal carcinomatosis and liver metastases from colorectal cancer. *Eur J Surg Oncol* 2006; **32**: 632-636
74 **Portier G**, Elias D, Bouche O, Rougier P, Bosset JF, Saric J, Belghiti J, Piedbois P, Guimbaud R, Nordlinger B, Bugat R, Lazorthes F, Bedenne L. Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial. *J Clin Oncol* 2006; **24**: 4976-4982
75 **Kokudo N**, Seki M, Ohta H, Azekura K, Ueno M, Sato T, Moroguchi A, Matsubara T, Takahashi T, Nakajima T, Aiba K. Effects of systemic and regional chemotherapy after hepatic resection for colorectal metastases. *Ann Surg Oncol* 1998; **5**: 706-712
76 **Benoist S**, Brouquet A, Penna C, Julié C, El Hajjam M, Chagnon S, Mitry E, Rougier P, Nordlinger B. Complete response of colorectal liver metastases after chemotherapy: does it mean cure? *J Clin Oncol* 2006; **24**: 3939-3945
77 **Choti MA**, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. *Ann Surg* 2002; **235**: 759-766
78 **Shaw IM**, Rees M, Welsh FK, Bygrave S, John TG. Repeat hepatic resection for recurrent colorectal liver metastases is associated with favourable long-term survival. *Br J Surg* 2006; **93**: 457-464
79 **Pessaux P**, Lermite E, Brehant O, Tuech JJ, Lorimier G, Arnaud JP. Repeat hepatectomy for recurrent colorectal liver metastases. *J Surg Oncol* 2006; **93**: 1-7
80 **Adam R**, Bismuth H, Castaing D, Waechter F, Navarro F, Abascal A, Majno P, Engerran L. Repeat hepatectomy for colorectal liver metastases. *Ann Surg* 1997; **225**: 51-60; discussion 60-62
81 **Adam R**, Pascal G, Azoulay D, Tanaka K, Castaing D, Bismuth H. Liver resection for colorectal metastases: the third hepatectomy. *Ann Surg* 2003; **238**: 871-883; discussion 883-884

S- Editor Tian L **L- Editor** Stewart GJ **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4076

World J Gastroenterol 2011 September 28; 17(36): 4076-4089
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Nitric oxide-releasing aspirin but not conventional aspirin improves healing of experimental colitis

Malgorzata Zwolinska-Wcislo, Tomasz Brzozowski, Agata Ptak-Belowska, Aneta Targosz, Katarzyna Urbanczyk, Slawomir Kwiecien, Zbigniew Sliwowski

Malgorzata Zwolinska-Wcislo, Gastroenterology and Hepatology Clinic, Jagiellonian University Medical College, 31-531 Cracow, Poland
Tomasz Brzozowski, Agata Ptak-Belowska, Aneta Targosz, Slawomir Kwiecien, Zbigniew Sliwowski, Department of Physiology, Jagiellonian University Medical College, 31-531 Cracow, Poland
Katarzyna Urbanczyk, Department of Pathomorphology, Jagiellonian University Medical College, 31-531 Cracow, Poland
Author contributions: Zwolinska-Wcislo M designed the study, performed the majority of the experiments and helped with writing of the manuscript; Ptak-Belowska A and Targosz A measured the plasma levels of cytokines, the generation of mucosal prostaglandins and assessed the expression of cytokines in the colonic tissue using molecular techniques; Urbanczyk K performed the histological evaluation of the colonic mucosa; Kwiecien S performed experiments in rats and measured the colonic blood flow; Sliwowski Z evaluated the lesion score, colonic tissue weight and MPO activity; Brzozowski T designed the study, wrote the manuscript and worked on the revised version of this paper.
Supported by The financial grant K/PBW/000067 of the Polish Ministry of Science and Higher Education
Correspondence to: Dr. Tomasz Brzozowski, Professor, Chairman, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka Street, 31-531 Cracow, Poland. mpbrzozo@cyf-kr.edu.pl
Telephone: +48-12-4211006 Fax: +48-12-4222014
Received: February 21, 2011 Revised: June 15, 2011
Accepted: June 22, 2011
Published online: September 28, 2011

Abstract

AIM: To determine the effect of non-selective cyclooxygenase (COX) inhibitors, selective COX-2 inhibitors and nitric oxide (NO)-releasing aspirin in the healing of ulcerative colitis.

METHODS: Rats with 2,4,6 trinitrobenzenesulfonic acid (TNBS)-induced colitis received intragastric (ig) treatment with vehicle, aspirin (ASA) (a non-selective COX inhibitor), celecoxib (a selective COX-2 inhibitor) or NO-releasing ASA for a period of ten days. The area of colonic lesions, colonic blood flow (CBF), myeloperoxidase (MPO) activity and expression of proinflammatory markers COX-2, inducible form of nitric oxide synthase (iNOS), IL-1β and tumor necrosis factor (TNF)-α were assessed. The effects of glyceryl trinitrate (GTN), a NO donor, and 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, onopotassium salt (carboxy-PTIO), a NO scavenger, administered without and with ASA or NO-ASA, and the involvement of capsaicin-sensitive afferent nerves in the mechanism of healing the experimental colitis was also determined.

RESULTS: Rats with colitis developed macroscopic and microscopic colonic lesions accompanied by a significant decrease in the CBF, a significant rise in colonic weight, MPO activity and plasma IL-1β and TNF-α levels. These effects were aggravated by ASA and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560), but not celecoxib and counteracted by concurrent treatment with a synthetic prostaglandin E_2 (PGE_2) analog. Treatment with NO-ASA dose-dependently accelerated colonic healing followed by a rise in plasma NO_x content and CBF, suppression of MPO and downregulation of COX-2, iNOS, IL-1β and TNF-α mRNAs. Treatment with GTN, the NO donor, significantly inhibited the ASA-induced colonic lesions and increased CBF, while carboxy-PTIO or capsaicin-denervation counteracted the NO-ASA-induced improvement of colonic healing and the accompanying increase in the CBF. These effects were restored by co-treatment with calcitonin gene related peptide (CGRP) and NO-ASA in capsaicin-denervated animals.

CONCLUSION: NO-releasing ASA, in contrast to ASA,

COX-1 inhibitors, and SC-560, accelerated the healing of colitis *via* a mechanism involving NO mediated improvement of microcirculation and activation of sensory nerves releasing CGRP.

Key words: Nitric oxide-releasing aspirin; Colitis; Cyclooxygenase-2; Aspirin; Celecoxib; Colonic blood flow; Interleukin-1β; Tumor necrosis factor-α

Peer reviewers: Dr. Paulino Martínez Hernández Magro, Department of Colon and Rectal Surgery, Hospital San José de Celaya, Eje Vial Norponiente No 200-509, Colonia Villas de la Hacienda, 38010 Celaya, México; **Benjamin Perakath**, Professor, Dr., Department of Surgery Unit 5, Christian Medical College, Vellore 632004, Tamil Nadu, India

Zwolinska-Wcislo M, Brzozowski T, Ptak-Belowska A, Targosz A, Urbanczyk K, Kwiecien S, Sliwowski Z. Nitric oxide-releasing aspirin but not conventional aspirin improves healing of experimental colitis. *World J Gastroenterol* 2011; 17(36): 4076-4089 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4076.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4076

INTRODUCTION

In humans inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn's disease, considered as chronically relapsing disorders. The pathogenesis of IBD is complex, with individuals displaying a genetic predisposition, specific immunological properties of the gastrointestinal (GI) mucosa and the type of GI microflora all being involved[1-4]. The features of animal models of colitis do not necessary mimic the human scenario of UC with the use of chemical stimuli and the magnitude of inflammatory changes. However, it is believed that an increase in mucosal prostaglandin (PG) synthesis, mainly cyclooxygenase (COX)-2 derived, correlates with the disease activity of human IBD and experimental colitis in rats[5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of pain and inflammation, but are also recommended prophylactically and used as therapeutic strategies against both neurological and cardiological disorders. The therapeutic effects and side effects of NSAIDs in the gut are the consequence of the inhibition of COX activity, which is the key enzyme in the biosynthesis of prostanoids from arachidonate[6]. It was reported that COX-2 is undetectable or expressed at very low levels in the healthy GI mucosa of humans and animals but is upregulated in the GI tract of individuals with inflammatory conditions[7]. Thus, it has been suggested that the anti-inflammatory action of NSAIDs depend on the inhibition of COX-2 activity, whereas side effects, such as gastrointestinal damage and renal toxicity, is a consequence of COX-1 inhibition[8,9]. It is believed that COX-2 derived PG play an important role in the healing process of colitis, which is similar to that observed for the mechanisms of gastric ulcers[10,11]. Results of studies on the influence of COX-2 inhibitors in experimental colitis and human IBD thus far have provided conflicting evidence for the exacerbation of colitis, and the attenuation of inflammation by COX-2 inhibitors[12,13].

The mechanism of ulcerogenic activity of NSAIDs on the healing of the intestinal and colonic lesions has not been fully explained. Reuter *et al*[12] reported that NSAIDs can exacerbate colitis by a mechanism attributable to the suppression of COX-2 derived PG synthesis. Tanaka *et al*[14] showed that the non-selective COX inhibitor indomethacin, suppressed the mucosal prostaglandin E_2 (PGE_2) level, which in turn caused intestinal damage that was accompanied by the upregulation of COX-2 expression. It should be emphasized that neither a selective COX-1 inhibitor [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560)], nor a COX-2 selective inhibitor (rofecoxib) when applied alone caused intestinal damage[8,15,16]. Recent evidence indicates that incorporation of a nitric oxide (NO) generating moiety into the basic structure of NSAIDs, such as aspirin, attenuates the ulcerogenic activity of native NSAID[17]. Under basal conditions, NO derived from the activity of constitutive NO synthase (cNOS) contributes to the maintenance of intestinal integrity and the control of intestinal motility[2,18].

In human IBD and experimental colitis, the inducible form of nitric oxide synthase (iNOS), excessive production of NO derived from iNOS and abundant amounts of toxic peroxynitrite are observed[2]. On the other hand, NO was shown to possesses anti-inflammatory properties and contributes to the resolution of intestinal inflammation[18]. Moreover, iNOS deficient mice with colitis were more susceptible to inflammation as compared to animals with normal iNOS expression[3]. The role of NO released from NO-NSAIDs in the mechanism of gastroprotection has been well documented[19-21], but the efficacy of NO-aspirin (ASA) in the healing of experimental colitis has yet to be determined.

In this study, we focused mainly on the mechanism of healing of colitis by NO-ASA and therefore we compared the effect of vehicle, conventional NSAIDs such as ASA and indomethacin, SC-560 (a selective COX-1 inhibitor), celecoxib (a selective COX-2 inhibitor) with the new derivative of ASA, NO-releasing ASA, on the intensity of inflammation and the accompanying alterations in the colonic blood flow (CBF), myeloperoxidase (MPO) activity and expression of mRNA for COX-2, IL-1β, tumor necrosis factor (TNF)-α and iNOS and their activities in rats with trinitrobenzenesulfonic acid (TNBS)-induced colitis. An attempt was made to determine the involvement of NO in the mechanism of healing of colitis by using NO-ASA and ASA-treated rats with 2-(4-carboxyphenyl)-4, 5-dihydro-4, 4, 5, 5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, onopotassium salt (carboxy-PTIO), which is a NO scavenger and glyceryl trinitrate (GTN), an NO donor, respectively.

Also, the importance of sensory nerve neurotransmitters in the healing of colitis were determined in rats by the functional ablation of sensory nerves by capsaicin, in the absence and the presence of NO-derivative of ASA.

MATERIALS AND METHODS

Animal studies were carried out on male Wistar rats weighing 180-220 g. The animals had free access to water and food and were adapted to laboratory conditions and 12 h day/night cycles for 10 d after TNBS administration. The study was approved by the local Ethical Committee at the Jagiellonian University Medical College in Cracow, Poland and run in accordance with the Helsinki declaration.

Colitis in rats was induced by rectal administration of TNBS (Sigma, Slough, United Kingdom) at a dose of 10 mg/kg, dissolved in 50% solution of ethanol as reported in our previous study[22]. Briefly, the animals were anaesthetized with phenobarbital (60 mg/kg ip) and TNBS was administered into the colon in a volume of 0.25 mL per rat at a depth of 8 cm from the rectum with the use of a soft polyethylene catheter. Until the moment of awakening the rats were positioned in the Trendelenburg position so as to avoid loss of the TNBS solution *via* the rectum. Animals in the control group were given 0.9% saline or in some cases 50% ethanol in the same volume, corresponding to the rats that were administered TNBS.

Animals with TNBS-induced colitis were randomized into 8 experimental groups (A-H), consisting of 6-10 rats per group. Rats received ig the following daily treatments: A: vehicle (saline); B: ASA (80 mg/kg) or indomethacin (5 mg/kg) suspended in 0.25% carboxy methylcellulose (CMC); C: SC-560 (5 mg/kg); D: celecoxib (10 mg/kg); E: NO-ASA (80 mg/kg; NicOx SA, Sophia Antipolis, France); F: PGE_2 (5 μg/kg) combined with non-selective COX-1 (ASA) and selective COX-2 (celecoxib) inhibitors; G: GTN (10 mg/kg ig), a NO donor combined with ASA and carboxy-PTIO (5 mg/kg ig) combined with NO-ASA, both NSAID administered in a dose of 80 mg/kg. In series H consisting of 30 rats, capsaicin was administered in a large neurotoxic dose of 125 mg/kg applied in three doses of 25, 50 and 50 mg/kg 14 d prior to TNBS administration to induce the functional ablation of sensory nerves as described previously[23]. On day 15, capsaicin-denervated rats received TNBS and were subsequently given NO-ASA (80 mg/kg ig) with or without calcitonin gene related peptide (CGRP) (10 μg/kg sc), and administration was similar to those treated with vehicle (saline). The doses of NO-ASA and selective COX-1 and COX-2 inhibitors were selected on the basis of our group and others published evidence[14,15,19-21]. In the doses used in this study, the COX-1 and COX-2 selective inhibitors failed to produce gastrointestinal lesions after single or prolonged administration. The dose of 80 mg/kg of ASA was selected based on our preliminary determination of the dose-dependency of this conventional ASA applied in graded doses starting from 10 mg/kg up to 160 mg/kg on healing of TNBS colitis. ASA given in a dose of 10 mg/kg failed to significantly affect the healing of colitis but when this NSAID was administered ig in graded doses of 40 mg/kg, 80 mg/kg and 160 mg/kg, the area of colonic damage was significantly increased by 23%, 42% and 74%, respectively, at day 10 upon TNBS administration (data not shown). We have previously published that 80 mg/kg of ASA is equimolar to 128 mg/kg of NO-ASA and this dose of NO-ASA by itself does not influence the gastrointestinal integrity but markedly increases organ blood flow[21].

After 1, 3, 10 and 14 d from induction of colonic lesions with TNBS, the animals were weighed and anaesthetized to determine CBF using the H_2-gas clearance technique[22,23]. The abdominal cavity was opened and after separation of the colon, the CBF in the areas of the mucosa not affected by inflammatory lesions was measured. CBF was expressed as a percentage of the CBF in the vehicle-control rats without TNBS administration.

At the termination of the experiment, the entire colon was removed, isolated from surrounding tissues, opened along the antimesenteric border, rinsed, weighed, and processed for gross and histology determinations. The areas of colonic damage were evaluated planimetrically (Morphomat, Carl Zeiss, Berlin, Germany) by two independent researchers. Subsequently, fragments of the colon (2 mm × 10 mm) with colonic lesions were sampled, fixed with formaldehyde, embedded in paraffin and routinely stained with haematoxilin and eosin for histological assessment.

The presence and intensity of histological changes was evaluated for the following criteria: presence, area and depth of ulceration, presence and intensity of inflammatory infiltrations, ulcerations and fibrosis[24].

Determination of plasma IL-1β and TNF-α levels, NO concentration the mucosal generation of prostaglandin E_2 and gastric mucosal MPO activity

Immediately after CBF measurements, a venous blood sample was drawn from the vena cava and placed into EDTA-containing vials and used for the determination of plasma IL-1β and TNF-α. Blood was collected and placed into sterile, plastic syringes, kept in ice till centrifugation. The blood samples were centrifuged at a speed of 1000 *g* for 10 min at a temperature of 15 ℃ temperature and the sera were stored at -80 ℃. The serum levels of proinflammatory cytokines IL-1β and TNF-α were evaluated with high sensitive enzyme-linked immunosorbent assay (ELISA) (Quantikine HS, R and D Systems, Minneapolis, Minn., United States) according to manufacturer's instructions. Intensity of the color reaction was estimated in the spectrophotometer Stat Fax 2100 (Awareness Technology Inc., Pal City, FL, United States) at 490 nm. The intra-and inter-assay coefficients of variation were 8.5% and 10.6%, respectively, for TNF-α, and 10.2% and 10.4%, respectively, for IL-1β. The plasma NO concentration was quantified indi-

Table 1 Primer sequence used in reverse transcription polymerase chain reaction determination of gene expression of proinflammatory factors

Gene		Primer
β-actin	Upstream	5′-TTG TAA CCA ACT GGG ACG ATA TGG-3′
	Downstream	5′-GAT CTT GAT CTT CAT GGT GCT AGG-3′
IL-1β	Upstream	5′-GCT ACC TAT GTC TTG CCC GT-3′
	Downstream	5′–GAC CAT TGC TGT TTC CTA GG-3′
TNF-α	Upstream	5′-TAC TGA ACT TCG GGG TGA TTG GTC C-3′
	Downstream	5′–CAG CCT TGT CCC TTG AAG AGA ACC-3′
COX-2	Upstream	5′–ACA ACA TTC CCT TCC TTC-3′
	Downstream	5′–CCT TAT TTC CTT TCA CAC C-3′
iNOS	Upstream	5′–CCA CAA TAG TAC AAT ACT ACT TGG–3′
	Downstream	5′–ACG AGG TGT TCA GCG TGC TCC ACG–3′

TNF: Tumor necrosis factor; COX: Cyclooxygenase; iNOS: Inducible form of nitric oxide synthase.

rectly as nitrate (NO_3^-) and nitrite (NO_2^-) levels using the nitrate/nitrite kit purchased from Cayman Lab, Michigan, United States as described before[25,26]. This method is based on the Griess reaction and the generation of a chromophore absorbing at 595 nm, according to the original procedure reported previously[26]. Since NO in the colonic mucosa is quickly transformed into NO_3^- and NO_3^-[25], the total nitrate and nitrite concentration (NO_x) is routinely used as an index of NO production. In order to determine NO_x, the blood was withdrawn and centrifuged for 10 min at 3000 r/min, the samples were mixed with Griess reagent from the commercially available kit.

In rats with colitis treated with or without concurrent COX-1 and COX-2 inhibitors, the mucosal samples were taken by biopsy (about 200 mg) from unchanged colon mucosa without mucosal lesions to determine PGE_2 generation by radioimmunoassay (RIA) as described previously[19,21]. Briefly, the mucosal samples were placed in preweighed Eppendorf vial with 1 mL of Tris buffer (50 mmol/L, at pH 9.5) added to each vial. The samples were finally minced (15 s) with scissors, washed and centrifuged for 10 s, with the pellet being resuspended again in 1 mL of Tris. Then, each sample was incubated on a vortex mixer for 1 min and centrifuged for 15 s. The pellets were weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mmol/L) and kept at -20 ℃ until the time for RIA. The capability of the mucosa to generate PGE_2 was expressed in nanograms of wet tissue weight.

Fragments of colonic tissue weighing about 200 mg were collected and frozen in -70 ℃ for the determination of MPO activity by ELISA as reported before[22].

Expression of COX-2, IL-1β, TNF-α and iNOS transcripts in the rat colonic mucosa determined by reverse transcriptase-polymerase chain reaction

The mRNA expression for COX-2, IL-1β, TNF-α and iNOS were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) in the unchanged colon mucosa of intact rats or those with TNBS colitis given vehicle, ASA and NO-ASA. Samples of the colon mucosa (about 200 mg) were scrapped off into ice using glass slides and then immediately snapped frozen in liquid nitrogen and stored at -80 ℃. The total RNA was isolated from the colon mucosa according to the technique using Trizol Reagent (Invitrogen, Carlsbad, United States) and the manufacturer's protocol[27]. The first strand of cDNA was synthesized from total cellular RNA (2 μg) using a Reverse Transcription System (Promega, Madison, United States). The PCR was carried out in an automatic DNA thermal cycler, using 1 μg of cDNA and Promega PCR reagents. For amplification of β-actin, COX-2, TNF-α, IL-1β and iNOS cDNA, and gene-specific primers (SIGMA-Aldrich St. Louis, United States) were used. The sequences for primers used in this study are presented in Table 1. Primer annealing was carried out as follows: at 56 ℃, 60 ℃, 60 ℃ and 58 ℃ for COX-2, IL-1β, TNF-α and iNOS, respectively. Amplification of the control rat β-actin was performed on the same samples to verify the RNA integrity. PCR products were separated by electrophoresis in 2% agarose gel containing 0.5 μg/mL ethidium bromide and then visualized under UV light. Location of the predicted PCR product was confirmed by using O'Gene Ruler 50 bp DNA ladder (Fermentas, Life Sciences, San Francisco, United States) as a standard marker. Comparison between different treatment groups was made by determination of COX-2, IL-1β, TNF-α and the iNOS/β-actin ratio of the immunoreactive area by densitometry (Gel-Pro Analyzer, Fotodyne Incorporated, Hartland, WI, United States).

Statistical analysis

Results are expressed as mean ± SE. Statistical analysis was done using Student *t* test or analysis of variance and two-way ANOVA test with Tukey *post hoc* test where appropriate. Differences of $P < 0.05$ were considered significant.

RESULTS

Effect of vehicle, non-selective and selective COX-1 and COX-2 inhibitors on TNBS-induced colitis and accompanying changes in CBF and MPO activity

Intrarectal administration of TNBS caused severe damage to the colonic mucosa manifested by inflammatory changes in the colon with extensive ulcerations of the mucosa. The area of these lesions was at a maximum at 24 h, it was not significantly decreased on day 3 but then it significantly declined at day 10 and day 14 (Figure 1). The CBF was decreased by about 46% and 43% on day 1 and 3, respectively, but it was significantly increased on day 10 and 14 (Figure 1). The 10 d administration of NO-ASA applied ig in gradual concentrations ranging from 20 mg/kg up to 120 mg/kg produced a dose-dependent decrease in the area of colonic lesions and this effect was accompanied by a significant rise in plasma NOx concentrations and CBF (Figure 2). In contrast, after 10 d of ASA, indomethacin and SC-560 administration, a significant aggravation of the area of colonic lesions and a significant fall in CBF when

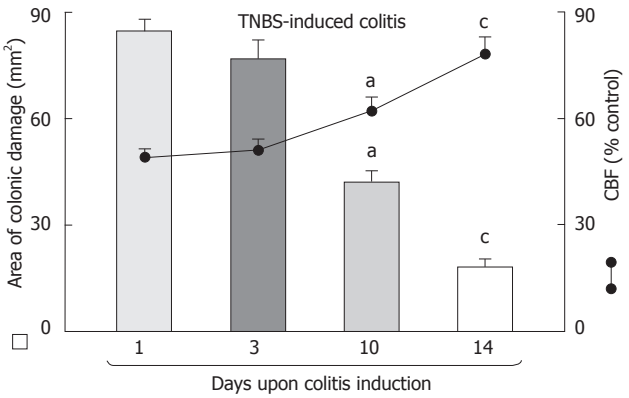


Figure 1 Time-sequence of the healing of trinitrobenzenesulfonic acid-induced colonic lesions, and accompanying changes in the colonic blood flow following day 1, 3, 10 and 14 upon induction of colitis. The area of trinitrobenzenesulfonic acid-induced lesions was maximal on day 1 and then it significantly declined on day 10 and day 14, respectively. Mean ± SE of 6-8 rats. $^{a}P < 0.05$ *vs* values obtained on day 3; $^{c}P < 0.05$ *vs* values on day 3 and day 10. TNBS: Trinitrobenzenesulfonic acid; CBF: Colonic blood flow.

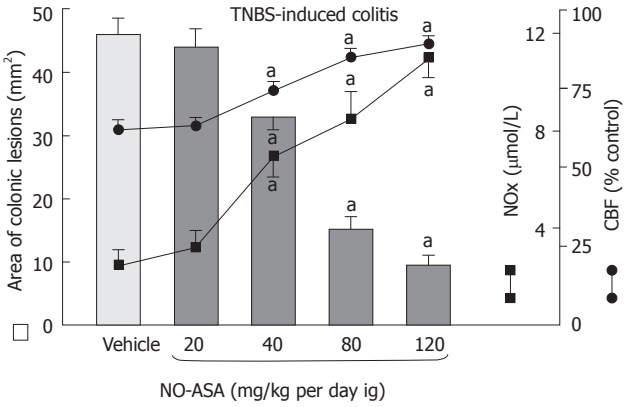


Figure 2 The effect of 10 d administration of nitric oxide-aspirin, applied ig in gradual concentrations ranging from 20 mg/kg up to 120 mg/kg on the area of colonic lesions, alterations in colonic blood flow and plasma NOx concentrations in rats with trinitrobenzenesulfonic acid-induced colitis on day 10. Mean ± SE of 6-8 rats. $^{a}P < 0.05$ *vs* vehicle (control) and animals treated with a dose of 20 mg/kg nitric oxide-aspirin (NO-ASA). TNBS: Trinitrobenzenesulfonic acid; CBF: Colonic blood flow.

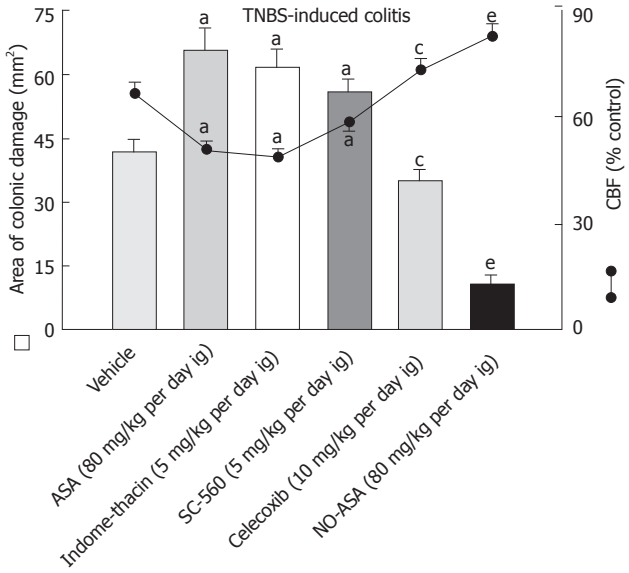


Figure 3 Effect of administration of vehicle, aspirin or indomethacin, non-selective cyclooxygenase inhibitors, a selective cyclooxygenase-1 inhibitor 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole, a selective cyclooxygenase-2 inhibitor (celecoxib) and nitric oxide releasing aspirin, on the area of colonic lesions and alterations in colonic blood flow on day 10 after colitis induction. Mean ± SE of 6-8 rats. $^{a}P < 0.02$ *vs* vehicle (control); $^{c}P < 0.05$ *vs* aspirin (ASA)-, indomethacin- and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560) groups; $^{e}P < 0.02$ *vs* vehicle, ASA, indomethacin, SC-560 and celecoxib groups. CBF: Colonic blood flow; NO-ASA: Nitric oxide-ASA; TNBS: Trinitrobenzenesulfonic acid.

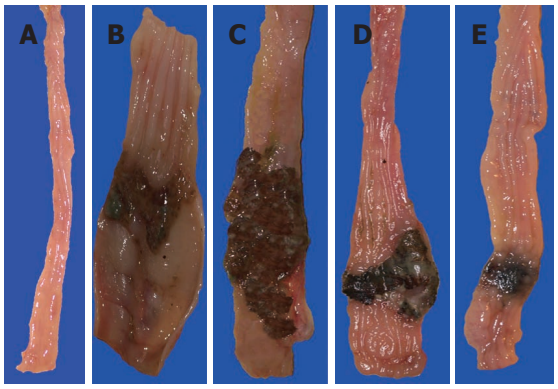


Figure 4 Gross appearance of the intact colon (A), and that of trinitrobenzenesulfonic acid-induced colitis rats treated with vehicle (B), aspirin (C), celecoxib (D) and nitric oxide-aspirin (E) at day 10 of colitis induction. In aspirin-treated rats (C) the area of colonic damage was larger than in the control trinitrobenzenesulfonic acid rats, which were treated with vehicle (B). In the celecoxib group (D), the area of colonic damage was significantly smaller when compared to the aspirin (ASA) and vehicle groups. The healing of colonic lesions was significantly improved in nitric oxide-ASA treated rats as documented by the small ulceration area and scar formation.

compared with vehicle was observed (Figure 3). Similarly as shown in Figure 2, the treatment with NO-ASA applied in a dose of 80 mg/kg ig produced a significant decrease in the area of colonic damage and significantly increased the CBF comparing to vehicle-control (Figure 3). Treatment with celecoxib also significantly decreased the area of colonic damage and produced a significant increase in CBF; however, these changes were significantly less pronounced as compared to those achieved with NO-releasing ASA (Figure 3). Ten days after colitis induced by TNBS treatment all animals had a significant reduction in body weight compared with the control rats without TNBS administration and this loss of body weight was counteracted by treatment with NO-ASA. In contrast, treatment with ASA and indomethacin failed to attenuate the loss of weight induced by TNBS administration (data not shown). As shown in Figure 4A, the intact colonic mucosa showed a normal macroscopic appearance. At day 10 after TNBS administration, colonic damage was still observed in vehicle-control animals (Figure 4B). This gross damage, as reflected by the area of ulceration, was exacerbated by treatment with ASA but significantly reduced by treatment with celecoxib. The area of TNBS-induced damage was significantly smaller in NO-ASA-treated animals as compared to that in rats treated with vehicle or celecoxib

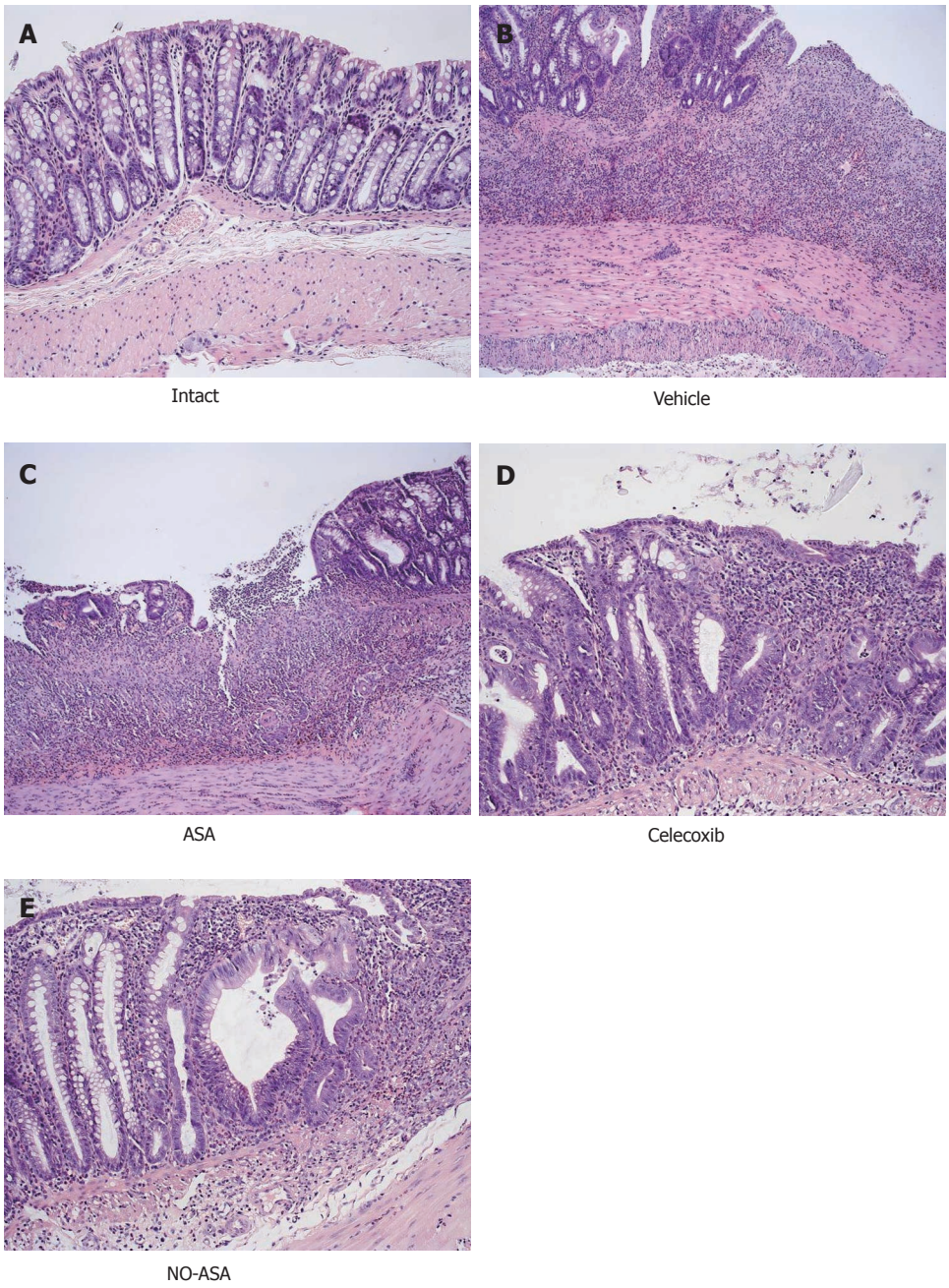


Figure 5 Histological appearance of the intact colonic mucosa (A) and that treated with vehicle (B), aspirin (C), celecoxib (D) and nitric oxide releasing aspirin on day 10 in rats with trinitrobenzenesulfonic acid-induced colitis. Intact rat colon shows regular colonic architecture and normal colonic mucosa continuity with no signs of inflammation (A). In trinitrobenzenesulfonic acid rats treated with vehicle, deep ulceration and an intense neutrophil infiltration with the presence of numerous neutrophils penetrating the muscularis mucosa and submucosa were observed. The features of regeneration adjacent to the ulcer margin are clearly visible. In aspirin (ASA)-treated rats with colitis (C), there is deep ulceration with necrosis and an intense inflammation followed by severe neutrophil infiltration and the formation of granulation tissue penetrating the muscle layer of the *muscularis propria*. Less regeneration is observed with ASA (C) than vehicle (control) animals (B). The partially healed epithelium and abnormal crypt architecture with much more pronounced regeneration was observed in the colonic mucosa of rats with colitis treated with celecoxib (D) as compared with other cyclooxygenase inhibitors. In nitric oxide (NO)-ASA treated rats with colitis (E), the most advanced healing process of colonic ulcers as reflected by scar formation, epithelial regeneration and significantly smaller neutrophil infiltration was observed. Part of the colonic crypts distant to the scar already shows a normal appearance.

(Figure 4E). By histology, intact colonic mucosa showed regular colonic architecture and continuity with no signs of inflammation (Figure 5A). In vehicle-treated TNBS rats on day 10 deep ulcerations and an intense infiltrate with the presence of numerous neutrophils penetrating the *muscularis mucosa* and submucosa were observed. In addition, features of regeneration, adjacent to the ulcer margin were clearly visible (Figure 5B). In ASA-treated rats with colitis (Figure 5C) there was a deep ulceration with necrosis and an intense inflammation followed by severe neutrophil infiltration and formation of granulation tissue penetrating the muscle layer of the *muscularis propria*. Less regeneration was observed with ASA (Figure 5C) than in vehicle (control) colonic mucosa (Figure 5B). The area of colonic damage

Table 2 Evaluation of related markers in trinitrobenzene-sulfonic acid rats treated ig with non-selective and selective cyclooxygenase-1 and cyclooxygenase-2 inhibitors or nitric oxide-aspirin throughout the period of 10 d in 6-10 rats per group (mean ± SE)

Treatment	Weight (mg)	MPO (ng/mL)	IL1-β (pg/mL)	TNF-α (pg/mL)
Vehicle	1150 ± 23.1	51 ± 8.1	42 ± 3.4	4.8 ± 0.6
ASA (80 mg/kg)	1580 ± 41.4[a]	78 ± 9.3[a]	65 ± 5.1[a]	8.5 ± 0.95[a]
Indomethacin (5 mg/kg)	1450 ± 45.8[a]	65 ± 5.2[a]	58 ± 4.1[a]	7.8 ± 0.62[a]
SC-560 (5 mg/kg)	1310 ± 32.4[a]	52 ± 6.8[a]	53 ± 3.9[a]	7.5 ± 0.46[a]
Celecoxib (10 mg/kg)	1082 ± 14.9[c]	44 ± 4.3[c]	36 ± 2.2[c]	3.8 ± 0.33[c]
NO-ASA (80 mg/kg)	710 ± 12.3[e]	35 ± 3.2[e]	18 ± 1.5[e]	2.4 ± 0.21[e]

MPO: Myeloperoxidase; TNF: Tumor necrosis factor; ASA: Aspirin; SC-560: 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole; NO: Nitric oxide. [a]$P < 0.05$ *vs* vehicle; [c]$P < 0.05$ *vs* vehicle, ASA, indomethacin or SC-560 groups; [e]$P < 0.05$ *vs* groups treated with non-selective and selective COX-1 or COX-2 inhibitors.

was significantly smaller in celecoxib-treated animals and in those treated with ASA and vehicle (Figure 5B and D). Partially healed epithelium and abnormal crypt architecture possessing a more pronounced regeneration were observed in colonic mucosa of the rat with colitis treated with celecoxib (Figure 5D) when compared to those receiving ASA. By histology, the area of colonic damage was significantly decreased in NO-ASA treated colitis rats as compared to all other COX inhibitors and vehicle-treated rats (Figure 5E). NO-ASA treated rats showed the most advanced healing of colitis, as reflected by the degree of epithelial regeneration and the significantly smaller infiltration of neutrophils. Part of the crypts, which were distant to the scar, displayed a more normal appearance (Figure 5E). In the indomethacin-group, similar to the ASA-group, the massive hemorrhagic lesions and transmural necrosis coexisting with acute inflammatory infiltrate were observed (data not shown).

As shown in Table 2, treatments with ASA, indomethacin and SC-560 were accompanied by a greater increase in the weight of colonic tissue and a significant rise in MPO activity in TNBS-treated animals as compared to vehicle control. In contrast, treatment with celecoxib decreased the weight of colonic tissue and MPO activity below those in the vehicle group; however, these changes were significantly smaller than those caused by NO-ASA (Table 2). In contrast, treatment with NO-ASA resulted in a significant decrease in the area of colonic lesions and colonic tissue weight, accompanied by a significant rise in CBF and a fall in MPO activity compared to the respective values in rats treated with vehicle or those treated with non-selective and selective COX-1 and COX-2 inhibitors (Table 2, Figure 3).

Effect of non-selective and selective COX-1 and COX-2 inhibitors on plasma proinflammatory cytokine levels and PGE$_2$ generation

Plasma levels of proinflammatory cytokines IL-1β and TNF-α, which were negligible in intact rats (data not shown), were markedly elevated in animals administered with ASA, indomethacin and SC-560, when compared to those treated with vehicle (Table 2). Plasma IL-1β and TNF-α levels were significantly attenuated by treatment with the COX-2 inhibitor (celecoxib) when compared to the levels of those cytokines achieved in animals treated with non-selective COX inhibitors (ASA and indomethacin). NO-ASA was administered at a similar dose to that of native ASA, which accelerated the healing of colonic damage, and significantly attenuated plasma IL-1β and TNF-α levels as compared to those achieved with non-selective COX inhibitors (Table 2).

Table 3 The generation of prostaglandin E$_2$ in intact rats and those treated rats (mean ± SE)

Group	PGE$_2$ generation in the colonic mucosa (pg/mg tissue weight)
Intact	182 ± 15.4
Vehicle	409 ± 23.2[a]
ASA (80 mg/kg per day)	130 ± 11.7[c]
Indomethacin (5 mg/kg per day)	124 ± 17.8[c]
SC-560 (5 mg/kg per day)	145 ± 15.6[c]
Celecoxib (10 mg/kg per day)	191 ± 18.3[e]
NO-ASA (80 mg/kg per day)	175 ± 17.3[e]

PGE$_2$: Prostaglandin E$_2$; ASA: Aspirin; SC-560: 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole; NO: Nitric oxide. [a]$P < 0.05$ *vs* intact colonic mucosa; [c]$P < 0.05$ *vs* vehicle (control); [e]$P < 0.05$ *vs* ASA, indomethacin and SC-560 groups.

As shown in Table 3, the intestinal level of PGE$_2$ increased in the colonic mucosa of animals with TNBS colitis as compared to that measured in vehicle-controls that did not receive TNBS. Administration of ASA and indomethacin significantly reduced the colonic PGE$_2$ content when compared to the respective value in vehicle-treated control animals. Treatment with ASA and SC-560, the selective COX-1 inhibitor, also significantly reduced PGE$_2$ generation; however this inhibition was less potent than in the case of ASA and indomethacin (Table 3). Administration of the selective COX-2 inhibitor celecoxib, and NO-ASA significantly inhibited the colonic PGE$_2$ generation when compared to the value measured in vehicle-control rats (Table 3).

Effect of NO donor, GTN and NO scavenger carboxy PTIO on area of colonic damage and alteration in CBF in rats with TNBS colitis

As shown in Figure 6, the area of colonic damage was significantly increased in animals treated for 10 d with ASA and this effect was accompanied by a significant fall in CBF as compared with the respective values observed in vehicle-control animals. Concurrent treatment with GTN, which by itself significantly attenuated the area of colonic damage markedly decreased these lesions and significantly improved the CBF.

Figure 7 shows the effect of 10 d administration of NO-ASA applied ig at a dose of 80 mg/kg on the area of colonic damage and accompanying alterations in CBF. Treatment with NO-ASA caused a similar decrease in

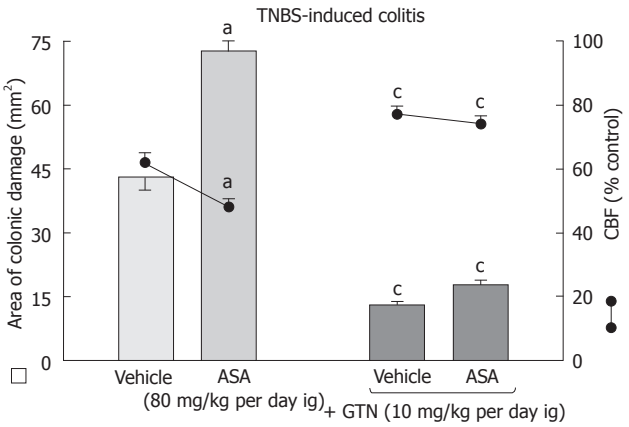


Figure 6 The effect of concurrent administration of nitric oxide donor glyceryl trinitrate on the area of colonic damage and alterations in colonic blood flow in rats with trinitrobenzenesulfonic acid-induced colitis, treated with vehicle or aspirin, on day 10. Mean ± SE of 6-8 rats. $^{a}P < 0.02$ *vs* vehicle (control); $^{c}P < 0.02$ *vs* vehicle or aspirin alone. TNBS: Trinitrobenzenesulfonic acid; ASA: Aspirin; GTN: Glyceryl trinitrate; CBF: Colonic blood flow.

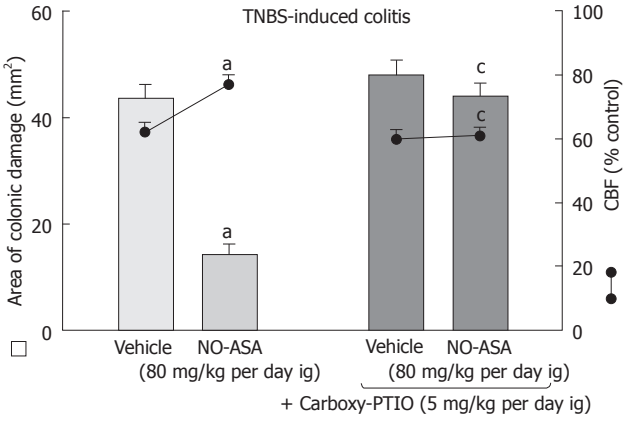


Figure 7 The effect of concurrent administration of nitric oxide scavenger 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, monopotassium salt on the area of colonic damage and alterations in colonic blood flow in rats with trinitrobenzenesulfonic acid-induced colitis, treated with vehicle or nitric oxide-aspirin (80 mg/kg per day ig) on day 10. Mean ± SE of 6-8 rats. $^{a}P < 0.05$ *vs* vehicle group (control); $^{c}P < 0.05$ *vs* vehicle or nitric oxide-aspirin (NO-ASA) without 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, monopotassium salt. TNBS: Trinitrobenzenesulfonic acid; CBF: Colonic blood flow.

area of colonic damage and a significant rise in CBF as presented in Figure 2. The concomitant treatment of carboxy-PTIO, the NO scavenger, which by itself had no influence on the CBF, completely abolished the beneficial effect of NO-ASA on healing of TNBS-induced colonic lesions and reversed an increase in CBF induced by this agent (Figure 7).

Effect of replacement therapy with exogenous PGE₂ on TNBS-induced colonic damage and changes in the weight of colonic tissue, CBF, plasma IL-1β and TNF-α levels in rats treated with COX inhibitors

The role of PGE_2 in the process of healing of colonic lesions in TNBS-induced colitis animals given ASA and celecoxib was determined using rats exogenously administered with PGE_2 in doses of 5 μg/kg added to both COX-1 and COX-2 inhibitors. An increase in both the area of colonic lesions and colonic weight as well as a significant fall in CBF induced by ASA and celecoxib were counteracted by concomitant treatment with exogenous PGE_2 (Figures 8 and 9). Moreover, the administration of this synthetic analogue of PGE_2 not only reduced the area of colonic damage but also significantly suppressed the rise in plasma IL-1β and TNF-α compared to those in ASA- or celecoxib-treated rats without PGE_2 administration (Figure 8).

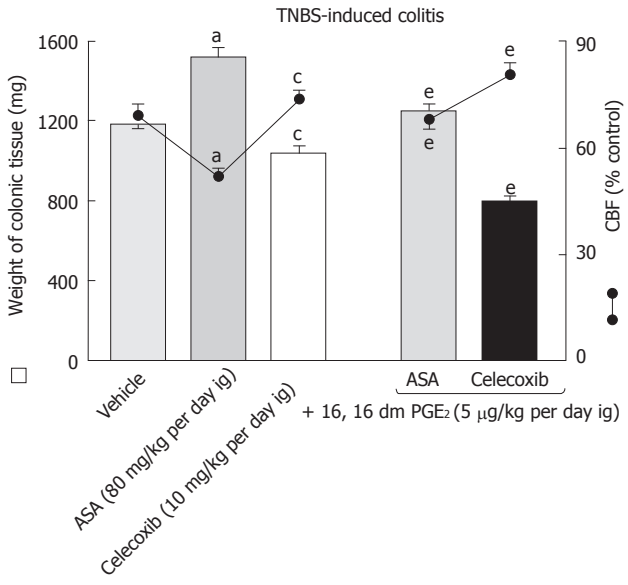


Figure 8 The weight of colonic tissue and alterations in colonic blood flow on day 10 after induction of trinitrobenzenesulfonic acid colitis in rats treated with aspirin or celecoxib with or without 16, 16 dm prostaglandin E_2 (5 μg/kg per day ig). Mean ± SE of 6-8 rats. $^{a}P < 0.02$ *vs* vehicle (control); $^{c}P < 0.05$ *vs* vehicle (control) and aspirin (ASA) groups; $^{e}P < 0.05$ *vs* ASA and celecoxib groups without concurrent prostaglandin E_2 (PGE_2) administration. TNBS: Trinitrobenzenesulfonic acid; CBF: Colonic blood flow.

Effect of NO-ASA on TNBS-induced colonic damage and alterations in CBF in rats with capsaicin induced sensory denervation

Capsaicin-deactivation of sensory nerves, which by itself increased the area of colonic lesions and produced a significant fall in CBF when compared to those in vehicle-controlled rats, significantly attenuated the NO-ASA induced acceleration of healing of these colonic lesions and the accompanying increase in CBF (Figure 10). Concurrent administration of CGRP (10 μg/kg sc) with NO-ASA restored the healing of colonic damage as reflected by the significant decrease in colonic damage and the increase in CBF induced by this NO-derivative of ASA in rats with capsaicin denervation.

Effect of vehicle, ASA and NO-ASA treatments on the mucosal expression of COX-2, IL-1β, TNF-α and iNOS in rats with colitis

As shown in Figure 11 (left panel), the signal for the expression of COX-2, IL-1β, TNF-α and iNOS was significantly increased in vehicle-treated colonic mucosa (lane 2) in rats

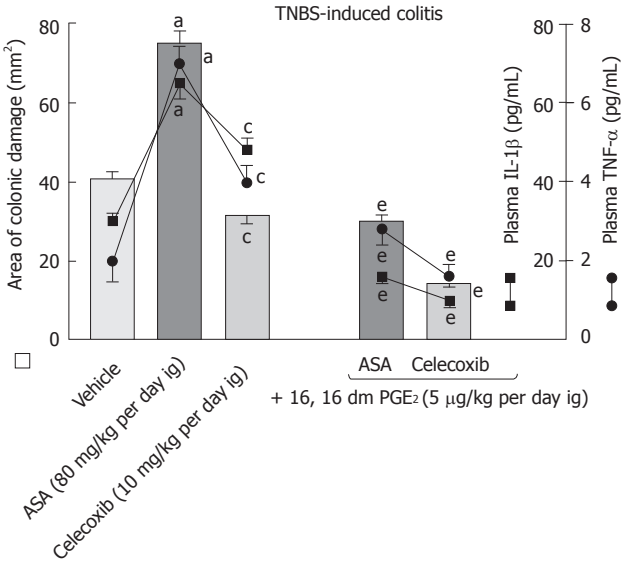


Figure 9 The area of colonic lesions and plasma levels of proinflammatory cytokines IL-1β and tumor necrosis factor-α on day 10 after induction of colitis in rats treated with aspirin or celecoxib with or without 16, 16 dm prostaglandin E_2 (5 μg/kg per day ig). Mean ± SE of 6-8 rats. $^aP < 0.02$ *vs* vehicle (control); $^cP < 0.02$ *vs* vehicle-treated and aspirin (ASA)-treated groups; $^eP < 0.02$ *vs* ASA and celecoxib groups without concurrent prostaglandin E_2 (PGE_2) administration. TNBS: Trinitrobenzenesulfonic acid; TNF: Tumor necrosis factor.

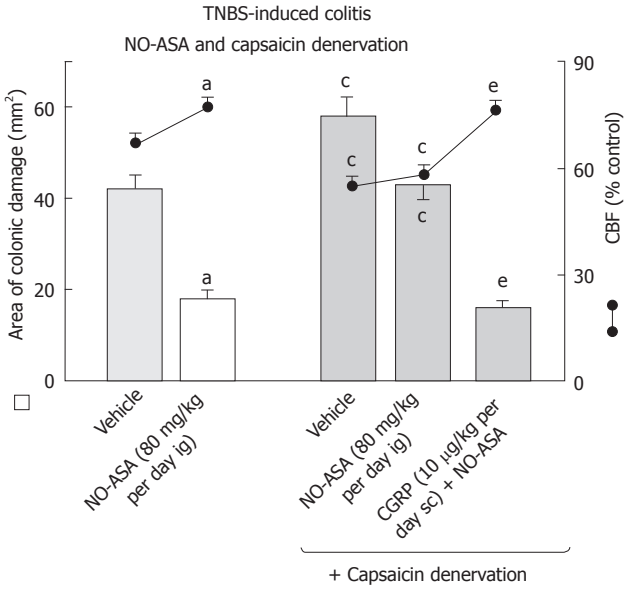


Figure 10 The area of colonic damage and changes in colonic blood flow on day 10 after colitis induction in rats with intact sensory nerves and in those with capsaicin-sensory denervation treated with vehicle (saline) or nitric oxide-aspirin (80 mg/kg per day ig) with or without administration of calcitonin gene related peptide (10 μg/kg per day sc). Mean ± SE of 6-8 rats. $^aP < 0.05$ *vs* vehicle (control); $^cP < 0.05$ *vs* trinitrobenzenesulfonic acid (TNBS) rats without capsaicin denervation; $^eP < 0.02$ *vs* rats with TNBS colitis treated with nitric oxide-aspirin (NO-ASA). CGRP: Calcitonin gene related peptide; CBF: Colonic blood flow.

with colitis when compared to that in the intact mucosa (lane 1). The ratio of COX-2, IL-1β, TNF-α and iNOS mRNA over β-actin mRNA, confirmed that expression of COX-2, IL-1β, TNF-α and iNOS mRNAs were significantly elevated in TNBS-treated animals (Figure 11, right panel). Treatment with ASA resulted in a strong signal of mRNAs for COX-2, IL-1β, TNF-α and iNOS. The semi-quantitative ratio of COX-2, IL-1β, TNF-α and iNOS (lane 3) confirmed that the expression of these inflammatory markers was significantly increased in the colonic mucosa of rats treated with ASA (Figure 11, right panel). In NO-ASA-treated animals the signal for COX-2, IL-1β, TNF-α and iNOS mRNAs was less pronounced (lane 4) and the determination of the ratio of COX-2, IL-1β, TNF-α and iNOS confirmed that expression of mRNAs for these inflammatory factors was significantly inhibited as compared to those recorded in ASA-treated animals (Figure 11, right panel).

DISCUSSION

Worldwide, NSAIDs are among the most widely prescribed medications, and are often the drugs of choice for the treatment of various inflammatory conditions. Currently, NSAIDs, including ASA, are recommended as a prophylactic therapy against neurological and cardiologic disorders including strokes and heart infarcts[28]. In humans, UC is a chronic relapsing disorder, characterized by colon mucosa inflammation, ulcerations, diarrhea, bloody stools and abdominal pain[1,3]. The inflamed mucosa of the lower GI tract produces a high amount of PG derived from COX-2 expression and activity in response to stimulation by proinflammatory cytokines and growth factors, also co-expressed at a site of inflammation[14]. Although the NSAIDs effect on the upper GI tract is well documented, the mechanisms by which NSAIDs and their new NO-releasing derivatives affect the course and the healing of colitis in humans and experimental animals has not been fully explored. In the present study, using a rodent model of colitis we determined the effect of the new ASA derivative NO-ASA, and selective and non-selective COX-1 and COX-1 inhibitors on the healing process of this colonic damage, and the effects on weight of colonic tissue, the CBF and MPO activity. Moreover, we assessed the colonic expression of COX-2 which in contrast to COX-1 expression is negligible in normal GI mucosa, but has been shown to be significantly upregulated in most GI-related disorders such as gastritis, gastric mucosal damage, ulcers and ulcerative colitis[3,11,14,29].

Interestingly, the inhibition of COX-2 activity by selective COX-2 inhibitors enhances gastric damage induced by stress and ischemia-reperfusion and delays the healing process of gastric ulcers in the GI tract[29,30]. Moreover, a single application of either COX-1 or COX-2 inhibitors does not cause GI damage, but concurrent treatment with inhibitors of COX-1 and COX-2 activity, resulted in both gastric and intestinal damage. For instance, administration of celecoxib alone or SC-560 alone failed to cause gastric damage, but administration of both selective COX-1 together with COX-2 inhibitors resulted in the formation of gastric mucosal injury. It was concluded that both COX-1 and COX-2 are essential for the maintenance of the integrity of the

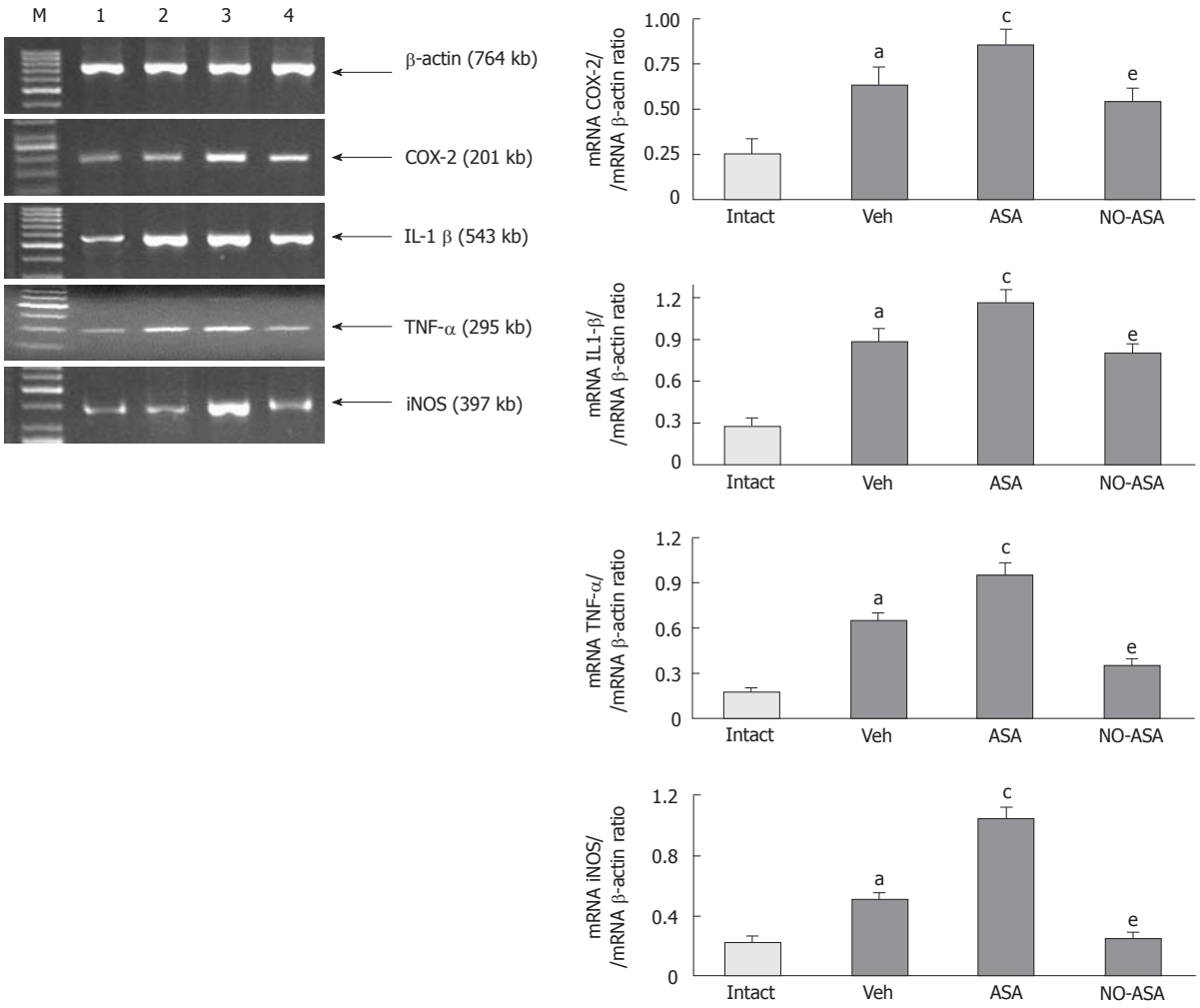


Figure 11 The expression of mRNA for cyclooxygenase-2, IL-1β, tumor necrosis factor-α and inducible form of nitric oxide synthase in colonic mucosa of intact rats (lane 1) those treated with vehicle (lane 2), aspirin (lane 3) and nitric oxide-aspirin (lane 4) at day 10 after induction of colitis. Mean ± SE of 4 determinations in 4 rats. [a]*P* < 0.05 *vs* intact colonic mucosa; [c]*P* < 0.05 *vs* rats with trinitrobenzenesulfonic acid colitis administered with vehicle (Veh); [e]*P* < 0.05 *vs* vehicle and aspirin (ASA) groups. COX: Cyclooxygenase; TNF: Tumor necrosis factor; iNOS: Inducible form of nitric oxide synthase; M: Marker.

upper GI-tract[7,31]. Tanaka *et al*[31] have shown that only TNF-α was influenced by the administration of SC-560 or celecoxib but other cytokines were not affected in mice models of dextran sulphate (DSS)-induced colitis. In their model of colitis produced by adding 3% DSS to drinking water, the inhibition of both COX-1 and COX-2 resulted in exacerbation by these NSAIDs of a widespread intestinal inflammation in mice. This model has, however, different histological appearance characteristics and time course of pathology[31] from that used in our present study[31]. We have utilized a colitis model with TNBS which rather mimics some features of human UC and Crohn's disease (CD) which do not readily apply to the DSS model.

Expression of TNF-α is considered an important pathogenic feature of human IBD, especially CD[13]. Another cytokine, IL-1β, plays an immunoregulatory role in amplifying the inflammatory response by inducing the cascade activation of immune cells. In high doses, IL-1β is responsible for the formation of epithelial cell necrosis, edema and neutrophil infiltration[24]. In our study TNBS-induced colonic damage was accompanied by a prominent increase in colon tissue weight as well as the rise in the MPO, the gene expression and the plasma levels of both IL-1β and TNF-α. This data is in keeping with previous observations[12] that pathogenesis of colitis is associated with an increase in expression and activity of TNF-α and IL-1β. Furthermore, treatment with the non-selective COX-1 and COX-2 inhibitors such as ASA or indomethacin in our study produced a further rise in plasma IL-1β and TNF-α levels. In contrast, the administration of celecoxib moderately improved the healing of colitis followed by a minor increase in plasma IL-1β and TNF-α levels, while a significant improvement of this healing, accompanied by the suppression of these proinflammatory cytokines, was observed in NO-ASA-treated animals. We have also found significant differences not only in macroscopic and microscopic appearance of the colonic mucosa treated with the selective COX-2 inhibitor and NO-releasing ASA *vs* the conventional NSAID (aspirin) on healing of colonic lesions but also in functional alterations such as CBF and MPO activity.

We documented that an increase in plasma levels of IL-1β and TNF-α in colitis was reduced to a greater extent by treatment with NO-ASA and to a lesser extent by a COX-2 inhibitor, which was not the case with the non-selective COX inhibitor, ASA. In ASA-treated rats, the expression and activity of these cytokines were both enhanced. The involvement of PG in healing of colitis is supported by our finding that an exogenously administered synthetic analog of PGE_2 reversed the delay in this healing, the increase in colonic weight and the rise in plasma levels of both IL-1β and TNF-α induced by ASA and celecoxib. It is concluded that the lack of intestinal PGE_2 plays a crucial role in the pathomechanism of exacerbation of inflammatory colonic lesions and the perturbations in CBF in animals administered non-selective COX inhibitors but not to the same extent as in the case of selective COX-2 inhibitors. This is in keeping with the observation that PGE_2 is essential to the process of regeneration of epithelial crypts during the time course of DSS-induced colitis[32]. In another report, PGE_2 was shown to inhibit production of proinflammatory cytokines, particularly that of TNF-α[33]. We found an apparent increase in MPO activity, which is considered to be an indicator of neutrophil-induced inflammatory infiltration, which was significantly elevated in colitis. The colonic MPO activity was further enhanced by non-selective COX inhibitors and selective COX-1 inhibitors such as ASA, indomethacin and SC-560, respectively, while both celecoxib and especially NO-ASA attenuated MPO activity in colitis rats treated with vehicle. This deleterious effect of non-selective COX-inhibitors, ASA and indomethacin, observed in our study could not be attributed to GI toxicity of these NSAIDs, since e.g. indomethacin was used in our study in a dose which by itself failed to induce gastric mucosal lesions but as shown before, prolonged the healing of acute and chronic gastric ulcers, mostly due to inhibition of protective PG[19,21]. Indomethacin was previously reported to induce experimental colitis at doses up to 10 mg/kg[34] that was higher than that in our present study.

We were particularly interested in exploring the mechanism of accelerated colonic healing of the new class of so-called "safer NSAID" such as NO-ASA. In our study the TNBS-induced colitis was associated with a fall in CBF, but this impairment in the colonic microcirculation was worsened by native ASA and indomethacin. Celecoxib moderately enhanced colonic healing and slightly increased CBF, suggesting that this COX-2 inhibitor exerts the opposite effect on colonic microcirculation than non-selective COX inhibitors. In clear contrast, NO-ASA greatly improved CBF and reduced both MPO activity and the expression of mRNAs and reduced plasma IL-1β and TNF-α levels thus contributing to the process of healing of inflammatory lesions. We conclude that these healing and anti-inflammatory effects could be attributed to NO being released from NO-ASA, which ultimately was responsible for the improvement of CBF observed in our study. This is supported by the fact that GTN which is an NO donor, when co-administered with ASA, significantly reduced colonic damage and counteracted the fall in CBF induced by this NSAID. Moreover, the mechanism of the acceleration of colonic healing involves the release of NO from NO-ASA. This notion is supported by the observation that NO-ASA dose-dependently accelerated healing of these lesions followed by an increase in plasma NOx levels. Second, carboxy-PTIO, an NO-scavenger, abolished the healing efficacy of this NO-derivative of ASA and the accompanying increase in CBF. In addition, NO released from NO-ASA could contribute to the intestinal elimination of bacteria, protozoa and fungi as reported previously[35]. In other studies, the addition of a NO-releasing moiety to mesalamine exerted an immunomodulatory effect and suppressed intestinal inflammation by inhibiting T-helper cells while stimulating the activity of the antiinflammatory cytokine IL-10, TGFβ and mucosal Treg pathway whereas standard mesalamine was less effective[36].

It is known that the maintenance of GI mucosal integrity depends on different protective mechanisms against injury, which involves the stimulation of two populations of afferent nerves, vagal and spinal. As shown previously, these sensory neurons are involved in GI protection *via* activation of vasodilatory and anti-inflammatory reflexes[37]. It was reported that the afferent neurons from the dorsal root ganglia play a role in the local regulation of GI circulation, secretion, motility and the process of mucosal repair and healing after injury[37]. CGRP, a neurotransmitter, is released from the peripheral fibers of sensory neurons and plays an important role in GI mucosal defense[37]. Capsaicin, an active ingredient of red pepper has been found to act on the capsaicin sensitive afferent nerves releasing CGRP[38]. When applied in small doses, capsaicin induces a protective response, but a large dose of this neurotoxin renders the gastric mucosa more susceptible to damage induced by indomethacin, ischemia and reperfusion and cold stress[23,26]. This has also been shown to result in a delay in the process of gastric ulcer healing, an event associated with decreased tissue levels of gastric CGRP[37].

In our study, capsaicin-induced functional ablation of sensory nerves with capsaicin, which by itself markedly prolonged the healing process of colonic lesions, attenuated the increase in the healing of these lesions and the accompanying rise in CBF induced by NO-ASA. The impaired healing and evident fall in CBF were restored by concurrent administration of CGRP with NO-ASA in capsaicin-denervated rats. These findings suggest that sensory nerve neuropeptides such as CGRP may contribute to the healing effect and hyperemia induced by NO-ASA. Both CGRP and NO contribute to hyperemia in the GI tract mucosa and facilitate other mechanisms of defense, such as bicarbonate secretion[21,23]. Existing evidence in the lower GI tract revealed an increased inflammatory reaction in experimental colitis of transient receptor potential vanilloid-1 (TRPV-1) knockout mice[37,38]. TRPV-1 is expressed by many afferent nerves and its activation results

in the release of vasodilatory peptides such as CGRP[38,39].

In summary, conventional NSAIDs, such as ASA and indomethacin or selective COX-1 inhibitors such as SC-560[40], delay the healing of experimental colitis and this effect is accompanied by a fall in CBF and an enhancement in gene expression and release of proinflammatory cytokines IL-1β and TNF-α. These deleterious effects are less pronounced with the use of the selective COX-2 inhibitor, celecoxib, which differs with respect to colonic healing with that of conventional ASA. NO-ASA exerts the opposite effects to those of native ASA and selective COX-1 inhibitors on the delay in healing of TNBS colitis and accompanying fall in colonic microcirculation induced by these agents. The beneficial healing action of NO-ASA, involves the NO mediated suppression of proinflammatory cytokines and the activation of sensory nerves resulting in a local release of sensory vasodilatatory neuropeptides such as CGRP.

ACKNOWLEDGMENTS

This article contains data from the paper presented in preliminary form by Zwolinska-Wcislo M, Kwiecien S, Sliwowski Z, Brzozowski T, Drozdowicz D, Mitis-Musiol M, Konturek SJ, Pawlik WW. (2008) "Classic aspirin, COX-2 selective inhibitor and NO derivative of aspirin in the mechanism of healing of ulcerative colitis" GUT 32: Abstract A124 awarded first prize at Gastroenterology XXV UEGW Meeting, Vienna 2008. Authors would like to acknowledge the careful reading of the manuscript by Mrs. Nily Osman.

COMMENTS

Background

Nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, are among the most prescribed drugs because of their anti-inflammatory, antipyretic, analgesic effects. Inhibition of prostaglandin synthesis seems to be the major mechanism implicated in both the beneficial and adverse effects of NSAIDs. Aside from the systemic activity of NSAIDs, resulting from the inhibition of mainly cyclooxygenase (COX)-1 activity, the NSAIDs exert a deleterious influence on the upper and lower gastrointestinal (GI) mucosal integrity by affecting the local inflammatory mechanisms, such as neutrophil recruitment, the activation of proinflammatory cytokines, impairment of microcirculation and the release of free oxygen metabolites. A new class of nitric oxide (NO)-releasing NSAIDs was developed to limit the serious adverse effects associated with NSAIDs ingestion, but their effect in lower GI healing with respect to colitis in animal models has not been carefully investigated. Sensory neurons releasing neuropeptides such as calcitonin gene related peptide (CGRP) are involved in the mechanism of GI protection and ulcer healing due to activation of vasodilatatory mediators such as CGRP. Currently, it is unknown whether these sensory neuropeptides could influence the healing of ulcerative colitis (UC) in animals treated with NO-releasing aspirin (ASA).

Research frontiers

Both UC and Crohn's disease belong to the category of inflammatory bowel diseases (IBD). It is believed that three major factors influence the pathogenesis of these two diseases: an individual's susceptibility, microflora of the gastrointestinal tract and immunological properties of the gastrointestinal mucosa. UC is a disease of the colon which is characterized in humans by chronic inflammation in both the mucosal and submucosal layer with a cellular and humoral immunological response. Patients suffering from UC frequently require anti-inflammatory analgesics such as NSAIDs because of inflammatory conditions such as arthritis, sacroilitis or osteoporosis-related fractures. On the other hand conventional NSAIDs may either induce development of colitis in the healthy colon or exacerbate preexisting colitis. Previous studies revealed that COX exists in the following two isoforms: the inducible COX-2, which is detected at the site of inflammation, and COX-1, which functions in a housekeeping fashion, is present in the majority of human tissues and organs, and is responsible for homeostasis and GI tract integrity. PGs induced by COX-2 are involved in maintaining the intestinal mucosa integrity, in the healing of gastrointestinal ulcers and the modulation of IBD. But the inhibition of both COX-1 and COX-2 isoforms seems to be necessary to induce significant intestinal damage. The new class of NSAIDs containing the NO moiety, such as NO-releasing ASA, could be an alternative strategy in the attenuation of GI side effects of conventional NSAIDs such as ASA. This is due to the fact that NO released from ASA possesses anti-inflammatory properties and contributes to the resolution of intestinal inflammation.

Innovations and breakthroughs

The authors attempted to determine the involvement of NO released from NO-ASA and its role in the mechanism of the healing of colitis. This was accomplished by using NO-ASA and glyceryl trinitrate (GTN), an NO donor, added to classic ASA to mimic the protective action of NO-ASA. Treatment with NO-ASA improved the healing of colitis, with these effects as well as the anti-inflammatory properties of NO-ASA being attributed to the NO released from NO-ASA, which was ultimately responsible for the improvement of colonic blood flow (CBF) observed in this study. Furthermore, the authors found that both NO and the activation of sensory nerve neuropeptides such as CGRP may contribute to the healing effects and the hyperemia induced by NO-ASA. This could be responsible for the attenuation of the expression and the release of pro-inflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α. This is supported by the fact that GTN, which is an NO donor, when co-administered with ASA, significantly decreased colonic damage and counteracted the decrease in CBF induced by this NSAID. The study demonstrates that NO-ASA is beneficial when compared to its parent drug ASA in the healing of experimental colitis. Furthermore, celecoxib, the selective COX-2 inhibitor, showed greater efficacy in healing colitis than was observed with non-selective COX-1 and COX-2 inhibitors (conventional ASA, indomethacin). However, the prolonged use of coxibs in clinical practice may be associated with prothrombotic action and increased risk of acute myocardial infarction. This significant finding of the authors with respect to selective COX-2 inhibitors and healing of ulcerative colitis requires further investigation and confirmation in clinical trials.

Applications

(1) Inhibition of both COX-1 and COX-2 isoforms by non-selective and selective COX-1 inhibitors exacerbates colonic damage and leads to functional impairment of the colonic mucosa blood flow during the process of healing of colitis; (2) The importance of PG inhibition by NSAIDs in the pathogenesis of colitis is confirmed by the finding that supplementation with exogenous PGs of animals concurrently treated with COX-1 or COX-2 inhibitors attenuated the colonic damage and the decrease of CBF induced by these agents; and (3) The NO released from ASA may be an alternative option to native ASA in patients with lower GI tract disorders such as UC.

Terminology

Incorporation of NO generating moiety into the basic structure of NSAIDs, such as aspirin (NO-ASA) attenuates the ulcerogenic activity of native NSAID. Under basal conditions, NO derived from the activity of constitutive NO synthase (cNOS) contributes to the maintenance of intestinal integrity and the control of intestinal motility. NO and CGRP exhibit a protective action against NSAIDs induced impairment of colonic healing. GTN, an NO donor, and 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, monopotassium salt, an NO scavenger were used for the evaluation of the involvement of NO in the process of colonic healing in rats with colitis. CGRP is a sensory neurotransmitter released from the peripheral endings of afferent sensory neurons implicated in the defense mechanism of the GI mucosa. Capsaicin, an active ingredient of red pepper, has been found to induce a functional ablation of the capsaicin-sensitive afferent nerves releasing CGRP. Previous studies revealed that when applied in small doses capsaicin exerts a protective action, but large doses of this neurotoxin render the GI mucosa more susceptible to damage induced by various ulcerogens and stressors.

Peer review

This work is good, with proper design of research and interesting results.

REFERENCES

1 **Shanahan F**. Inflammatory bowel disease: immunodiagnostics, immunotherapeutics, and ecotherapeutics. *Gastroenterology* 2001; **120**: 622-635
2 **Porras M**, Martín MT, Torres R, Vergara P. Cyclical upregulated iNOS and long-term downregulated nNOS are the bases for relapse and quiescent phases in a rat model of IBD. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G423-G430
3 **Dudhgaonkar SP**, Tandan SK, Kumar D, Raviprakash V, Kataria M. Influence of simultaneous inhibition of cyclooxygenase-2 and inducible nitric oxide synthase in experimental colitis in rats. *Inflammopharmacology* 2007; **15**: 188-195
4 **Kefalakes H**, Stylianides TJ, Amanakis G, Kolios G. Exacerbation of inflammatory bowel diseases associated with the use of nonsteroidal anti-inflammatory drugs: myth or reality? *Eur J Clin Pharmacol* 2009; **65**: 963-970
5 **Cipolla G**, Crema F, Sacco S, Moro E, de Ponti F, Frigo G. Nonsteroidal anti-inflammatory drugs and inflammatory bowel disease: current perspectives. *Pharmacol Res* 2002; **46**: 1-6
6 **Vane JR**, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* 1998; **38**: 97-120
7 **Wallace JL**. COX-2: a pivotal enzyme in mucosal protection and resolution of inflammation. *ScientificWorldJournal* 2006; **6**: 577-588
8 **Wallace JL**, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000; **119**: 706-714
9 **Bertolini A**, Ottani A, Sandrini M. Dual acting anti-inflammatory drugs: a reappraisal. *Pharmacol Res* 2001; **44**: 437-450
10 **Cuzzocrea S**, Mazzon E, Serraino I, Dugo L, Centorrino T, Ciccolo A, Sautebin L, Caputi AP. Celecoxib, a selective cyclo-oxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats. *Eur J Pharmacol* 2001; **431**: 91-102
11 **Schmassmann A**, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi B, Halter F. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br J Pharmacol* 1998; **123**: 795-804
12 **Reuter BK**, Asfaha S, Buret A, Sharkey KA, Wallace JL. Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. *J Clin Invest* 1996; **98**: 2076-2085
13 **Matuk R**, Crawford J, Abreu MT, Targan SR, Vasiliauskas EA, Papadakis KA. The spectrum of gastrointestinal toxicity and effect on disease activity of selective cyclooxygenase-2 inhibitors in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 352-356
14 **Tanaka A**, Hase S, Miyazawa T, Ohno R, Takeuchi K. Role of cyclooxygenase (COX)-1 and COX-2 inhibition in nonsteroidal anti-inflammatory drug-induced intestinal damage in rats: relation to various pathogenic events. *J Pharmacol Exp Ther* 2002; **303**: 1248-1254
15 **Takeuchi K**, Tanaka A, Ohno R, Yokota A. Role of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. *J Physiol Pharmacol* 2003; **54** Suppl 4: 165-182
16 **Yokota A**, Taniguchi M, Takahira Y, Tanaka A, Takeuchi K. Rofecoxib produces intestinal but not gastric damage in the presence of a low dose of indomethacin in rats. *J Pharmacol Exp Ther* 2005; **314**: 302-309
17 **Wallace JL**, Zamuner SR, McKnight W, Dicay M, Mencarelli A, del Soldato P, Fiorucci S. Aspirin, but not NO-releasing aspirin (NCX-4016), interacts with selective COX-2 inhibitors to aggravate gastric damage and inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G76-G81
18 **McCafferty DM**, Mudgett JS, Swain MG, Kubes P. Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation. *Gastroenterology* 1997; **112**: 1022-1027
19 **Brzozowski T**, Konturek PC, Konturek SJ, Sliwowski Z, Pajdo R, Drozdowicz D, Ptak A, Hahn EG. Classic NSAID and selective cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers. *Microsc Res Tech* 2001; **53**: 343-353
20 **Konturek SJ**, Brzozowski T, Pajdo R, Konturek PC, Kwiecień S, Sliwowski Z, Pawlik M, Ptak A, Drozdowicz D, Hahn EG. Gastric preconditioning induced by short ischemia: the role of prostaglandins, nitric oxide and adenosine. *Med Sci Monit* **2001; 7**: 610-621
21 **Konturek PC**, Brzozowski T, Ptak A, Kania J, Kwiecień S, Hahn EG, Konturek SJ. Nitric oxide releasing aspirin protects the gastric mucosa against stress and promotes healing of stress-induced gastric mucosal damage: role of heat shock protein 70. *Digestion* 2002; **66**: 160-172
22 **Zwolinska-Wcislo M**, Brzozowski T, Budak A, Kwiecien S, Sliwowski Z, Drozdowicz D, Trojanowska D, Rudnicka-Sosin L, Mach T, Konturek SJ, Pawlik WW. Effect of Candida colonization on human ulcerative colitis and the healing of inflammatory changes of the colon in the experimental model of colitis ulcerosa. *J Physiol Pharmacol* 2009; **60**: 107-118
23 **Brzozowski T**, Konturek SJ, Sliwowski Z, Pytko-Polończyk J, Szlachcic A, Drozdowicz D. Role of capsaicin-sensitive sensory nerves in gastroprotection against acid-independent and acid-dependent ulcerogens. *Digestion* **1996; 57**: 424-432
24 **Vilaseca J**, Salas A, Guarner F, Rodriguez R, Malagelada JR. Participation of thromboxane and other eicosanoid synthesis in the course of experimental inflammatory colitis. *Gastroenterology* 1990; **98**: 269-277
25 **Green LC**, Tannenbaum SR, Goldman P. Nitrate synthesis in the germfree and conventional rat. *Science* 1981; **212**: 56-58
26 **Pajdo R**, Brzozowski T, Konturek PC, Kwiecien S, Konturek SJ, Sliwowski Z, Pawlik M, Ptak A, Drozdowicz D, Hahn EG. Ischemic preconditioning, the most effective gastroprotective intervention: involvement of prostaglandins, nitric oxide, adenosine and sensory nerves. *Eur J Pharmacol* 2001; **427**: 263-276
27 **Chomczynski P**, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159
28 **Laine L**. The gastrointestinal effects of nonselective NSAIDs and COX-2-selective inhibitors. *Semin Arthritis Rheum* 2002; **32**: 25-32
29 **Brzozowski T**, Konturek PC, Konturek SJ, Sliwowski Z, Drozdowicz D, Stachura J, Pajdo R, Hahn EG. Role of prostaglandins generated by cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia-reperfusion-induced gastric lesions. *Eur J Pharmacol* 1999; **385**: 47-61
30 **S Kwiecien S**, Pawlik MW, Brzozowski T, Konturek PC, Sliwowski Z, Pawlik WW, Konturek SJ. Nitric oxide (NO)-releasing aspirin and (NO) donors in protection of gastric mucosa against stress. *J Physiol Pharmacol* 2008; **59** Suppl 2: 103-115
31 **Tanaka K**, Suemasu S, Ishihara T, Tasaka Y, Arai Y, Mizushima T. Inhibition of both COX-1 and COX-2 and resulting decrease in the level of prostaglandins E2 is responsible for non-steroidal anti-inflammatory drug (NSAID)-dependent exacerbation of colitis. *Eur J Pharmacol* 2009; **603**: 120-132
32 **Cohn SM**, Schloemann S, Tessner T, Seibert K, Stenson WF. Crypt stem cell survival in the mouse intestinal epithelium is regulated by prostaglandins synthesized through cyclooxygenase-1. *J Clin Invest* 1997; **99**: 1367-1379
33 **Kabashima K**, Saji T, Murata T, Nagamachi M, Matsuoka T, Segi E, Tsuboi K, Sugimoto Y, Kobayashi T, Miyachi Y, Ichikawa A, Narumiya S. The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest* 2002; **109**: 883-893
34 **Kankuri E**, Vaali K, Korpela R, Paakkari I, Vapaatalo H, Moilanen E. Effects of a COX-2 preferential agent nimesu-

lide on TNBS-induced acute inflammation in the gut. *Inflammation* 2001; **25**: 301-310
35 **Vazquez-Torres A**, Jones-Carson J, Warner T, Balish E. Nitric oxide enhances resistance of SCID mice to mucosal candidiasis. *J Infect Dis* 1995; **172**: 192-198
36 **Santucci L**, Wallace J, Mencarelli A, Farneti S, Morelli A, Fiorucci S. Different sensitivity of lamina propria T-cell subsets to nitric oxide-induced apoptosis explains immunomodulatory activity of a nitric oxide-releasing derivative of mesalamine in rodent colitis. *Gastroenterology* 2005; **128**: 1243-1257
37 **Holzer P**. Role of visceral afferent neurons in mucosal inflammation and defense. *Curr Opin Pharmacol* 2007; **7**: 563-569
38 **Mózsik G**, Szolcsányi J, Dömötör A. Capsaicin research as a new tool to approach of the human gastrointestinal physiology, pathology and pharmacology. *Inflammopharmacology* 2007; **15**: 232-245
39 **Evangelista S**. Role of sensory neurons in restitution and healing of gastric ulcers. *Curr Pharm Des* 2006; **12**: 2977-2984
40 **Lanas A**, Scarpignato C. Microbial flora in NSAID-induced intestinal damage: a role for antibiotics? *Digestion* 2006; **73** Suppl 1: 136-150

S- Editor Sun H **L- Editor** O'Neill M **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4090

World J Gastroenterol 2011 September 28; 17(36): 4090-4098
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Inhibition of high-mobility group box 1 expression by siRNA in rat hepatic stellate cells

Wen-Song Ge, Jian-Xin Wu, Jian-Gao Fan, Yao-Jun Wang, Ying-Wei Chen

Wen-Song Ge, Jian-Xin Wu, Jian-Gao Fan, Ying-Wei Chen, Department of Gastroenterology, Shanghai Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China
Yao-Jun Wang, Department of Gastroenterology, General Hospital of Jinan Military Region, Jinan 250031, Shandong Province, China
Author contributions: Ge WS, Wu JX, Fan JG, Wang YJ and Chen YW designed research; Ge WS, Wang YJ and Chen YW performed research; Wang YJ and Chen YW contributed new reagents/analytic tools; Ge WS and Chen YW analyzed data; and Ge WS, Wang YJ and Chen YW wrote the paper.
Supported by The Select and Train Outstanding Young Teachers Foundation of Shanghai, No. jdy08086 and WUJieping Experimental Diagnosis of Liver Disease Medical Foundation, No. LDWMF-SY-2011B009
Correspondence to: Ying-Wei Chen, Vice-professor, Department of Gastroenterology, Shanghai Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China. way_01chen@hotmail.com
Telephone: +86-21-25076431 Fax: +86-21-25071316
Received: July 5, 2011 Revised: September 5, 2011
Accepted: September 12, 2011
Published online: September 28, 2011

Abstract

AIM: To explore the role of high-mobility group box 1 (HMGB1) protein during liver fibrogenesis and investigate the functional effects of *HMGB1* gene silencing in hepatic stellate cells (HSCs) using siRNA.

METHODS: Hepatic fibrosis in rats was induced through serial subcutaneous injections of dimethylnitrosamine, and expression of HMGB1 was detected by immunohistochemistry. HMGB1 siRNAs were developed and transiently transfected into HSC-T6 cells using Lipofectamine 2000. HMGB1 expression was evaluated by real-time polymerase chain reaction (PCR) and Western blotting analysis. Expression of α-smooth muscle actin (α-SMA) and collagen types Ⅰ and Ⅲ was evaluated by real-time PCR. Cell proliferation and the cell cycle were determined using the methyl thiazolyl tetrazolium method. Finally, collagen content in HSC supernatant was evaluated by an enzyme-linked immunosorbent assay.

RESULTS: The results showed that HMGB1 was up-regulated during liver fibrosis and that its expression was closely correlated with the deposition of collagen. siRNA molecules were successfully transfected into HSCs and induced inhibition of HMGB1 expression in a time-dependent manner. Moreover, HMGB1 siRNA treatment inhibited synthesis of α-SMA and collagen types Ⅰ and Ⅲ in transfected HSCs.

CONCLUSION: This study suggests a significant functional role for HMGB1 in the development of liver fibrosis. It also demonstrates that downregulation of HMGB1 expression might be a potential strategy to treat liver fibrosis.

Key words: Hepatic fibrosis; High-mobility group box 1; Hepatic stellate cells; RNA interference

Peer reviewers: Ekihiro Seki, MD, PhD, Department of Medicine, University of California San Diego, Leichag Biomedical Research Building Rm 349H, 9500 Gilman Drive MC#0702, La Jolla, CA 92093-0702, United States; London Lucien Ooi, Professor, Chairman, Division of Surgery, Singapore General Hospital, 1 Hospital Drive, 169608, Singapore

Ge WS, Wu JX, Fan JG, Wang YJ, Chen YW. Inhibition of high-mobility group box 1 expression by siRNA in rat hepatic stellate cells. *World J Gastroenterol* 2011; 17(36): 4090-4098 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4090.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4090

INTRODUCTION

Hepatic fibrosis is a major medical problem associated with significant morbidity and mortality. Regardless of

the underlying aetiology[1], hepatic fibrosis is characterized by the accumulation of excess extracellular matrix (ECM). The amount of matrix deposition depends on the balance between its synthesis and degradation. When synthesis of ECM exceeds its degradation, the pathological accumulation of ECM leads to liver fibrosis. Therefore, a critical balance must be achieved between maintaining the proper amount of ECM for homeostasis, while at the same time, providing a means of ensuring that excess or improper accumulation does not occur.

High-mobility group box 1 (HMGB1) protein was originally identified as a nuclear nonhistone protein with DNA-binding domains, and it has been implicated as an important endogenous danger signaling molecule. In addition, it can be secreted from cells and exert extracellular functions as a proinflammatory cytokine[2,3]. Increasing evidence now points to multiple functions of HMGB1 in infection, tissue injury, inflammation, apoptosis, and the immune response[4]. HMGB1 can be released both through active secretion from various cells, including activated monocytes/macrophages, neutrophils, and endothelial cells, and through passive release from necrotic cells[3-7]. HMGB1 can directly promote the secretion of proinflammatory cytokines [tumor necrosis factor (TNF), interleukin (IL)-1A/B, IL-6 and IL-8] and chemokines (macrophage inflammatory protein-1A/B) by peripheral blood mononuclear cells (PBMCs)[8,9]. In turn, PBMCs also produce different cytokines that are potentially involved in virus-induced liver damage. HMGB1 acts as a chemoattractant for fibroblasts and endothelial and smooth muscle cells, which are cell types that significantly contribute to wound repair[9,10]. Consequently, HMGB1 can directly stimulate fibroblast proliferation and participate in fibrogenesis[4]. Additionally, inhibitors of HMGB1 significantly reduce tissue damage[5,6]. Moreover, Hamada *et al*[4] have reported that inhibition of HMGB1 may be beneficial in pulmonary fibrosis. Therefore, we postulated that inhibiting the upregulation of HMGB1 during liver fibrogenesis could be a potential strategy for treating liver fibrosis.

RNA interference is known as a powerful tool for post-transcriptional gene silencing[11] and has opened new avenues in gene therapy. In this study, we induced hepatic fibrosis in rats through serial subcutaneous injections of dimethylnitrosamine (DMN) for 4 wk and evaluated the expression of HMGB1 during the process of hepatic fibrogenesis. Additionally, siRNA molecules targeting the sequences within the rat *HMGB1* gene were transfected into hepatic stellate cell (HSC)-T6 cells. The results show that the expression of HMGB1 was correlated with collagen deposition during hepatic fibrosis and that downregulating HMGB1 expression could prohibit collagen production and enhance collagen degradation.

MATERIALS AND METHODS

Animal models

Thirty-two 6-wk-old male Sprague-Dawley rats (230-260 g) were purchased from the Shanghai Laboratory Animal Centre of Chinese Academy of Sciences and fed *ad libitum* with standard laboratory chow. All rats received humane care according to the Guide for the Care and Use of Laboratory Animals by the Chinese Academy of Sciences. Hepatic fibrosis was induced by intraperitoneal injections of 1% DMN (1 mL/kg body weight) for three consecutive days per week for up to 4 wk[11]. Rats were sacrificed at 1, 2 and 3 wk from the first DMN injection. Liver tissues were either snap-frozen in liquid nitrogen or fixed in 10% formalin for histology and immunostaining.

Histological and immunohistochemical examination

Liver tissue sections were stained with hematoxylin-eosin (HE) for histopathological examination. Immunohistochemical examination was performed to detect the expression of HMGB1 and collagen types I and Ⅲ in liver tissues. Briefly, the paraffin sections of left median hepatic lobes were incubated with 3% H_2O_2 in methanol at 37 ℃ for 10 min to quench endogenous peroxidase activity. After blocking at room temperature for 20 min, the sections were incubated with antibodies against HMGB1 (R and D Systems, Germany), collagen type I or collagen type Ⅲ (Boster, Wuhan, China) overnight at 4 ℃ followed by incubation with horseradish-peroxidase-conjugated secondary antibody (Dako, Kyoto, Japan) at 37 ℃ for 20 min. Finally, the signals were detected using the Diaminobenzidine Substrate Kit (Vector Laboratories, Burlingame, CA, United States), and a positive outcome was indicated by brown staining in the cytoplasm or nucleus. For the semiquantitative analysis of HMGB1 and collagen expression, the brown-stained tissues in immunohistostaining sections were measured on an image analyzer by a technician blinded to the samples. Five fields were selected randomly from each of two sections, and six rats from each group were examined.

Double immunostaining of HMGB1 and α-smooth muscle actin

Liver sections were blocked with 5% normal goat serum after fixing and then simultaneously incubated with both monoclonal anti-HMGB1 (R and D Systems, Germany) and polyclonal α-smooth muscle actin (α-SMA) (Fremont, CA, United States) antibodies prepared in phosphate-buffered saline (PBS). The sections were incubated overnight at 4 ℃ or 1 h at room temperature and then washed with PBS. Sections were then simultaneously incubated with fluorescein-isothiocyanate-conjugated secondary antibody and rhodamine-conjugated secondary antibody for 30 min at 37 ℃ in the dark. Both primary antibodies were produced in different species. Antibody labeling was examined under a Zeiss LSM-510 laser scanning confocal microscope.

Cell culture

The HSC-T6 cell line, an immortalized rat HSC line, which has a stable phenotype and biochemical characteristics, was kindly provided by Dr. SL Friedman (Divi-

sion of Liver Diseases, Mount Sinai School of Medicine, New York, NY, United States). All cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum and 5% antibiotics and incubated at 37 ℃ in a humidified atmosphere of 5% CO_2. Cells were seeded at 2×10^5 per well in six-well plates 24 h before transfection. The amount of siRNA and transfection reagent was calculated according to the manufacturer's instructions.

Immunofluorescence study

HSC-T6 cells were cultured for 24 h on glass coverslips and fixed in 4% formaldehyde for 30 min at room temperature prior to detergent extraction with 0.1% Triton X-100 for 10 min at 4 ℃. Coverslips were saturated with PBS containing 2% bovine serum albumin (BSA) for 1 h at room temperature. Next, cells were incubated with the specific primary antibody for HMGB1 (R and D Systems, Germany) in 1% BSA for 1 h, washed, and incubated with secondary antibody (TRITC AffiniPure Goat Anti-Rabbit IgG, EarthOx, LLC, United States). Finally, cells were stained for 30 min at room temperature with 4,6-diamidino-2-phenylindole. Slides were viewed with a Zeiss LSM-510 laser scanning confocal microscope.

Preparation of siRNA, construction of siRNA expression vector and transfection assay

The siRNAs for rat HMGB1 mRNA were designed and synthesised by Invitrogen Life Technologies. We prepared three siRNAs, and the most effective one was selected for construction of the siRNA expression vector. The siRNA sequences used are shown in Table 1. Negative control siRNAs were used to assess non-specific gene silencing effects, and the mock group was the non-transfection group. Cells were transfected with a mixture of plasmid DNA and Lipofectamine 2000 (Invitrogen) in Opti-MEM I medium without serum as recommended by the manufacturer. The medium was then replaced with standard RPMI medium (containing 10% FBS and gentamicin) 24 h post-transfection.

Real-time quantitative polymerase chain reaction

Total RNA was extracted at different time points after siRNA transfection using the Trizol kit (Gibco/Life Technologies) according to the manufacturer's protocol. The mixture of RNA and primers was loaded into the polymerase chain reaction (PCR) amplifier. The PCR protocol was as follows: predenaturate setting at 95 ℃ for 5 min, 94 ℃ for 45 s, annealing at 50 ℃ for 1 min, and extension at 72 ℃ for 1 min. The PCR was performed for 40 cycles followed by a final extension at 72 ℃ for 10 min. We then visualized the PCR product by running it on a 1.5% agarose gel and quantitatively analysed it with Lab Works 4.5 analysis software.

Western blotting

The same quantities of cells were collected from the four groups, and the protein was extracted from the cells at the 24, 48 and 72 h after transfection. The protein content in the supernatant was detected using the bicinchoninic acid method. An equal amount of protein was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis electrophoresis and transferred onto a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was incubated overnight at 4 ℃ with monoclonal anti-human HMGB1 (1:300) and was then incubated for 2 h with a secondary antibody (1:5000). Finally, after staining and fixing, the film was analyzed using the Image Analysis System.

Table 1 Design of small interfering RNA sequences for high-mobility group box 1

Plasmid constructs	Target sequence in mRNA(5′-3′)
HMGB1-1 (shRNAH1)	GCAAATGACTCAATCTGATT
HMGB1-2 (shRNAH2)	AATAGGAAAAGGATATTGCT
HMGB1-3 (shRNAH3)	ACCCGGATGCTTCTGTCAAC

HMGB1: High-mobility group box 1.

Enzyme-linked immunosorbent assay

Commercial kits (Sigma, St. Louis, MO, United States) were used to quantitate the amount of collagen types Ⅰ and Ⅲ in the culture supernatant of HSCs at different time points after siRNA transfection.

Methyl thiazolyl tetrazolium used for observing cell proliferation

The cell suspension was inoculated into 96-well plates at 1000 cells per well with eight ambi-wells and incubated for 1, 2, 3, 4 and 5 d after transfection. Cells were incubated with 20 μL methyl thiazolyl tetrazolium for 4 h. After centrifugation, 150 μL dimethyl sulfoxide was added to the precipitate, and the absorbance of the enzyme was measured at 490 nm. Cell growth rates (average absorbance of each transfected group/non-transfected group) were then calculated.

Statistical analysis

Continuous data were expressed as the mean ± SD and were analyzed using the Student's *t* test. Correlations among the study variables were tested using Pearson's correlation coefficients. $P < 0.05$ were considered statistically significant. All calculations were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Histological and immunohistochemical assessment

To investigate the expression of HMGB1 during liver fibrosis, liver sections were analysed by HE staining and immunohistochemistry. We localized HMGB1 and collagen types Ⅰ and Ⅲ in liver specimens by immunohistochemistry. None of these proteins were observed in control rat livers. In fibrotic rat livers, HMGB1 was markedly increased during liver fibrogenesis and was correlated with

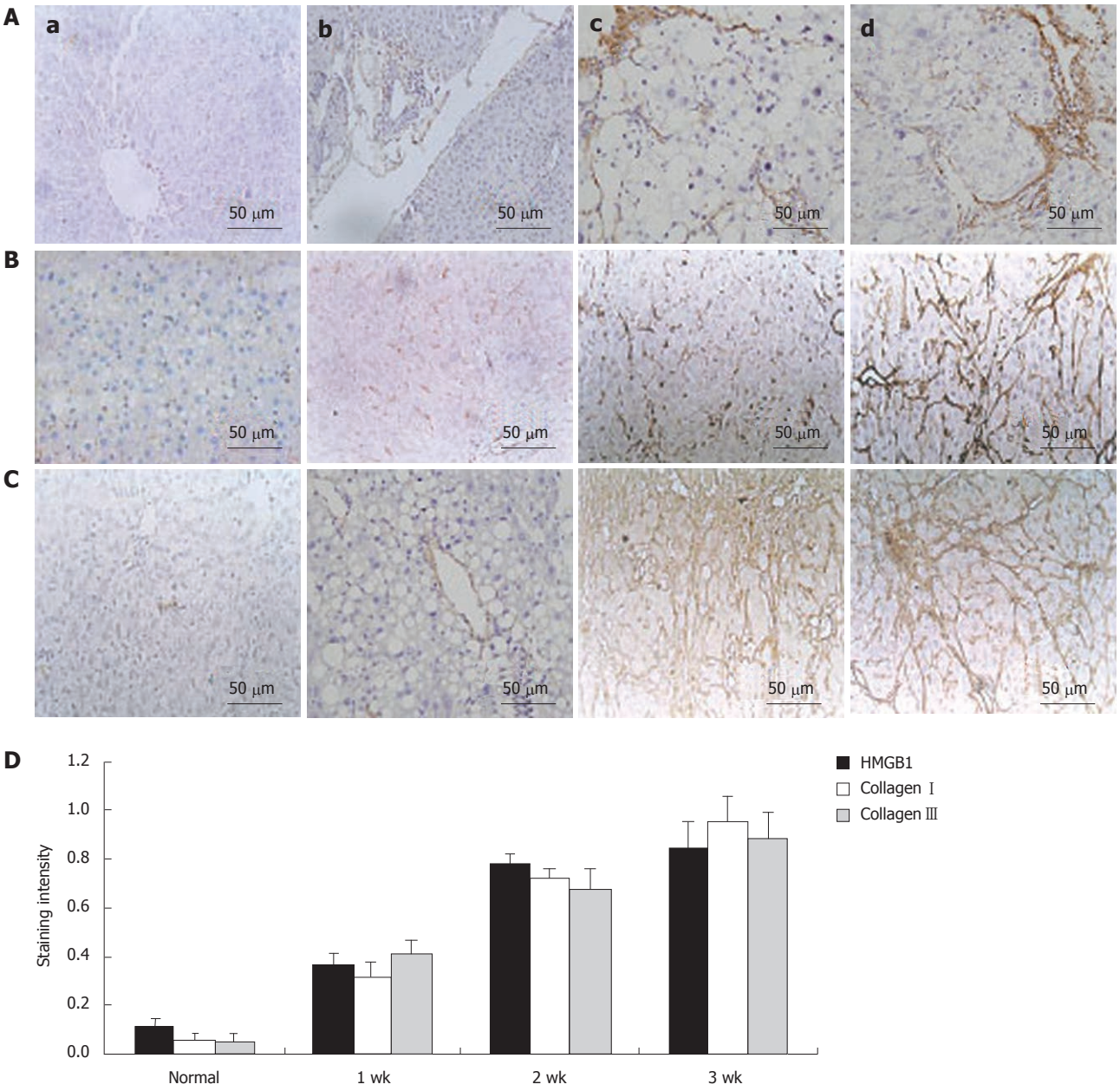


Figure 1 High-mobility group box 1 protein was upregulated after dimethylnitrosamine injection. A: Immunohistochemical study of high-mobility group box 1 (HMGB1) distribution and expression in liver fibrosis specimens (original magnification, × 400). Brown color displays the positive expression. a: There was no immunoreactivity in the normal liver tissue; b: Weak staining in liver fibrosis tissue at 1 wk after the first dimethylnitrosamine (DMN) injection; c: Moderate staining in liver fibrosis tissue at 2 wk after the first DMN injection; d: Strong staining in liver fibrosis tissue at 3 wk after the first DMN injection; B: Immunohistochemical study of collagen type Ⅰ in liver fibrosis specimens (original magnification, × 400). Brown color displays the positive expression. Collagen type Ⅰ was markedly increased during liver fibrogenesis; C: Immunohistochemical study of collagen type Ⅲ in liver fibrosis specimens (original magnification, × 400). Brown color displays the positive expression. Collagen type Ⅲ was markedly increased during liver fibrogenesis; D: The amount of HMGB1, collagen types Ⅰ and Ⅲ staining in liver tissue was measured using an image analyzer during liver fibrosis. HMGB1 was markedly increased during liver fibrogenesis, correlated with the expression of collagen types Ⅰ and Ⅲ ($r = 0.90$, $P < 0.05$ and $r = 0.89$, $P < 0.05$).

the expression of collagen types Ⅰ and Ⅲ. Immunohistochemistry indicated that the intensity of HMGB1 immunostaining was stronger in the fibrotic samples (DMN week 1) than in the control group. After DMN injection for 2-3 wk, greater HMGB1 staining was found around the portal tracts and fibrotic septa (Figure 1A). With the development of hepatic fibrosis, there was an enhanced expression of HMGB1, correlating with collagen types Ⅰ and Ⅲ expression, which was mainly located within the mesenchymal (Figure 1B and C). Statistical analysis showed that the expression of HMGB1 was completely correlated with the expression of collagen types Ⅰ and Ⅲ during the development of hepatic fibrosis (Figure 1D) ($P < 0.05$).

Cellular localization of HMGB1 in DMN-treated rats

α-SMA, a typical marker of activated HSCs, was selected to determine the cellular localization of HMGB1 in hepat-

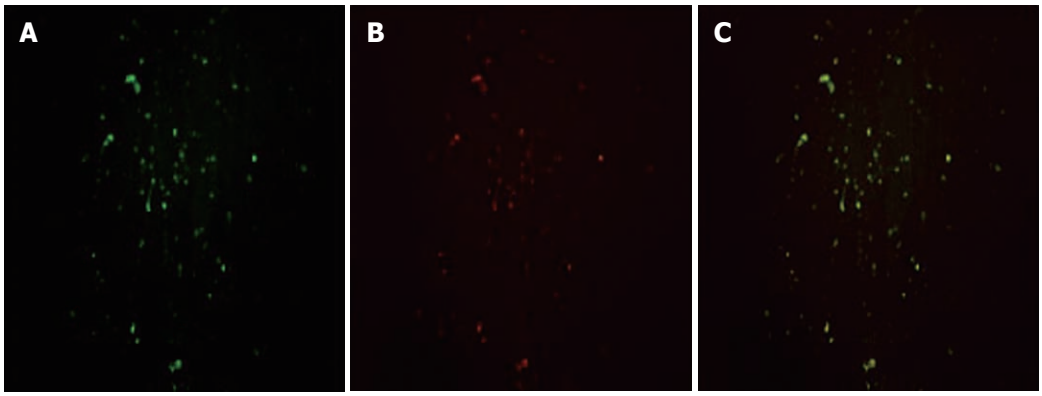


Figure 2 Double immunostaining was used to analyze the cellular localization of high-mobility group box 1 protein and α-smooth muscle actin in hepatic fibrosis tissue (original magnification, × 200). A: α-smooth muscle actin (α-SMA) was stained with polyclonal α-SMA antibody and secondarily by rhodamine -conjugated anti-rabbit antibody (green); B: High-mobility group box 1 (HMGB1) was stained with monoclonal anti-HMGB1 antibody and secondarily by fluoresceinisothiocyanate-conjugated anti-rabbit antibody (red); C: The yellow areas on the merged image show co-localization of α-SMA and HMGB1.

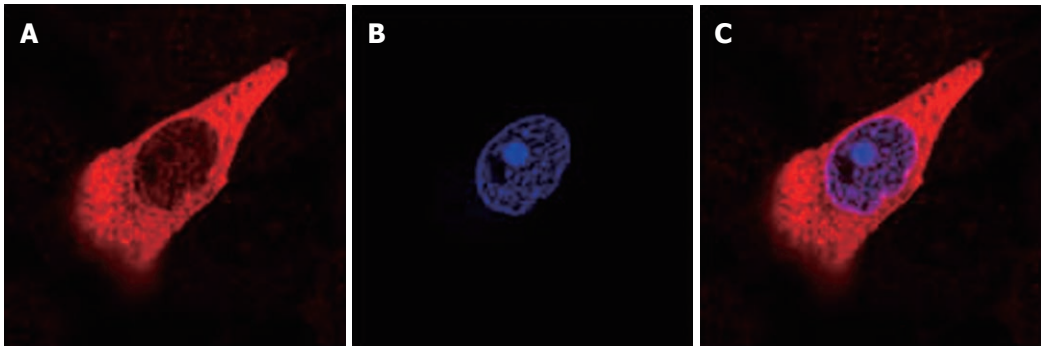


Figure 3 High-mobility group box 1 protein expression in hepatic stellate cell-T6 cells by immunofluorescence staining (original magnification, × 200). A: High-mobility group box 1 (HMGB1) protein was stained with monoclonal anti-HMGB1 antibody and secondarily by fluoresceinisothiocyanate-conjugated anti-rabbit antibody (red); B: Nuclei were labelled with 4',6-diamidino-2-phenylindole (blue); C: The merge picture.

ic fibrosis tissue. The localization of HMGB1 and α-SMA was visualized by immunofluorescent double labeling and laser scanning confocal microscopy. The image analysis showed a diffused distribution of HMGB1 throughout the hepatic fibrosis tissue (Figure 2A), and a similar distribution was observed for α-SMA (Figure 2B). When the two images were merged, there was a very high degree of co-localization of HMGB1 with α-SMA throughout the hepatic fibrosis tissue (Figure 2C).

Intracellular localization of HMGB1 in activated HSC-T6 cells

An immunofluorescence study of HSC-T6 cells after 24 h of culture demonstrated the intracellular localization of HMGB1. We evaluated the subcellular localization of HMGB1 by separating bulk nuclei and cytosolic fractions, and HMGB1 was detected primarily within the cytosol of activated HSC-T6 cells (Figure 3).

Selection of HMGB1 mRNA sequence target

As shown in Table 1, a total of three candidate siRNA sequences were chosen to be complementary to various regions of the rat *HMGB1* gene. In a set of preliminary experiments designed to identify the most appropriate sequence for further study, these sequences were transfected into HSC-T6 using Lipofectamine. Forty-eight hours after transfection, HMGB1 transcript and protein levels were reduced in transfected cells. This *HMGB1* gene-silencing effect was reproducible and was specific in that it failed to knock down the expression of an unrelated protein, β-actin. All three HMGB1 shRNAs tested in this study were able to reduce the HMGB1 expression in HSC-T6 cells compared with the negative control (NC) siRNA transfectants. Although all three HMGB1 shRNA constructs were effective, shRNAH3 was more efficient in reducing the HMGB1 transcript levels than shRNAH2 and shRNAH1 (Figure 4A). Western blotting analysis (Figure 4C) further confirmed the shRNAH3 silencing of the HMGB1 protein in HSC-T6 cells. Semiquantitative analysis of the real-time (RT)-PCR and Western blot results (Figure 4B and D) also showed that HMGB1 shRNAH3 decreased the expression of HMGB1 in HSC-T6 cells more efficiently than shRNAH2 and shRNAH1. Accordingly, we chose shRNAH3 for the subsequent experiments.

HMGB1 siRNA downregulated mRNA expression of α-SMA and types Ⅰ and Ⅲ collagen in HSC-T6

To investigate the effect of HMGB1 siRNA on HSCs and its potential molecular mechanisms, we detected the

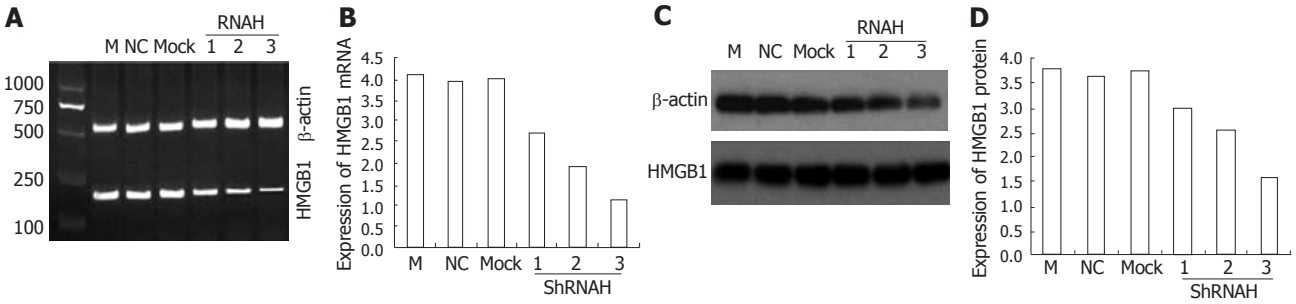


Figure 4 Screening the most effective high-mobility group box 1 siRNA sequence. Total RNA and protein were obtained from hepatic stellate cell-T6 transfected with negative control (NC), mock and three different high-mobility group box 1 (HMGB1) siRNA molecules (shRNAH1, shRNAH2 and shRNAH3). A: Real-time polymerase chain reaction (RT-PCR) for the effect of three different HMGB1 siRNA molecules on HMGB1 mRNA level 48 h after transfection. The expression was normalized against β-actin; B: Semiquantitative analysis of the RT-PCR result; C: Western blotting analyzed HMGB1 protein expression 48 h after transfection; D: Semiquantitative analysis of the western blotting results. Data represent results from one of three similar experiments. Results show that all three HMGB1 shRNA constructs were effective, but shRNAH3 was more efficient in reducing the HMGB1 mRNA and protein levels than shRNAH2 and shRNAH1.

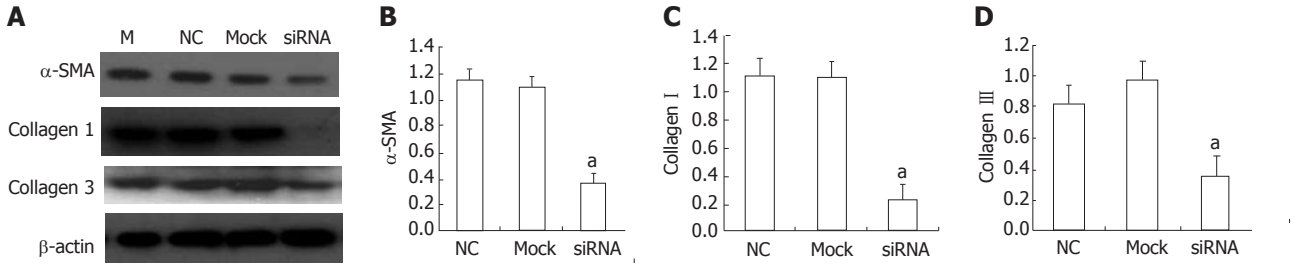


Figure 5 High-mobility group box 1 siRNA inhibited α-smooth muscle actin, collagen types Ⅰ and Ⅲ mRNA expression in hepatic stellate cell-T6 cells. A: Real-time polymerase chain reaction (RT-PCR) analysis for α-smooth muscle actin (α-SMA), collagen types Ⅰ and Ⅲ mRNA expression in hepatic stellate cell-T6 cells after siRNA high-mobility group box 1 transfection. β-actin was used as the internal loading control; B-D: Semiquantitative analysis of the RT-PCR result. [a]$P < 0.05$ *vs* negative controls (NC) or mock.

Table 2 Effect of high-mobility group box 1 siRNA on the cell cycle

Cell cycle phases(%)	ShRNAH3 group	NC group
G0/G1 phase	58.31% ± 0.48%[a]	44.25% ± 0.63%
S phase	29.12% ± 1.26%[a]	41.32% ± 1.58%
G2/M phase	12.57% ± 1.04%	14.53% ± 1.28%

[a]$P < 0.05$ *vs* negataive controls (NC) group.

mRNA expression of some profibrogenic markers, including α-SMA and collagen types Ⅰ and Ⅲ, in transfected HSC-T6. As shown in Figure 5, HMGB1 siRNA reduced the mRNA levels of α-SMA and collagen types Ⅰ and Ⅲ.

HMGB1 siRNA reduced the collagen content in the HSC-T6 supernatant

To confirm the effect of HMGB1 siRNA on collagen secretion and degradation, we examined the amount of collagen types Ⅰ and Ⅲ in HSCs 48 and 72 h after transfection with shRNAH3 using an ELISA. The results reveal that the content of both collagen types Ⅰ and Ⅲ was decreased after transfection with HMGB1 siRNA. Compared with the NC group, the content of collagen types Ⅰ and Ⅲ was reduced to 63% and 61%, respectively, 72 h after shRNAH3 transfection (Figure 6).

HMGB1 siRNA inhibited HSC-T6 cells proliferation

The trypan blue dye test showed that there were no significant differences in the number of cells in the three-groups 2 d after transfection ($P > 0.05$), but the proliferation in the shRNAH3 group was less than that in the NC group and non-transfection group (Mock group) 3, 4 and 5 d after transfection ($P < 0.05$, Figure 7). A cell cycle study also indicated that cells were arrested in the G0/G1 phase and that the proportion of cells in the S phase was significantly reduced after downregulation of HMGB1 in HSCs (Table 2).

DISCUSSION

Liver fibrosis is highly associated with chronic hepatocellular injury and the subsequent inflammatory response that produces inflammatory cytokines and recruits inflammatory leukocytes to the injured site. This inflammatory circumstance in the liver drives the activation of HSCs through various fibrogenic mediators[12,13]. Activated HSCs transdifferentiate into myofibroblasts, which then produce excessive amounts of ECM proteins, including collagen types Ⅰ, Ⅲ and Ⅳ. This leads to irreversible collagen deposition, resulting in liver fibrosis[12,13]. Many studies have suggested that enhancement of matrix degradation may prove particularly valuable in response to injury caused by matrix deposition[14-17]. Some studies have shown that HMGB1 can stimulate proinflammatory cytokine synthesis and directly stimulate fibroblast proliferation and participate in fibrogenesis[8-10].

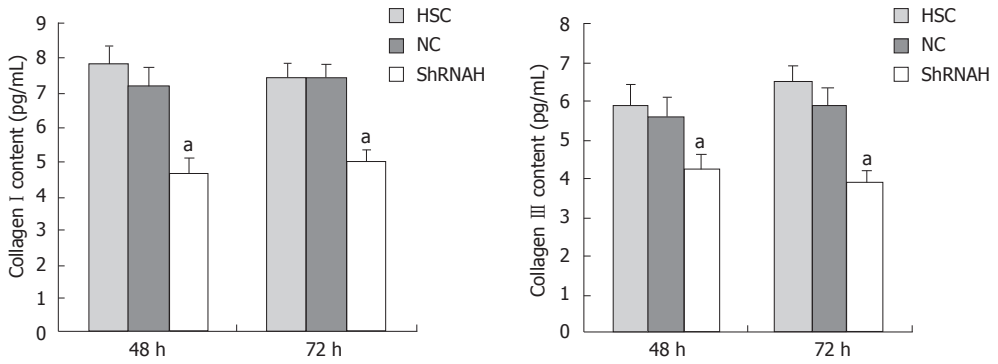


Figure 6 Determination of content of collagen types I and III after shRNAH3 transfection. Enzyme-linked immunosorbent assays were used for quantitative determination of collagen types I and III content hepatic stellate cells (HSCs) culture supernatant at 48 and 72 h after shRNAH3 transfection using Lipofectamine 2000. Values are presented as mean ± SD. $^{a}P < 0.05$ *vs* negative controls (NC) and HSC group.

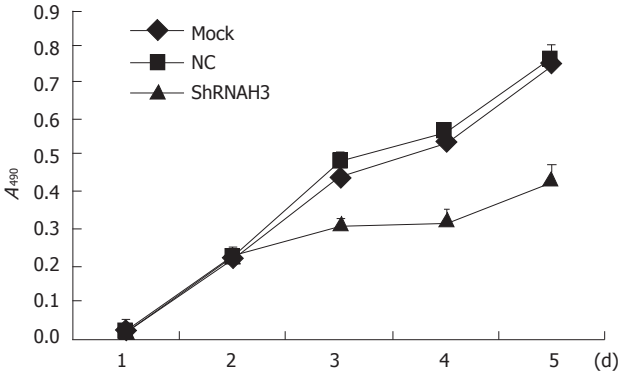


Figure 7 High-mobility group box 1 siRNA suppressed hepatic stellate cell-T6 proliferation. Cell growth curves of hepatic stellate cell-T6 transfected with shRNAH3 were analyzed by methyl thiazolyl tetrazolium conversion. Each sample was tested in triplicate and error bars were included. Compared with negative controls (NC) group and non-transfection group, proliferation of shRNAH3 group was less at the 3-5 d after transfection ($P < 0.05$).

Increased expression of HMGB1 has been reported in several liver diseases, including Con A-induced hepatitis[18], hepatic ischemia[2], and orthotopic liver transplantation (OLT)[19]. In the present study, we evaluated HMGB1 expression in the DMN rat model. We found that the level of HMGB1 was upregulated during DMN injection. Moreover, the expression of HMGB1 was closely correlated with the expression of collagen types I and III and was mainly localized to the nonparenchymal cells, especially HSCs. These results suggest that HMGB1 is involved in hepatic fibrogenesis and may play a critical role in the reversal process of liver fibrosis.

HMGB1 was originally identified as a nuclear nonhistone protein with DNA-binding domains and was implicated as an important endogenous danger signaling molecule. Although predominantly located in the nucleus of quiescent cells, HMGB1 can be actively secreted in response to exogenous and endogenous inflammatory stimuli such as endotoxin, TNF-α, IL-1, and interferon-γ[20,21]. In addition, extracellular HMGB1 mediates a wide range of inflammatory responses and promotes cell proliferation, migration, and differentiation[10,22]. The cytoplasmic localization of HMGB1 in our study may suggest that HMGB1 plays extra nuclear roles in liver fibrosis and that HSC-T6 cells may even secrete HMGB1 to promote extracellular functions. The subcellular location of HMGB1 in monocytic cells is known to be dependent on the acetylation status of the nuclear localization signal (NLS) of the HMGB1 protein[23]. Inflammatory signals promote acetylation of the NLS, leading to cytoplasmic accumulation of HMGB1 in secretory lysosomes in the monocytic cells[24]. These secretory lysosomes are subsequently exocytosed when the monocytic cells are triggered by a second inflammatory stimulus. Whether the subcellular location of HMGB1 in HSC-T6 cells is regulated in a similar way remains to be investigated.

It has become apparent in recent years that HMGB1 is instrumental in mediating a response to tissue damage and infection. HMGB1 released from necrotic or damaged cells not only triggers inflammation as a nonspecific proinflammatory cytokine but also triggers the adaptive immune response[25,26]. Extracellular HMGB1 functions as a damage-associated molecular pattern molecule and activates proinflammatory signaling pathways by activating pattern-recognition receptors including toll-like receptor 4 (TLR4) and the receptor for advanced glycation end-products (RAGE)[27,28]. A previous report showed that RAGE expression in fibrotic livers is restricted to HSCs; its expression is up regulated during cellular activation and transition to myofibroblasts[29], strongly suggesting that HMGB1 is involved in the pathogenesis of liver fibrosis. TLR4 has been suggested to be a receptor for extracellular HMGB1[30,31], and previous studies have indicated that the interaction of HMGB1 with TLR4 plays a critical role in hepatic fibrosis[32]. To date, little has been reported about the pathogenic interactions between HMGB1 and HSCs in terms of profibrogenic propensity. Kao provided evidence that HMGB1 up regulates α-SMA expression and suppresses the activity of the collagen-degrading enzyme matrix metalloproteinase-2[33]. That study also implied that HMGB1, once it is released during rejection of OLT, activates HSCs and exhibits profibrogenic effects either by increasing the HSC population and ECM deposition

in liver grafts or by transforming HSCs into myofibroblasts. In contrast, neutralization with an anti-HMGB1 antibody may be a therapeutic modality to prevent fibrogenesis in post-OLT liver grafts[33].

siRNA has become a powerful tool for functional genetic studies and gene therapy in mammals[34,35]. Although gene knockdown by siRNA is highly effective, the off-target effect of siRNA may represent a major obstacle for therapeutic applications. However, the potential off-target effects could be minimized by choosing an siRNA with maximal sequence divergence from the list of genes with partial sequence identity to the intended mRNA target[36]. Software was used to choose a maximal sequence identity of HMGB1 siRNA, and three siRNA sequences were designed. In preliminary experiments, we identified the fact that shRNAH3 had certain interference effects. Our results show that this sequence was more efficient in reducing the HMGB1 transcript levels.

In the present study, we found that after HMGB1 was downregulated in HSCs by siRNA, there was an inhibitory effect on the mRNA levels of α-SMA and collagen types Ⅰ and Ⅲ, suggesting that inhibition of HMGB1 could directly result in suppression of HSC activation and collagen production. We also discovered that HMGB1 siRNA prohibited HSC proliferation, and a cell cycle analysis revealed that downregulation of HMGB1 arrested cells at the G0/G1 phase, which confirmed the effect of HMGB1 on cell proliferation; however, the definitive mechanism responsible is still uncertain because HMGB1 is multifunctional and has multiple molecular interactions.

In conclusion, HMGB1 was upregulated during liver fibrogenesis, and downregulating HMGB1 expression in HSCs by siRNA prohibited the activity of HSCs and collagen synthesis and enhanced collagen degradation. The results of our study indicate a significant functional role for HMGB1 in the development of liver fibrosis, and downregulating HMGB1 expression with siRNA could be an effective way to treat liver fibrosis.

COMMENTS

Background

Hepatic fibrosis is a response to injury in the liver. It is characterized by both a quantitative and qualitative change in the extracellular matrix (ECM). The activated hepatic stellate cell (HSC) is primarily responsible for excessive ECM deposition during liver fibrosis. It has been shown that high-mobility group box 1 (HMGB1) expression is up regulated during myofibroblast cellular activation and involved in the pathogenesis of hepatic fibrosis. This suggests that HMGB1 is a promising molecular target for hepatic fibrosis gene therapy. Inhibition of abnormal expression of HMGB1 may be an effective strategy for biological therapy of hepatic fibrosis.

Research frontiers

HMGB1 is a major component of mammalian chromatin endowed with an architectural function. Increasing evidence now points to multiple functions of HMGB1 in infection, tissue injury, inflammation, apoptosis and the immune response. It has been reported in several liver diseases, including hepatitis, hepatic ischemia, and orthotopic liver transplantation. HMGB1 has been implicated in the pathogenesis of several liver diseases, including Con-A-induced hepatitis, hepatic ischemia, and orthotopic liver transplantation. However, the role of HMGB1 and how to inhibit its expressiong in hepatic fibrosis has yet to be fully elucidated. In this study, the authors demonstrate that the overexpression of HMGB1 could be a potential mechanism for mediating collagen expression and downregulating HMGB1 expression might present as a potential strategy to treat liver fibrosis.

Innovations and breakthroughs

Studies of targeting *in vitro* and *in vivo* over expressed genes in hepatic fibrosis by RNA interference, including transforming growth factor-β, connective tissue growth factor and p90RSK, have been reported. However, there has been still no report about targeting HMGB1 by siRNA in hepatic fibrosis. In the present study, the authors used siRNA approach to block HMGB1 expression in HSC-T6 cells, to determine the role of constitutively activated HMGB1 during hepatic fibrosis pathogenesis, and to explore the role and molecular mechanism of targeting HMGB1 in hepatic fibrosis therapy.

Applications

By investigating the effect of silencing HMGB1 expression by siRNA on the collagen synthesis and proliferation of HSC-T6 cells, this study may provides a new strategy for biological therapy of liver fibrosis by targeting HMGB1.

Terminology

HMGB1 was originally identified as a nuclear nonhistone protein with DNA-binding domains and implicated as an important endogenous danger signaling molecule. But it can also be secreted from cells and exert extracellular functions as a proinflammatory cytokine. HSCs are a minor and quiescent cell type in the liver that usually reside in the space of Disse, but which undergo activation after hepatic injury to produce large quantities of fibrillar collagens.

Peer review

The authors demonstrated the increase of HMGB1 expression in fibrotic livers. Then, they investigated the effect of HMGB1 silencing by siRNA on stellate cell activation and proliferation. The results show that siRNA for HMGB1 significantly inhibits collagen expression and stellate cell proliferation.

REFERENCES

1 **Moreira RK**. Hepatic stellate cells and liver fibrosis. *Arch Pathol Lab Med* 2007; **131**: 1728-1734

2 **Tsung A**, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005; **201**: 1135-1143

3 **Wang H**, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999; **285**: 248-251

4 **Hamada N**, Maeyama T, Kawaguchi T, Yoshimi M, Fukumoto J, Yamada M, Yamada S, Kuwano K, Nakanishi Y. The role of high mobility group box1 in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2008; **39**: 440-447

5 **Ilmakunnas M**, Tukiainen EM, Rouhiainen A, Rauvala H, Arola J, Nordin A, Mäkisalo H, Höckerstedt K, Isoniemi H. High mobility group box 1 protein as a marker of hepatocellular injury in human liver transplantation. *Liver Transpl* 2008; **14**: 1517-1525

6 **Scaffidi P**, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002; **418**: 191-195

7 **Ito I**, Fukazawa J, Yoshida M. Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. *J Biol Chem* 2007; **282**: 16336-16344

8 **Andersson U**, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H, Janson A, Kokkola R, Zhang M, Yang H, Tracey KJ. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J Exp Med* 2000; **192**: 565-570

9 **Raucci A**, Palumbo R, Bianchi ME. HMGB1: a signal of necrosis. *Autoimmunity* 2007; **40**: 285-289

10 **Mitola S**, Belleri M, Urbinati C, Coltrini D, Sparatore B, Pedrazzi M, Melloni E, Presta M. Cutting edge: extracellular high mobility group box-1 protein is a proangiogenic cyto-

kine. *J Immunol* 2006; **176**: 12-15
11 **Chen SW**, Chen YX, Zhang XR, Qian H, Chen WZ, Xie WF. Targeted inhibition of platelet-derived growth factor receptor-beta subunit in hepatic stellate cells ameliorates hepatic fibrosis in rats. *Gene Ther* 2008; **15**: 1424-1435
12 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218
13 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669
14 **Hu YB**, Li DG, Lu HM. Modified synthetic siRNA targeting tissue inhibitor of metalloproteinase-2 inhibits hepatic fibrogenesis in rats. *J Gene Med* 2007; **9**: 217-229
15 **Uchinami H**, Seki E, Brenner DA, D'Armiento J. Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. *Hepatology* 2006; **44**: 420-429
16 **Roderfeld M**, Weiskirchen R, Wagner S, Berres ML, Henkel C, Grötzinger J, Gressner AM, Matern S, Roeb E. Inhibition of hepatic fibrogenesis by matrix metalloproteinase-9 mutants in mice. *FASEB J* 2006; **20**: 444-454
17 **González-Cuevas J**, Bueno-Topete M, Armendariz-Borunda J. Urokinase plasminogen activator stimulates function of active forms of stromelysin and gelatinases (MMP-2 and MMP-9) in cirrhotic tissue. *J Gastroenterol Hepatol* 2006; **21**: 1544-1554
18 **Gong Q**, Zhang H, Li JH, Duan LH, Zhong S, Kong XL, Zheng F, Tan Z, Xiong P, Chen G, Fang M, Gong FL. High-mobility group box 1 exacerbates concanavalin A-induced hepatic injury in mice. *J Mol Med* (Berl) 2010; **88**: 1289-1298
19 **Nakano T**, Goto S, Lai CY, Hsu LW, Kao YH, Lin YC, Kawamoto S, Chiang KC, Ohmori N, Goto T, Sato S, Jawan B, Cheng YF, Ono K, Chen CL. Experimental and clinical significance of antinuclear antibodies in liver transplantation. *Transplantation* 2007; **83**: 1122-1125
20 **Rendon-Mitchell B**, Ochani M, Li J, Han J, Wang H, Yang H, Susarla S, Czura C, Mitchell RA, Chen G, Sama AE, Tracey KJ, Wang H. IFN-gamma induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. *J Immunol* 2003; **170**: 3890-3897
21 **Wang H**, Vishnubhakat JM, Bloom O, Zhang M, Ombrellino M, Sama A, Tracey KJ. Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. *Surgery* 1999; **126**: 389-392
22 **Palumbo R**, Sampaolesi M, De Marchis F, Tonlorenzi R, Colombetti S, Mondino A, Cossu G, Bianchi ME. Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J Cell Biol* 2004; **164**: 441-449
23 **Bonaldi T**, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, Rubartelli A, Agresti A, Bianchi ME. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J* 2003; **22**: 5551-5560
24 **Gardella S**, Andrei C, Ferrera D, Lotti LV, Torrisi MR, Bianchi ME, Rubartelli A. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep* 2002; **3**: 995-1001
25 **Dumitriu IE**, Baruah P, Valentinis B, Voll RE, Herrmann M, Nawroth PP, Arnold B, Bianchi ME, Manfredi AA, Rovere-Querini P. Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. *J Immunol* 2005; **174**: 7506-7515
26 **Yang D**, Chen Q, Yang H, Tracey KJ, Bustin M, Oppenheim JJ. High mobility group box-1 protein induces the migration and activation of human dendritic cells and acts as an alarmin. *J Leukoc Biol* 2007; **81**: 59-66
27 **Hori O**, Brett J, Slattery T, Cao R, Zhang J, Chen JX, Nagashima M, Lundh ER, Vijay S, Nitecki D. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. *J Biol Chem* 1995; **270**: 25752-25761
28 **Park JS**, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* 2004; **279**: 7370-7377
29 **Fehrenbach H**, Weiskirchen R, Kasper M, Gressner AM. Up-regulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. *Hepatology* 2001; **34**: 943-952
30 **Yang H**, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, Li J, Lu B, Chavan S, Rosas-Ballina M, Al-Abed Y, Akira S, Bierhaus A, Erlandsson-Harris H, Andersson U, Tracey KJ. A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. *Proc Natl Acad Sci USA* 2010; **107**: 11942-11947
31 **Han J**, Zhong J, Wei W, Wang Y, Huang Y, Yang P, Purohit S, Dong Z, Wang MH, She JX, Gong F, Stern DM, Wang CY. Extracellular high-mobility group box 1 acts as an innate immune mediator to enhance autoimmune progression and diabetes onset in NOD mice. *Diabetes* 2008; **57**: 2118-2127
32 **Tsung A**, Klune JR, Zhang X, Jeyabalan G, Cao Z, Peng X, Stolz DB, Geller DA, Rosengart MR, Billiar TR. HMGB1 release induced by liver ischemia involves Toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling. *J Exp Med* 2007; **204**: 2913-2923
33 **Kao YH**, Jawan B, Goto S, Hung CT, Lin YC, Nakano T, Hsu LW, Lai CY, Tai MH, Chen CL. High-mobility group box 1 protein activates hepatic stellate cells in vitro. *Transplant Proc* 2008; **40**: 2704-2705
34 **Jackson AL**, Burchard J, Schelter J, Chau BN, Cleary M, Lim L, Linsley PS. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *RNA* 2006; **12**: 1179-1187
35 **Tschuch C**, Schulz A, Pscherer A, Werft W, Benner A, Hotz-Wagenblatt A, Barrionuevo LS, Lichter P, Mertens D. Off-target effects of siRNA specific for GFP. *BMC Mol Biol* 2008; **9**: 60
36 **De Paula D**, Bentley MV, Mahato RI. Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. *RNA* 2007; **13**: 431-456

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4099

World J Gastroenterol 2011 September 28; 17(36): 4099-4103
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Antioxidative potential of a combined therapy of anti TNFα and Zn acetate in experimental colitis

Michela Barollo, Valentina Medici, Renata D'Incà, Antara Banerjee, Giuseppe Ingravallo, Marco Scarpa, Surajit Patak, Cesare Ruffolo, Romilda Cardin, Giacomo Carlo Sturniolo

Michela Barollo, Renata D'Incà, Antara Banerjee, Surajit Patak, Cesare Ruffolo, Romilda Cardin, Giacomo Carlo Sturniolo, Department of Surgical and Gastroenterological Sciences, University of Padova, Padova 35128, Italy
Valentina Medici, Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of California, Davis, CA 95616, United States
Giuseppe Ingravallo, Department of Pathology, University of Bari, Bari 70121, Italy
Marco Scarpa, Department of Oncological Surgery, Veneto Oncological Institute, Padova 35128, Italy
Author contributions: Barollo M and Medici V designed the study and wrote the manuscript; Scarpa M and Ruffolo C performed the experiments and the statistical analysis; Banerjee A, Patak S revised the manuscript; D'Incà R and Sturniolo GC designed the study and revised the manuscript; Cardin R performed the biochemical analysis; Ingravallo G carried out the histological analysis.
Supported by MIUR 40% University of Padova
Correspondence to: Giacomo Carlo Sturniolo, MD, Professor of Gastroenterology, Department of Gastroenterology, Via Giustiniani 2, Padova 35128, Italy. gc.sturniolo@unipd.it
Telephone: +39-49-8218726 Fax: +39-49-8760820
Received: December 15, 2010 Revised: February 19, 2011
Accepted: February 26, 2011
Published online: September 28, 2011

Abstract

AIM: To evaluate whether combination therapy with anti-tumour necrosis factor α (TNFα) antibody and Zn acetate is beneficial in dextran sodium sulphate (DSS) colitis.

METHODS: Colitis was induced in CD1-Swiss mice with 5% DSS for 7 d. The experimental mice were then randomised into the following subgroups: standard diet + DSS treated (induced colitis group); standard diet + DSS + subcutaneous 25 μg anti-TNFα treated group; Zn acetate treated group + DSS + subcutaneous 25 μg anti-TNFα; standard diet + DSS + subcutaneous 6.25 μg anti-TNFα treated group and Zn acetate treated group + DSS + subcutaneous 6.25 μg anti-TNFα. Each group of mice was matched with a similar group of sham control animals. Macroscopic and histological features were scored blindly. Homogenates of the colonic mucosa were assessed for myeloperoxidase activity as a biochemical marker of inflammation and DNA adducts (8OH-dG) as a measure of oxidative damage.

RESULTS: DSS produced submucosal erosions, ulcers, inflammatory cell infiltration and cryptic abscesses which were reduced in both groups of mice receiving either anti-TNFα alone or combined with zinc. The effect was more pronounced in the latter group (*vs* Zn diet, $P < 0.02$). Myeloperoxidase activity (*vs* controls, $P < 0.02$) and DNA adducts, greatly elevated in the DSS fed colitis group (*vs* controls, $P < 0.05$), were significantly reduced in the treated groups, with a more remarkable effect in the group receiving combined therapy (*vs* standard diet, $P < 0.04$).

CONCLUSION: DSS induces colonic inflammation which is modulated by the administration of anti-TNFα. Combining anti-TNFα with Zn acetate offers marginal benefit in colitis severity.

Key words: Anti tumor necrosis factor α; Experimental colitis; Inflammatory bowel disease; Oxidative damage; Zinc

Peer reviewers: Dr. Tamara Vorobjova, MD, PhD, Scimed. Senior Researcher in Immunology, Department of Immunology, Institute of General and Molecular Pathology, University of Tartu, Ravila, 19, Tartu 51014, Estonia; Jay Pravda, MD, Inflammatory Disease Research Center, West Palm Beach, FL 33420, United States

Scarpa M, Patak S, Ruffolo C, Cardin R, Sturniolo GC. Anti-oxidative potential of a combined therapy of anti TNFα and Zn acetate in experimental colitis. *World J Gastroenterol* 2011; 17(36): 4099-4103 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4099.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4099

INTRODUCTION

Ulcerative colitis and Crohn's disease are chronic diseases of the gastrointestinal tract characterized by activation of the immune system with production of several inflammatory cytokines[1,2]. Altered T cell apoptosis[3,4] and abnormal production of the pro-inflammatory cytokine tumour necrosis factor α (TNFα) play a central role in intestinal inflammation of inflammatory bowel disease patients[5].

Novel treatment strategies based on the inhibition of TNFα have shown to be effective both in experimental models of colitis[6] and in inducing and maintaining remission in humans affected with inflammatory bowel disease[7]. However, as these therapies are very expensive they may represent an important and unaffordable economic burden in the near future.

Trace element metabolism is altered during inflammatory processes of the gastrointestinal tract. Zinc is essential for intestinal homeostasis, since there are several zinc-dependent antioxidant enzymes such as superoxide dismutase which converts superoxide to hydrogen peroxide and metallothionein which can neutralize free radical production. Moreover, zinc status affects gene expression of the inflammatory cytokines TNF, IL-1B and IL-8. Zinc deficiency causes functional defects in T cells, neutrophils and macrophages, and positive modulatory responses are produced following zinc supplementation[8]. In the model of acetic acid-induced ulcerations, zinc reduced mucosal damage[9]. In models of experimental colitis both oral and topical zinc treatment were found to decrease intestinal inflammation, to favour mucosal healing and to improve immune function[10], We therefore thought that zinc may be useful if added to conventional anti-TNFα therapy in modulating the symptoms of dextran sodium sulphate (DSS)-induced colitis in mice and in decreasing oxidative stress.

MATERIALS AND METHODS

Animals

Male CD1 Swiss mice, 4 wk old, weighing 20-25 g purchased from Charles River (Calco, Italy) were used in this study. The animals were kept in plastic platform cages in a temperature controlled room (22 ℃) under a 12-h light-dark cycle, with free access to water and standard chow containing 125 mg/kg zinc oxide. The experimental protocol was approved by the Veterinary and Health Committee of the University of Padua.

Experimental protocol

Mice were fed 5% DSS (5% dextran sulphate solution purchased from ICN Pharmaceuticals, SRL, Italy) dissolved in drinking water in one single cycle to induce acute colitis. The cycle consisted of administering 5% DSS for 7 d which caused loose stools in all animals and the presence of gross rectal bleeding in about 50% of the animals.

The animals were randomised into the following six groups each with 6 mice: (1) healthy untreated mice receiving standard diet; (2) induced colitis group, i.e., mice receiving standard diet + 5% DSS for 7 d; (3) mice receiving standard diet + 675 mg/kg Zn acetate supplement starting 7 d before induction of colitis; (4) mice receiving standard diet + 25 μg anti-TNFα intraperitoneally after 1 wk of DSS administration; (5) mice receiving standard diet + 675 mg/kg Zn acetate supplement + 25 μg anti-TNFα intraperitoneally after 1 wk of DSS administration; and (6) mice receiving standard diet + 675 mg/kg Zn acetate supplement + 6.25 μg anti-TNFα intraperitoneally after 1 wk of DSS administration. The three groups receiving anti-TNFα treatment were sacrificed 48 h after initiation of treatment. Anti-TNFα monoclonal antibody (rat anti-mouse TNFα) was purchased from Biosource International Inc. (United States) and Zn Acetate 675 mg/kg diet, from Mucedola SRL, (Milano, Italy).

Macroscopic and histologic features of colitis

Damage was assessed macroscopically by scoring the number and extent of ulcers, adhesions, and thickness of the colonic wall[11] and histologically by scoring cryptitis, crypt abscesses and epithelial injury. Colonic tissue samples were obtained and processed for myeloperoxidase and 8-hydroxydeoxyguanosine (8-OHdG) in order to quantify inflammation and DNA damage.

Colonic samples were immediately fixed in buffered formalin (10%). After fixation, the specimens were routinely processed and embedded in paraffin. Serial histology sections of 4 μm thickness were obtained from each paraffin block and mounted on poly-L-lysine coated slides. Sections were stained with haematoxylin-eosin and examined blindly.

Cryptitis was defined as the presence of polymorphonuclear cells within crypt epithelium, while crypt abscesses were defined as the presence of polymorphonuclear cells within the crypt lumens. Epithelial injury included changes such as crypt regeneration, mucodepletion, cuboidal shape, nuclear enlargement, loss of surface cells, erosion, and ulceration. Each of the features, defined above, was scored on a 0 to 3+ scale based on the severity and degree of involvement[12,13].

Mean colonic activity scores for cryptitis, crypt abscesses and epithelial injury were marked for each slide on the following basis: 0 (no activity); 1-2 (mild activity); 3-4 (moderate activity); 5-6 (severe activity).

Assessment of myeloperoxidase activity

Assessment of myeloperoxidase (MPO) activity was as-

Table 1 Biochemical and morphological parameters of colitis severity among the study groups

	Macroscopic score	Colonic activity index	Myeloperoxidase activity (U/g)
Controls	0 (0-0)	0 (0-0)	1.9 (1.34-1.1)
Colitis	1 (1-1)[b]	4 (1-1)[a]	5.69 (0.04-0.21)[b]
Colitis + Zinc	2 (2-2)[b]	5 (0-0)[a]	7.8 (0-0.8)[e]
Colitis + anti-TNFα (25 μg)	0.5 (0-1)	4 (1-3)[a]	4.85 (1.81-3)[d]
Colitis + Zinc + anti-TNFα (25 μg)	0 (0-0)[c]	1 (2-0)[a,d]	3.88 (2.9-2.87)
Colitis + anti-TNFα (6.25 μg)	1 (0.25-1)	5 (0-2)	5.44 (0.63-0)
Colitis + Zinc + anti-TNFα (6.25 μg)	0.5 (0-1)	3 (2-0)	4.42 (0.34-0.33)

[a]$P < 0.01$ *vs* controls; [b]$P < 0.03$ *vs* controls; [c]$P < 0.03$ *vs* colitis; [d]$P < 0.02$ *vs* colitis+Zinc; [e]$P < 0.02$ *vs* controls. TNF: Tumour necrosis factor.

sessed according to the method previously described[14]. Briefly, colonic tissue samples were minced in 1 mL of 50 mmol/L potassium phosphate buffer (pH = 6.0) containing 14 mmol/L hexadecyltrimethylammonium bromide (Fluka), homogenized and sonicated. The lysates were frozen and thawed thrice, then centrifuged for 2 min in cold at 1 5000 *g*. Aliquots of the supernatants were mixed with potassium phosphate buffer containing o-dianisidine-HCl (Sigma-Aldrich, St. Louis, MO, United States) and 0.0005% H_2O_2. MPO activity was expressed as units/g of wet tissue. The enzyme unit was defined as the conversion of 1 mol of H_2O_2 per min at 25.

8-OHdG determination

Oxidative DNA damage was assessed following previously described methods[15]. Briefly, colonic biopsy specimens were thawed, homogenized in a separation buffer and approximately 20 μg of purified DNA per sample was injected into the HPLC system (Shimadzu, Kyoto, Japan). The 8-OHdG was detected using an electrochemical detector (ESA Coulochem Ⅱ 5200A, Bedford, MA, Untied States). The levels of 8-OHdG were expressed as the number of 8-OHdG adducts per 10^5 dG bases. The coefficient of variation was < 10%; 100 μg of DNA were required for the determination.

Statistical analysis

Data are expressed as median (interquartile range). Statistical data were analyzed with Mann-Whitney *U* test for comparison of the groups and Spearman's rank correlation test. *P* values less than 0.05 were considered significant.

RESULTS

Macroscopic evaluation of colitis

The macroscopic score was increased significantly in untreated colitic mice. Groups treated with anti-TNFα or anti-TNFα and zinc acetate showed a decreased macroscopic score which was more evident in the combined diet. Chronic feeding of DSS significantly increased the colonic activity score. The administration of anti-TNFα alone or combined with zinc acetate significantly reduced this index. The effect appeared to be significantly more evident in the group receiving anti-TNFα and zinc acetate than in the group receiving anti-TNFα alone. The administration of a reduced dose of anti-TNFα (6.25 μg) was effective only if combined with zinc acetate (Table 1).

Myeloperoxidase activity

Myeloperoxidase activity was increased in all colitic mice. However, there was a significant reduction in this activity in the groups treated with anti-TNFα alone and anti-TNFα + Zn supplementation, with a slightly better effect in the group receiving the combination therapy. A lower dose of anti-TNFα was associated with reduced MPO activity only in the group receiving both zinc and anti-TNFα (Table 1).

Determination of oxidative damage as measured by 8-OHdG mucosal levels

Oxidative damage was significantly increased in colitic mice. Anti-TNFα significantly reduced DNA adducts, OH-dG levels were similar in the group receiving both anti-TNFα and zinc acetate (Figure 1). Anti-TNFα treatment significantly reduced DNA adducts at both doses used. In both groups receiving the combination therapy, DNA adducts were reduced compared to anti-TNFα therapy alone, but no significant effect was demonstrated with respect to the groups receiving anti-TNFα alone (Figure 1).

DISCUSSION

Chemically induced models of intestinal inflammation are widely used as surrogate models of chronic inflammatory bowel disease and oral DSS administration effectively resembles human inflammatory bowel disease with similar clinical features (bloody diarrhoea) and endoscopic/histological findings (ulcerations and neutrophil infiltration). DSS is believed to be directly toxic to gut epithelial cells of the basal crypts and affects the integrity of the mucosal barrier.

Zinc metabolism has been reported to be reduced in about 65% of patients with Crohn's disease. In an experimental model of colitis we also reported that zinc supplementation induced metallothionein expression, while having little effect on the short-term course of colitis[16]. Zinc has several potential mechanisms of action which can benefit the inflammatory process. It regulated tight junction permeability in an experimental model of colitis[17] and in Crohn disease[18]. Sturniolo *et al*[19] reported

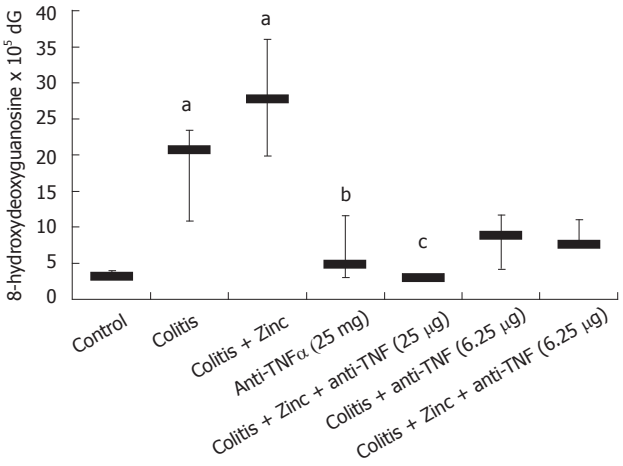


Figure 1 8-hydroxydeoxyguanosine. [a]*P* < 0.05 *vs* controls; [b]*P* < 0.02 *vs* colitis; [c]*P* < 0.04 *vs* colitis. TNF: Tumour necrosis factor.

that zinc sulphate enemas exert an anti-inflammatory action on experimental colitis.

In the last few years, biological therapies have changed the pharmacological armamentarium of inflammatory bowel disease therapy: the first and still most widely used drug is the anti-TNFα monoclonal antibody, infliximab[20]. Even in experimental models of colitis, the subcutaneous administration of infliximab reduced the inflammatory activity as well as tissue TNFα concentration[21]. Our experimental approach which added zinc acetate to the diet while administering anti-TNFα monoclonal antibody, aimed to examine the effects on DSS-induced colitis in mice.

The mucosa did not show complete healing probably because the treatment effects were evaluated 48 h after treatment. Nevertheless, therapy with anti-TNFα ameliorated the macroscopic and histological aspects and decreased myeloperoxidase concentration. Similar results were reported by Videla *et al*[22] who found that anti-TNFα significantly reduced the release of inflammatory mediators and induced histopathological remission in a model of experimental colitis. Zinc alone had little effect in ameliorating the severity of acute colitis induced by intra-rectal instillation of dinitrobenzene-sulphonic acid in rats, even though Tran *et al*[23] and Luk *et al*[24] recently reported some therapeutic effects of zinc supplementation in DSS-induced colitis in mice.

Zinc supplementation alone worsened the histopathological and biochemical aspects of colitis compared to colitis alone and this can be explained by the fact that superoxide dismutase by itself is a pro-oxidant enzyme by virtue of its ability to generate hydrogen peroxide[25,26]. This may explain why a worsening of colitis was recorded when zinc was added alone. However, in our study zinc allowed us to reduce the dose of anti-TNFα maintaining the same biochemical and morphological effects.

Acute colitis is characterised by an increased production of free radicals which contribute to protein, DNA chain and lipid damage. As the antioxidant potential of colonic epithelial cells is quite low, this results in tissue injury[27]. The administration of antioxidants thus has the potential to improve the outcome of experimental colitis by scavenging free radicals. In our experimental conditions, the oxidative damage, expressed by DNA adducts was significantly reduced in the groups treated with anti-TNFα confirming the findings of Popivanova *et al*[28]. In our study, the effect of anti-TNFα on oxidative stress appeared to be dose-dependent with the highest dose having the strongest effect in reducing oxidative damage, and the combination of anti-TNFα and zinc supplementation added little effect.

Obermeier *et al*[29] reported that excess nitric oxide formation occurs in experimental colitis and can be decreased by treatment with rat anti-mouse TNF and interferon gamma monoclonal antibodies. In several studies, zinc supplementation ameliorated antioxidant concentrations thus reducing the production of oxidative species[27-30]. In the present study, zinc supplementation allowed a reduction in the dose of anti-TNFα antibody, while maintaining the same level of reduced intestinal inflammation observed with a higher dose of anti-TNFα antibody alone, as quantified by the four parameters of tissue inflammation utilized in the study. This effect is in accordance with the described capability of zinc to increase antioxidant concentration and reduce oxidative species.

In conclusion, the combined administration of zinc acetate in the diet along with the systemic administration of anti-TNFα had a positive effect in reducing the severity of DSS-induced colitis in mice, with reduced production of DNA adducts. Moreover, the same effect was demonstrated with the reduced anti-TNFα dose combined with zinc. This experimental approach offers the advantage of reducing the potential side effects of anti-TNFα and costs, while ameliorating oxidative stress and inflammation in patients with inflammatory bowel disease.

COMMENTS

Background

Dextran sodium sulphate (DSS) colitis is a well-known model of inflammatory bowel disease in which the authors tested the effect of the well-known drug anti-tumour necrosis factor α (TNFα) combined with zinc with the aim of evaluating the possibility of lowering the dose of anti-TNFα.

Research frontiers

In this article the authors explore the possibility of a combination therapy in inflammatory bowel disease (IBD) in order to reduce potential side effects and costs.

Innovations and breakthroughs

In this article, zinc was added to a biological therapy in order to evaluate the effects of this combination therapy. There are no articles in the literature exploring this combination therapy.

Applications

The potential applications include the possibility of adding zinc to anti-TNFα therapy. Moreover, future perspectives include the application of other combination therapies in inflammatory bowel disease.

Peer review

This study considers the investigation of the role of combined administration of Zinc acetate in the diet with systemic administration of anti TNF alfa on the effect of the severity of experimental colitis in mice induced by DSS. The study is set up correctly. The paper is written sufficiently good, the Introduction give a good overview about the study background and the authors raised clearly the hypoth-

esis of the study. The description of methods used is accurate. The results are presented clearly and have been discussed well, the table and figure give good overview about the results.

REFERENCES

1 **Torres MI**, Rios A. Current view of the immunopathogenesis in inflammatory bowel disease and its implications for therapy. *World J Gastroenterol* 2008; **14**: 1972-1980
2 **Sanchez-Munoz F**, Dominguez-Lopez A, Yamamoto-Furusho JK. **Role of cytokines in inflammatory bowel disease.** *World J Gastroenterol* 2008; **14**: 4280-4288
3 **Nielsen OH**, Vainer B, Madsen SM, Seidelin JB, Heegaard NH. Established and emerging biological activity markers of inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 359-367
4 **Shanahan F**. Crohn's disease. *Lancet* 2000; **359:** 62-69
5 **Papadakis KA**, Targan SR. Tumor necrosis factor: biology and therapeutic inhibitors. *Gastroenterology* 2000; **119**: 1148-1157
6 **Worledge KL**, Godiska R, Barrett TA, Kink JA. Oral administration of avian tumor necrosis factor antibodies effectively treats experimental colitis in rats. *Dig Dis Sci* 2000; **45**: 2298-2305
7 **Akobeng AK**, Zachos M. Tumor necrosis factor-alpha antibody for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2004; CD003574
8 **Filippi J**, Al-Jaouni R, Wiroth JB, Hébuterne X, Schneider SM. Nutritional deficiencies in patients with Crohn's disease in remission. *Inflamm Bowel Dis* 2006; **12**: 185-191
9 **Troskot B**, Simicevic VN, Dodig M, Rotkvic I, Ivankovic D, Duvnjak M. The protective effect of zinc sulphate pretreatment against duodenal ulcers in the rat. *Biometals* 1997; **10**: 325-329
10 **Bucci I**, Napolitano G, Giuliani C, Lio S, Minnucci A, Di Giacomo F, Calabrese G, Sabatino G, Palka G, Monaco F. Zinc sulfate supplementation improves thyroid function in hypozincemic Down children. *Biol Trace Elem Res* 1999; **67**: 257-268
11 **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
12 **Jenkins D**, Balsitis M, Gallivan S, Dixon MF, Gilmour HM, Shepherd NA, Theodossi A, Williams GT. Guidelines for the initial biopsy diagnosis of suspected chronic idiopathic inflammatory bowel disease. The British Society of Gastroenterology Initiative. *J Clin Pathol* 1997; **50**: 93-105
13 **Tanaka M**, Riddell RH, Saito H, Soma Y, Hidaka H, Kudo H. Morphologic criteria applicable to biopsy specimens for effective distinction of inflammatory bowel disease from other forms of colitis and of Crohn's disease from ulcerative colitis. *Scand J Gastroenterol* 1999; **34**: 55-67
14 **Krawisz JE**, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; **87**: 1344-1350
15 **Helbock HJ**, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC, Ames BN. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc Natl Acad Sci USA* 1998; **95**: 288-293
16 **Di Leo V**, D'Incà R, Barollo M, Tropea A, Fries W, Mazzon E, Irato P, Cecchetto A, Sturniolo GC. **Effect of zinc supplementation on trace elements and intestinal metallothionein concentrations in experimental colitis in the rat.** *Dig Liver Dis* 2001; **33**: 135-139
17 **Sturniolo GC**, Di Leo V, Ferronato A, D'Odorico A, D'Incà R. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis* 2001; **7**: 94-98
18 **Chen BW**, Wang HH, Liu JX, Liu XG. Zinc sulphate solution enema decreases inflammation in experimental colitis in rats. *J Gastroenterol Hepatol* 1999; **14**: 1088-1092
19 **Sturniolo GC**, Fries W, Mazzon E, Di Leo V, Barollo M, D'inca R. Effect of zinc supplementation on intestinal permeability in experimental colitis. *J Lab Clin Med* 2002; **139**: 311-315
20 **Blam ME**, Stein RB, Lichtenstein GR. Integrating anti-tumor necrosis factor therapy in inflammatory bowel disease: current and future perspectives. *Am J Gastroenterol* 2001; **96**: 1977-1997
21 **Triantafillidis JK**, Papalois AE, Parasi A, Anagnostakis E, Burnazos S, Gikas A, Merikas EG, Douzinas E, Karagianni M, Sotiriou H. Favorable response to subcutaneous administration of infliximab in rats with experimental colitis. *World J Gastroenterol* 2005; **11**: 6843-6847
22 **Videla S**, García-Lafuente A, Antolín M, Vilaseca J, Guarner F, Crespo E, González G, Salas A, Malagelada JR. Antitumor necrosis factor therapy in rat chronic granulomatous colitis: critical dose-timing effects on outcome. *J Pharmacol Exp Ther* 1998; **287**: 854-859
23 **Tran CD**, Ball JM, Sundar S, Coyle P, Howarth GS. The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. *Dig Dis Sci* 2007; **52**: 2113-2121
24 **Luk HH,** Ko JK, Fung HS, Cho CH. Delineation of the protective action of zinc sulfate on ulcerative colitis in rats. *Eur J Pharmacol* 2002; **443:** 197-204
25 **Koningsberger JC**, van Asbeck BS, van Faassen E, Wiegman LJ, van Hattum J, van Berge Henegouwen GP, Marx JJ. Copper, zinc-superoxide dismutase and hydrogen peroxide: a hydroxyl radical generating system. *Clin Chim Acta* 1994; **230**: 51-61
26 **Yim MB**, Chock PB, Stadtman ER. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. *Proc Natl Acad Sci USA* 1990; **87**: 5006-5010
27 **Lih-Brody L**, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, Weissman GS, Katz S, Floyd RA, McKinley MJ, Fisher SE, Mullin GE. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci* 1996; **41**: 2078-2086
28 **Popivanova BK**, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 2008; **118**: 560-570
29 **Obermeier F**, Kojouharoff G, Hans W, Schölmerich J, Gross V, Falk W. Interferon-gamma (IFN-gamma)- and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. *Clin Exp Immunol* 1999; **116**: 238-245
30 **Mulder TP**, van der Sluys Veer A, Verspaget HW, Griffioen G, Peña AS, Janssens AR, Lamers CB. Effect of oral zinc supplementation on metallothionein and superoxide dismutase concentrations in patients with inflammatory bowel disease. *J Gastroenterol Hepatol* **1994; 9**: 472-477

S- Editor Sun H **L- Editor** Webster JR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4104

World J Gastroenterol 2011 September 28; 17(36): 4104-4108
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Helicobacter species and gut bacterial DNA in Meckel's diverticulum and the appendix

Peren H Karagin, Unne Stenram, Torkel Wadström, Åsa Ljungh

Peren H Karagin, Torkel Wadström, Åsa Ljungh, Department of Medical Microbiology, Lund University, Sölvegatan 23, SE-223 62 Lund, Sweden
Unne Stenram, Department of Pathology, Lund University, SE-22185 Lund, Sweden
Author contributions: Karagin PH did the all molecular analyses and wrote most of the manuscript; Stenram U did the pathological analyses and wrote part of the manuscript; Stenram U, Ljungh Å, and Wadström T designed the project and helped in writing the manuscript.
Supported by A grant from the University Hospital of Lund (ALF) to Torkel Wadström and a grant from the John Forssman's foundation, the Royal Physiographic Society in Lund to Peren Karagin
Correspondence to: Peren H Karagin, PhD, Department of Medical Microbiology, Lund University, Sölvegatan 23, SE-223 62 Lund, Sweden. perenbaglan@yahoo.com
Telephone: +46-46-173298 Fax: +46-46-189117
Received: February 1, 2011 Revised: March 4, 2011
Accepted: March 11, 2011
Published online: September 28, 2011

Abstract

AIM: To analyse the possible association of various *Helicobacter* species and certain common gut bacteria in patients with Meckel's diverticulum and appendicitis.

METHODS: A nested-polymerase chain reaction (PCR), specific to 16S rRNA of the *Helicobacter* genus, was performed on paraffin embedded samples, 50 with acute appendicitis, 50 normal appendixes, and 33 Meckel's diverticulum with gastric heterotopia and/or ulcer. *Helicobacter* genus positive samples were sequenced for species identification. All samples were also analysed for certain gut bacteria by PCR.

RESULTS: *Helicobacter pullorum* DNA was found in one out of 33 cases and *Enterobacteria* in two cases of Meckel's diverticulum. *Helicobacter pylori* (*H. pylori*) was found in three, *Enterobacter* in 18, and *Bacteroides* in 19 out of 100 appendix samples by PCR. *Enterococcus* was not found in any MD or appendix samples. All *H. pylori* positive cases were from normal appendixes.

CONCLUSION: *Helicobacter* is not an etiological agent in the pathogenesis of symptomatic Meckel's diverticulum or in acute appendicitis.

Key words: Meckel's diverticulum; *Helicobacter*; Appendix; Polymerase chain reaction

Peer reviewers: Tamara Vorobjova, Senior Researcher in Immunology, Department of Immunology, Institute of General and Molecular Pathology, University of Tartu, Ravila, 19, Tartu 51014, Estonia; Gopal Nath, MD, PhD, Professor, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Karagin PH, Stenram U, Wadström T, Ljungh Å. *Helicobacter* species and gut bacterial DNA in Meckel's diverticulum and the appendix. *World J Gastroenterol* 2011; 17(36): 4104-4108 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4104.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4104

INTRODUCTION

Although the stomach is the most frequent site of *Helicobacter pylori* (*H. pylori*) infection, *H. pylori* and enterohepatic *Helicobacter spp.* (EHS) have also been associated with extragastric diseases[1].

Meckel's diverticulum (MD) is the most common developmental anomaly of the gastrointestinal tract and is present in 1%-2% of the general population. It often contains ectopic tissue, notably gastric and pancreatic tissue[2]. Gastric mucosa is found in 10%-25% of MD and may be associated with inflammation, ulceration, gastrointestinal bleeding, and perforation[3-5]. *H. pylori*

has been demonstrated in the ectopic gastric epithelium within MD[6]. *Campylobacter-like* organisms in MD were first reported in 1989[7-9]. However, conflicting results were reported concerning colonisation by *H. pylori* of such ectopic mucosa[10,11]. There has been no study that investigated EHS in MD.

Acute appendicitis is the most common abdominal surgical emergency and can be seen in all ages, especially in those younger than 30 years[12]. However, the aetiology of acute appendicitis is uncertain, and diagnosis is often difficult. There have been some investigations of *H. pylori* in appendix tissue[13-15], but none that investigated non-*pylori Helicobacters*.

We hypothesized that non-*pylori Helicobacters*, such as enterohepatic *Helicobacters*, might be associated with these diseases. Most studies have investigated only *H. pylori* in MD and the appendix and mostly used non-molecular biological techniques; therefore, we aimed to analyse gastric, EHS and certain common gut bacteria in appendicitis and MD patients by genus specific polymerase chain reaction (PCR) and sequencing.

MATERIALS AND METHODS

Patients and histology

We re-examined all MD patients from 1990-2009 taken from the files of the Department of Pathology, Lund University Hospital. Thirty-three MD patients (two cases of ulcer without heterotopia, 31 cases with gastric heterotopia, of which seven also had an ulcer) (mean age: 11 years; range: 4 wk-73 years; 26 male, 7 female) were included in our study. Abdominal pain was the reason for operation in 16 cases, two of whom had acute appendicitis and one enlarged lymph nodes, nine were operated upon because of gastrointestinal bleeding and six for other abdominal diseases. No indication was given in two of the cases. Histological sections from stored paraffin blocks were stained with Alcian blue-periodic acid Schiff (AB-PAS) pH 2.5, Whartin-Starry silver stain and immunostained with an anti-*H. pylori* antibody (DAKO, Glostrup, Denmark, diluted 1:300).

We also re-examined mucosa from 50 cases of acute appendicitis (26 male, 24 female, median age: 30 years; range: 9-87 years) and 50 cases of normal appendix (16 male, 34 female, median age: 34 years; range: 10 d-80 years) from 2008-2009. Of the latter patients 26 were operated for a suspected appendicitis (8 male, 18 female, median age: 21 years; range: 8-77 years), 12 for intestinal diseases (8 male, 4 female, median age: 59 years; range: 10 d-80 years), and 12 for female genital disorders (median age: 38 years; range: 11-75 years). Histological sections from the *Helicobacter* positive cases were stained as mentioned above.

From the cases of MD, heterotopic mucosa of the gastric as well as the antral type were obtained from the paraffin blocks for PCR-assay with the tip of a scalpel. Ulcers were examined separately. In the case positive for *Helicobacter* DNA, the intestinal type mucosa surrounding the heterotopia was also studied. Corresponding areas from appendix samples of mucosa, or of necrotic appendicitis were sampled for PCR. It was not possible to avoid including material from the appendical lumen in these samples. In the cases positive for *Helicobacter* DNA, other tissues removed at the same operation were also examined by PCR.

DNA extraction

DNA was extracted from the paraffin-embedded tissue samples by de-embedding, as previously described[1]. DNA was extracted by a QIAamp DNA Mini Kit Tissue protocol (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Helicobacter specific PCR

DNA extracts were amplified in a GeneAmp 2700 Thermocycler (Applied Biosystems, Foster City, CA, United States) using a semi-nested PCR assay specific for *Helicobacter spp.* 16S rDNA, as previously described[1]. *H. pylori* (CCUG 17874) was used as a positive control in all PCR reactions. The 416-bp PCR products were visualized by 1.3% agarose gel electrophoresis.

Amplification of non-Helicobacter bacteria

PCR specific for *Enterobacteriaceae,* the *Bacterioides-Prevotella* group, and *Enterococcus* were performed. The reaction mixture and amplification conditions, except for annealing temperatures, for non-*Helicobacter* PCR assays were the same as in the first step of the semi-nested *Helicobacter* PCR. The annealing temperatures and primers used for detection of *Enterobacteriaceae*, *Bacterioides-prevotella* group, and *Enterococcus* were as described by Karagin *et al*[1] 2010. As positive controls, *Escherchia coli* (CCUG 17620), *Bacteroides fragilis* (CCUG 4856), and *Enterococcus faecalis* (CCUG 9997) were used in all PCR reactions. The 112-bp PCR product of *Enterococcus*, the 418-bp product of *Bacteroides*, and the 195-bp product of *Enterobacteriaceae* were visualized by 1.3% agarose gel electrophoresis.

DNA sequence analysis

Helicobacter specific PCR products were purified from agarose gels using the Montage DNA Gel Extraction Kit (Millipore, Bedford, MA, United States), according to the manufacturer's instructions. DNA sequence reactions were performed using the ABI PRISM™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit version 3.0 (Applied Biosystems) as described by Tolia *et al*[16]. Products of the sequencing reaction were aligned and the closest homologous DNA was identified by BLASTn-analysis.

The study was approved by the Research Ethics Committee at Lund University, permit number 588/2006.

RESULTS

Histology

The most dominant heterotopia seen in MD was of the

corpus type with, in most cases, small areas of antral heterotopia. It was therefore easy to include both types of heterotopia, if present, in the same sample. No *Helicobacter* was found by histological and immunohistological examinations, neither in heterotopia nor in ulcus. In 3/33 of the MD cases, a mild chronic inflammation in the heterotopic area with slightly increased amounts of lymphocytes was seen. The ulcer was infiltrated with polymorphonuclear cells but there was no general, active gastritis. The normal intestinal mucosa in the MD outside the heterotopia did not have an increased amount of lympathic tissue, in except the *Helicobacter pullorum (H. pullorum)* positive case. The nine ulcers and their surrounding mucosa were negative for *Helicobacter* DNA.

One of the heterotopic mucosa specimens was positive for *Helicobacter* DNA, namely that from a 44-year-old male. He was operated on for acute appendicitis. The appendix was not sent for histological analysis. The MD was also removed. Histology displayed a few gastric glands of the corpus type and a small strip of surface epithelium of the gastric type. There were a moderate number of lymphocytes and plasma cells in the heterotopic area. The surrounding mucosa of the intestinal type displayed an unusually well developed lymphatic tissue with germinal centres, a predominance of lymphocytes, and very few polymorphonuclear cells (Figure 1). There was no *Helicobacter* DNA detected by PCR in this sample.

Three normal appendixes were positive for *Helicobacter* DNA: from one an 18-year-old female with suspicion of appendicitis, one from a 63-year-old male with colon adenoma, one from a 55-year-old male with colon diverticulitis. Adenoma, diverticulitis, and normal colon tissue removed from the two latter patients were negative for *Helicobacter*. No tissue other than the appendix was removed from the first patient. All cases revealed *H. pylori* on sequence analysis. There was no gastric metaplasia in any of the appendixes, and no immunopositive *H. pylori* structures in the mucosa of the samples that were PCR-positive for *Helicobacter*.

Helicobacter specific PCR assay and sequencing results

Using the *Helicobacter* specific PCR assay and agarose gel electrophoresis, *Helicobacter spp.* was detected in 1/33 (3%) of specimens from patients with MD by genus specific nested-PCR. The sequenced PCR amplicon showed 98% similarity to *H. pullorum*. There were 3/50 (6%) samples that were positive for *Helicobacter spp.*, among normal appendixes. All of them showed 98%-99% sequence similarity to *H. pylori*. However *Helicobacter spp.* was not found in any samples of acute appendicitis.

PCR detection of bacterial DNA other than *Helicobacter*

Using the *Enterobacteria* specific PCR assay and agarose gel electrophoresis, *Enterobacteria spp.* was detected in 10/50 (20%) acute appendicitis cases and 8/50 (16%) normal appendixes. There were 7/50 (14%) and 12/50 (24%) samples that were positive for *Bacteroides spp.*, among the acute appendicitis and normal appendix sample, respectively. However, all MD and appendix samples were negative for *Enterococcus spp.* MD samples were also negative for *Bacteroides*; however, 2/33 (6%) were positive for *Enterobacteria spp.*

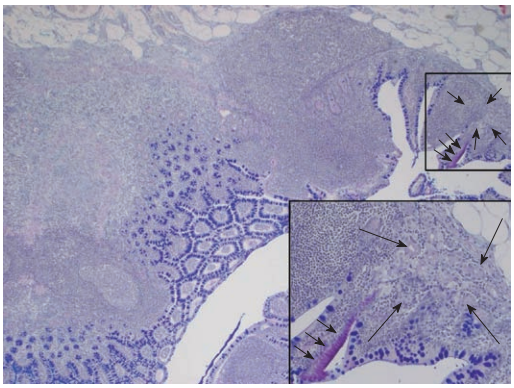


Figure 1 Microscopic analysis of the *Helicobacter pullorum* positive Meckel´s diverticulum case. Low power view of a histological section from Meckel´s diverticulum, positive for *Helicobacter pullorum* (*H. pullorum)* DNA, in the heterotopic area. There is an unusually well developed lymphatic tissue with germinal centres and a predominance of lymphocytes in the non-heterotopic area. Plasma cells were mainly found in the heterotopic area. The small arrows in the big square, point to a small strip of gastric heterotopia. This area displays at higher magnification in the inset. Large arrows point to gastric glands of corpus type. Blue without arrows in the photo indicates intestinal mucus. Alcian blue-PAS staining pH 2.5.

DISCUSSION

In this study, we screened for the presence of DNA of *Helicobacter spp.* and certain common intestinal bacteria by PCR in MD with gastric heterotopia and in appendix samples. We detected *H. pullorum* DNA in one out of 33 MD cases (3%) and *Enterobacteria* in two (6%). No *Enterococus* or *Bacteroides* were found in the MD cases.

The *H. pullorum* case was positive only in the heterotopic area, not in the surrounding diverticulum mucosa of the intestinal type. No luminal contents were seen in these samples. This argues for the interpretation that the *H. pullorum* DNA originated from the heterotopic mucosa and not from the lumen. This assumption is further strengthened by the very low prevalence of other bacterial DNA in the MD samples. *H. pullorum* has, however, been described in stools from cases with gastroenteritis[17], but our patient did not have such symptoms.

No *Helicobacter* was seen by immunohistochemistry. However, PCR is a more sensitive method and does not require intact bacteria. Our PCR technique is considered to be highly reliable for genus identification of *Helicobacter spp.*[18,19]. Some authors have found *H. pylori* by immunohistochemistry in MD with active gastritis, implying the presence of polymorphonuclear cells; the prevalence varied between 2 and 28%[6-8,20-23]. We had no cases with such inflammation and found no *H. pylori* DNA.

Interestingly, there was an increased amount of lymphatic tissue in the intestinal type mucosa of the *H.*

pullorum positive case. However, no conclusions can be drawn from just one case.

EHS are known to cause inflammatory bowel diseases[24,25]. We have previously found *H. pullorum* DNA in cholecystitis samples with gastric metaplasia[1]. Perhaps *H. pullorum* has some preference for the gastric epithelium.

We found *H. pylori* DNA in three out of 50 normal appendixes (6%) and none in the 50 cases of acute appendicitis. Other bacterial DNA was found in up to 24% of samples. We could not avoid including some luminal material in the appendix samples and therefore *H. pylori* DNA in the appendixes might be a contamination. Pavlidis *et al*[14] found *H. pylori* by PCR in two out of 46 samples (4%) of acute appendicitis. However, most authors have failed to demonstrate the presence of *H. pylori* in the appendix[13,15,26]. *H. pylori* commonly colonises the gastrointestinal tract. However, our results suggest that *Helicobacter* is without importance in the etiology of acute appendicitis.

In conclusion, *H. pullorum* has, for the first time, been detected by PCR in MD patients with gastric heterotopias. However, there is no association between *H. pullorum* and MD pathogenesis. Moreover, *H. pylori* has no role in the aetiology of acute appendicitis. Its presence might have been that of a passenger.

ACKNOWLEDGMENTS

Hans-Olof Nilsson's expert knowledge on PCR and specimen handling and PCR inhibitor removal and Ingrid Nilsson's valuable expertise on *Helicobacters* are highly appreciated. We also thank Roland Andersson, MD PhD, Department of Surgery, for contributing the case story of the *H. pullorum* positive patient.

COMMENTS

Research frontiers

Helicobacter-like bacteria in Meckel´s diverticulum (MD) have been reported by histological methods. However, no study has reported *Helicobacter* DNA in such specimens by polymerase chain reaction(PCR) and there is some doubt as to the presence of *Helicobacter* in patients with appendicitis.

Innovations and breakthroughs

Most studies have analyzed only *Helicobacter pylori (H. pylori)* in Meckel's diverticulum and appendix samples. However, enterohepatic Helicobacter species might also be important in the etiology of such diseases. The authors demonstrated the presence of *Helicobacter pullorum* in Meckel's diverticulum for the first time and concluded that *Helicobacter* might be a passenger in such patients.

Applications

By understanding the role of *Helicobacters* in the pathology of Meckel's diverticulum and appendicitis, this study could represent a future strategy for further pathological studies.

Terminology

Enterohepatic *Helicobacter spp.* (EHS) are the species of the genus *Helicobacter* that colonize the hepatobiliary tract and can cause extragastric diseases in humans or in animals.

Peer review

This work has been had the objective of seeking any association between Helicobacter species other than *H. pylori* with Meckel's diverticulum by very sensitive method, i.e., Nested PCR. Most previous studies were based on conventional methods, such as culture isolation, whereas in this study, the authors have used molecular techniques. Although they did not find any association between MD and *Helicobacter* species, it does not undermine the importance of the study.

REFERENCES

1 **Karagin PH**, Stenram U, Wadström T, Ljungh A. Helicobacter species and common gut bacterial DNA in gallbladder with cholecystitis. *World J Gastroenterol* 2010; **16**: 4817-4822
2 **Mackey WC**, Dineen P. A fifty year experience with Meckel' s diverticulum. *Surg Gynecol Obstet* 1983; **156**: 56-64
3 **Diamond T**, Russell CF. Meckel's diverticulum in the adult. *Br J Surg* 1985; **72**: 480-482
4 **Leijonmarck CE**, Bonman-Sandelin K, Frisell J, Räf L. Meckel's diverticulum in the adult. *Br J Surg* 1986; **73**: 146-149
5 **Vane DW**, West KW, Grosfeld JL. Vitelline duct anomalies. Experience with 217 childhood cases. *Arch Surg* 1987; **122**: 542-547
6 **Bemelman WA**, Bosma A, Wiersma PH, Rauws EA, Brummelkamp WH. Role of Helicobacter pylori in the pathogenesis of complications of Meckel's diverticula. *Eur J Surg* 1993; **159**: 171-175
7 **de Cothi GA**, Newbold KM, O'Connor HJ. Campylobacter-like organisms and heterotopic gastric mucosa in Meckel's diverticula. *J Clin Pathol* 1989; **42**: 132-134
8 **Morris A**, Nicholson G, Zwi J, Vanderwee M. Campylobacter pylori infection in Meckel's diverticula containing gastric mucosa. *Gut* 1989; **30**: 1233-1235
9 **Stolte M**, Lauer E. [Campylobacter pylori in heterotopic gastric mucosa in Meckel's diverticulum]. *Leber Magen Darm* 1989; **19**: 209-210
10 **Ergün O**, Celik A, Akarca US, Sen T, Alkanat M, Erdener A. Does colonization of Helicobacter pylori in the heterotopic gastric mucosa play a role in bleeding of Meckel's diverticulum? *J Pediatr Surg* 2002; **37**: 1540-1542
11 **Tuzun A**, Polat Z, Kilciler G, Turan I, Kilic A, Ozcan A, Uygun A. Evaluation for Helicobacter pylori in Meckel's diverticulum by using real-time PCR. *Dig Dis Sci* 2010; **55**: 1969-1974
12 **Simpson J**, Samaraweera AP, Sara RK, Lobo DN. Acute appendicitis--a benign disease? *Ann R Coll Surg Engl* 2008; **90**: 313-316
13 **Fanning NF**, Horgan PG, Tanner WA, Keane FB. Helicobacter pylori does not play a role in the aetiology of acute appendicitis. *Ir J Med Sci* 1998; **167**: 39-40
14 **Pavlidis TE**, Atmatzidis KS, Papaziogas BT, Souparis A, Koutelidakis IM, Papaziogas TB. Helicobacter pylori infection in patients undergoing appendectomy. *Swiss Surg* 2002; **8**: 110-112
15 **Kell MR**, Winter DC, Ryan D, Lynch M, Brew B, Rajpal P, Kirwan WO, Redmond HP. Nitric oxide synthetase and Helicobacter pylori in patients undergoing appendicectomy. *Br J Surg* 1999; **86**: 1538-1542
16 **Tolia V**, Nilsson HO, Boyer K, Wuerth A, Al-Soud WA, Rabah R, Wadström T. Detection of Helicobacter ganmani-like 16S rDNA in pediatric liver tissue. *Helicobacter* 2004; **9**: 460-468
17 **Ceelen L**, Decostere A, Verschraegen G, Ducatelle R, Haesebrouck F. Prevalence of Helicobacter pullorum among patients with gastrointestinal disease and clinically healthy persons. *J Clin Microbiol* 2005; **43**: 2984-2986
18 **Moyaert H**, Pasmans F, Ducatelle R, Haesebrouck F, Baele M. Evaluation of 16S rRNA gene-based PCR assays for genus-level identification of Helicobacter species. *J Clin Microbiol* 2008; **46**: 1867-1869
19 **Al-Soud WA**, Ouis IS, Li DQ, Ljungh S, Wadström T. Characterization of the PCR inhibitory effect of bile to optimize real-time PCR detection of Helicobacter species. *FEMS Immunol Med Microbiol* 2005; **44**: 177-182

20 **Hill P**, Rode J. Helicobacter pylori in ectopic gastric mucosa in Meckel's diverticulum. *Pathology* 1998; **30**: 7-9
21 **Finn LS**, Christie DL. Helicobacter pylori and Meckel's diverticula. *J Pediatr Gastroenterol Nutr* 2001; **32**: 150-155
22 **Ackerman Z**, Peston D, Cohen P. Role of Helicobacter pylori infection in complications from Meckel's diverticulum. *Dig Dis Sci* 2003; **48**: 1068-1072
23 **Oğuzkurt P**, Talim B, Tanyel FC, Cağlar M, Senocak ME, Büyükpamukçu N. The role of heterotopic gastric mucosa with or without colonization of Helicobacter pylori upon the diverse symptomatology of Meckel's diverticulum in children. *Turk J Pediatr* 2001; **43**: 312-316
24 **Laharie D**, Asencio C, Asselineau J, Bulois P, Bourreille A, Moreau J, Bonjean P, Lamarque D, Pariente A, Soulé JC, Charachon A, Coffin B, Perez P, Mégraud F, Zerbib F. Association between entero-hepatic Helicobacter species and Crohn's disease: a prospective cross-sectional study. *Aliment Pharmacol Ther* 2009; **30**: 283-293
25 Wadström T, Hanninen M-L. Other helicobacters in the digestive tract. *Curr Opin in Gastroenterol* 1999; **15** (suppl 1): S53-S56
26 **Paredes Esteban RM**, Muñoz Villanueva JR, Velasco Sánchez B, González Mariscal M, Rodríguez Vargas J, Martínez Sánchez M, García Ruiz M. [Role of the Helicobacter pylori in the aetiology of acute appendicitis. Preliminary studies]. *Cir Pediatr* 2007; **20**: 156-158

S- Editor Tian L **L- Editor** Stewart GJ **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4109

World J Gastroenterol 2011 September 28; 17(36): 4109-4112
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Epinephrine plus argon plasma or heater probe coagulation in ulcer bleeding

Ahmet Karaman, Mevlut Baskol, Sebnem Gursoy, Edip Torun, Alper Yurci, Banu Demet Ozel, Kadri Guven, Omer Ozbakir, Mehmet Yucesoy

Ahmet Karaman, Mevlut Baskol, Sebnem Gursoy, Edip Torun, Alper Yurci, Banu Demet Ozel, Kadri Guven, Omer Ozbakir, Mehmet Yucesoy, Department of Gastroenterology, Erciyes University, 38030 Kayseri, Turkey
Author contributions: Karaman A, Ozel BD, Torun E, Yurci A researched the data; Baskol M and Gursoy S analyzed the data; Karaman A and Baskol M wrote the manuscript; Yucesoy M, Ozbakir O, and Guven K edited the manuscript.
Correspondence to: Dr. Ahmet Karaman, Department of Gastroenterology, Erciyes University, 38030 Kayseri, Turkey. drkaraman@hotmail.com
Telephone: +90-533-4834197 Fax: +90-352-4375273
Received: January 4, 2011 Revised: February 28, 2011
Accepted: March 7, 2011
Published online: September 28, 2011

Abstract

AIM: To compare the effectiveness of argon plasma coagulation (APC) and heater probe coagulation (HPC) in non-variceal upper gastrointestinal bleeding.

METHODS: Eighty-five (18 female, 67 male) patients admitted for acute gastrointestinal bleeding due to gastric or duodenal ulcer were included in the study. Upper endoscopy was performed and HPC or APC were chosen randomly to stop the bleeding. Initial hemostasis and rebleeding rates were primary and secondary end-points of the study.

RESULTS: Initial hemostasis was achieved in 97.7% (42/43) and 81% (36/42) of the APC and HPC groups, respectively ($P < 0.05$). Rebleeding rates were 2.4% (1/42) and 8.3% (3/36) in the APC and HPC groups, respectively, at 4 wk ($P > 0.05$).

CONCLUSION: APC is an effective hemostatic method in bleeding peptic ulcers. Larger multicenter trials are necessary to confirm these results.

Key words: Upper gastrointestinal bleeding; Argon plasma coagulation; Heater probe coagulation; Duodenal ulcer; Gastric ulcer

Peer reviewer: Yoshio Yamaoka, MD, PhD, Associate Professor, Department of Medicine/Gastroenterology, Baylor College of Medicine and VA Medical Center (111D), 2002 Holcombe Blvd, Houston, TX 77030, United States

Karaman A, Baskol M, Gursoy S, Torun E, Yurci A, Ozel BD, Guven K, Ozbakir O, Yucesoy M. Epinephrine plus argon plasma or heater probe coagulation in ulcer bleeding. *World J Gastroenterol* 2011; 17(36): 4109-4112 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4109.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4109

INTRODUCTION

Upper gastrointestinal bleeding (UGIB) is a common and life-threatening medical emergency. UGIB is defined as bleeding proximal to the ligament of Treitz. At least 80% of patients admitted to hospital because of acute bleeding have an excellent prognosis; generally, bleeding stops spontaneously and circulatory supportive therapy is adequate. Endoscopic therapy has been shown to reduce the rate of rebleeding, blood transfusion and surgery[1]. Endoscopic therapy is indicated in the following situations: (1) bleeding esophageal varices; (2) peptic ulcer with major stigmata of recent hemorrhage (active spurting bleeding, non-bleeding visible vessel or non-adherent blood clot); (3) vascular malformations including actively bleeding arteriovenous malformation, gastric antral vascular ectasia, and Dieulafoy malformation; and (4) active bleeding from a Mallory-Weiss tear.

Endoscopic hemostasis has significantly improved the outcome of patients with gastrointestinal bleeding.

Contact thermal coagulation with heater probe and argon plasma coagulation (APC) are among the hemostatic methods for bleeding peptic ulcers. Devices are applied directly to the bleeding point to cause coagulation and thrombosis in heater probe coagulation (HPC). The heater probe is pushed firmly on to the bleeding lesion to apply tamponade and deliver defined pulses of heat energy. APC is a non-contact method of delivering high-frequency monopolar current through ionized and electrically conductive argon gas[2].

The aim of this study was to compare these two methods for UGIB due to gastric and duodenal ulcers. The primary outcome measure was initial hemostasis and secondary outcome measure was recurrence of bleeding. This study was approved by Erciyes University Ethical Committee.

MATERIALS AND METHODS

All patients admitted for acute gastrointestinal bleeding due to gastric and duodenal ulcers were included in the study. Gastrointestinal bleeding was diagnosed only if medical staff witnessed hematemesis or melena, or detected black, tarry material on digital examination of the rectum. Patients with actively bleeding peptic ulcers, ulcers with adherent clots, or ulcers with non-bleeding visible vessels were randomly assigned to epinephrine injection plus HPC or epinephrine injection plus APC. Informed consent was obtained before the procedure and this was solely for the procedure itself. Patients with previous malignant ulcers, and unidentifiable ulcers because of torrential bleeding were excluded.

Randomization of patients was carried out by means of sealed numbered envelopes. Informed consent was obtained for therapeutic endoscopic intervention. Patients were blind to the study. All patients underwent endoscopy within 24 h of admission. All procedures were performed by experienced gastroenterologists (experienced endoscopist as a specialist in gastroenterology with a minimum of 3 years of post-training experience) with a Fujinon-2200 endoscope. An Olympus HPU-20 heater probe system with 10 F probes was used with power settings of 30-40 J. The heater probe was pushed firmly on to the bleeding lesion to apply compression and to cause coagulation and thrombosis. We used an Erbe 200 D APC unit, which consists of an argon gas source, a high-frequency electrosurgical unit, an APC probe, and foot switches to activate the argon gas source and current generator. Operative distance between the probe and tissue was adjusted to 2-10 mm by sense of proportion (Technically, APC cannot be activated unless the tip of the probe is at least 2-10 mm distant from the ulcer region. An automated switch-off system has been integrated into the system to avoid damage to the endoscope when this distance increases). Power/gas flow settings were 50 W and 2 L/min. All endoscopists used the same settings on the instrument. Epinephrine injection (5-6 mL, 1/10 000 dilution) was applied around the ulcer in all patients, before both of these two methods.

Table 1 Demographic data and randomization of the groups

	APC	HPC	Total	*P* value
Male	33	34	67	0.418
Female	10	8	18	0.418
Duodenal ulcer	23	25	48	0.366
Gastric ulcer	20	17	37	0.366
Age (yr)	57	52	54	0.19
Forrest				
1a	4	9	13	
1b	39	33	72	0.291

Randomization of the groups was homogeneous (All *P* values > 0.05). APC: Argon plasma coagulation; HPC: Heater probe coagulation.

Initial hemostasis was defined as cessation of active bleeding. All the patients were treated with the same protocol after the endoscopic procedure. A policy of early feeding was adopted, and intravenous omeprazole was prescribed at a dose of 40 mg/d. Primary failure was defined as failure to stop bleeding during initial endoscopy. Recurrent bleeding was defined by one of the following: 2 g/dL drop in hemoglobin value compared to that when the patient was discharged from hospital; fresh hematemesis; hypotension (systolic blood pressure < 90 mm Hg) with tachycardia (pulse > 110 beats/min); or melena after endoscopic treatment. Patients who did not have initial hemostasis were excluded during evaluation of rebleeding rates. Patients were followed for the next 4 wk after initial hemostasis to monitor rebleeding. Distribution of bleeding focus and severity of bleeding (by using Forrest Classification) was found to be similar by χ^2 analysis (Table 1).

Analysis of data was performed using SPSS version 16.0 (SPSS, Chicago, IL, United States). $P < 0.05$ was regarded as significant. We calculated the power of the study with PASS 2008 software, with $\alpha = 0.05$, $n = 85$, degrees of freedom = 1, and power of study = 99.9%.

RESULTS

Eighty-five (18 female, 67 male) patients were included in the study between February 2008 and November 2009 in the Gastroenterology Department, Erciyes University School of Medicine. Forty-two patients received HPC and 43 received APC. Forty-eight bleeding duodenal ulcers (25 received HPC therapy and 23 APC) and 37 bleeding gastric ulcers (17 received HPC therapy and 20 APC) were included. A consort flow diagram was designed and is presented in Figure 1.

Initial hemostasis was achieved in 97.7% (42/43) and 81% (36/42) of the APC and HPC groups, respectively ($P < 0.05$). There were significant differences in initial hemostasis rates ($P = 0.015$). One patient died, two had surgery, and three had hemoclips applied in the HPC group; one patient had hemoclips applied in the APC group, who did not have initial hemostasis. Rebleeding rates were 2.4% (1/42) and 8.3% (3/36) in the APC and

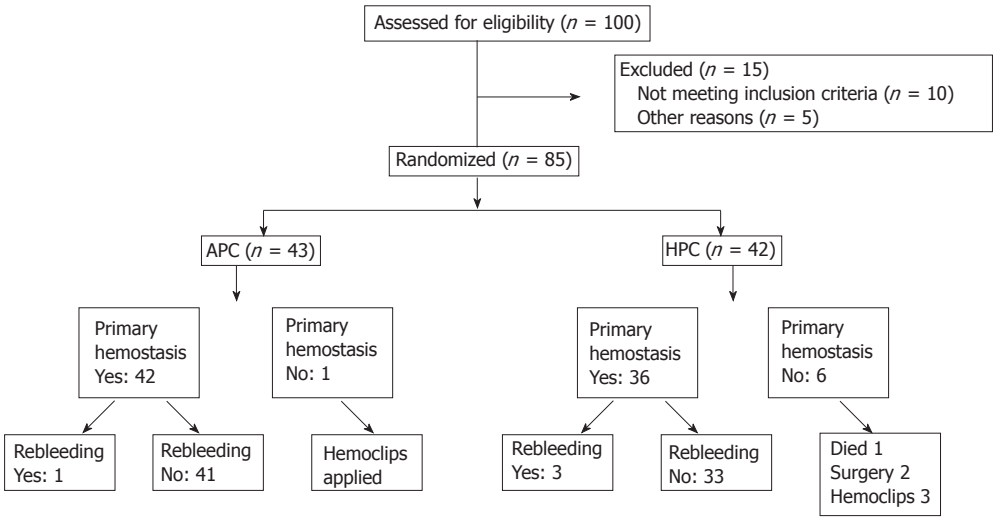


Figure 1 Consort flow diagram of the study. APC: Argon plasma coagulation; HPC: Heater probe coagulation.

Table 2 Initial hemostasis and rebleeding rates of the groups

	No. of cases (gastric/duodenal ulcer)	Initial hemostasis (%)	Rebleeding (%)
APC	43 (20-23)	42/43 (97.7)	1/42 (2.4)
HPC	42 (17-25)	36/42 (81)	3/36 (8.3)
Total	85 (37-48)	78 (91.7)	4/78 (5.1)

There was a significant difference ($P < 0.05$) in initial hemostasis but not in rebleeding rates between the groups. APC: Argon plasma coagulation; HPC: Heater probe coagulation.

HPC groups, respectively at 4 wk after index bleeding ($P = 0.25$). Although rebleeding rate was greater in the HPC group, there was no significant difference between the groups (Table 2).

DISCUSSION

Acute UGIB is a common medical emergency that carries hospital mortality in excess of 10%[3]. Bleeding stops spontaneously in about 80% of patients with non-variceal UGIB. Various modalities of endoscopic therapy have been used to reduce recurrent bleeding, surgery, and mortality rates, and therefore, endoscopic intervention is a preferable procedure in acute gasrointestinal (GI) bleeding. In the past few decades, several endoscopic methods have been developed for hemostasis of gastrointestinal bleeding. Some of these are HPC, direct injection of fluids (e.g., diluted epinephrine or distilled water) into the bleeding lesion using disposable needles, mechanical devices such as endoclips, and APC. There have been several studies about the efficiency of these methods in gastrointestinal bleeding. Although APC is used especially for chronic radiation proctitis[4], watermelon stomach and ablation of Barrett's esophagus, there are no current clinical studies about the application of APC in bleeding ulcers and for other causes of UGIB. APC therapy has various theoretical merits over contact-type thermal coagulation. First, depending on different power/flow settings, the burn depth can be preset between 0.5 mm and 3 mm, which is a particularly appropriate range for hemostasis in thin-walled duodenum and colon. Second, some bleeding points, such as those in the posterior wall or lesser curvature of the upper gastric body or the posterior wall of the duodenal bulb, may be difficult to approach. The no-touch technique in APC can make the approach easier by the arcing effect[2,5]. Wang *et al*[6] have compared APC and distilled water injection in treating high-risk bleeding ulcers, and they have reported that bleeding recurrence was 11% in the APC group and 27% in the distilled water injection group. They have concluded that endoscopic therapy is more effective than distilled water injection for preventing rebleeding in these patients, and no significant differences were observed between the two groups in terms of surgery and mortality. There have been a few studies in which APC and HPC were compared for treatment of peptic ulcer bleeding[7]. Although HPC is the more commonly used method in active bleeding lesions[8], APC is not used as much as HPC for stopping gastrointestinal bleeding. In our study, APC appeared to be more effective in initial hemostasis, but rebleeding rates were similar with both techniques. Bleeding recurrence has consistently been identified as the most important prognostic factor for mortality[9]. Arresting recurrence of bleeding can decrease the rate of morbidity and mortality from UGIB. Cipolletta *et al*[7] have reported that initial hemostasis rates were 95% and 95.2%, and recurrent bleeding rates were 21% and 15% in HPC and APC group, respectively, and there was no significant difference between the groups in the rate of recurrent bleeding[2]. Although rebleeding rates were much lower in the APC group, we found no significant difference in the rebleeding rates between the groups. Skok *et al*[10] have reported that clinically and endoscopically diagnosed bleeding recurred in 14% of patients in the APC group, and 18% of patients in the sclerotherapy group. Although these controlled trials had similar hemostatic efficacy, the patients treated

with APC had noticeably lower rebleeding rates.

The theoretical advantages of APC include its ease of application, speedy treatment of multiple lesions in the case of arteriovenous malformations or wide areas (the base of resected polyps or tumor bleeding), and safety due to reduced depth of penetration[11]. Superficial ulceration occurs following APC, which typically heals within 2-3 wk. Despite theoretical safety advantages due to reduced depth of penetration, all of the complications that have been reported with other thermal hemostasis techniques may occur. The first series of clinical applications of APC in gastrointestinal endoscopy was published in 1994[12]. Although no specific data were provided to assess the outcome for GI bleeding, the authors have described the technique as successful[12]. Several centers have subsequently reported experience with this technique in the management of GI bleeding[2,12]. However, few randomized studies comparing with APC with other hemostasis techniques have been performed, and our study is believed to be the first comparison of these two techniques in UGIB in recent years. APC application had better rates of initial hemostasis than HPC in our study.

The heater probe has a thermocouple at the tip of the probe that can heat up quickly and achieve tissue coagulation. As a result, deep coagulation is feasible with the heater probe, but this effect may risk perforation. As mentioned above, few studies have directly compared APC to other methods, especially HPC, for achieving hemostasis. In conclusion, APC is one of the effective hemostatic methods in bleeding peptic ulcers. Larger multicenter trials are necessary to confirm these data.

COMMENTS

Background

Contact thermal coagulation with heater probe coagulation (HPC) and argon plasma coagulation (APC) are the hemostatic methods used for the treatment of bleeding peptic ulcers. Few studies have directly compared the use of epinephrine injection plus APC versus epinephrine injection plus HPC for achieving hemostasis.

Research frontiers

Upper gastrointestinal bleeding (UGIB) is a common and life-threatening medical emergency. Contact thermal coagulation with HPC and APC are among the hemostatic methods for treatment of bleeding peptic ulcers. In this study, the authors demonstrate that there is a higher initial hemostasis rate in APC when compared with HPC in ulcer bleeding.

Innovations and breakthroughs

Few randomized studies have compared APC with other hemostasis techniques, and the study is believed to be the first comparison of these two techniques with additional use of epinephrine injections for UGIB in recent years. This study suggests that APC could be used instead of HPC, and that APC could provide clinicians with an effective alternative method to stop bleeding ulcers, due to its high rate of primary hemostasis and low rate of rebleeding.

Applications

This study may encourage clinicians without experience in APC to use the technique for treatment of ulcer bleeding.

Terminology

APC is a non-contact method of delivering a high-frequency monopolar current through ionized and electrically conductive argon gas. Devices are applied directly to the bleeding point to cause coagulation and thrombosis in HPC. The heater probe is pushed firmly onto the bleeding lesion to apply tamponade and deliver defined pulses of heat energy.

Peer review

The authors have compared the effectiveness of APC and HPC for ulcer bleeding. They have concluded that APC is superior to HPC for initial hemostasis. This was a well-designed study because the patients were assigned to two groups at random.

REFERENCES

1 **Cappell MS**, Friedel D. Acute nonvariceal upper gastrointestinal bleeding: endoscopic diagnosis and therapy. *Med Clin North Am* 2008; **92**: 511-550, vii-viii

2 **Vargo JJ**. Clinical applications of the argon plasma coagulator. *Gastrointest Endosc* 2004; **59**: 81-88

3 **Palmer K**. Management of haematemesis and melaena. *Postgrad Med J* 2004; **80**: 399-404

4 **Karamanolis G**, Triantafyllou K, Tsiamoulos Z, Polymeros D, Kalli T, Misailidis N, Ladas SD. Argon plasma coagulation has a long-lasting therapeutic effect in patients with chronic radiation proctitis. *Endoscopy* 2009; **41**: 529-531

5 **Malick KJ**. Clinical applications of argon plasma coagulation in endoscopy. *Gastroenterol Nurs* **2006; 29**: 386-391; quiz 392-393

6 **Wang HM**, Hsu PI, Lo GH, Chen TA, Cheng LC, Chen WC, Lin CK, Yu HC, Chan HH, Tsai WL, Wang EM, Lai KH. Comparison of hemostatic efficacy for argon plasma coagulation and distilled water injection in treating high-risk bleeding ulcers. *J Clin Gastroenterol* **2009; 43**: 941-945

7 **Cipolletta L**, Bianco MA, Rotondano G, Piscopo R, Prisco A, Garofano ML. Prospective comparison of argon plasma coagulator and heater probe in the endoscopic treatment of major peptic ulcer bleeding. *Gastrointest Endosc* 1998; **48**: 191-195

8 **Barkun AN**, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M, Sinclair P. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010; **152**: 101-113

9 **Turner IB**, Jones M, Piper DW. Factors influencing mortality from bleeding peptic ulcers. *Scand J Gastroenterol* 1991; **26**: 661-666

10 **Skok P**, Krizman I, Skok M. Argon plasma coagulation versus injection sclerotherapy in peptic ulcer hemorrhage--a prospective, controlled study. *Hepatogastroenterology* **2004; 51**: 165-170

11 **Freeman ML**. New and old methods for endoscopic control of nonvariceal upper gastrointestinal bleeding. *Rev Gastroenterol Mex* 2003; **68** Suppl 3: 62-65

12 **Grund KE**, Storek D, Farin G. Endoscopic argon plasma coagulation (APC) first clinical experiences in flexible endoscopy. *Endosc Surg Allied Technol* 1994; **2**: 42-46

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4113

World J Gastroenterol 2011 September 28; 17(36): 4113-4117
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Role of *cyclooxygenase-2* gene polymorphisms in pancreatic carcinogenesis

Renata Talar-Wojnarowska, Anita Gasiorowska, Marek Olakowski, Pawel Lampe, Beata Smolarz, Hanna Romanowicz-Makowska, Ewa Malecka-Panas

Renata Talar-Wojnarowska, Anita Gasiorowska, Ewa Malecka-Panas, Department of Digestive Tract Diseases, Medical University, 22 Kopcinskiego, 90-153 Lodz, Poland
Marek Olakowski, Paweł Lampe, Department of Digestive Tract Surgery, Silesian Medical University, 14 Medykow, 40-752 Katowice, Poland
Beata Smolarz, Hanna Romanowicz-Makowska, Laboratory of Molecular Genetics, Institute of Polish Mother's Memorial Hospital, 281/289 Rzgowska, 93-338 Lodz, Poland
Author contributions: Talar-Wojnarowska R and Gasiorowska A provided the protocols and performed the research; Olakowski M and Lampe P provided the collection of human material and analyzed the data; Smolarz B and Romanowicz-Makowska H carried out the molecular analysis; and Talar-Wojnarowska R and Malecka-Panas E designed the study and wrote the paper.
Correspondence to: Renata Talar-Wojnarowska, MD, PhD, Department of Digestive Tract Diseases, Medical University of Lodz, Kopcinskiego 22, 90-153 Lodz, Poland. renata.talar-wojnarowska@umed.lodz.pl
Telephone: +48-42-6786480 Fax: +48-42-6786480
Received: December 20, 2010 Revised: March 26, 2011
Accepted: April 2, 2011
Published online: September 28, 2011

Abstract

AIM: To evaluate the clinical significance of *-765G/C* and *-1195G/A cyclooxygenase-2 (COX-2)* gene polymorphisms in patients with pancreatic cancer (PC).

METHODS: The study included 201 patients: 85 with PC and 116 healthy controls. *-765G/C* and *-1195G/A COX-2* gene polymorphisms were studied in DNA isolated from blood samples. The associations of the analyzed genotypes and clinical data at diagnosis were evaluated.

RESULTS: We found an increased frequency of the homozygous *-1195AA COX-2* genotype in patients with PC (53.7%) compared with the control group (21%) ($P < 0.01$). In contrast, the distribution of genotype and allele frequencies of the *-765G/C COX-2* polymorphism in the PC patients were not different from those in control groups. A correlation between presence of homozygous *-1195AA COX-2* genotype and tumor size > 3 cm was observed ($P < 0.05$). Analyzed polymorphisms were unrelated to the patients' sex and age, nor to the presence of regional or distant metastases.

CONCLUSION: These preliminary results indicate that the *-1195G/A COX-2* polymorphism may play an important role in PC prognosis and carcinogenesis.

Key words: *Cyclooxygenase-2*; Polymorphisms; Pancreatic cancer; Carcinogenesis

Peer reviewer: Kotaro Miyake, MD, PhD, Department of Surgery, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan

Talar-Wojnarowska R, Gasiorowska A, Olakowski M, Lampe P, Smolarz B, Romanowicz-Makowska H, Malecka-Panas E. Role of *cyclooxygenase-2* gene polymorphisms in pancreatic carcinogenesis. *World J Gastroenterol* 2011; 17(36): 4113-4117 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4113.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4113

INTRODUCTION

Cyclooxygenase-2 (COX-2), also known as prostaglandin endoperoxide synthase, is a key enzyme in the arachidonic acid pathway, initiating the synthesis of biologically important prostanoids and eicosanoids. *COX-2* is involved in many biologic processes, such as cell proliferation, invasion, angiogenesis and inhibition of apoptosis, which are all relevant to cancer development and progression[1].

COX-2 is expressed under certain extracellular or intracellular stimuli, such as mitogens, growth factors, hormones, infectious agents and proinflammatory cytokines[1,2]. Numerous studies have demonstrated increased expression of *COX-2* in various cancers, including pancreatic tumors[3-6]. In several studies, the overexpression of *COX-2* in pancreatic cancer (PC) cells has been shown to be an independent prognostic factor and to increase the clinical aggressiveness of this disease[3,4,7].

The *COX-2* gene was demonstrated to be genetically polymorphic, which may affect the expression or activity of this enzyme and consequently contribute to variation in individual susceptibility to cancer through aberrant arachidonic acid metabolism. Genetic variants represented by single nucleotide polymorphisms (SNPs) of the *COX-2* gene, among them *-765G/C* and *-1195G/A* in the promoter region, have been identified. The functional effects of these SNPs have been recently confirmed[8-10]. The *-765G* allele is associated with heightened *COX-2* transcription by creating a transcriptional factor c-MYB-binding site and with significantly higher promoter activity compared with *-765C* allele[8,9]. In the study of Zhang *et al*[10], the *-1195A*-containing haplotypes also had significantly increased *COX-2* messenger RNA levels in esophageal tissues compared with the *-1195G*-containing counterparts.

COX-2 has been the object of prevention/intervention strategies in many clinical trials, including being a potential therapeutic target for chemoprevention and therapy of PC[1,2,11]. Selective inhibition of *COX-2* results in variable responses in individual patients. Information regarding the functional significance of *COX-2* polymorphisms with risk-modulating ability would have significant implications, not only for risk identification, but also for pharmacological management of the disease.

The purpose of this study was to evaluate the clinical significance of *-765G/C* and *-1195G/A COX-2* gene polymorphisms in patients with PC.

MATERIALS AND METHODS

The study included 201 Caucasian patients: 85 with PC (41 men and 44 women, aged 44-84 years) and 116 gender- and age-matched healthy volunteers. Analyzed patients were hospitalized in the Department of Digestive Tract Diseases, Medical University of Lodz Hospital or in the Department of Digestive Tract Surgery of Silesian Medical University in Katowice between 2004 and 2009. Only patients with a confirmed pathological diagnosis of ductal pancreatic adenocarcinoma were included in the study. The pathological diagnosis was confirmed after surgical treatment or after pancreatic tissue biopsy in patients who qualified for palliative chemotherapy. Twenty-nine patients (34.1%) with PC underwent Whipple resection or distal pancreatectomy, 33 patients (38.8%) underwent palliative surgery and 23 patients (27.1%) underwent palliative chemotherapy and/or endoscopic treatment. Tumor grade was classified into G1 (well differentiated), G2 (moderately differentiated) and G3 (poorly differentiated). The study protocol was approved by the ethical committee of Lodz Medical University.

Peripheral venous blood samples were obtained from all analyzed patients at the time of hospital admission. *-765G/C* and *-1195G/A COX-2* gene polymorphisms were studied in DNA isolated from blood samples using the QIAmp DNA Mini Kit (Qiagen). Polymerase chain reaction (PCR) products for the *COX-2* variants were analyzed by the restriction fragment length polymorphism method. The primers used to amplify the *COX-2* promoter region were 5'-TAT TAT GAG GAG AAT TTA CCT TTC GC-3' and 5'-GCT AAG TTG CTT CAA CAG AAG AAT-3' for the *-765G/C* variant; and 5'-CCC TGA GCA CTA CCC ATG AT-3' and 5'-GCC CTT CAT AGG AGA TAC TGG-3' for the *-1195G/A* polymorphism. PCR amplification was per-formed in a final volume of 25 μL containing 30-100 ng of DNA, 10 mmol/L Tris-HCl (pH 8.3), 4 μL of 25 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.5 μL dNTP (10 mmol/L), each primer at 1.0 μmol/L and 1.0 unit of Taq polymerase (BIOKOM, Takara, Japan) in a GeneAmp PCR system 9700 Thermocycler (Applied Biosystems).

Ten microliters of the PCR product were digested with 2 units of restriction enzymes HhaI or Pvu Ⅱ (Bio-Laps, New England) using the manufacturer's recommended protocol. PCR products were visualized on 8% polyacrylamide gels with 10% ethidium bromide. *COX-2* genotypes that could be detected were respectively: *-765CC* (100 bp fragment), *-765GC* (100 and 74 and 26 bp fragments), *-765GG* (74 and 26 bp fragments), *-1195AA* (273 bp fragment), *-1195GA* (273 and 220 and 53 bp fragments) and *-1195GG* (220 and 53 bp fragments).

The serum concentrations of CA19-9 were measured by an enzyme-linked immunoassay (DRG International, United States), according to the manufacturer's recommendations. The associations of the analyzed genotypes and patient characteristics at PC diagnosis were evaluated. The following demographic and clinical data were analyzed: age, gender, tumor size, lymph node involvement, histological grade, CA19-9 levels, weight loss and history of smoking. The cut-off point of CA19-9 was set at 37 U/mL.

Statistics analysis

The results were analyzed using StatSoft Statistica for Windows, release 6.0 (StatSoft, Inc., Tulsa, United States). To determine differences between groups, Mann-Whitney *t* tests were used. Clinical significance of analyzed polymorphisms was determined using logistic regression analysis and presented in tables as odds ratios (OR) with their 95% confidence intervals. The deviations from Hardy-Weinberg equilibrium were analyzed using the χ^2 test. Differences with a *P* value less than 0.05 were considered significant.

RESULTS

All patients involved in the study were Caucasians. Mean ages were not significantly different for patients with PC

Table 1 Distribution of *-1195 G/A* and *-765 G/C COX-2* genotype in the analyzed group of patients *n* (%)

Genotype	PC patients (*n* = 85)	Control group (*n* = 116)	OR (95% CI)
-1195 G/A			
GG	13 (15.7)	44 (37.9)	Reference
GA	26 (30.6)	48 (41.4)	1.83 (0.84-4.00)
AA	46 (53.7)	24 (21.0)	6.48 (2.93-14.31)
- 765 G/C			
GG	47 (55.4)	44 (37.9)	Reference
GC	27 (31.7)	40 (34.5)	0.63 (0.33-1.19)
CC	11 (12.9)	32 (27.6)	0.32 (0.14-1.71)

PC: Pancreatic cancer; OR: Odds ratios.

Table 2 Relationship between *-1195 G/C COX-2* polymorphism and clinical data of patients with pancreatic cancer *n* (%)

	Group	G^+ allele (GA and GG) (*n* = 39)	*P* value	G^- allele (AA) (*n* = 46)	*P* value
Age	< 65	20 (51.3)	NS	19 (41.3)	NS
	⩾ 65	19 (48.7)		27 (58.7)	
Gender	Male	18 (46.2)	NS	23 (50.0)	NS
	Female	21 (53.8)		23 (50.0)	
Tumor size	⩽ 3 cm	21 (53.8)	NS	13 (28.3)	*P* < 0.05
	> 3 cm	18 (46.2)		33 (71.7)	
Tumor	G1 + G2	21 (53.8)	NS	27 (58.7)	NS
Differentiation	G3	17 (43.8)		18 (39.2)	
Lymph node	Absent	23 (58.9)	NS	23 (50.0)	NS
Metastases	Present	16 (41.1)		23 (50.5)	
Weight loss	< 10 %	19 (48.7)	NS	25 (54.4)	NS
	⩾ 10%	20 (51.3)		21 (45.6)	
Smoking	Yes	21 (53.8)	NS	19 (41.3)	NS
	No	18 (46.2)		27 (58.7)	
CA19-9	< 37 U/mL	10 (25.6)	NS	13 (28.3)	NS
	⩾ 37 U/mL	29 (74.4)		33 (71.7)	

NS: Not significant.

Table 3 Relationship between *-765 G/C COX-2* polymorphism and clinical data of patients with pancreatic cancer *n* (%)

	Group	G^+ allele (GC and GG) (*n* = 47)	*P* value	G^- allele (CC) (*n* = 38)	*P* value
Age	< 65	23 (48.9)	NS	16 (42.1)	NS
	⩾ 65	24 (51.1)		22 (57.9)	
Gender	Male	22 (46.8)	NS	19 (50.0)	NS
	Female	25 (53.2)		19 (50.0)	
Tumor size	⩽ 3 cm	17 (40.4)	NS	15 (39.5)	NS
	> 3 cm	28 (59.6)		23 (60.5)	
Tumor	G1 + G2	27 (57.4)	NS	21 (55.3)	NS
Differentiation	G3	20 (40.4)		16 (42.1)	
Lymph node	Absent	28 (59.8)	NS	18 (47.4)	NS
Metastases	Present	19 (40.4)		20 (52.6)	
Weight loss	< 10 %	30 (63.8)	NS	21 (55.3)	NS
	⩾ 10%	17 (36.2)		17 (44.7)	
Smoking	Yes	23 (48.9)	NS	17 (44.7)	NS
	No	24 (51.1)		21 (55.3)	
CA19-9	< 37 U/mL	12 (25.5)	NS	12 (31.6)	NS
	⩾ 37 U/mL	35 (74.5)		26 (68.4)	

NS: Not significant.

(mean 66.8 ± 4.1 years) and controls (63.1 ± 4.7 years, $P > 0.05$). In patients with pancreatic adenocarcinoma, the tumor size ranged from 2 cm to 7 cm (mean 3.7 ± 2.3). As for histological differentiation, 19, 29 and 35 patients were classified into G1, G2 and G3 respectively, whereas 2 patients had missing data. Lymph node metastases were observed in 39 patients with PC (45.9%) and liver metastases in 16 of them (18.8%). Serum levels of CA19-9 were higher in patients with PC compared to control group ($P < 0.001$; respectively 101.2 ± 21.4 U/mL *vs* 17.6 ± 3.2 U/mL; data shown in our previously published work[22]).

The genotype distributions of analyzed *-765G/C* and *-1195G/A COX-2* gene polymorphisms are summarized in Table 1. We found an increased frequency of the homozygous *-1195AA COX-2* genotype in patients with PC compared with control group [OR 6.48 (2.93-14.31), $P < 0.01$]. In contrast, the distribution of genotype and allele frequencies of the *-765G/C COX-2* polymorphism in the PC patients did not differ from those in control groups (Table 1). Each of the *COX-2* polymorphisms in the controls was consistent with Hardy-Weinberg equilibrium.

The potential relationship between *COX-2* genotype distribution and clinical data of the PC patients was investigated. *COX-2 -1195AA* genotype showed a significant association with tumor size > 3 cm in patients with PC ($P < 0.05$, Table 2). This analyzed polymorphism was unrelated to the patients' sex and age, weight loss, history of smoking, CA19-9 levels, nor with the presence of regional or distant metastases. In contrast, the *-765G/C COX-2* polymorphism was not associated with any clinical data (Table 3).

DISCUSSION

The overexpression of COX-2 has been shown to induce angiogenesis by increased synthesis of vascular endothelial growth factor and to inhibit apoptosis by activation of proto-oncogene Bcl-2[2]. It is known that the expression of COX-2 is increased in the majority of PC cells and may represent a target for adjuvant therapy of PC. However, little is known about the role of *COX-2* gene polymorphisms in pancreatic carcinogenesis. We investigated the clinical significance of the *-765G/C* and *-1195G/A COX-2* gene polymorphisms, as well as their potential association with the risk of developing PC.

In our study, the presence of the *-1195AA* genotype was found more frequently in patients with PC compared to the control group. Similarly, Zhao *et al*[8] observed that subjects carrying the *COX-2 -1195A* allele had significantly increased risk for developing PC compared with subjects carrying the *-1195G* allele. In previous studies, the association of the *-1195A* allele with an increased risk of lung, oral and esophageal cancers was also demonstrated[12-14]. In the study of Bi *et al*[13], *-1195AA* genotype was significantly correlated with worse overall survival (15.7 mo *vs* 20.2 mo, $P = 0.006$) and with shorter

progression-free survival (9.5 mo *vs* 11.9 mo, $P = 0.0034$) in patients with unresectable locally advanced non-small cell lung cancer.

However, others authors observed opposite results. In the study of Kristinsson *et al*[15], the *-1195GG* genotype resulted in a higher risk of developing esophageal adenocarcinom. On the other hand, Pereira *et al*[16] observed that men carrying the *-1195G* allele appeared to have a nine-fold increased risk for colorectal cancer. Racial and ethnical differences in the studies' populations may explain these contradictory results, because the distribution of *COX-2* polymorphisms may differ considerably between populations.

The published data about clinical significance of the second analyzed polymorphism of *COX-2* gene, *-765G/C*, are also controversial. In the study of Hoff *et al*[17], the *-765GG* genotype was present more often in patients with colorectal cancer compared to control group. Similarly, Coskunpinar *et al*[14] observed increased risk of lung carcinoma in Turkish patients carrying the *-765G* allele. In contrast, the *-765C* allele was associated with an increased risk for developing PC and urinary bladder cancer[8,18]. This is not in line with our findings, since we could not demonstrate a significant difference in *-765G/C* genotype distribution in patients with PC. Similarly, Dong *et al*[19] in a meta-analysis of 47 case-control studies did not find a convincing association between *-765G/C COX-2* gene polymorphism and the risk of cancer in diverse populations.

Another important aspect of our analysis was to assess the potential association of *-765G/C* and *-1195G/A COX-2* gene polymorphisms with clinical data of patients with PC. In the current study, the homozygous *-1195AA* was found to be present more frequently in patients with larger tumor size. To the best of our knowledge there are no available data about relationships between *-1195G/A COX-2* polymorphism and clinical characteristics of patients with PC. Earlier, Tan *et al*[20] demonstrated that the *COX-2 -1195A* allele was associated with the presence of distant metastases in patients with colorectal cancer. They suggested that COX-2 may play a role not only in colorectal tumorigenesis but also in cancer progression by stimulating cell proliferation and spread.

According to our data, the second analyzed *COX-2* polymorphism, *-765G/C*, was not associated with clinical parameters. Similarly, in other studies the *-765G/C* variant was not correlated with clinical stage of patients with cervical and colorectal cancers[17,21].

Overexpression of *COX-2* may be an important cellular mechanism in smoking-related PC development. Numerous studies have shown that smoking induces *COX-2* expression, but the exact signal pathways remain to be elucidated. Zhao *et al*[8] suggested that *COX-2* genetic polymorphisms may determine interindividual variation in the inducibility of *COX-2* expression. They observed that smoking remarkably increased *COX-2* promoter activity, especially in patients with PC carrying the *-765C* allele. In contrast, in our study, there was no association between analyzed polymorphisms and smoking. Similarly, Pandey *et al*[21] did not find a correlation between smoking and *COX-2* polymorphisms in patients with cervical cancer. This lack of association could be due to a relatively small number of subjects and certainly needs further validation.

In summary, we found a significant difference in the *-1195G/A COX-2* gene polymorphism distribution between patients with PC and the control group. The presence of *-1195AA* genotype was associated with an increased PC risk; however, further studies are needed to investigate its possible association with PC prognosis. Our results are consistent with the biological function of the polymorphisms and support the hypothesis that aberrant arachidonic acid metabolism may play an important role in pancreatic carcinogenesis.

COMMENTS

Background

Despite improved diagnostic and therapeutic capabilities, pancreatic cancer (PC) still has a very poor prognosis. Numerous studies suggest a role for *cyclooxygenase-2 (COX-2)* in pancreatic carcinogenesis. *COX-2* is involved in many biologic processes, such as cell proliferation, invasion, angiogenesis and inhibition of apoptosis, which are all relevant to cancer development and progression.

Research frontiers

The *COX-2* gene was demonstrated to be genetically polymorphic, which may affect the expression or activity of this enzyme and consequently contribute to variation in individual susceptibility and aggressiveness of PC.

Innovations and breakthroughs

This study analyzed the clinical significance of *-765G/C* and *-1195G/A COX-2* gene polymorphisms in patients with PC. In the study, the presence of the *-1195AA* genotype was associated with an increased risk of PC; however, further studies are needed to investigate its possible association with PC prognosis.

Applications

The results are consistent with the biological function of the polymorphisms and support the hypothesis that aberrant arachidonic acid metabolism may play an important role in pancreatic carcinogenesis.

Terminology

COX-2 is a key enzyme in the arachidonic acid pathway, initiating the synthesis of biologically important prostaglandin H2 (the precursor of other prostaglandins), prostacyclin and tromboxanes.

Peer review

In this manuscript, the authors demonstrate that *COX-2* gene polymorphisms might be associated with carcinogenesis of PC. A series of experiments are well-planned and well-performed and this manuscript is well written.

REFERENCES

1 **Sobolewski C**, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol* 2010; **2010:** 215158

2 **Ghosh N**, Chaki R, Mandal V, Mandal SC. COX-2 as a target for cancer chemotherapy. *Pharmacol Rep* 2010; **62**: 233-244

3 **Juuti A**, Louhimo J, Nordling S, Ristimäki A, Haglund C. Cyclooxygenase-2 expression correlates with poor prognosis in pancreatic cancer. *J Clin Pathol* 2006; **59**: 382-386

4 **Hermanova M**, Karasek P, Tomasek J, Lenz J, Jarkovsky J, Dite P. Comparative analysis of clinicopathological correlations of cyclooxygenase-2 expression in resectable pancreatic cancer. *World J Gastroenterol* 2010; **16**: 1879-1884

5 **Bergmann F**, Moldenhauer G, Herpel E, Gaida MM, Strobel O, Werner J, Esposito I, Müerköster SS, Schirmacher P, Kern

MA. Expression of L1CAM, COX-2, EGFR, c-KIT and Her2/neu in anaplastic pancreatic cancer: putative therapeutic targets? *Histopathology* 2010; **56**: 440-448
6 **Abe T**, Fukushima N, Brune K, Boehm C, Sato N, Matsubayashi H, Canto M, Petersen GM, Hruban RH, Goggins M. Genome-wide allelotypes of familial pancreatic adenocarcinomas and familial and sporadic intraductal papillary mucinous neoplasms. *Clin Cancer Res* 2007; **13**: 6019-6025
7 **Matsumoto G**, Muta M, Tsuruta K, Horiguchi S, Karasawa K, Okamoto A. Tumor size significantly correlates with postoperative liver metastases and COX-2 expression in patients with resectable pancreatic cancer. *Pancreatology* 2007; **7**: 167-173
8 **Zhao D**, Xu D, Zhang X, Wang L, Tan W, Guo Y, Yu D, Li H, Zhao P, Lin D. Interaction of cyclooxygenase-2 variants and smoking in pancreatic cancer: a possible role of nucleophosmin. *Gastroenterology* 2009; **136**: 1659-1668
9 **Papafili A**, Hill MR, Brull DJ, McAnulty RJ, Marshall RP, Humphries SE, Laurent GJ. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1631-1636
10 **Zhang X**, Miao X, Tan W, Ning B, Liu Z, Hong Y, Song W, Guo Y, Zhang X, Shen Y, Qiang B, Kadlubar FF, Lin D. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. *Gastroenterology* 2005; **129**: 565-576
11 **Lurje G**, Nagashima F, Zhang W, Yang D, Chang HM, Gordon MA, El-Khoueiry A, Husain H, Wilson PM, Ladner RD, Mauro DJ, Langer C, Rowinsky EK, Lenz HJ. Polymorphisms in cyclooxygenase-2 and epidermal growth factor receptor are associated with progression-free survival independent of K-ras in metastatic colorectal cancer patients treated with single-agent cetuximab. *Clin Cancer Res* 2008; **14**: 7884-7895
12 **Chiang SL**, Chen PH, Lee CH, Ko AM, Lee KW, Lin YC, Ho PS, Tu HP, Wu DC, Shieh TY, Ko YC. Up-regulation of inflammatory signalings by areca nut extract and role of cyclooxygenase-2 -1195G& gt; a polymorphism reveal risk of oral cancer. *Cancer Res* 2008; **68**: 8489-8498
13 **Bi N**, Yang M, Zhang L, Chen X, Ji W, Ou G, Lin D, Wang L. Cyclooxygenase-2 genetic variants are associated with survival in unresectable locally advanced non-small cell lung cancer. *Clin Cancer Res* 2010; **16**: 2383-2390
14 **Coskunpinar E,** Eraltan IY, Turna A, Agachan B. Cyclooxygenase-2 gene and lung carcinoma risk. *Med Oncol* 2010; In press
15 **Kristinsson JO**, van Westerveld P, te Morsche RH, Roelofs HM, Wobbes T, Witteman BJ, Tan AC, van Oijen MG, Jansen JB, Peters WH. Cyclooxygenase-2 polymorphisms and the risk of esophageal adeno- or squamous cell carcinoma. *World J Gastroenterol* 2009; **15**: 3493-3497
16 **Pereira C**, Pimentel-Nunes P, Brandão C, Moreira-Dias L, Medeiros R, Dinis-Ribeiro M. COX-2 polymorphisms and colorectal cancer risk: a strategy for chemoprevention. *Eur J Gastroenterol Hepatol* 2010; **22**: 607-613
17 **Hoff JH**, te Morsche RH, Roelofs HM, van der Logt EM, Nagengast FM, Peters WH. COX-2 polymorphisms -765G--& gt; C and -1195A--& gt; G and colorectal cancer risk. *World J Gastroenterol* 2009; **15**: 4561-4565
18 **Gangwar R**, Mandhani A, Mittal RD. Functional polymorphisms of cyclooxygenase-2 (COX-2) gene and risk for urinary bladder cancer in North India. *Surgery* 2011; **149**: 126-134
19 **Dong J**, Dai J, Zhang M, Hu Z, Shen H. Potentially functional COX-2-1195G& gt; A polymorphism increases the risk of digestive system cancers: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1042-1050
20 **Tan W**, Wu J, Zhang X, Guo Y, Liu J, Sun T, Zhang B, Zhao D, Yang M, Yu D, Lin D. Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. *Carcinogenesis* 2007; **28**: 1197-1201
21 **Pandey S**, Mittal RD, Srivastava M, Srivastava K, Mittal B. Cyclooxygenase-2 gene polymorphisms and risk of cervical cancer in a North Indian population. *Int J Gynecol Cancer* 2010; **20**: 625-630
22 **Talar-Wojnarowska R**, Gasiorowska A, Olakowski M, Lekstan A, Lampe P, Malecka-Panas E. Clinical value of serum neopterin, tissue polypeptide-specific antigen and CA19-9 levels in differential diagnosis between pancreatic cancer and chronic pancreatitis. *Pancreatology* 2010; **10**: 689-694

S- Editor Tian L **L- Editor** Logan S **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4118

World J Gastroenterol 2011 September 28; 17(36): 4118-4123
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Does the bile duct angulation affect recurrence of choledocholithiasis?

Dong Beom Seo, Byoung Wook Bang, Seok Jeong, Don Haeng Lee, Shin Goo Park, Yong Sun Jeon, Jung Il Lee, Jin-Woo Lee

Dong Beom Seo, Byoung Wook Bang, Seok Jeong, Jung Il Lee, Jin-Woo Lee, Division of Gastroenterology, Department of Internal Medicine, Inha University School of Medicine, Incheon 400-711, South Korea
Don Haeng Lee, Division of Gastroenterology, Department of Internal Medicine and Center for Advanced Medical Education by BK21 Project, Inha University School of Medicine, and Utah-Inha DDS and Advanced Therapeutics Research Center, Incheon 400-711, South Korea
Shin Goo Park, Department of Occupational and Environmental Medicine, Inha University School of Medicine, Incheon 400-711, South Korea
Yong Sun Jeon, Department of Radiology, Inha University School of Medicine, Incheon, 400-711, South Korea
Author contributions: Seo DB and Bang BW wrote the paper; all authors performed research and collected the data; Park SG analyzed the data; Jeong S and Lee DH reviewed the paper; Jeon YS, Lee JI, Lee JW provided technical support and advice.
Supported by An Inha University Research Grant
Correspondence to: Don Haeng Lee, MD, Department of Internal Medicine, Inha University Hospital, 7-206, 3-Ga, Sinheung-Dong, Jung-Gu, Incheon 400-711, South Korea. ldh@inha.ac.kr
Telephone: +82-32-8902548 Fax: +82-32-8902549
Received: September 26, 2010 Revised: March 24, 2011
Accepted: March 31, 2011
Published online: September 28, 2011

Abstract

AIM: To investigate whether bile duct angulation and T-tube choledochostomy influence the recurrence of choledocholithiasis.

METHODS: We conducted a retrospective study including 259 patients who underwent endoscopic sphincterotomy and cholecystectomy for choledocholithiasis between 2000 and 2007. The imaginary line was drawn along the center of the bile duct and each internal angle was measured at the two angulation sites of the bile duct respectively. The values of both angles were added together. We then tested our hypothesis by examining whether T-tube choledochostomy was performed and stone recurrence occurred by reviewing each subject's medical records.

RESULTS: The overall recurrence rate was 9.3% (24 of 259 patients). The mean value of sums of angles in the recurrence group was 268.3° ± 29.6°, while that in the non-recurrence group was 314.8° ± 19.9° ($P < 0.05$). Recurrence rate of the T-tube group was 15.9% (17 of 107), while that of the non T-tube group was 4.6% (7 of 152) ($P < 0.05$). Mean value of sums of angles after T-tube drainage was 262.5° ± 24.6° and that before T-tube drainage was 298.0° ± 23.9° in 22 patients ($P < 0.05$).

CONCLUSION: The bile duct angulation and T-tube choledochostomy may be risk factors of recurrence of bile duct stones.

Key words: Choledocholithiasis; Common bile duct; Cholecystectomy; Recurrence; Endoscopic retrograde cholangio pancreatography

Peer reviewers: Giuseppe Currò, MD, University of Messina, Via Panoramica, 30/A, 98168 Messina, Italy; Beata Jolanta Jablońska, MD, PhD, Department of Digestive Tract Surgery, University Hospital of Medical University of Silesia, Medyków 14 St. 40-752 Katowice, Poland

Seo DB, Bang BW, Jeong S, Lee DH, Park SG, Jeon YS, Lee JI, Lee JW. Does the bile duct angulation affect recurrence of choledocholithiasis? *World J Gastroenterol* 2011; 17(36): 4118-4123 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4118.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4118

INTRODUCTION

Since its introduction in 1974, endoscopic sphincterotomy (EST) has become a well established therapeutic method for the extraction of common bile duct (CBD) stones[1,2]. In previous studies of EST, 4%-24% of patients experienced recurrent CBD stones during follow-up intervals of up to 15 years[3-5]. The suggested causes of recurrent bile duct stones after EST are bile duct inflammation, papillary stenosis, dilated common bile duct, peripapillary diverticulum, reflux of the duodenal contents into the bile duct, and foreign bodies within the bile duct[6,7]. After EST, the biliary sphincter is rendered permanently insufficient[8]. The loss of this physiologic barrier between duodenum and biliary tract results in duodenocholedochal reflux and bacterial colonization of the biliary tract[9,10]. The presence of bacteria in the biliary system might lead to late complications after EST. These complications may include recurrence of CBD stones from deconjugation of bilirubin by bacterial enzymes[11], inflammatory changes of the biliary and/or hepatic system[12,13], recurrent ascending cholangitis[14], and even malignant degeneration[13,15].

Bile stasis may be one of the possible mechanisms of stone recurrence[16]. If bile flow can be decreased by the bile duct angulation, it may be a risk factor of stone recurrence. In a previous study, it was disclosed that in treatment of CBD stones, a T-tube drainage group had a higher recurrence rate of stones than either a choledochoduodenostomy group or an EST group, although their accurate mechanism was not elucidated[17]. It was also reported that the angulation of the extrahepatic bile duct-so called "the elbow sign"-occurred as a sequela of T-tube drainage[18]. Therefore, we investigated whether draining the T-tube changes configuration of the extrahepatic bile duct significantly and then whether bile duct deformity affects recurrence of CBD stones.

MATERIALS AND METHODS

Patient selection

All patients who were treated for choledocholithiasis by means of endoscopic retrograde cholangiopancreatography (ERCP) with EST between January 2000 and December 2007 in our institution were recruited. Their medical records were retrospectively reviewed. Among them, patients who met the following criteria were selected: (1) complete clearance of the bile duct stones was achieved; (2) cholecystectomy performed; (3) absence of bile duct stricture; (4) absence of concurrent hepatolithiasis; (5) absence of coexisting malignant neoplasm; and (6) absence of coexisting severe medical disease. Consequently, in total 259 patients were enrolled in the study.

For all the patients, the following data were collected: gender, age, recurrence, presence of periampullary diverticulum, maximal CBD diameter and presence of T-tube drainage. We divided them into two groups; recurrent and non-recurrent groups, and the sum of extrahepatic bile duct angles and maximal CBD diameter between both groups was compared. EST was performed completely by traction-type or needle-knife sphincterotome and the bile duct stones were extracted with basket and/or stone-retrieval balloon catheters. After extraction of CBD stones, we performed a regular check-up on each patient at the outpatient department base, and considered absence of symptoms of biliary colic or cholangitis episode as absence of recurrence by using telephone interviews in patients who were missed.

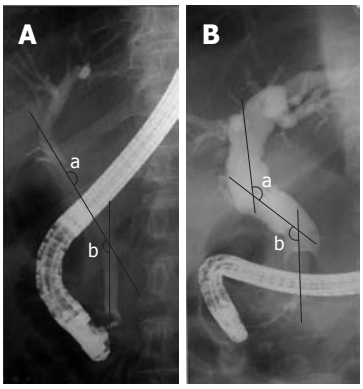


Figure 1 Cholangiographic configuration of (A) usual extrahepatic bile duct and (B) deformed bile duct. The imaginary line was drawn along the center of the bile duct and each internal angle was measured at the angulation of the proximal (a) and distal (b) bile duct level respectively. The values of both angles were added together (a + b).

Periampullary diverticulum

Periampullary diverticulum was defined as the presence of a diverticulum within a 2-cm radius from ampulla of Vater. We could identify whether periampullary diverticulum was present or not in 231 of the subjects. The information was missing from the medical records of the remaining patients. We divided patients into two groups: non-periampullary diverticulum and periampullary diverticulum groups, and the recurrence rate between the two groups was compared.

Measurement of bile duct angulation

There were films of acceptable quality, taken in the prone position during ERCP. When the exrahepatic bile duct was deformed, the number of its angulation site was one or two in most patients. The angles were measured at the intersection of imaginary lines drawn down the center of the bile duct. Its intersection did not necessarily occur within the lumen of the duct, particularly where angulation took the form of a gentle curve. Each internal angle was measured at the two angulation sites of the proximal and distal bile duct level respectively by one experienced radiologist (Figure 1). The values of both angles were added together to estimate the grade of angulation. The smaller sum of angles implies more angulation.

T-tube choledochostomy

T-tube drainage was performed during the cholecystectomy when the bile duct was injured surgically or when a

Table 1 Univariate analysis of risk factors for recurrent bile duct stones *n* (%)

Variable		Non recurrence group (*n* = 235)	Recurrence group (*n* = 24)	*P* value
Age (yr)		57.5 ± 15.2	62.4 ± 11.6	0.064
Gender (M/F)		116/119	11/13	0.742
Common bile duct diameter (mm)[1]		15.8 ± 6.2	20.6 ± 7.6	0.001
Sum of angle (°)[1]		314.9 ± 20.0	268.3 ± 29.6	0.001
T-tube drainage	No	145 (95.4)	7 (4.6)	0.002
	Yes	90 (84.1)	17 (15.9)	
Periampullary diverticulum[2]	No	111 (93.3)	8 (6.7)	0.091
	Yes	97 (86.6)	15 (13.4)	

M: Male; F: Female. [1]Values expressed as mean ± SD; [2]231 patients identified by whether periampullary diverticulum was present or not were included.

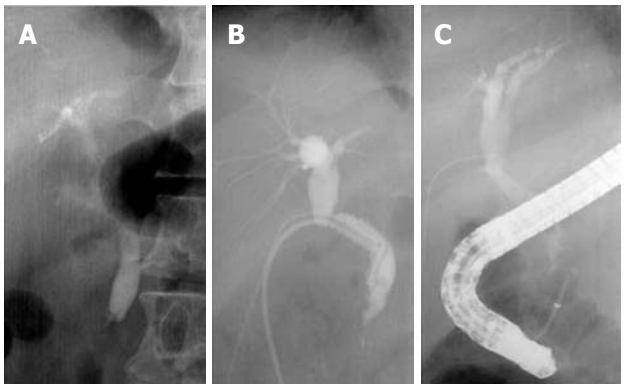


Figure 2 The bile duct angulation caused by T-tube choledochostomy. A: Extrahepatic bile duct configuration before T-tube choledochostomy; B: Bile duct configuration during T-tube drainage; C: Bile duct configuration altered after T-tube drainage. This change of cholangiographic findings showed that T-tube drainage would have induced bile duct angulation.

bile duct stone remained after EST. We divided patients into two groups; T-tube drainage (107 patients) and non-T-tube drainage group (152 patients). The recurrence rate in each group and the sum of angles, especially the sum of the angles before and after T-tube drainages in 22 patients whose films of ERCP before and after T-tube drainage were available among T-tube drainage group, were determined. We compared the recurrence rate and sum of angles between the T-tube drainage group and the non-T-tube drainage group, and evaluated the change of the sum of angles before and after the T-tube drainages in 22 patients to find the influence of T-tube drainage on bile duct configuration (Figure 2).

Maximal transverse common bile duct diameter

We defined transverse diameter of the CBD as a diameter measured in the line which forms a right angle to an imaginary line drawing down along the center of the CBD lumen. We measured the maximal transverse diameter of the CBD in all subjects and corrected for the magnification, with the external diameter of the distal end of the duodenoscope as a reference. We also evaluated the recurrence rate according to the CBD diameter.

Statistical analysis

Categorical variables (gender, presence of periampullary diverticulum and presence of T-tube drainage) were compared by chi-square test or Fisher exact test. Continuous variables (age, CBD diameter, sum of angle) were analyzed using the Student *t* test when the variables were normally distributed or by using the Mann Whitney *U* test for non-normal distribution. Significant predictors for choledocholithiasis recurrence identified by univariate analyses were included in a multiple logistic regression model to determine the most significant risk factors for recurrence of the bile duct stones. For multivariate analysis, we categorized continuous variables into two subgroups: age (< 65 years *vs* ≥ 65 years), sum of angle (< 270° *vs* ≥ 270°), and maximal transverse CBD diameter (< 13 mm *vs* ≥ 13 mm).

Statistical analysis were conducted using SPSS (Statistical Package for the Social Sciences) computer program (version 14.00).

RESULTS

Two hundred and fifty-nine patients were included in the study. Median age was 58 years (range, 17-85 years). There were 132 (50.9%) women and 127 (49.1%) men. Median follow-up period was 47.9 mo (6-101 mo). The results of univariate analyses for recurrence of bile duct stones in relation to each factor were presented in Table 1.

Recurrence rate

The overall recurrence rate of CBD stones was 9.3% (24 of 259 patients). The recurrence rate was 8.7% (11 of 127 patients) in males and 9.9% (13 of 132 patients) in females respectively.

A total of 13.4% (15 of 112) of the patients with periampullary diverticulum developed recurrent stones, while 6.7% (8 of 119) of those without diverticulum showed recurrence. There was no evidence that the periampullary diverticulum exerted a significant influence on the recurrence of bile duct stones statistically (P = 0.091). Seventeen of 107 patients (15.9%) who underwent T-tube drainage had a recurrence, compared with 7 of 152 patients (4.6%) without T-tube drainage, and the presence of T-tube drainage was shown to be statistically associated with the recurrence of CBD stones (P = 0.002).

Bile duct angulation

The mean value of the sum of angles in the non-recur-

Table 2 Adjusted odds ratio of risk factors for recurrent bile duct stones

Variables	Recurrence of bile duct stones		
	Odds ratio	*P* value	95% CI[1]
Periampullary diverticulum	2.648	0.107	0.809-8.668
Common bile duct diameter	3.011	0.152	0.564-11.700
T-tube drainage	2.578	0.119	0.785-8.470
Sum of angle (°)	17.897	0.000	5.083-63.015
Age ≥ 65 years	0.372	0.102	0.113-1.219

[1]95% CI: Expressed as 95% confidence interval.

rence group was 314.9° ± 20.0°, while the mean sum of angles in the recurrence group was 268.3° ± 29.6° (P = 0.001). These results mean that the more angulation in the extrahepatic bile duct could develop, the greater recurrence of the stone.

common bile duct diameter

The mean maximal transverse CBD diameter in the non-recurrence group was 15.8 ± 6.2 mm while the mean value of CBD in the recurrence group was 20.6 ± 7.6 mm. CBD diameter was a significant risk factor for recurrence on univariate analysis (P = 0.001).

T-tube drainage

The mean value of sums of angles at the cholangiogram before T-tube choledochostomy was 311.7° ± 22.4° in T-tube group, while that in the non T-tube group was 314.9° ± 19.3° (P = 0.232). On the other hand, the mean value of sums of angles before T-tube drainage was 298.0° ± 3.9° , while after T-tube drainage, it was 262.5° ± 24.6° in some patients (n = 22) who underwent ERCP after removal of the T-tube among members of the T-tube group (P = 0.001). Consequently, these results suggested that T-tube drainage may affect bile duct angulation.

Multivariate analysis

A univariate analysis revealed that CBD diameter, T-tube drainage and sum of angle were significant risk factors for the recurrence of choledocholithiasis (P < 0.05). But multivariate analyses of all variables that reached a P value of less than 0.1 in univariate analysis were performed. On multivariate analysis, angulation was the only independent risk factor for the recurrence of choledocholithiasis (Table 2).

DISCUSSION

ERCP with EST is the therapeutic procedure of choice for CBD stones. Early complications of EST include acute pancreatitis, bleeding, duodenal perforation, and acute cholangitis. On the other hand, its late complications include recurrence of stones and papillary stenosis[19,20]. In long-term follow-up studies after EST, the recurrence rate of CBD stones in patients with cholecystectomy has been in the range of 4% to 24%[3-5]. However, the theory that loss of sphincter function after EST leads to formation of recurrent CBD stones seems to need validation. Several authors have demonstrated that the biliary tree becomes infected with bacteria after EST[9,10] and there is substantial evidence that bacteria play an essential role in the formation of brown pigment stones. Some bacterial species, especially *Escherichia coli*[21], produce enzymes such as β-glucuronidase that are known to precipitate bilirubin and calcium[11], the main components of brown pigment stone[22]. Furthermore, electron microscopy studies have demonstrated that bacteria are present in the core of brown pigment stone whereas they are absent in cholesterol and black pigment stone[14,23,24].

Apart from bacterial infection, biliary stasis is thought to be an important factor in the pathogenesis of recurrent bile duct stones[6,16,25]. The markedly dilated bile duct is often contaminated with bacteria and also leads to bile stasis, which is thought to be an important factor in the pathogenesis of recurrent stones. The association between dilated CBD and stone recurrence has been reported in previous studies of post-EST patients[26,27]. In the univariate analysis of our study, in the recurrence group (n = 21, mean CBD diameter = 20.6 mm ± 7.6 mm) there was greater dilation in the extrahepatic bile duct than in the non-recurrence group (n = 200, mean CBD diameter = 15.8 mm ± 6.2 mm) (P = 0.001). Therefore dilated bile ducts may be another important risk factor of CBD stone recurrence by causing impaired bile flow.

Previous studies of the peripapillary diverticulum in relation to recurrence of bile duct stone have disclosed inconsistent results[28-31]. Biliary stasis in patients with diverticulum could be caused by either mechanical factors or the presence of coexisting motility disorders involving the sphincter of Oddi[28-31]. Theoretically, the effect of the diverticulum on bile flow might not completely disappear after the EST. In our study, the recurrence rate of the diverticulum group (n = 112, 13.4%) was higher than that of the non-diverticulum group (n = 119, 6.7%), but the result was not statistically significant (P = 0.91). Hence further studies on periampullary diverticulum as a risk factor of stone recurrence might be needed.

Bile duct angulation may cause stasis of bile flow. Warren suggested that angulation of CBD is associated with choledocholithiasis[32]. Mean cholangiographic angulation of CBD differed significantly between patients with cholelithiasis only and those with choledocholithiasis in this previous series. The degree of ductal angulation may be a useful consideration in development of bile duct stones. Recently two reports showed angulation of the CBD contributes to biliary stasis, and hence predisposes to recurrent choledocholithiasis[33,34]. In the current study, the recurrence group (n = 24, mean of sums of angles = 268.3° ± 29.6°) was more angulated in the extrahepatic bile duct than the non-recurrence group (n = 235, mean of sum of angles = 314.9° ± 20.0°) (P < 0.05). In multivariate analysis, the sum of angles was the only independent risk factor of CBD stone recurrence (Table 2).

There was a previous study describing long-term results of medical or surgical treatment in patients with choledocholithiasis[17]. Two hundred and thirteen patients were treated for CBD stones, and then the patients were divided into 3 groups based on the treatment modality: group 1, choledocholithotomy and T-tube choledochostomy; group 2, choledochoduodenostomy; and group 3, endoscopic sphincterotomy. Recurrence of choledocholithiasis was examined for each type of treatment modality. This study suggested that patients treated by T-tube drainage had more recurrent bile duct stones than those in the choledochoduodenostomy or EST groups, but accurate mechanisms were not clearly demonstrated.

Lee and Burhenne suggested that extrahepatic bile duct angulation was caused by T-tube drainage[18]. They observed lateral distortion in the shape of the bile ducts in a considerable number of patients with an indwelling T-tube such that an angle measured between the proximal and distal parts of the duct, centered at the site of T-tube drainage insertion, decreased to a range of 60 to 158. They have called this finding the "elbow sign". In the current study, the mean sum of angles before T-tube drainage was 298.0° ± 23.9°, while the mean value of angles after T-tube drainage was 262.5° ± 24.6° in the same patients. The sum of the angles in the CBD changed after T-tube drainage by 35.5° and was statistically significant ($P < 0.05$). Furthermore, the extrahepatic bile duct deformity caused by T-tube drainage influenced the recurrence rate of CBD stones. The recurrence rate of the non-T-tube drainage group was 4.6%, while the recurrence rate of the T-tube drainage group was 15.9% ($P < 0.05$). It is presumed that T-tube placement could introduce local adhesion[33], which, in turn, influences the angulation of the bile duct and then leads to recurrence of bile duct stones as one of the mechanisms.

Our study has some limitations. First, this study is retrospective. Second, we did not prove recurrence with repeat ERCP or cholangiogram in every patient. It may have ascertainment bias. Third, we should estimate the three dimensional image, but we measure bile duct angulation by two-dimensional fluoroscopic imaging. This may show some difference from real angulation. Practically, it is difficult to get three dimensional bile duct images. Fourth, there may be several possible confounders which we did not consider. Medication such as ursodeoxycholic acid and the time of drainage of contrast from the bile duct were not reported in our series. On account of these limitations, it is difficult to be absolutely certain that our specific risk factors were solely responsible for the recurrence of choledocholithiasis.

In conclusion, subsequent to endoscopic biliary sphincterotomy and clearance of bile duct stones, three significant risk factors for the recurrence of the stone were identified on univariate analyses, although bile duct angulation was the only risk factor for recurrence of choledocholithiasis in the multivariate analysis. Patients with a more angulated and dilated bile duct, and a history of T-tube choledochostomy developed stone recurrence more frequently. T-tube choledochostomy performed after cholecystectomy were prone to recurrence of stones by an influence on bile duct angulation.

COMMENTS

Background

After endoscopic sphincterotomy (EST), 4%-24% of patients might experience recurrent choledocholithiasis. The risk of stone recurrence is an important issue, especially for relatively young patients. Bile duct angulation may be a risk factor of stone recurrence by decreasing bile flow.

Research frontiers

The risk factors for true recurrence of bile duct stones after EST are suboptimally defined. Until now, infection along the bile duct and bile stasis were thought to contribute to the recurrence.

Innovations and breakthroughs

Recent several studies showed the association between bile duct angulation and recurrence of stones. The authors added more information about that. In addition, the authors suggested that T-tube choledochostomy influences the recurrence of choledocholithiasis by angulating the bile duct.

Applications

This article provides important data about the risk factors of choledocholithiasis recurrence. By identifying risk factors for stone recurrence, people can improve outcomes by prophylactic treatments or earlier intervention.

Peer review

This is an interesting study that investigated the risk factors of recurrence of common bile duct stones. This study suggested an association between T-tube drainage and recurrence of choledocholithiasis by analyzing the angulation of the common bile duct before and after T-tube drainage.

REFERENCES

1 **Classen M**, Demling L. Endoscopic sphincterotomy of the papilla of vater and extraction of stones from the choledochal duct (author's transl). *Dtsch Med Wochenschr* 1974; **99**: 496-497

2 **Kawai K**, Akasaka Y, Murakami K, Tada M, Koli Y. Endoscopic sphincterotomy of the ampulla of Vater. *Gastrointest Endosc* 1974; **20**: 148-151

3 **Seifert E**. Long-term follow-up after endoscopic sphincterotomy (EST). *Endoscopy* 1988; **20** Suppl 1: 232-235

4 **Hawes RH**, Cotton PB, Vallon AG. Follow-up 6 to 11 years after duodenoscopic sphincterotomy for stones in patients with prior cholecystectomy. *Gastroenterology* 1990; **98**: 1008-1012

5 **Ikeda S**, Tanaka M, Matsumoto S, Yoshimoto H, Itoh H. Endoscopic sphincterotomy: long-term results in 408 patients with complete follow-up. *Endoscopy* 1988; **20**: 13-17

6 **Geenen JE**, Toouli J, Hogan WJ, Dodds WJ, Stewart ET, Mavrelis P, Riedel D, Venu R. Endoscopic sphincterotomy: follow-up evaluation of effects on the sphincter of Oddi. *Gastroenterology* 1984; **87**: 754-758

7 **Cheon YK**, Lehman GA. Identification of risk factors for stone recurrence after endoscopic treatment of bile duct stones. *Eur J Gastroenterol Hepatol* 2006; **18**: 461-464

8 **Thistle JL**. Pathophysiology of bile duct stones. *World J Surg* 1998; **22**: 1114-1118

9 **Gregg JA**, De Girolami P, Carr-Locke DL. Effects of sphincteroplasty and endoscopic sphincterotomy on the bacteriologic characteristics of the common bile duct. *Am J Surg* 1985; **149**: 668-671

10 **Sand J**, Airo I, Hiltunen KM, Mattila J, Nordback I. Changes in biliary bacteria after endoscopic cholangiography and sphincterotomy. *Am Surg* 1992; **58**: 324-328

11 **Nakai K**, Tazuma S, Nishioka T, Chayama K. Inhibition of cholesterol crystallization under bilirubin deconjugation: partial characterization of mechanisms whereby infected bile accelerates pigment stone formation. *Biochim Biophys*

Acta 2003; **1632**: 48-54
12 **Greenfield C**, Cleland P, Dick R, Masters S, Summerfield JA, Sherlock S. Biliary sequelae of endoscopic sphincterotomy. *Postgrad Med J* 1985; **61**: 213-215
13 **Kurumado K**, Nagai T, Kondo Y, Abe H. Long-term observations on morphological changes of choledochal epithelium after choledochoenterostomy in rats. *Dig Dis Sci* 1994; **39**: 809-820
14 **Goldman LD**, Steer ML, Silen W. Recurrent cholangitis after biliary surgery. *Am J Surg* 1983; **145**: 450-454
15 **Kinami Y**, Ashida Y, Seto K, Takashima S, Kita I. Influence of incomplete bile duct obstruction on the occurrence of cholangiocarcinoma induced by diisopropanolnitrosamine in hamsters. *Oncology* 1990; **47**: 170-176
16 **Lai KH**, Peng NJ, Lo GH, Cheng JS, Huang RL, Lin CK, Huang JS, Chiang HT, Ger LP. Prediction of recurrent choledocholithiasis by quantitative cholescintigraphy in patients after endoscopic sphincterotomy. *Gut* 1997; **41**: 399-403
17 **Uchiyama K**, Onishi H, Tani M, Kinoshita H, Kawai M, Ueno M, Yamaue H. Long-term prognosis after treatment of patients with choledocholithiasis. *Ann Surg* 2003; **238**: 97-102
18 **Lee SH**, Burhenne HJ. Extrahepatic bile duct angulation by T-tube: the elbow sign. *Gastrointest Radiol* 1991; **16**: 157-158
19 **Prat F**, Malak NA, Pelletier G, Buffet C, Fritsch J, Choury AD, Altman C, Liguory C, Etienne JP. Biliary symptoms and complications more than 8 years after endoscopic sphincterotomy for choledocholithiasis. *Gastroenterology* 1996; **110**: 894-899
20 **Freeman ML**, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, Moore JP, Fennerty MB, Ryan ME, Shaw MJ, Lande JD, Pheley AM. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909-918
21 **Leung JW**, Sung JY, Costerton JW. Bacteriological and electron microscopy examination of brown pigment stones. *J Clin Microbiol* 1989; **27**: 915-921
22 **Cetta F**. The role of bacteria in pigment gallstone disease. *Ann Surg* 1991; **213**: 315-326
23 **Kaufman HS**, Magnuson TH, Lillemoe KD, Frasca P, Pitt HA. The role of bacteria in gallbladder and common duct stone formation. *Ann Surg* 1989; **209**: 584-591; discussion 591-592
24 **Smith AL**, Stewart L, Fine R, Pellegrini CA, Way LW. Gallstone disease. The clinical manifestations of infectious stones. *Arch Surg* 1989; **124**: 629-633
25 **Cetta F**. The possible role of sphincteroplasty and surgical sphincterotomy in the pathogenesis of recurrent common duct brown stones. *HPB Surg* 1991; **4**: 261-270
26 **Kim DI**, Kim MH, Lee SK, Seo DW, Choi WB, Lee SS, Park HJ, Joo YH, Yoo KS, Kim HJ, Min YI. Risk factors for recurrence of primary bile duct stones after endoscopic biliary sphincterotomy. *Gastrointest Endosc* 2001; **54**: 42-48
27 **Ueno N**, Ozawa Y, Aizawa T. Prognostic factors for recurrence of bile duct stones after endoscopic treatment by sphincter dilation. *Gastrointest Endosc* 2003; **58**: 336-340
28 **Kim MH**, Myung SJ, Seo DW, Lee SK, Kim YS, Lee MH, Yoo BM, Min MI. Association of periampullary diverticula with primary choledocholithiasis but not with secondary choledocholithiasis. *Endoscopy* 1998; **30**: 601-604
29 **Chandy G**, Hart WJ, Roberts-Thomson IC. An analysis of the relationship between bile duct stones and periampullary duodenal diverticula. *J Gastroenterol Hepatol* 1997; **12**: 29-33
30 **Hall RI**, Ingoldby CJ, Denyer ME. Periampullary diverticula predispose to primary rather than secondary stones in the common bile duct. *Endoscopy* 1990; **22**: 127-128
31 **Kennedy RH**, Thompson MH. Are duodenal diverticula associated with choledocholithiasis? *Gut* 1988; **29**: 1003-1006
32 **Warren BL**. Association between cholangiographic angulation of the common bile duct and choledocholithiasis. *S Afr J Surg* 1987; **25**: 13-15
33 **Keizman D**, Shalom MI, Konikoff FM. An angulated common bile duct predisposes to recurrent symptomatic bile duct stones after endoscopic stone extraction. *Surg Endosc* 2006; **20**: 1594-1599
34 **Keizman D**, Ish Shalom M, Konikoff FM. Recurrent symptomatic common bile duct stones after endoscopic stone extraction in elderly patients. *Gastrointest Endosc* 2006; **64**: 60-65

S- Editor Sun H **L- Editor** O'Neill M **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4124

World J Gastroenterol 2011 September 28; 17(36): 4124-4129
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Assessment of participant satisfaction with upper gastrointestinal endoscopy in South Korea

Hoo-Yeon Lee, Sun Mi Lim, Mi Ah Han, Jae Kwan Jun, Kui Son Choi, Myung-Il Hahm, Eun-Cheol Park

Hoo-Yeon Lee, Department of Social Medicine, College of Medicine, Dankook University, 201, Manghyang-ro, Dongnam-gu, Cheonan-si, Chungcheongnam-do 330-714, South Korea
Hoo-Yeon Lee, Sun Mi Lim, Jae Kwan Jun, Kui Son Choi, National Cancer Control Institute, National Cancer Center, Gyeonggi-do 410-769, South Korea
Mi Ah Han, Department of Preventive Medicine, College of Medicine, Chosun University, Seoseok-dong, Dong-gu, Gwangju 501-759, South Korea
Myung-Il Hahm, Department of Health Administration and Management, College of Medical Science, Soonchunhyang University, Shinchang-myun, Asan-si, Chungcheongnam-do 336-745, South Korea
Eun-Cheol Park, Department of Preventive Medicine and Institute of Health Services Research, College of Medicine, Yonsei University, Seoul 120-752, South Korea
Author contributions: Lee HY and Park EC contributed to the concept and study design; Lee HY and Lim SM contributed to execution of the work; Lee HY, Han MA and Hahm MI drafted the article and revise it critically for important intellectual content; Lee HY, Jun JK and Choi KS conducted the data analysis and interpretation; all authors approved the final version of the article.
Supported by The National Cancer Center Research Fund (grant No. 1010200)
Correspondence to: Eun-Cheol Park, MD, PhD, Department of Preventive Medicine and Institute of Health Services Research, College of Medicine, Yonsei University, 250 Seongsan-no, Seodaemun-gu, Seoul 120-752, South Korea. ecpark@yuhs.ac
Telephone: +82-2-22281862 Fax: +82-2-3928133
Received: November 12, 2010 Revised: May 19, 2011
Accepted: May 26, 2011
Published online: September 28, 2011

Abstract

AIM: To measure the perceived satisfaction with gastric cancer screening as part of the National Cancer Screening Program (NCSP) in South Korea.

METHODS: Data were derived from the participants in a satisfaction survey of the Quality Evaluation of National Cancer Screening in 2009. This is a population-based nationwide telephone survey of participants who were screened by the NCSP between May and October 2009. This study included 4412 participants who provided full sets of data and who had upper endoscopies for the purpose of gastric cancer screening.

RESULTS: The negative appraisal percentages for each of the seven questions were as follows: explanation in preparation for the upper endoscopy, 12.3%; explanation about the process and procedure of the upper endoscopy, 13.8%; explanation about any pain or discomfort related to the upper endoscopy, 27.5%; level of pain during the procedure, 30.3%; physical environment, 16.2%; manner of the staff, 11.2%, and privacy protection, 8.8%.

CONCLUSION: The critical issues identified by the Pareto analysis include the adequacy of the explanation about any pain or discomfort associated with the upper endoscopy and the level of pain experienced during the procedure.

Key words: Gastric cancer; Cancer screening; Upper endoscopy; Satisfaction

Peer reviewer: Sung Kim, MD, PhD, Professor, Department of Surgery, Sansung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-Dong, Gangnam-Gu, Seoul, South Korea

Lee HY, Lim SM, Han MA, Jun JK, Choi KS, Hahm MI, Park EC. Assessment of participant satisfaction with upper gastrointestinal endoscopy in South Korea. *World J Gastroenterol* 2011; 17(36): 4124-4129 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4124.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4124

INTRODUCTION

In recent years, patient satisfaction with endoscopic procedures has become an important outcome measure of gastrointestinal (GI) endoscopies[1]. Patient satisfaction not only establishes performance standards, but also increases the accountability of physicians and staff, and most importantly, can lead to improvement in the quality of care. Few studies have assessed the factors associated with patient satisfaction with upper endoscopies. Factors that have been significantly and positively associated with patient satisfaction included the adequacy of analgesia during the procedure, low patient anxiety before the endoscopic procedure, respectful personal manner of endoscopists, respectful personal manner of nurses, patient positive perceptions of endoscopists' technical skills, pleasant physical environment in the endoscopy unit, and adequate time spent by physicians explaining the procedure[1-3].

Understanding the level of satisfaction with upper endoscopies performed for gastric cancer screening may provide information necessary for optimising adherence to screening protocols. The satisfaction or procedure-related discomfort in upper endoscopies has rarely been given priority because the procedure can be done quickly and is associated with few complications. Despite a low rate of medical complications, the procedure is associated with substantial pre-procedural anxiety and procedure-related discomfort. Endoscopists tend to underestimate patient discomfort or dissatisfaction[4].

Gastric cancer is the second most common type of cancer worldwide. With estimated 934 000 new cases in 2002 (8.6%), gastric cancer fell to the 4th place behind cancers of the lung, breast, and colon and rectum[5]. However, gastric cancer remains the most frequently diagnosed cancer in South Korea[6-8]. Gastric cancer screening is an increasingly important activity in the effort to early detect and control gastric cancer[9,10]. Countries, such as Japan and South Korea, where gastric cancer is highly prevalent, have conducted gastric cancer screenings for people at average risk. Although there is debate over the value and risk of screening asymptomatic individuals, interest has shifted to determining the preferred screening strategy and discerning the most effective ways of implementing screening procedures for the general population[11].

In South Korea, screening for gastric cancer started in 1999 as a part of the National Cancer Screening Program (NCSP) for low-income groups. Currently, the NCSP provides Medical Aid recipients and National Health Insurance beneficiaries in the 50% of income brackets with free screening services for five common cancers: gastric, liver, colorectal, breast, and cervical. The NCSP recommends a biennial upper gastrointestinal series (UGIS) or an upper endoscopy for men and women over 40 years of age. In recent years, mass screenings using upper endoscopies have replaced upper GI X-rays in several cities in South Korea. In 2002, 75.0% of the participants underwent upper-GI X-rays, and 25.0% received upper endoscopies. In 2008, 50.9% received upper-GI X-rays, and 49.1% received upper endoscopies[12].

The objectives of this study were to analyze the data obtained by questionnaires measuring the perceived satisfaction with gastric cancer screening as part of the NCSP and to identify and ameliorate the issues that contribute most to patient dissatisfaction.

MATERIALS AND METHODS

Data source

Quality Evaluation of National Cancer Screening (QENCS) programs to improve the quality of NCSP were established in 2008. QENCS programs in an upper endoscopy evaluate all aspects of cancer screening, including the structure, process and outcome. It also includes the general items, such as the indication for the endoscopic examination, informed consent, patient risk stratification, and sedation practice. Outcome indicators are participant satisfaction and accuracy such as cancer detection rate, false positive rate, and interval caner.

Based on a previously validated questionnaire[13], we conducted a population-based nationwide telephone survey among participants who were screened by the NCSP between May and October 2009. A sample of 43 157 participants was randomly chosen and stratified according to age, gender and gastric cancer unit. We evaluated participants' satisfaction with gastric cancer screening performed in hospitals. In total, 12 922 calls were successful, and 9090 participants (70.3%) agreed to complete the survey. Participants who received gastric cancer screening with an upper gastrointestinal series were excluded from the study. Finally, 4412 participants who provided full sets of data and who had upper endoscopies for purposes of gastric cancer screening were included in this study. This research was approved by the Institutional Review Board Committee.

Questionnaire

The questionnaire addressed six specific aspects of participant satisfaction with the screening experience: the adequacy of explanations (questions 1, 2 and 3), the manner adopted by doctors and nurses (question 4), privacy protection (question 5), physical surroundings (question 6), pain or discomfort during the procedure (question 7), and overall satisfaction (question 8). The items and scoring method are presented in Table 1. Participants answered each question, except the question regarding overall satisfaction, with a numerical score on a Likert scale, ranging from 1 (poor) to 4 (excellent). Overall satisfaction was scored on a 10-point scale.

Statistical analysis and graphic representation

We defined the problem rate as the percentage of "fair" or "poor" responses given by all participants to all questions[14]. The problem rate was calculated by adding all poor or fair responses on all questionnaires, dividing this figure by the total number of questions, and multiplying

Table 1 Items and scoring methods

Questions	Poor	Fair	Good	Excellent
I received adequate information/explanations in preparation for the upper endoscopy	1	2	3	4
I received adequate information/explanations about the process and procedure of the upper endoscopy	1	2	3	4
I received adequate information/explanations about any pain or discomfort related to the upper endoscopy	1	2	3	4
I was satisfied with the respectfulness of the staff and the manner of doctors and nurses	1	2	3	4
I had adequate privacy during the procedure	1	2	3	4
I was satisfied with the pleasantness of the physical environment	1	2	3	4
I did not experience too much pain/discomfort during the procedure	1	2	3	4
	Strongly disagree			Strongly agree
I am satisfied with my overall experience with gastric cancer screening	1 2 3 4 5 6 7 8 9 10			

Table 2 Characteristics of study population (n = 4412)

Variable group	Frequency (%)
Gender	
Male	40.1
Female	59.9
Age	
≥ 40 yr, < 50 yr	49.9
≥ 50 yr, < 60 yr	33.6
≥ 60 yr	17.5
Education level	
Elementary school or none	17.3
Middle school	19.7
High school	44.3
University or above	18.7
Sedation	
Yes	48.0
No	52.0

this result by 100. This calculation can be expressed by the following formula:

$$\frac{\sum \text{poor and fair answers}}{\sum \text{times each question was evaluated}} \times 100$$

The percentage of poor or fair responses was also calculated for each question.

Quality assessment uses special methods for graphic representation and analysis, and is considered to be as important as the statistical analysis itself. Of the various graphic analysis methods used currently, the Pareto chart was adopted in this study[14]. This chart presents the relative importance assigned by all participants to each question included in the calculation of the problem rate and produces a bar chart representing the percentage of problems for each question over the total number of problems emerging from the data. Bars are shown from the left to right in order of decreasing level of importance. Figures in the column represent the percentages of poor and fair responses over the total of poor and fair responses. In addition, we drew a line over the bars to represent the accumulated percentage of problems

RESULTS

Of the total 4412 participants in the study population, men accounted for 40.1%, and those aged 40-49 years

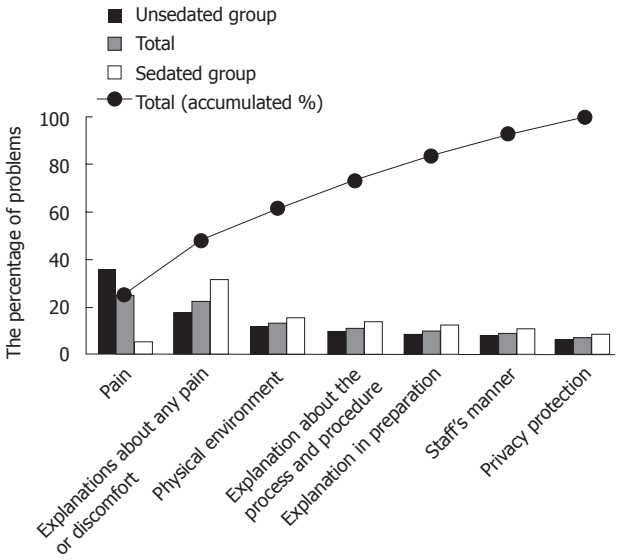


Figure 1 Relative importance of each question for the problem rate among all participants.

accounted for 49.9%. Additionally, 48.0% underwent upper endoscopies while sedated (Table 2). In general, the mean and median satisfaction scores were high (> 3) for all questions, with the exception of the question addressing the level of pain (Table 3). We observed a trend toward higher mean satisfaction scores among the sedated group with respect to seven of the eight questions; the exception was the question addressing the adequacy of the explanation of any pain or discomfort associated with the procedure.

The critical few issues identified by the Pareto analysis included the question about the adequacy of the explanation about any pain or discomfort associated with the procedure and the question regarding the level of pain experienced during the procedure (Table 3, Figure 1). The problem rate was 17.2% (5298 poor or fair responses to a total of 30 884 questions) in the total study population. The critical few with a 30.3% problem rate, included the question about the level of pain experienced during the procedure (Table 3). The problem rate was 12.8% (1900 poor or fair responses to a total of 14 812 questions) in the sedated group and 21.1% (3398 poor or fair responses to a total of 16 072 questions) in the unsedated group.

Table 3 Comparison of satisfaction scores between sedated and unsedated groups

Question label	Total (n = 4412)			Sedated group(n = 2116)			Unsedated group (n = 2296)		
	Mean (SD)	Median (25Q-75Q)	Poor and fair (%)	Mean (SD)	Median (25Q-75Q)	Poor and fair (%)	Mean (SD)	Median (25Q-75Q)	Poor and fair (%)
Adequacy of explanations in preparation for upper endoscopy (%)	3.3 (0.8)	3 (3-4)	12.3	3.4 (0.8)	4 (3-4)	11.4	3.3 (0.8)	3 (3-4)	13.2
Adequacy of explanations about process and procedure of upper endoscopy (%)	3.3 (0.8)	3 (3-4)	13.8	3.3 (0.8)	4 (3-4)	12.6	3.2 (0.8)	3 (3-4)	14.9
Adequacy of explanations about any pain or discomfort related to upper endoscopy (%)	3.0 (1.0)	3 (2-4)	27.5	3.0 (1.0)	3 (2-4)	28.7	3.0 (0.9)	3 (2-4)	26.3
Staff's manner (%)	3.3 (0.7)	3 (3-4)	11.2	3.4 (0.7)	3 (3-4)	10	3.3 (0.7)	3 (3-4)	12.2
Privacy protection (%)	3.4 (0.7)	3 (3-4)	8.8	3.4 (0.7)	4 (3-4)	7.9	3.3 (0.7)	3 (3-4)	9.6
Physical environment (%)	3.2 (0.7)	3 (3-4)	16.2	3.2 (0.7)	3 (3-4)	14	3.1 (0.7)	3 (3-4)	18.3
Level of pain experienced during the procedure (%)	3.0 (1.0)	3 (2-4)	30.3	3.7 (0.6)	4 (4-4)	5.2	2.4 (0.8)	2 (2-3)	53.5
Overall satisfaction	7.8 (1.8)	8 (7-10)		8.0 (1.8)	8 (7-10)		7.6 (1.7)	8 (7-8)	
Problem rate			17.2			12.8			21.1

DISCUSSION

We used the overall score on questions 1-8 as an indicator of participants' satisfaction with the upper endoscopy offered by the NCSP. In general, the mean and median satisfaction scores were high (> 3) for all questions, with the exception of the question concerning the level of pain. We observed a trend toward higher mean satisfaction scores in the sedated group. Our research shows that the level of pain experienced during the procedure and the adequacy of the explanation of any pain or discomfort associated with the procedure constituted the factors that contributed most to the problem rate among participants.

In terms of quality, these two concerns comprise the critical few. The implementation of measures to improve these two main problem areas would probably reduce the rate of problems among participants. The level of pain was an important determinant of satisfaction with the upper endoscopic procedure used for gastric cancer screening, especially in the unsedated group. This finding is consistent with the results of previous studies, which reported that higher levels of pain or discomfort were correlated with lower patient satisfaction with the procedure[15,16]. A second reason for dissatisfaction identified by our study was the adequacy of explanations of any pain or discomfort associated with the procedure. A large percentage of complaints in both sedated and unsedated groups was related to communication problems[14]. The total problem rates identified by the three questions regarding the adequacy of explanations were greater than 50% in both sedated and unsedated groups, indicating that dissatisfaction with explanations and communication is the most important contributor to the problem rate. This suggests that screenings are often performed without sufficient explanation, even though participants acquire information before this procedure[17,18]. Before screening, both the preparation for and the actual process of the endoscopy should be explained. It would also be helpful to explain the possible discomfort associated with this procedure. Additionally, efforts to ensure privacy and improve the interactional style of staff members may improve population screening programmes.

The total gastric cancer-screening rates have been increasing steadily in South Korea, and the rate of upper endoscopic examination has also been increasing[12]. However, the rate of participation in gastric cancer screening programs is still not optimal[19]. Previous results of a population-based survey have identified the upper endoscopy as the preferred gastric cancer screening method. Interestingly, respondents with higher income levels were more likely to have had an endoscopic examination compared with those in lower income levels. Under the South Korean NCSP, endoscopic examination, like UGI tests, are free of charge. Despite these programmes, the use of endoscopy varies with household income, suggesting the possible impact of barriers other than the cost of endoscopy *per se*[6]. Indeed, under this NCSP, participants have to pay for all procedure-related costs associated with sedation, which may represent one of the barriers associated with income disparities.

Patient satisfaction is a crucial parameter in the management of the quality of endoscopies because it directly reflects patient acceptance of procedures and possibly reflects patient compliance with screening and monitoring[20], thus, dissatisfaction with the screening experience may lead to non-compliance[13,21-24]. Satisfaction is particularly important when targeting asymptomatic individuals because they have no obvious reason to seek the services of a screening program. Moreover, levels of satisfaction are also important indicators of the quality of care, and feedback from participants can be used to modify program operations[21]. To increase compliance with the NCSP, we addressed issues related to improving satisfaction with screening services.

Upper endoscopy is a safe and quick procedure, and can be performed without sedation[25]. However, it can also evoke anxiety, feelings of vulnerability, embarrass-

ment, and discomfort, and the fears and concerns associated with endoscopic procedures decrease patient compliance. Conscious sedation is the method most widely used, and good tolerance or conscious sedation has been related to the acceptance of and higher satisfaction with endoscopic procedures[1,15,16,26,27]. Although usually safe, gastric cancer screening tests have tradeoffs in terms of efficacy, complications, discomfort, time, and cost[6,12,28]. The performance of upper endoscopies using sedation is more costly but remains an efficacious strategy because it increases the rates of successful endoscopies, patient satisfaction, and willingness to undergo repeat procedures[29].

For these reasons, the NCSP should support the cost of sedation for upper endoscopies and let patients choose whether to undergo this procedure with or without sedation. Such decisions should be based on patient preferences and the clinical judgment of endoscopists, which, in turn, are based on patient age, sex, and tolerance for the procedure[6,16]. Providers' assessment of individuals' screening preferences, in combination with intervention strategies to promote the preferred screening method, may increase compliance with gastric cancer screening recommendations[28,30]. It will be imperative to consider these results when making decisions about population-based screening strategies[31].

This study has several limitations, including the possibility of a degree of participant bias. Respondents who completed the follow-up questionnaire were often those who reported discomfort on the post-procedure questionnaire; thus, this group may have been more likely to respond. The study was conducted among healthy individuals (i.e., participants in the National Gastric Cancer Screening Program), and the results may not be applicable to other patient populations. Indeed, participants might have different values, expectations about the procedure, and pain tolerance, which would potentially influence their satisfaction with the endoscopic procedure. Additionally, our study was conducted at a hospital, which may influence the extent to which our findings are generalizable to non-hospital settings such as clinics.

Despite these limitations, the present study used a reliable and valid survey methodology to evaluate satisfaction with the NCSP. The assessment of satisfaction with the NCSP is useful, as the degree of satisfaction with screening programmes is correlated with adherence patterns. The present study serves as a basis for future interventions to improve satisfaction with upper endoscopic procedures, including using sedation or establishing a program for training staff in communication skills and interpersonal interactions. These findings may be used to develop strategies to promote participation in and adherence to the South Korean Gastric Cancer Screening Program.

COMMENTS

Background

In recent years, patient satisfaction with endoscopic procedures has become an important outcome measure of gastrointestinal (GI) endoscopies. Patient satisfaction not only establishes performance standards, but also increases the accountability of physicians and staff, and most importantly, can lead to improvement in the quality of care.

Research frontiers

National gastric cancer-screening rates have been increasing steadily in South Korea. However, the rate of participation in gastric cancer screening programs is still not optimistic. The authors suggest measuring the perceived satisfaction with gastric cancer screening as part of the National Cancer Screening Program so as to identify and ameliorate the issues that contribute most to participant dissatisfaction.

Innovations and breakthroughs

This study shows that the level of pain experienced during the endoscopic procedure and the adequacy of the explanation of any pain or discomfort associated with the procedure constituted the factors that contributed most to the problem rate among participants. The implementation of measures to improve these two main problem areas would probably reduce the rate of problems among participants.

Applications

This study serves as a basis for future interventions to improve the satisfaction with upper endoscopic procedures, including using sedation or establishing a program for training staff in communication skills and interpersonal interactions.

Peer review

The study is well done both number and selection of cases, and the results quite clear.

REFERENCES

1 **Ko HH**, Zhang H, Telford JJ, Enns R. Factors influencing patient satisfaction when undergoing endoscopic procedures. *Gastrointest Endosc* 2009; **69**: 883-891, quiz 891.e1

2 **Yacavone RF**, Locke GR, Gostout CJ, Rockwood TH, Thieling S, Zinsmeister AR. Factors influencing patient satisfaction with GI endoscopy. *Gastrointest Endosc* 2001; **53**: 703-710

3 **Peña LR**, Mardini HE, Nickl NJ. Development of an instrument to assess and predict satisfaction and poor tolerance among patients undergoing endoscopic procedures. *Dig Dis Sci* 2005; **50**: 1860-1871

4 **Seip B**, Huppertz-Hauss G, Sauar J, Bretthauer M, Hoff G. Patients' satisfaction: an important factor in quality control of gastroscopies. *Scand J Gastroenterol* 2008; **43**: 1004-1011

5 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108

6 **Choi KS**, Kwak MS, Lee HY, Jun JK, Hahm MI, Park EC. Screening for gastric cancer in Korea: population-based preferences for endoscopy versus upper gastrointestinal series. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1390-1398

7 **Lee HY**, Park EC, Jun JK, Choi KS, Hahm MI. Comparing upper gastrointestinal X-ray and endoscopy for gastric cancer diagnosis in Korea. *World J Gastroenterol* 2010; **16**: 245-250

8 **Leung WK**, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, Wu KC, Wu DC, Sollano J, Kachintorn U, Gotoda T, Lin JT, You WC, Ng EK, Sung JJ. Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008; **9**: 279-287

9 **Kim YS**, Park HA, Kim BS, Yook JH, Lee MS. Efficacy of screening for gastric cancer in a Korean adult population: a case-control study. *J Korean Med Sci* 2000; **15**: 510-515

10 Cancer Fact **and Figures 2008 in Korea: Ministry for Health** and Welfare. **National Cancer Center; 2008**

11 **Pisani P**, Oliver WE, Parkin DM, Alvarez N, Vivas J. Case-control study of gastric cancer screening in Venezuela. *Br J Cancer* 1994; **69**: 1102-1105

12 Annual report of national cancer screening program in Korea. **National Cancer Center; 2009**

13 **Yoon NH**, Lee HY, Kwak MS, Choi KS, Jun JK, Kim MK, Park EC. Comparison of satisfaction with cancer screening at mobile van and static sites: National Cancer Screening

Program in Korea. *Jpn J Clin Oncol* 2009; **39**: 169-174
14 **Del Río AS**, Baudet JS, Fernández OA, Morales I, Socas Mdel R. Evaluation of patient satisfaction in gastrointestinal endoscopy. *Eur J Gastroenterol Hepatol* 2007; **19**: 896-900
15 **Schutz SM**, Lee JG, Schmitt CM, Almon M, Baillie J. Clues to patient dissatisfaction with conscious sedation for colonoscopy. *Am J Gastroenterol* 1994; **89**: 1476-1479
16 **Froehlich F**, Thorens J, Schwizer W, Preisig M, Köhler M, Hays RD, Fried M, Gonvers JJ. Sedation and analgesia for colonoscopy: patient tolerance, pain, and cardiorespiratory parameters. *Gastrointest Endosc* 1997; **45**: 1-9
17 **Doyle C,** Stanton M. Significant factors in patient satisfaction ratings of screening mammography. *Radiol* 2002; **8**: 159-172
18 **Engelman KK**, Ellerbeck EF, Mayo MS, Markello SJ, Ahluwalia JS. Mammography facility characteristics and repeat mammography use among Medicare beneficiaries. *Prev Med* 2004; **39**: 491-497
19 **Kwon YM**, Lim HT, Lee K, Cho BL, Park MS, Son KY, Park SM. Factors associated with use of gastric cancer screening services in Korea. *World J Gastroenterol* 2009; **15**: 3653-3659
20 **Eckardt AJ**, Swales C, Bhattacharya K, Wassef WY, Phelan NP, Zubair S, Martins N, Patel S, Moquin B, Anwar N, Leung K, Levey JM. Open access colonoscopy in the training setting: which factors affect patient satisfaction and pain? *Endoscopy* 2008; **40**: 98-105
21 **Decker KM**, Harrison M, Tate RB. Satisfaction of women attending the Manitoba breast screening program. *Prev Med* 1999; **29**: 22-27
22 **Peipins LA**, Shapiro JA, Bobo JK, Berkowitz Z. Impact of women's experiences during mammography on adherence to rescreening (United States). *Cancer Causes Control* 2006; **17**: 439-447
23 **Somkin CP**, McPhee SJ, Nguyen T, Stewart S, Shema SJ, Nguyen B, Pasick R. The effect of access and satisfaction on regular mammogram and Papanicolaou test screening in a multiethnic population. *Med Care* 2004; **42**: 914-926
24 **Orton M**, Fitzpatrick R, Fuller A, Mant D, Mlynek C, Thorogood M. Factors affecting women's response to an invitation to attend for a second breast cancer screening examination. *Br J Gen Pract* 1991; **41**: 320-322
25 **Trevisani L**, Sartori S, Gaudenzi P, Gilli G, Matarese G, Gullini S, Abbasciano V. Upper gastrointestinal endoscopy: are preparatory interventions or conscious sedation effective? A randomized trial. *World J Gastroenterol* 2004; **10**: 3313-3317
26 **Ladas SD**, Aabakken L, Rey JF, Nowak A, Zakaria S, Adamonis K, Amrani N, Bergman JJ, Boix Valverde J, Boyacioglu S, Cremers I, Crowe J, Deprez P, Díte P, Eisen M, Eliakim R, Fedorov ED, Galkova Z, Gyokeres T, Heuss LT, Husic-Selimovic A, Khediri F, Kuznetsov K, Marek T, Munoz-Navas M, Napoleon B, Niemela S, Pascu O, Perisic N, Pulanic R, Ricci E, Schreiber F, Svendsen LB, Sweidan W, Sylvan A, Teague R, Tryfonos M, Urbain D, Weber J, Zavoral M. Use of sedation for routine diagnostic upper gastrointestinal endoscopy: a European Society of Gastrointestinal Endoscopy Survey of National Endoscopy Society Members. *Digestion* 2006; **74**: 69-77
27 **Ladas SD**, Satake Y, Mostafa I, Morse J. Sedation practices for gastrointestinal endoscopy in Europe, North America, Asia, Africa and Australia. *Digestion* 2010; **82**: 74-76
28 **Meissner HI**, Breen N, Klabunde CN, Vernon SW. Patterns of colorectal cancer screening uptake among men and women in the United States. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 389-394
29 **Abraham NS**, Fallone CA, Mayrand S, Huang J, Wieczorek P, Barkun AN. Sedation versus no sedation in the performance of diagnostic upper gastrointestinal endoscopy: a Canadian randomized controlled cost-outcome study. *Am J Gastroenterol* 2004; **99**: 1692-1699
30 **Leard LE**, Savides TJ, Ganiats TG. Patient preferences for colorectal cancer screening. *J Fam Pract* 1997; **45**: 211-218
31 **Lee YC**, Lin JT, Wu HM, Liu TY, Yen MF, Chiu HM, Wang HP, Wu MS, Hsiu-Hsi Chen T. Cost-effectiveness analysis between primary and secondary preventive strategies for gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 875-885

S- Editor Sun H **L- Editor** Ma JY **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4130

World J Gastroenterol 2011 September 28; 17(36): 4130-4134
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Anti-hepatitis A seroprevalence among chronic viral hepatitis patients in Kelantan, Malaysia

Fazlina Ahmad, Nor Aizal Che Hamzah, Nazri Mustaffa, Siew Hua Gan

Fazlina Ahmad, Nor Aizal Che Hamzah, Nazri Mustaffa, Department of Medicine, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
Siew Hua Gan, Human Genome Centre, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
Author contributions: Che Hamzah NA, Ahmad F, Mustaffa N and Gan SH designed the study; Ahmad F performed the research and analysed the data; Ahmad F and Che Hamzah NA wrote the paper.
Supported by Short term grant No. 304/PPSP/61310014 from the Universiti Sains Malaysia
Correspondence to: Nor Aizal Che Hamzah, MRCP (UK), Senior Lecturer in Internal Medicine and Gastroenterology, Department of Medicine, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia. aizalchehamzah@hotmail.com
Telephone: +60-9-7676590 Fax: +60-9-7673949
Received: February 14, 2011 Revised: April 15, 2011
Accepted: April 22, 2011
Published online: September 28, 2011

Abstract

AIM: To determine the seroprevalence of anti-hepatitis A virus (HAV) antibodies in patients with chronic liver disease (CLD) and to justify the need for hepatitis A vaccination.

METHODS: Patients (n = 119) were enrolled between July and September 2009. The diagnosis of CLD was based on the presence of viral markers for more than 6 mo. The diagnosis of liver cirrhosis was based on clinical, biochemical and radiological profiles. Patient serum was tested for anti-HAV IgG.

RESULTS: The overall anti-HAV seroprevalence was 88.2%. The aetiology of CLD was hepatitis B in 96 patients (80.7%) and hepatitis C in 23 patients (19.3%). Mean age was 44.4 ± 14 years. Patients were grouped according to age as follows: 24 (20.2%) patients in the 21-30 years age group, 22 (18.5%) in the 31-40 years age group, 31 (26.1%) in the 41-50 years age group, 23 (19.3%) in the 51-60 years age group and 19 (16.0%) patients aged greater than 60 years, with reported seroprevalences of 66.7%, 95.5%, 93.5%, 91.3% and 94.7%, respectively. There was a marked increase of seroprevalence in subjects older than 30 years (P = 0.001).

CONCLUSION: Our study demonstrated that patients aged greater than 30 years of age were likely to have natural immunity to hepatitis A. Therefore, hepatitis A vaccination may not be routinely required in this age group.

Key words: Hepatitis A seroprevalence; Chronic viral hepatitis; Malaysia; Hepatitis A vaccination

Peer reviewer: Yoshiaki Iwasaki, MD, PhD, Associate Professor, Health Service Center, Okayama University, 2-1-1, Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan

Ahmad F, Che Hamzah NA, Mustaffa N, Gan SH. Anti-hepatitis A seroprevalence among chronic viral hepatitis patients in Kelantan, Malaysia. *World J Gastroenterol* 2011; 17(36): 4130-4134 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4130.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4130

INTRODUCTION

Hepatitis A remains a significant problem in Malaysia. Malaysia is among those countries reported to be of intermediate endemicity, along with Thailand and Sri Lanka[1-3]. The hepatitis A virus (HAV) has been reported to be the main cause of symptomatic clinical hepatitis (up to 66.4% in 1996) in the Eastern region of Peninsular Malaysia when compared to other causes of viral hepatitis[4]. Kelantan is one of the states situated in the Eastern region of Peninsular Malaysia. However, over the last 20 years, pat-

terns of endemicity in South-East Asia have changed due to improvements in living standards, with some countries shifting from high to intermediate or intermediate to low endemicities[1,3,5-7]. The prevalence of HAV in Malaysia is expected to fall with time; this means, however, that reintroduction of the virus to a non-immune population could produce a community-level outbreak, which may lead to an increase in morbidity and mortality[2,8].

HAV super-infections in patients with underlying chronic liver disease (CLD) may lead to decompensation of the liver. Acute HAV super-infection is associated with higher morbidity and mortality than are isolated cases of acute HAV infection, leading to an increase in the likelihood of developing a fulminant liver failure[9-14]. Based on epidemiological studies of large hepatitis A outbreaks in Shanghai in the late 1980s, acute hepatitis A in patients with chronic hepatitis B has an even more severe clinical course and higher risk of death[15]. The fatality rates for acute hepatitis A were 5.6 times higher among hepatitis B surface antigen (HBsAg) carriers when compared to HBsAg-negative patients[16]. A similar scenario may also be true for super-infection of hepatitis A in chronic hepatitis C patients. An observational study conducted over a 7-year period among 432 Italian patients with chronic hepatitis C reported that amongst the 17 patients (3.9%) with acute hepatitis A super-infection, 41% progressed into fulminant hepatic failure[17]. This emphasises the need for vaccination in CLD patients without natural immunity.

In the West, hepatitis A vaccination has been recommended in all patients with CLD to prevent super-infection with HAV, which may cause high morbidity and mortality in this group of patients[18-20]. However, in countries where hepatitis A is still endemic, such as Malaysia, the utility of this vaccine must be examined, as it is possible that most patients have already acquired natural immunity[21-23].

Therefore, we aimed to determine the seroprevalence of anti-HAV antibodies in patients with CLD in our region.

MATERIALS AND METHODS

Sample population

Patients with CLD (n = 119) attending the Gastroenterology Clinic of Universiti Sains Malaysia, Kelantan between July and September 2009 were enrolled after having signed written informed consents. Diagnosis of CLD was based on the presence of HBsAg or anti-hepatitis C virus antibody (anti-HCV) in serum for a period of at least 6 mo. The underlying liver diseases were classified into either liver cirrhosis (LC) or non-cirrhotic CLD. LC was evidenced by previous ultrasonography (i.e., coarse liver architecture, nodular liver surface and blunt liver edges) as well as confirmation of hypersplenism (i.e., splenomegaly on ultrasonography with a platelet count < 100 000/mm^3)[3]. Patients with underlying CLD of non-viral origin were excluded.

The patients were classified into the following five groups according to age: (1) Group A: 21 to 30 years; (2) Group B: 31 to 40 years; (3) Group C: 41 to 50 years; (4) Group D: 51 to 60 years; and (5) Group E: greater than 60 years of age. The study was approved by the local university's Research and Ethics Committee and complied with the Declaration of Helsinki.

Detection of HAV IgG, HBV and HCV infections

Immunity towards hepatitis A was established by detection of anti-HAV IgG using commercially available immunoassay kits for anti-HAV IgG (Abbot Laboratories, Chicago, Illinois, United States) that rely on microparticle enzyme immunoassay methods. Presence of hepatitis B virus (HBV) and hepatitis C virus infection was determined by detection of HBsAg and anti-HCV antibodies, respectively.

Statistical analysis

All data analyses were carried out using SPSS statistical software (Version 12.0.1). Continuous variables were expressed as mean and standard deviation for normally distributed data while categorical variables were expressed as frequency and percentage. A chi-square (χ^2) test was used to determine whether significant differences exist between two categorical variables. Results were reported as significant when $P < 0.05$. For multivariate analyses, a stepwise multivariate logistic regression model was employed to assess the relative importance of variables showing a significant association ($P < 0.05$) or any other clinically important variables in univariate analysis ($P < 0.10$). Results of all multivariable analyses were reported as adjusted odds ratio, 95% CI and exact P value.

RESULTS

The mean age at presentation was 44.4 ± 14 years (range 21-76 years). Males comprised the majority of the study population (62.2%). The Malay constituted the highest proportion of the study subjects (80.7%). This distribution reflects the current ethnic diversity in our population.

The aetiology of the underlying liver disease was chronic HBV infection in 96 (80.7%) patients, while chronic HCV infection was present in the remainder of the population (19.3%). The distribution of disease status was LC (14.3%), while others were in the non-cirrhotic group (Table 1).

The overall prevalence of anti-HAV was 88.2% (105/119), while seroprevalence differed greatly based on age group: 66.7% in Group A, 95.5% in Group B, 93.5% in Group C, 91.3% in Group D and 94.7% in Group E (Figure 1). The anti-HAV prevalence was significantly lower in patients younger than 30 years of age when compared to those who were in the older age groups (Table 2). Multivariate analysis of age category variables also showed a significant difference between patients younger than 30 years when compared to those who were in the older age groups, with the exception of

Table 1 Patient demographic and clinical data

Characteristics	Values (%)
Mean age (yr)	44.4 ± 14.0
Male	74 (62.2)
Aetiology of liver disease	
HBV	96 (80.7)
HCV	23 (19.3)
Status of liver disease	
Non-cirrhotic	102 (85.7)
Liver cirrhosis	17 (14.3)
Prevalence of IgG HAV	105/119 (88.2)

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

Table 2 Univariate analysis of demographic data and anti-hepatitis A virus IgG positivity

Variables	Anti-HAV IgG *n* (%)		χ^2 value	*P* value
	Positive	Negative		
Age (yr), mean ± SD	45.7 ± 13.4	35.1 ± 15.8		
Age less than 30 yr	16 (66.7)	8 (33.3)	13.473	0.001[1]
Age more than 30 yr	89 (93.7)	6 (6.3)		
Race				
Malay	84 (87.5)	12 (12.5)	0.259	0.611[1]
Non-Malay	21 (91.3)	2 (8.7)		
Gender				
Male	68 (91.9)	6 (8.1)	2.521	0.112[2]
Female	37 (82.2)	8 (17.8)		
Aetiology of liver disease				
Hepatitis B	83 (86.5)	13 (13.5)	1.511	0.219[1]
Hepatitis C	22 (95.7)	1 (4.3)		
Status of CLD				
Non cirrhotic	88 (86.3)	14 (13.7)	2.644	0.216[1]
Liver cirrhosis	17 (100.0)	0 (0.0)		
Education level				
No formal education	5 (71.4)	2 (28.6)	2.024	0.191[1]
Primary	12 (100.0)	0 (0.0)	1.779	0.356[1]
Secondary	61 (89.7)	7 (10.3)	0.331	0.565[2]
Tertiary	27 (84.4)	5 (15.6)	0.628	0.522[1]
Salary category (RM)				
< 1000	48 (84.2)	9 (15.8)	1.707	0.257[2]
1001-2000	31 (88.6)	4 (11.4)	0.005	1.000[1]
2001-3000	14 (93.3)	1 (6.7)	0.43	1.000[1]
> 3000	12 (11.4)	0 (0.0)	1.779	0.356[1]
Comorbidities				
Diabetes	21 (100)	0 (0.0)	3.4	0.127[1]
Hypertension	19 (100.0)	0 (0.0)	3.015	0.122[1]
Ischaemic heart	6 (100.0)	0 (0.0)	0.842	1.000[1]

[1]Fisher's exact test; [2]Pearson χ^2 Test. $P < 0.05$ was considered as significant at the 95% confidence level. HAV: Hepatitis A virus; CLD: Chronic liver disease.

group D ($P = 0.053$) (Table 3).

The overall prevalence of hepatitis A was 80.5% in the 96 patients with chronic HBV infection and 95.7% in the 23 patients with chronic HCV infection. Anti-HAV was more frequently (100%) detected in LC patients when compared to non-cirrhotic patients (86.3%)

DISCUSSION

A study by Ton *et al*[24] investigated 100 healthy individuals from Kuala Lumpur in 1983 and reported a seroprevalence of hepatitis A of 78.2%. In 1985, a study reported that 100% of a Malaysian population were anti-HAV positive by 30 years of age[25]. However, in 1992, only 45% of the same age group were antibody-positive, indicating a shifting epidemiology, most probably due to improvements in living standards[25]. In the present study, we found that the overall anti-HAV seropositivity was high, at 88.2%. The higher seroprevalence of hepatitis A in our chronic viral hepatitis patients compared to previously reported seroprevalence for normal individuals was most likely related to the characteristics of the studied population in this region. This study was conducted in Kelantan, which is located in the north-eastern corner of the peninsula facing the South China Sea, with a chiefly agrarian economy. The population has diverse socioeconomic status and large income inequalities. The previous study was conducted in Malaysia's capital, Kuala Lumpur[24], which has higher living standards, while the other included paediatric age groups with lower expected natural immunity[26]. Therefore, it is possible that the seroprevalence of HAV in other parts of Malaysia might not be similar to our findings, and we recommend further study in each locality to determine this. It is also possible that CLD patients have a higher prevalence of HAV positivity than the normal population. Contradicting this, Joshi and colleagues have revealed similar hepatitis A seroprevalence differences between CLD and normal populations in India[21].

We also show that the main factor that influences the rate of positivity is the age group. The seroprevalence

Table 3 Multivariate analysis of age category variables

Variables (age)	Walds (df)	Adjusted OR	*P* value	95% CI	
				Lower	Upper
Group A: 21 to 30 yr	11.021 (4)		0.026[a]		
Group B: 31 to 40 yr	4.664 (1)	11	0.031[a]	1.248	96.951
Group C: 41 to 50 yr	5.236 (1)	7	0.022[a]	1.322	37.066
Group D: 51 to 60 yr	3.741 (1)	5.25	0.053	0.978	28.182
Group E: > 60 yr	3.884 (1)	9	0.049[a]	1.012	28.182

[a]$P < 0.05$ was considered as significant at the 95% confidence level. OR: Odds ratio.

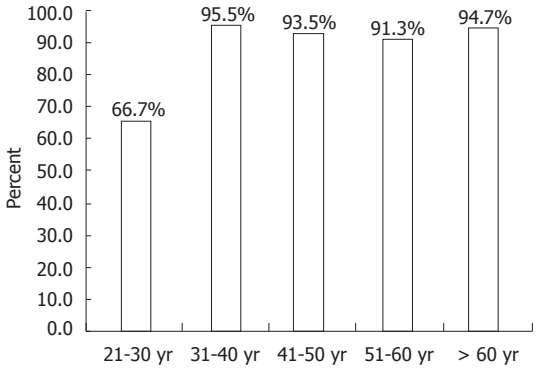


Figure 1 Seropositivity of anti-hepatitis A virus IgG according to age groups.

rates in patients in Groups B, C, D and E were greater than 90%, compared to only 66.7% in patients belonging to Group A. These data imply that most patients with chronic viral liver disease who are greater than 30 years of age may have been exposed to HAV infection and have therefore already acquired natural immunity towards the disease. Therefore, routine HAV vaccination cannot be recommended in this age group. However, there remains the need to vaccinate patients aged less than 30 years with chronic liver disease.

Malaysia is a multiracial country consisting of three major ethnic groups: Malay, Chinese and Indian. Because the Indian population is generally very small in Kelantan, we did not manage to enrol any Indian patients, and only the Malay and Chinese races could be compared. There was no significant difference in the seroprevalence rate between these two ethnicities. However, this should be interpreted with caution, as various sociocultural behaviours may also play a role in influencing viral transmission rates.

The anti-HAV seropositive rate was 86.5% in hepatitis B and 95.7% in hepatitis C. Even though chronic hepatitis C is a different disease entity from chronic hepatitis B, there was no significant difference in anti-HAV positivity according to aetiology of underlying chronic liver disease ($P > 0.05$). This could be due to the small sample size of patients with hepatitis C infection ($n = 23$) in our study. Notably, the seroprevalence of hepatitis A was higher in hepatitis C patients than in hepatitis B patients, which is consistent with Korean data, where 100% of chronic hepatitis C patients were anti-HAV IgG positive compared to only 86.1% of hepatitis B patients[27]. Similarly, the Korean and Indian studies also failed to demonstrate any significance in hepatitis A seropositivity in relation to chronic liver disease aetiology[21,27].

All cirrhotic patients were anti-HAV positive, compared to only 86.3% in non-cirrhotic liver disease cases. The higher prevalence in cirrhotics was due to these patients falling into an older age group (mean age 52.4 ± 13.4) than non-cirrhotics (mean age 43.06 ± 13.7), as shown by multiple logistic regression analyses. Various studies, particularly those in highly endemic regions such as India, have demonstrated that the majority of cirrhotic patients of any aetiology are positive for anti-HAV IgG. A study from New Delhi revealed that 97.6% of cirrhotics (248/288) were found to be positive for anti-HAV[28]. Another study from South India demonstrated that 51 out of 52 patients with cirrhosis had antibodies towards HAV[29]. All these studies proposed that the higher seroprevalence of anti-HAV IgG in cirrhotic patients was actually related to increased age. Our findings are in agreement with the results of these studies.

Our study demonstrated that the overall hepatitis A seroprevalence was higher in CLD patients in Kelantan compared to the previously determined prevalence in normal individuals in other parts of Malaysia. Age was the most important factor in determining anti-HAV positivity, and most patients greater than 30 years of age were likely to have natural immunity.

ACKNOWLEDGMENTS

We would like to thank Dr. Habsah Hasan for her skilful help in conducting the anti-HAV IgG tests.

COMMENTS

Background

Populations in developed countries may not have had prior exposure to hepatitis A virus (HAV) and, therefore, no natural immunity; thus, there is a need for hepatitis A vaccination in selected high-risk groups. **In view of this, Western** guidelines advocate hepatitis A vaccination for those with chronic liver disease (CLD), as infection may lead to further deterioration of liver function, which can then cause significant morbidity and mortality amongst these patients.

Research frontiers

Over the last 20 years, patterns of endemicity in South East Asia have changed due to improvements in living standards, with some countries shifting from high to intermediate or intermediate to low endemicity. **The question remains** as to the importance of hepatitis A vaccination amongst hepatitis B and C CLD patients in Malaysia, a country of intermediate endemicity for hepatitis A. To answer this, a research team from Universiti Sains Malaysia determined the prevalence and associated factors of natural immunity towards hepatitis A amongst these patients in the eastern region of Peninsular Malaysia.

Innovations and breakthroughs

The study demonstrated that the overall prevalence of natural immunity towards hepatitis A was high (88%). **There was a statistically significant difference when** the data were broken down according to age. Results revealed that hepatitis B and C CLD patients less than 30 years of age were significantly less likely to have a natural immunity towards hepatitis A. **This implies that although hepatitis** A vaccination is not needed for the majority of CLD patients, a subset of patients (particularly patients who are younger than 30 years old) will still benefit from being vaccinated.

Applications

Study results show that for north-eastern Peninsular Malaysia, there is currently no need for routine hepatitis A vaccination amongst hepatitis B and C CLD patients. **However, CLD patients who are younger than 30 years of age will still** benefit from being vaccinated. Patterns of endemicity in South East Asian countries are expected to change; in Malaysia, the prevalence of hepatitis A viral exposure is expected to fall with time. **Thus, there is a need to repeat this study** in the future, as it is expected that the prevalence of natural immunity towards hepatitis A will fall as the country becomes more developed.

Peer review

The authors investigated the seroprevalence of anti-HAV antibodies in patients with CLD and the need for vaccination in the region of Kelantan, Malaysia.

REFERENCES

1 **Baaten GG**, Sonder GJ, Dukers NH, Coutinho RA, Van den Hoek JA. Population-based study on the seroprevalence of hepatitis A, B, and C virus infection in Amsterdam, 2004. *J Med Virol* 2007; **79**: 1802-1810
2 **Barzaga BN**. Hepatitis A shifting epidemiology in South-East Asia and China. *Vaccine* 2000; **18** Suppl 1: S61-S64
3 **Kunasol P**, Cooksley G, Chan VF, Isahak I, John J, Loleka S, Villar EP, Poovorawan Y, Seong NH, Sulaiman HA, Wah LB. Hepatitis A virus: declining seroprevalence in children and adolescents in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1998; **29**: 255-262
4 **Saat Z**, Sinniah M, Kin TL, Baharuddin R, Krishnasamy M. A four year review of acute viral hepatitis cases in the east coast of peninsular Malaysia (1994-1997). *Southeast Asian J Trop Med Public Health* 1999; **30**: 106-109
5 **Lee SD**. Asian perspectives on viral hepatitis A. *J Gastroenterol Hepatol* 2000; **15** Suppl: G94-G99
6 **Jacobsen KH**, Koopman JS. The effects of socioeconomic de-

velopment on worldwide hepatitis A virus seroprevalence patterns. *Int J Epidemiol* 2005; **34**: 600-609
7 **Jacobsen KH**, Koopman JS. Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect* 2004; **132**: 1005-1022
8 **Khairullah NS**, Merican DI. Hepatitis disease management programs in Malaysia. *J Gastroenterol Hepatol* 2004; **19** Suppl: S13-S16
9 **Keeffe EB**. Acute hepatitis A and B in patients with chronic liver disease: prevention through vaccination. *Am J Med* 2005; **118** Suppl 10A: 21S-27S
10 **Song HJ**, Kim TH, Song JH, Oh HJ, Ryu KH, Yeom HJ, Kim SE, Jung HK, Shim KN, Jung SA, Yoo K, Moon IH, Chung KW. Emerging need for vaccination against hepatitis A virus in patients with chronic liver disease in Korea. *J Korean Med Sci* 2007; **22**: 218-222
11 **Saab S**, Lee C, Shpaner A, Ibrahim AB. Seroepidemiology of hepatitis A in patients with chronic liver disease. *J Viral Hepat* 2005; **12**: 101-105
12 **Cooksley G**. The importance and benefits of hepatitis A prevention in chronic liver disease patients. *J Gastroenterol Hepatol* 2004; **19** Suppl: S17-S20
13 **Keeffe EB**. Is hepatitis A more severe in patients with chronic hepatitis B and other chronic liver diseases? *Am J Gastroenterol* 1995; **90**: 201-205
14 **Hadler SC**. Global impact of hepatitis A virus infection changing patterns. In: Hollinger FB, Lemon SM, Margolis H, editors. **Viral Hepatitis and Liver Disease. Baltimore: Lippincott Williams and Wilkins, 1990: 14-20**
15 **Reiss G**, Keeffe EB. Review article: hepatitis vaccination in patients with chronic liver disease. *Aliment Pharmacol Ther* 2004; **19**: 715-727
16 **Yao G**. Clinical spectrum and natural history of viral hepatitis in a 1988 Shanghai epidemic In : Viral Hepatitis and Liver Disease. Baltimore: Lippincott Williams and Wilkins, 1991
17 **Vento S**, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, Ferraro T, Concia E. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998; **338**: 286-290
18 **Fiore AE**, Wasley A, Bell B P. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWRRecomm Rep* 2006; **55**(RR07**): 1-23**
19 **Centers for Disease Control and Prevention.** Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999; **48**: 1-37
20 **National Institutes of Health**. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: June 10-12, 2002. *Hepatology* 2002; **36**: S3-S20
21 **Joshi N**, Rao S, Kumar A, Patil S, Rani S. Hepatitis A vaccination in chronic liver disease: is it really required in a tropical country like India? *Indian J Med Microbiol* 2007; **25**: 137-139
22 **Wasley A**, Fiore A, Bell BP. Hepatitis A in the era of vaccination. *Epidemiol Rev* 2006; **28**: 101-111
23 **Hollinger FB**, Eickhoff T, Gershon A, Jong EC, Koff RS. Discussion: who should receive hepatitis A vaccine? A strategy for controlling hepatitis A in the United States. *J Infect Dis* 1995; **171** Suppl 1: S73-S77
24 **Ton SH**, Thiruselvam A, Lopez CG, Noriah R. Prevalence of hepatitis A virus infection in normal individuals and hospital patients in Kuala Lumpur. *Med J Malaysia* 1983; **38**: 279-281
25 **Malik YA**, Baharin R. Changing prevalence of Hepatitis A in Malaysia. In: Proceedings of the 4th Western Pacific Congress on Chemotherapy and Infectious Diseases**; 1994 Dec** 4-7; **Manila, Philippines. Singapore:** MediMedia Asia, 1994
26 **Tan DS**, Fang R, Collett D, Ooi BG. A seroepidemiologic study of hepatitis A in Malaysia. *Southeast Asian J Trop Med Public Health* 1986; **17**: 201-204
27 **Kim do Y**, Ahn SH, Lee HW, Kim SU, Kim JK, Paik YH, Lee KS, Han KH, Chon CY. Anti-hepatitis A virus seroprevalence among patients with chronic viral liver disease in Korea. *Eur J Gastroenterol Hepatol* 2007; **19**: 923-926
28 **Acharya SK**, Batra Y, Saraya A, Hazari S, Dixit R, Kaur K, Bhatkal B, Ojha B, Panda SK. Vaccination for hepatitis A virus is not required for patients with chronic liver disease in India. *Natl Med J India* **2002; 15**: 267-268
29 **Xavier S**, Anish K. Is hepatitis A vaccination necessary in Indian patients with cirrhosis of liver? *Indian J Gastroenterol* 2003**; 22**: 54-55

S- Editor Sun H **L- Editor** Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4135

World J Gastroenterol 2011 September 28; 17(36): 4135-4142
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Viral kinetics of Enterovirus 71 in human habdomyosarcoma cells

Jing Lu, Ya-Qing He, Li-Na Yi, Hong Zan, Hsiang-Fu Kung, Ming-Liang He

Jing Lu, Li-Na Yi, Hsiang-Fu Kung, Ming-Liang He, Stanley Ho Center for Emerging Infectious Diseases, and Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
Ya-Qing He, Shenzhen Center for the Disease Control and Prevention, Shenzhen 518055, Guangdong Province, China
Hong Zan, Institute for Immunology, University of California at Irvine, CA 92697, United States
Author contributions: Lu J and He YQ contributed equally to this paper; Lu J, Yi LN, He YQ performed the experiments; Zan H contributed to analysis; He ML designed the research; He ML, Kung HF and Zan H wrote the paper.
Supported by Research Grant Council (RGC, CUHK4428/06M); and a commissioned grant of the Research Fund for Control of Infectious Diseases (CU-09-02-02), Food and Health Bureau, the Government of Hong Kong Special Administration Region (HKSAR)
Correspondence to: Ming-Liang He, Professor, Rm 708, Li Ka Shing Medical Science Bldg, Prince Wales Hospital, The Chinese University of Hong Kong,
Hong Kong, China. mlhe7788@gmail.com
Telephone: +852-37636096 Fax: +852-21458013
Received: January 13, 2011 Revised: May 19, 2011
Accepted: May 26, 2011
Published online: September 28, 2011

Abstract

AIM: To characterise the viral kinetics of enterovirus 71 (EV71).

METHODS: In this study, human rhabdomyosarcoma (RD) cells were infected with EV71 at different multiplicity of infection (MOI). After infection, the cytopathic effect (CPE) was monitored and recorded using a phase contrast microscope associated with a CCD camera at different time points post viral infection (0, 6, 12, 24 h post infection). Cell growth and viability were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in both EV71 infected and mock infected cells at each time point. EV71 replication kinetics in RD cells was determined by measuring the total intracellular viral RNA with real-time reverse-transcription polymerase chain reaction (qRT-PCR). Also, the intracellular and extracellular virion RNA was isolated and quantified at different time points to analyze the viral package and secretion. The expression of viral protein was determined by analyze the levels of viral structure protein VP1 with Western blotting.

RESULTS: EV71 infection induced a significant CPE as early as 6 h post infection (p.i.) in both RD cells infected with high ratio of virus (MOI 10) and low ratio of virus (MOI 1). In EV71 infected cells, the cell growth was inhibited and the number of viable cells was rapidly decreased in the later phase of infection. EV71 virions were uncoated immediately after entry. The intracellular viral RNA began to increase at as early as 3 h p.i. and the exponential increase was found between 3 h to 6 h p.i. in both infected groups. For viral structure protein synthesis, results from western-blot showed that intracellular viral protein VP1 could not be detected until 6 h p.i. in the cells infected at either MOI 1 or MOI 10; and reached the peak at 9 h p.i. in the cells infected with EV71 at both MOI 1 and MOI 10. Simultaneously, the viral package and secretion were also actively processed as the virus underwent rapid replication. The viral package kinetics was comparable for both MOI 1 and MOI 10 infected groups. It was observed that at 3 h p.i, the intracellular virions obviously decreased, thereafter, the intracellular virions began to increase and enter into the exponential phase until 12 h p.i. The total amounts of intracellular virons were decreased from 12 to 24 h p.i. Consistent with this result, the increase of virus secretion occurred during 6 to 12 h p.i.

CONCLUSION: The viral kinetics of EV71 were established by analyzing viral replication, package and secretion in RD cells.

Key words: Enterovirus 71; Quantitative reverse transcription polymerase chain reaction; Viral kinetics; Western blotting

Peer reviewer: Dr. Mohamed Hassan, Department of Tumour Therapy, University Hospital of Dusseldorf, 40225 Dusseldorf, Germany

Lu J, He YQ, Yi LN, Zan H, Kung HF, He ML. Viral kinetics of Enterovirus 71 in human rhabdomyosarcoma cells. *World J Gastroenterol* 2011; 17(36): 4135-4142 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4135.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4135

INTRODUCTION

Enterovirus 71 (EV71) is a member of human enterovirus species which belongs to the *Picornaviridae* family. It is one of the major causative agents for herpangina and hand, foot and mouth disease (HFMD)[1,2]. Among young children, acute EV71 infection may also cause severe neurological diseases such as encephalitis and meningitis that lead to significant mortality[3,4]. Recently, outbreaks of EV71 infection have been frequently reported throughout the world[5-10]. In China, a recent outbreak of EV71 infected more than 1.4 million children and caused 760 deaths from January to July, 2010. It is also noted that an adult died due to EV71 infection in Hong Kong in May, 2010 (http://www.hkcd.com.hk/content/2010-05/28/content_2531606.htm).

EV71 is a small RNA virus containing a non-enveloped capsid and a single-stranded positive genomic RNA (about 7400 bp)[11]. The life cycle of EV71 was speculated according to studies on other enteroviruses. EV71 would attach to the host cell *via* its specific receptors[12] and then the viral genomic RNA is released into the cytoplasm, where it directly translates into a polyprotein. This precursor protein can subsequently be cleaved into four structural (VP1, VP2, VP3 and VP4) and seven non-structural (2A, 2B, 2C, 3A, 3B, 3C and 3D) proteins[4]. For virus RNA replication, a complementary minus-strand RNA is synthesised in the cytoplasm and then this minus-strand RNA can serve as a template for new plus-strand RNA molecules. Newly synthesized virus RNA may go into another round of translation and replication, or is packaged into capsid proteins to produce infectious viral particles (see review in Ref.[13]). Studies on other viruses demonstrate that the knowledge of virus kinetics is important for clarifying viral pathogenesis and exploring effective treatments. The knowledge of the viral kinetics on human immunodeficiency virus, hepatitis B virus and hepatitis C virus has greatly improved the understanding of the cell response to these viruses and mechanisms of related antiviral therapy[14-16]. In the *Picornavirus* family, the kinetics of swine vesicular disease virus (SVDV) and foot-and-mouth disease virus (FMDV) have been described in several studies[17,18]. However, little information is known about EV71 infection. In this study, we used rhabdomyosarcoma (RD) cells as an *in vitro* model and intensively investigated the viral kinetics of EV71, including the kinetics of viral replication, viral protein synthesis, packaging and secretion.

MATERIALS AND METHODS

Cell culture and virus propagation

RD cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. EV71 strain SHZH98 (GenBank accession number AF302996.1) was obtained from Shen-zhen Center for Disease Control and Prevention, Shenzhen, China. To prepare virus stocks, viruses were propagated on a 90% confluent cell monolayer in DMEM with 2% FBS. The viral titres were measured by the cytopathic effect (CPE) microtitration assays and expressed as 50% tissue culture infective dose (TCID50) per millilitre (mL) according to the Kärber formula[19].

Viral infection and cytopathic effects assay

RD cells were cultured in 12-well plates and infected with nil or EV71 at multiplicity of infection (MOI) 1 and 10, respectively. Briefly, plated cells were washed twice with phosphate buffered saline (PBS) and infected with EV71. Time was set as zero after adsorption for 1 h. The culture media were removed and cells were washed twice with PBS to remove unattached virus before adding 1 mL of DMEM medium containing 10% FBS to each well. The cells were cultured at 37 ℃ in 5% CO_2. The cell morphology was monitored and recorded using a phase-contrast microscope associated with a CCD camera and computer at different time points. The infected cells and culture supernatants were harvested to isolate RNA and proteins.

MTT assay

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays were performed to determine the cell viability upon EV71 infection. Briefly, RD cells were set in 96-well plates at 1×10^4 cells per well 24 h before infection with EV71 at MOI 1 or MOI 10. The medium was then replaced with 0.5 mg/mL MTT medium at different time points and incubated for another 4 h. The MTT solution was removed from the wells and the formazan crystals were dissolved in DMSO. Absorbances of the formazan products were measured at 550 nm with the reference wavelength at 690 nm.

RNA isolation

For intracellular viral RNA quantification, the total cellular RNA was isolated from EV71 infected cells using TRIzol reagent (Invitrogen, United States) according to the manufacturer's instructions. To quantitate the extracellular virions, we first isolated the virions from the culture media of infected cells. The media were harvested and briefly centrifuged to remove cell debris. Viral core

particles were then precipitated with 10% polyethylene glycol 8000 containing 0.5 mol/L NaCl at 4 ℃ overnight. After centrifuging for 30 min at 16 000 *g*, viral particles were pelleted and treated with 100 μg/mL of RNase A (Sigma, United States). To isolate the intracellular virions, EV71 infected cells were lysed with lysis buffer (1% Triton 100 and 1 × Roche protease inhibitor cocktail in PBS). Then the cell lysates were used to isolate viral particles as described above. The virion-associated RNA was then isolated by using TRIzol reagent. To set up the standard curve of infectious viruses, the viral titres were first determined by CPE assay. Then the viral RNA was extracted from those infectious EV71 viruses. RNA was diluted at ten-fold serial and used to reflect the calculated PFU from 10 to 1 × 10^7 live virions.

Quantitative reverse transcription-polymerase chain reaction

The one-step quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using the ABI 7500 Real-Time PCR system (Applied Biosystems) with QuantiTect SYBR Green RT-PCR Kit (Qiagen) and specific forward EV71-VP1F (5'-GCAGCCCAAAAGAACTTCAC-3') and reverse EV71-VP1R (5'-ATTTCAGCAGCTTGGAGTGC-3') primers targeting a conserved region of the *VP1* gene[20]. PCR assay was carried out in a 20 μL volume consisting of 10 μL of 2 × Quantitect SYBR green RT-PCR Master Mix, containing HotStarTaq DNA polymerase, 1 μL of 10 μmol/L of each oligonucleotide primer, 0.2 μL of 100 × QuantiTect RT Mix (containing Omniscript and Sensiscript reverse transcriptases) and 2 μL of RNA extracted from samples or from ten-folds serial diluted virus RNA standard (from 10^7 to 10 copies). The target fragment amplification was carried out as follows: reverse transcription at 50 ℃ for 30 min; initial activation of HotStar Taq DNA Polymerase at 95 ℃ for 15 min; 45 cycles in four steps: 94 ℃ for 10 s, 56 ℃ for 30 s, and 72 ℃ for 30 s. At the end of the amplification cycles, melting temperature analysis was carried out by a slow increase in temperature (0.1 ℃/s) up to 95 ℃.

Western blotting

To prepare total cellular protein extracts, the cells were lysed with RIPA buffer (50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 0.1% SDS, 1 × Roche protease inhibitor cocktail) with occasional vortexing. Lysates were collected by centrifugation at 14 000 *g* for 10 min at 4 ℃ and protein concentrations were measured by the Bradford method (Bio-Rad, United States). Equal amounts (20 μg) of proteins from each sample were separated through 12% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences). Membranes were blocked by 5% skim milk in Tris-Buffered Saline Tween-20 (TBST) (20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.1% Tween 20) followed by incubation with specific antibodies against VP1 (PAB7631-D01P, Abnova) or GAPDH (Santa Cruz Biotechnology). Target proteins were visualized with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) and a chemiluminescence detection system (Amersham Biosciences). Each immunoblotting was carried out at least three times.

Statistical analysis

Data are depicted as mean ± SD. All statistical analyses were carried out with SPSS 14.0 software (SPSS Inc.). Two-tailed Student's *t* test was applied for two-group comparison. $P < 0.05$ was considered statistically significant.

RESULTS

Cytopathic effects and the kinetic of cell viability

Morphological changes were observed as early as 6 h p.i. when RD cells were infected with EV71 at either MOI 1 or 10 (Figure 1A, panels f and j). Initially, the cells rounded up and became more refractile. As the culture progressed, some infected cells detached from the culture plate and floated into the medium (Figure 1A, panels g, h, k and l). Compared with the cells infected at MOI 1, more cells were unhealthy at 6 h p.i. when infected at MOI 10 (Figure 1A, panel j *vs* f). Later on, the cells infected with EV71 both at MOI 1 and 10 underwent significant cell death and detached from the surface of culture dishes (Figure 1A, panels g and k). At 24 h p.i, most of the cells were detached from the surface of the plate in both infected groups (Figure 1A, panels h and l). The MTT assay showed that the viability of the cells infected with EV71 at MOI 1 did not significantly decrease at 12 h p.i., but slightly reduced in cells infected at MOI 10 (Figure 1B). At 18 and 24 h p.i., the viability of the cells infected with EV71 either at MOI 1 or 10 significantly decreased.

The kinetics of viral replication

To examine the kinetics of viral replication, the levels of intracellular viral RNA were measured by qRT-PCR at each time point. As shown in Figure 2, the intracellular viral RNA began to increase as early as 3 h p.i. and the exponential phase was from 3 to 6 h p.i in both infected groups. In the case of infection at MOI 1, the intracellular viral RNA continually increased from 6 to 12 h p.i., and then gradually decreased until 24 h p.i. In the case of MOI 10, the intracellular viral RNA reached a peak between 6 and 9 h p.i., and then began to decrease.

The kinetics of viral protein synthesis

The intracellular viral protein VP1 was not detected until 6 h p.i. in the cells infected at MOI 1 and10 (Figure 3). The VP1 protein level reached a peak at 9 h p.i. in the cells infected with EV71 at both MOI 1 and MOI 10. Obviously, the VP1 levels were much higher in the cells infected at MOI 10 than at MOI 1. In the case of infection at MOI 1, the VP1 level was maintained until 12 h p.i.; whereas the

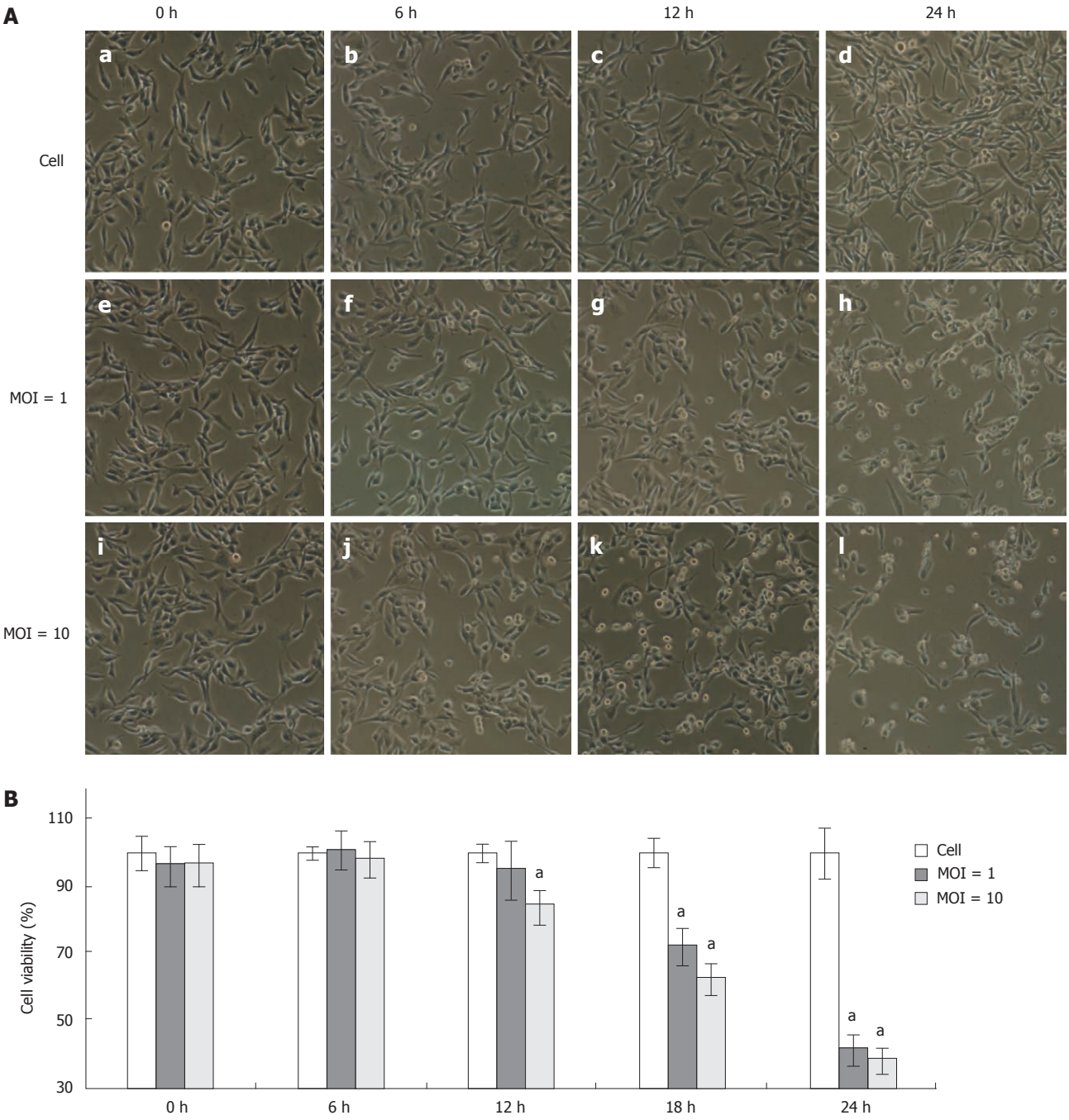


Figure 1 Cytopathic effects and kinetics of cell viability upon enterovirus 71 infection. Rhabdomyosarcoma cells were infected with enterovirus 71 (EV71) at multiplicity of infection (MOI) 1 or MOI 10. A: The cytopathic effects were shown by cell morphological changes (original magnification, × 100); B: Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays at different time points after EV71 infection. Data are the mean ± SD of three independent experiments, each carried out in triplicate. $^{a}P < 0.05$.

VP1 protein level rapidly decreased after 9 h p.i. when the cells were infected with EV71 at MOI 10.

The kinetics of viral package

To determine the kinetics of virus package, the intracellular EV71 virions were quantified at different time points p.i. At 3 h p.i., the intracellular virions significantly decreased. Thereafter, the intracellular virions began to increase and enter into the exponential phase until 12 h p.i. when the amount of intracellular virions reached a peak. The viral package kinetics were comparable for both the MOI 1 and MOI 10 infected groups (Figure 4). The total amounts of intracellular virions then decreased from 12 to 24 h p.i.

The kinetics of viral secretion

To determine the kinetics of virus secretion, the extracellular EV71 virions were quantitated. The EV71 virions began to be released from the cells infected either at MOI 1 or 10 3 h p.i. (Figure 5). The amounts of extra-

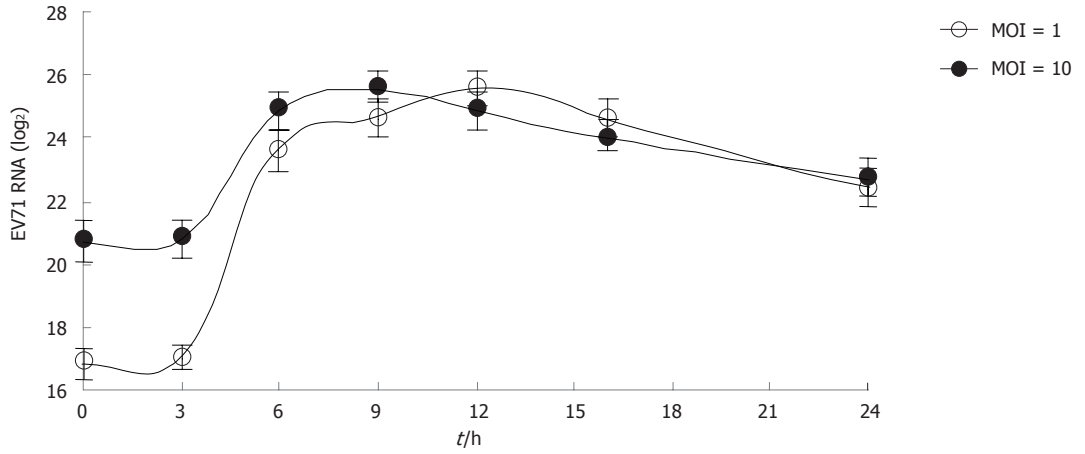


Figure 2 The kinetics of enterovirus 71 Replication. Rhabdomyosarcoma cells were infected with enterovirus 71 (EV71) virus at multiplicity of infection (MOI) = 1 or MOI = 10. At the indicated time points, the levels of total intracellular viral RNA were measured by quantitative real-time polymerase chain reaction. Data are the mean ± SD of three independent experiments; each carried out in triplicate.

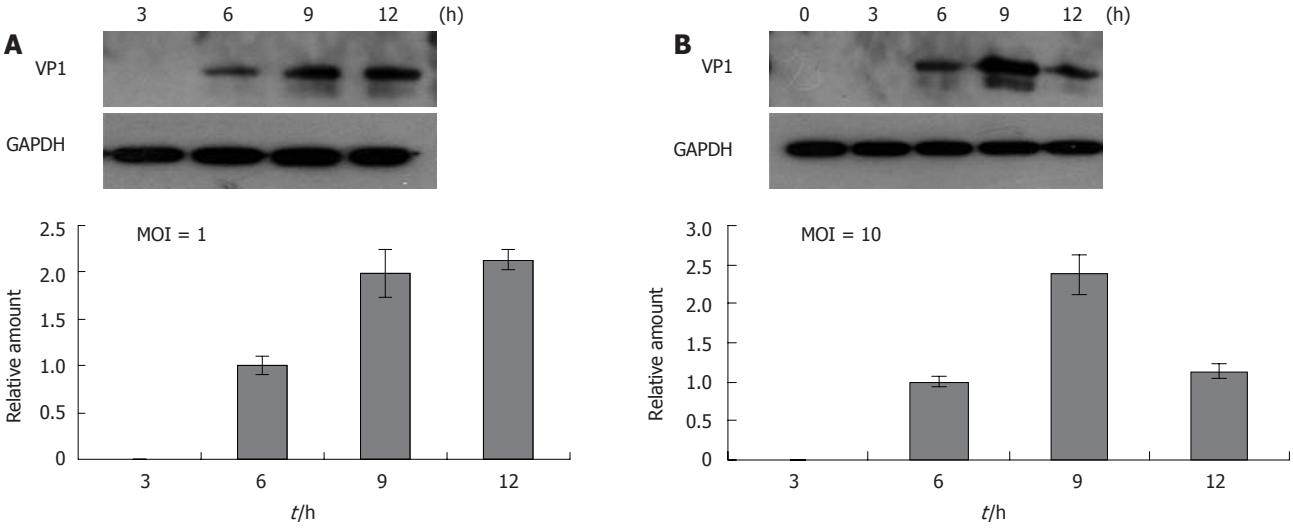


Figure 3 The kinetics of virus VP1 protein synthesis. Rhabdomyosarcoma cells were infected with enterovirus 71 (EV71) virus at multiplicity of infection (MOI) = 1 (A) and MOI = 10 (B). The intracellular viral protein VP1 was measured by Western blotting. The relative VP1 levels (the density of VP1/GAPDH) were calculated and are shown as solid bars.

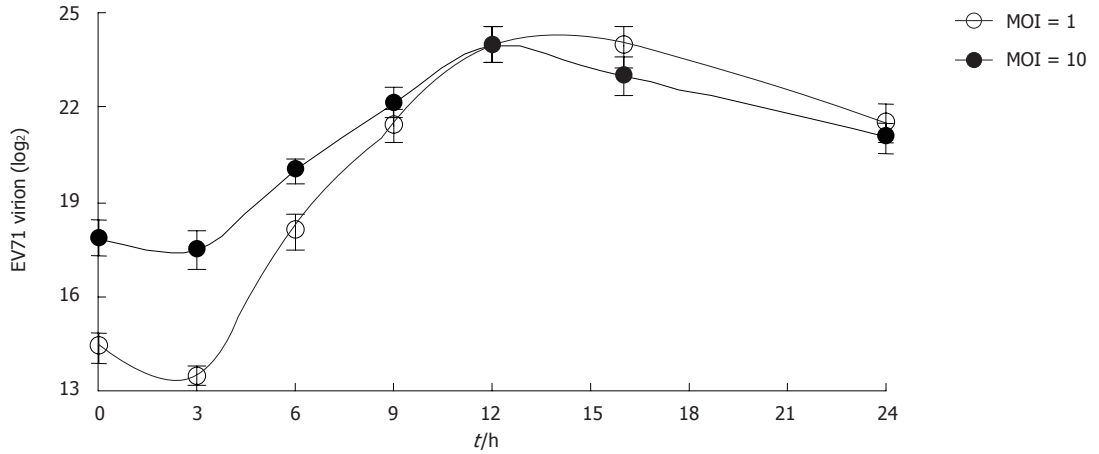


Figure 4 The kinetics of enterovirus 71 virus package. Rhabdomyosarcoma cells were infected with enterovirus 71 (EV71) virus at multiplicity of infection (MOI) = 1 or MOI = 10. The intracellular virus particles were isolated to measure the virion RNA by quantitative real-time polymerase chain reaction. Data are the mean ± SD of three independent experiments; each carried out in triplicate.

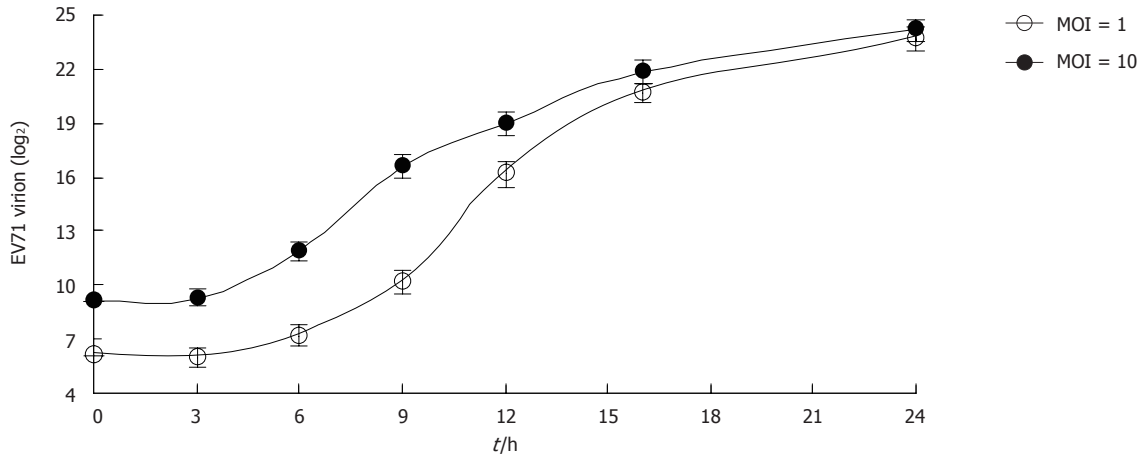


Figure 5 The kinetics of enterovirus 71 virus secretion. Rhabdomyosarcoma cells were infected with enterovirus 71 (EV71) virus at multiplicity of infection (MOI) = 1 or MOI = 10. Extracellular EV71 virions in the culture media were measured by quantitative real-time polymerase chain reaction at different time points post infection. Data are the mean ± SD of three independent experiments; each carried out in triplicate.

cellular EV71 virions in the cultures of the two groups were constitutively increased. From 3 to 6 h p.i., the virions were slowly secreted into the culture media, and the virus secretion entered into the exponential phase from 6 to 12 h p.i. At 12 h p.i., the rate of increase declined and the total amount of extracellular virions reached a maximum at 24 h p.i. For cells infected at MOI 1 or 10, the virions in the culture media were similar at 24 h p.i.

DISCUSSION

Viral kinetics is an important parameter for demonstrating viral activities in the host cells and provides basic information on viral-host interactions and pathogenesis. The kinetics of some picornaviruses such as SVDV and FMDV have been described in several studies[17,18]. However, little information on EV71 is available. Some studies provided brief descriptions on EV71 RNA replication and the growth kinetics of EV71 infected cells, however, the infection ratios used in these studies were too low (MOI $\leqslant$ 0.01) to guarantee the synchronicity of infection[21,22]. In this situation, some cells were undergoing cell death, whereas others just had a chance to be infected by new EV71 viruses secreted from the first round infected cells. Therefore, the viral life cycle could not be accurately examined. In addition, with the exception of RNA synthesis, no information was provided on viral protein expression, virus package and secretion. Our study, for the first time, comprehensively described the detailed viral kinetics in human RD cells. As RD cells infected by EV71 would develop cellular pathogenesis (CPE), these cells have been extensively used to investigate the viral activities of EV71 and host responses to EV71 infection[23-26]. To obtain a synchronized infection, RD cells were pulse infected at high MOIs (MOI 1 and 10) to ensure that the majority of the cells were primarily infected in our study. Following infection, the unattached viruses were removed by washing the cells twice with PBS. This would minimize the interference of non-infectious virions. In this study, the kinetics of viral replication, gene expression, package and secretion as well as the effects of viral activities on host cells were carefully examined at different time points.

We showed here that the intracellular virions significantly decreased by over 90% at 3 h p.i. (Figure 4), while the total intracellular RNA copies remained almost at the same levels (Figure 2). These results suggested that the virions were immediately uncoated after entry and virus replication was inactive within the first 3 h after infection. During this phase, the viral RNA could be translated to generate viral proteins essential for viral replication. From 3 to 6 h p.i., the virus underwent fast replication and the total intracellular viral RNA was rapidly accumulated (Figure 2). Similar results were also reported in other poliovirus infection[27]. The total intracellular viral RNA increased by more than 64-fold within this period. In the meantime, viral gene expression was also initiated along with viral replication, as viral VP1 proteins in the host cells were clearly detected at 6 h (Figure 3). The viral package was also started (Figure 4) but very few virions were secreted (Figure 5). At 6 h p.i., about 1% (MOI 1) to 3% (MOI 10) of viral RNA was packaged into virions (Figures 2 and 4). Although the virus was rapidly replicating, the host cells were generally healthy during this period. From 6 to 9 h p.i., some cells became unhealthy (Figure 1), the viral replication entered a static stage as the total intracellular viral RNA only increased by about 2-fold in the MOI 1 and MOI 10 group. In the case of MOI 1, the total intracellular viral RNA increased a further 2-fold from 9 to 12 h p.i., but began to decrease 9 h p.i. in the cells infected at MOI 10. This suggested that viral RNA in cells infected with higher MOI reached maximal levels earlier. The viral gene expression and package were also actively processed in this period. The viral protein VP1 levels reached a peak at 9 h p.i. and rapidly decreased at 12 h p.i. in the MOI 10 group. In the case of MOI 1, the VP1 levels also reached a maximum at 9 h p.i. and maintained the same levels at 12 h p.i. Following viral protein synthesis, the intracellular virions also rapidly increased over 16-fold (MOI 10) or

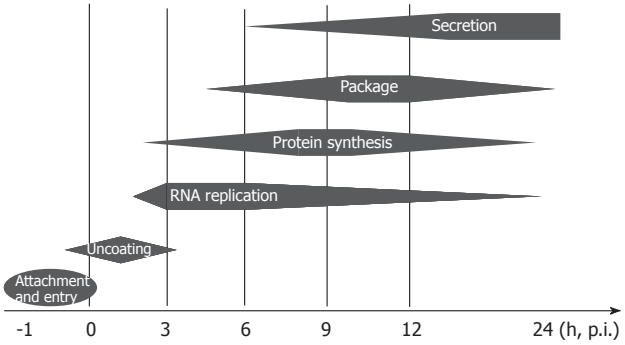


Figure 6 Schematic view of enterovirus 71 activities in rhabdomyosarcoma cells. Within one hour of inoculation, enterovirus 71 would first attach and enter into the host cell *via* its specific receptors. The virus was then uncoated in the first 3 h and started to synthesize the essential viral proteins for replication. From 3 to 6 h p.i., the virus rapidly replicated its genomic RNA and initiated structure protein synthesis. Fast viral package was observed from 6 to 12 h p.i. and virion secretion increased from 6 h p.i. to the end of the observation period.

64-fold (MOI 1). In both cases, the intracellular virions reached a peak and about 30% of viral RNA was packaged into the virions at 12 h p.i. (Figure 4 *vs* Figure 2). In the case of MOI 1, the extracellularly accumulated virions increased 8- and 64-fold from 6 to 9 h, and 9 to 12 h, respectively; while the extracellularly accumulated virions increased 30- and 5-fold during the same periods in the MOI 10 group. From 12 to 24 h p.i., as more and more infected cells became unhealthy and died, the intracellular viral RNA levels significantly decreased and viral replication became less active. These findings suggested that the cells could no longer sustain further viral replication and died[28-30]. This was further supported by the data on intracellular and extracellular virion levels. We showed here that the intracellular virions maintained high levels at 16 h p.i. although more and more virions were secreted into the culture media. After that, the ratio of packaged viral RNA to total viral RNA was constant at about 50% in both the MOI 1 and MOI 10 group.

In summary, we have established a viral kinetics model of EV71 in human RD cells (Figure 6). We showed that upon infection, the virus uncoated within the first 3 h and started to synthesize the essential viral proteins for replication. From 3 to 6 h p.i., the virus rapidly replicated its genomic RNA and initiated viral package. The fast viral package displayed from 3 to 12 h p.i. and virion secretion from 6 h p.i. continued until death of the host cells. Host cells started to become unhealthy as early as 6 h p.i. but still supported viral replication, package and secretion until death. Thus, our study provides important information for further investigations into virus-host interactions and host pathogenesis.

COMMENTS

Background

Enterovirus 71 (EV71) infection causes hand-foot-and-mouth disease (HFMD) and neurological disease. Recently, EV71 infection has become a major health threat in China. However, the mechanisms of these diseases caused by EV71 infection are still largely unknown.

Innovations and breakthroughs

Studies on other human viruses such as human immunodeficiency virus, hepatitis C virus and hepatitis B virus have highlighted the importance of understanding viral kinetics. In this study, for the first time, the authors fully described the viral kinetics of EV71 in rhabdomyosarcoma (RD) cells and characterized the activities during each step of viral replication in detail.

Applications

Accurate information on viral kinetics will provide a valuable reference for investigating EV71-host interactions and the pathogenic mechanisms of diseases caused by EV71 infection.

Terminology

EV71 is a small positive RNA virus. During viral replication, the complementary minus-strand RNA is synthesised first and serves as a template for the next round of translation and replication. Thus, the viral RNA levels in cells, viral particles and culture supernatants represent the relative levels of viral replication, package and secretion.

Peer review

The authors provided detailed information on EV71 replication, package, secretion and viral protein expression in RD cells. The rapid increase of intracellular EV71 viral RNA and viral particles revealed that EV71 RNA was synthesized soon after infection and the viral particles could immediately package and released. These results will provide important information for further understanding the viral pathogenesis and EV71-host interactions.

REFERENCES

1 **Fowlkes AL**, Honarmand S, Glaser C, Yagi S, Schnurr D, Oberste MS, Anderson L, Pallansch MA, Khetsuriani N. Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. *J Infect Dis* 2008; **198**: 1685-1691
2 **Hamaguchi T**, Fujisawa H, Sakai K, Okino S, Kurosaki N, Nishimura Y, Shimizu H, Yamada M. Acute encephalitis caused by intrafamilial transmission of enterovirus 71 in adult. *Emerg Infect Dis* 2008; **14**: 828-830
3 **Ishimaru Y**, Nakano S, Yamaoka K, Takami S. Outbreaks of hand, foot, and mouth disease by enterovirus 71. High incidence of complication disorders of central nervous system. *Arch Dis Child* 1980; **55**: 583-588
4 **McMinn PC**. An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiol Rev* 2002; **26**: 91-107
5 **Huang SW**, Hsu YW, Smith DJ, Kiang D, Tsai HP, Lin KH, Wang SM, Liu CC, Su IJ, Wang JR. Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. *J Clin Microbiol* 2009; **47**: 3653-3662
6 **Yang F**, Ren L, Xiong Z, Li J, Xiao Y, Zhao R, He Y, Bu G, Zhou S, Wang J, Qi J. Enterovirus 71 outbreak in the People's Republic of China in 2008. *J Clin Microbiol* 2009; **47**: 2351-2352
7 **AbuBakar S**, Chee HY, Al-Kobaisi MF, Xiaoshan J, Chua KB, Lam SK. Identification of enterovirus 71 isolates from an outbreak of hand, foot and mouth disease (HFMD) with fatal cases of encephalomyelitis in Malaysia. *Virus Res* 1999; **61**: 1-9
8 **McMinn P**, Lindsay K, Perera D, Chan HM, Chan KP, Cardosa MJ. Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. *J Virol* 2001; **75**: 7732-7738
9 **Bible JM**, Iturriza-Gomara M, Megson B, Brown D, Pantelidis P, Earl P, Bendig J, Tong CY. Molecular epidemiology of human enterovirus 71 in the United Kingdom from 1998 to 2006. *J Clin Microbiol* 2008; **46**: 3192-3200
10 **Singh S**, Chow VT, Phoon MC, Chan KP, Poh CL. Direct detection of enterovirus 71 (EV71) in clinical specimens from a hand, foot, and mouth disease outbreak in Singapore by reverse transcription-PCR with universal enterovirus and EV71-specific primers. *J Clin Microbiol* 2002; **40**: 2823-2827

11 **Muir P**, Kämmerer U, Korn K, Mulders MN, Pöyry T, Weissbrich B, Kandolf R, Cleator GM, van Loon AM. Molecular typing of enteroviruses: current status and future requirements. The European Union Concerted Action on Virus Meningitis and Encephalitis. *Clin Microbiol Rev* 1998; **11**: 202-227

12 **Yamayoshi S**, Yamashita Y, Li J, Hanagata N, Minowa T, Takemura T, Koike S. Scavenger receptor B2 is a cellular receptor for enterovirus 71. *Nat Med* 2009; **15**: 798-801

13 **Zoll J**, Heus HA, van Kuppeveld FJ, Melchers WJ. The structure-function relationship of the enterovirus 3'-UTR. *Virus Res* 2009; **139**: 209-216

14 **Herrmann E**, Lee JH, Marinos G, Modi M, Zeuzem S. Effect of ribavirin on hepatitis C viral kinetics in patients treated with pegylated interferon. *Hepatology* 2003; **37**: 1351-1358

15 **Layden-Almer JE**, Ribeiro RM, Wiley T, Perelson AS, Layden TJ. Viral dynamics and response differences in HCV-infected African American and white patients treated with IFN and ribavirin. *Hepatology* 2003; **37**: 1343-1350

16 **Zeuzem S**, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. *Gastroenterology* 2001; **120**: 1438-1447

17 **Belsham GJ**, Normann P. Dynamics of picornavirus RNA replication within infected cells. *J Gen Virol* 2008; **89**: 485-493

18 **Egger D**, Bienz K. Intracellular location and translocation of silent and active poliovirus replication complexes. *J Gen Virol* 2005; **86**: 707-718

19 **Cohen BJ**, Audet S, Andrews N, Beeler J. Plaque reduction neutralization test for measles antibodies: Description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. *Vaccine* 2007; **26**: 59-66

20 **Yi L**, He Y, Chen Y, Kung HF, He ML. Potent inhibition of human enterovirus 71 replication by type I interferon subtypes. *Antivir Ther* 2011; **16**: 51-58

21 **Leong WF**, Chow VT. Transcriptomic and proteomic analyses of rhabdomyosarcoma cells reveal differential cellular gene expression in response to enterovirus 71 infection. *Cell Microbiol* 2006; **8**: 565-580

22 **Shih SR**, Stollar V, Lin JY, Chang SC, Chen GW, Li ML. Identification of genes involved in the host response to enterovirus 71 infection. *J Neurovirol* 2004; **10**: 293-304

23 **Kok TW**, Pryor T, Payne L. Comparison of rhabdomyosarcoma, buffalo green monkey kidney epithelial, A549 (human lung epithelial) cells and human embryonic lung fibroblasts for isolation of enteroviruses from clinical samples. *J Clin Virol* 1998; **11**: 61-65

24 **She RC**, Crist G, Billetdeaux E, Langer J, Petti CA. Comparison of multiple shell vial cell lines for isolation of enteroviruses: a national perspective. *J Clin Virol* 2006; **37**: 151-155

25 **Johansson ES**, Xing L, Cheng RH, Shafren DR. Enhanced cellular receptor usage by a bioselected variant of coxsackievirus a21. *J Virol* 2004; **78**: 12603-12612

26 **Pérez-Ruiz M**, Navarro-Marí JM, Palacios del Valle E, Rosa-Fraile M. Human rhabdomyosarcoma cells for rapid detection of enteroviruses by shell-vial assay. *J Med Microbiol* 2003; **52**: 789-791

27 **Pliaka V**, Dedepsidis E, Kyriakopoulou Z, Papadi G, Tsakogiannis D, Pratti A, Levidiotou-Stefanou S, Markoulatos P. Growth kinetic analysis of bi-recombinant poliovirus vaccine strains. *Virus Genes* 2010; **40**: 200-211

28 **Chen LC**, Shyu HW, Chen SH, Lei HY, Yu CK, Yeh TM. Enterovirus 71 infection induces Fas ligand expression and apoptosis of Jurkat cells. *J Med Virol* 2006; **78**: 780-786

29 **Liang CC**, Sun MJ, Lei HY, Chen SH, Yu CK, Liu CC, Wang JR, Yeh TM. Human endothelial cell activation and apoptosis induced by enterovirus 71 infection. *J Med Virol* 2004; **74**: 597-603

30 **Chang SC**, Lin JY, Lo LY, Li ML, Shih SR. Diverse apoptotic pathways in enterovirus 71-infected cells. *J Neurovirol* 2004; **10**: 338-349

S- Editor Lv S **L- Editor** Webster JR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4143

World J Gastroenterol 2011 September 28; 17(36): 4143-4148
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Radiofrequency ablation *vs* hepatic resection for solitary colorectal liver metastasis: A meta-analysis

Yun-Zi Wu, Bin Li, Tao Wang, Shuang-Jia Wang, Yan-Ming Zhou

Yun-Zi Wu, Bin Li, Tao Wang, Shuang-Jia Wang, Yan-Ming Zhou, Department of Hepato-Biliary-Pancreato-Vascular Surgery, the First Affiliated Hospital of Xiamen University, Xiamen 361003, Fujian Province, China

Author contributions: Zhou YM participated in the design and coordination of the study, carried out the critical appraisal of studies and wrote the manuscript; Wu YZ and Li B developed the literature search, carried out the extraction of data, assisted in the critical appraisal of included studies and assisted in writing up; Wang T and Wang SJ carried out the statistical analysis of studies; Zhou YM interpreted data, corrected and approved the manuscript; All authors read and approved the final manuscript.

Correspondence to: Yan-Ming Zhou, MD, Department of Hepato-Biliary-Pancreato-Vascular Surgery, the First Affiliated Hospital of Xiamen University, 55 Zhenhai Road, Xiamen 361003, Fujian Province, China. zhouyms@yahoo.com.cn

Telephone: +86-592-2139708 Fax: +86-592-2137289

Received: December 22, 2010 Revised: January 19, 2011
Accepted: January 26, 2011
Published online: September 28, 2011

Abstract

AIM: To evaluate the comparative therapeutic efficacy of radiofrequency ablation (RFA) and hepatic resection (HR) for solitary colorectal liver metastases (CLM).

METHODS: A literature search was performed to identify comparative studies reporting outcomes for both RFA and HR for solitary CLM. Pooled odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using either the fixed effects model or random effects model.

RESULTS: Seven nonrandomized controlled trials studies were included in this analysis. These studies included a total of 847 patients: 273 treated with RFA and 574 treated with HR. The 5 years overall survival rates in the HR group were significantly better than those in the RFA group (OR: 0.41, 95% CI: 0.22-0.90, P = 0.008). RFA had a higher rate of local intrahepatic recurrence compared to HR (OR: 4.89, 95% CI: 1.73-13.87, P = 0.003). No differences were found between the two groups with respect to postoperative morbidity and mortality.

CONCLUSION: HR was superior to RFA in the treatment of patients with solitary CLM. However, the findings have to be carefully interpreted due to the lower level of evidence.

Key words: Hepatic resection; Colorectal liver metastases; Radiofrequency ablation; Efficacy; Meta-analysis

Peer reviewer: Selin Kapan, Dr., Associate Professor of General Surgery, Dr., Sadi Konuk Training and Research Hospital, Department of General Surgery, Kucukcekmece, Istanbul 34150, Turkey

Wu YZ, Li B, Wang T, Wang SJ, Zhou YM. Radiofrequency ablation *vs* hepatic resection for solitary colorectal liver metastasis: A meta-analysis. *World J Gastroenterol* 2011; 17(36): 4143-4148 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4143.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4143

INTRODUCTION

Colorectal cancer continues to be one of the most common human malignancies, afflicting nearly one million individuals worldwide every year[1]. Approximately 50% of patients with colorectal cancer develop hepatic metastases during the course of their disease. Survival without treatment is very limited, with a median of 7.4 to 11 mo[2]. Hepatic resection (HR) is the only chance of cure for patients with colorectal liver metastases (CLM) and 5 years survival rates after radical resection are about 27%-58%[3]. However, the great majority of

patients with CLM present with unresectable disease, mainly due to the extent or distribution of their disease, or concurrent medical disability, so only up to 20% of patients are candidates for HR[4,5]. So, many nonsurgical ablative methods have been developed. The most widely utilized modality is radiofrequency ablation (RFA), which includes generation of high-frequency alternating current which causes ionic agitation and conversion to heat, with subsequent evaporation of intracellular water which leads to irreversible cellular changes, including intracellular protein denaturation, melting of membrane lipid bilayers, and coagulative necrosis of individual tumor cells.

Although RFA has established its role in the treatment algorithm of patients with inoperable CLM as a safe, well tolerated, easily repeated and less invasive procedure[2,6,7], the therapeutic efficacy of RFA for those with resectable CLM remains controversial, especially for solitary lesions. For example, Oshowo *et al*[8] reported equivalent median (41 mo *vs* 37 mo) and 3 years overall survival rates (55.4% *vs* 52.6%) between HR and RFA groups, whereas White *et al*[3] reported better 5 years (71% *vs* 27%) and overall median survival (56 mo *vs* 36 mo) for resection *vs* RFA.

Meta-analysis can be used to evaluate the existing literature in both a qualitative and quantitative way by comparing and integrating the results of different studies and taking into account variations in characteristics that can influence the overall estimate of the outcome of interest[9]. Therefore, we evaluated the available evidence comparing the clinical efficacy and safety of RFA and HR for treatment of solitary CLM using meta-analysis.

MATERIALS AND METHODS

Study selection

A MEDLINE, EMBASE, OVID, and Cochrane database search was performed on all studies between 1996 and 2010 to compare RFA and HR for solitary CLM. The following MeSH search headings were used: "colorectal liver metastases", "hepatic resection", "radiofrequency ablation" and "comparative study". Only studies on humans and in English language were considered for inclusion. Reference lists of all retrieved articles were manually searched for additional studies.

Data extraction

Two reviewers (BL and TW, respectively) independently extracted the following parameters from each study: (1) first author and year of publication; (2) number of patients, patients' characteristics, study design; and lastly (3) treatment outcome. All relevant text, tables and figures were reviewed for data extraction. Discrepancies between the two reviewers were resolved by discussion and consensus.

Criteria for inclusion and exclusion

For inclusion in the meta-analysis, a study had to fulfill the following criteria: (1) compare the initial therapy effects of RFA and HR for the treatment of solitary CLM; (2) report on at least one of the outcome measures mentioned below; (3) clearly document indications for RFA and HR; and (4) if dual (or multiple) studies were reported by the same institution and/or authors, the one of higher quality or the most recent publication was included in the analysis.

Abstracts, letters, editorials and expert opinions, reviews without original data, case reports and studies lacking control groups were excluded. The following studies were also excluded: (1) those dealing with multiple CLM; (2) those with no clearly reported outcomes of interest; and (3) those evaluating patients with primary liver cancer.

Study objectives

The primary outcome was efficacy, including 5 years overall survival, local intrahepatic recurrence or 5 years disease-free survival. The secondary outcome was safety, including the morbidity and mortality.

Statistical analysis

The meta-analysis was performed using the Review Manager (RevMan) software, version 4.2.7. We analysed dichotomous variables using estimation of odds ratios (OR) with a 95% confidence interval (95% CI). Pooled effect was calculated using either the fixed effects model or random effects model. Heterogeneity was evaluated by χ^2 and I^2. We considered heterogeneity to be present if the I^2 statistic was > 50%. $P < 0.05$ was considered significant.

RESULTS

Selection of trials

After initial screening, 13 potentially relevant clinical trials were identified[3,8,10-20]. Of these, in three trials including patients with multiple metastases, it was impossible to extract or calculate the appropriate data regarding solitary CLM[10-12], two trials included patients with non-colorectal cancer[13,14], and one trial lacked information concerning 5 years overall survival[15]; all 6 studies were excluded. Finally, a total of 7 nonrandomized studies published between 2003 and 2009 matched the inclusion criteria and were therefore included[3,8,16-20].

The characteristics of these 7 studies are summarized in Table 1. The 7 studies included a total of 847 patients: 273 in the RFA group and 574 in the HR group. Four studies were conducted in United States[3,16,17,20], two in Korea[18,19], and one in United Kingdom[3]. The sample size of each study varied from 45 to 192 patients. The proportion of men ranged from 46.6% to 66.6%. Median duration of follow-up ranged from 17 to 68 mo.

Efficacy

The pooled analysis of the 7 studies furnishing data demonstrated a significant improvement in 5 years overall survival favoring HR over RFA (OR: 0.41, 95% CI:

Table 1 Baseline characteristics of studies included in the meta-analysis

Author/(yr)	Country	Group	*n*	M/F	Mean age (yr)	Mean tumor size (cm)	Median follow-up (mo)
Oshowo[8]	United Kingdom	RFA	25	11/14	57 (34-80)	3 (1-10)[1]	37 (9-67)
2003		HR	20	10/10	63 (52-77)	4 (2-7)	41 (0-97)
Aloia[16]	United States	RFA	30	23/7	-	3.0 (1.0-7.0)[1]	31.3 (4-138)
2006		HR	150	85/65	-	3.5 (0.5-17.0)	31.3 (4-138)
White[3]	United States	RFA	22	8/14	62 ± 7.5	2.4 ± 1.0	17
2007		HR	30	20/10	63 ± 9.6	2.7 ± 1.1	68
Berber[17]	United States	RFA	68	43/25	67 ± 1.4	3.7 ± 0.2	23 (2-86)
2008		HR	90	57/33	63.7 ± 1.3	3.8 ± 0.2	33 (2-132)
Lee[18]	Korea	RFA	37	26/11	59.0 (28-75)[1]	2.25 (0.8-5.0)	48.2 (0.9-133.9)
2008		HR	116	76/40	58.0 (26-79)	3.29 (0.5-18.0)	48.2 (0.9-133.9)
Hur[19]	Korea	RFA	25	15/10	62.6 (33-82)	2.5 (0.8-3.6)	42 (13-120)
2009		HR	42	27/15	58 (42-75)	2.8 (0.6-8)	42 (13-120)
Reuter[20]	United States	RFA	66	46/20	63.5	3.2	20
2009		HR	126	69/57	61.9	5.3	20

RFA: Radiofrequency ablation; HR: Hepatic resection; M: Male; F: Female. [1]Median.

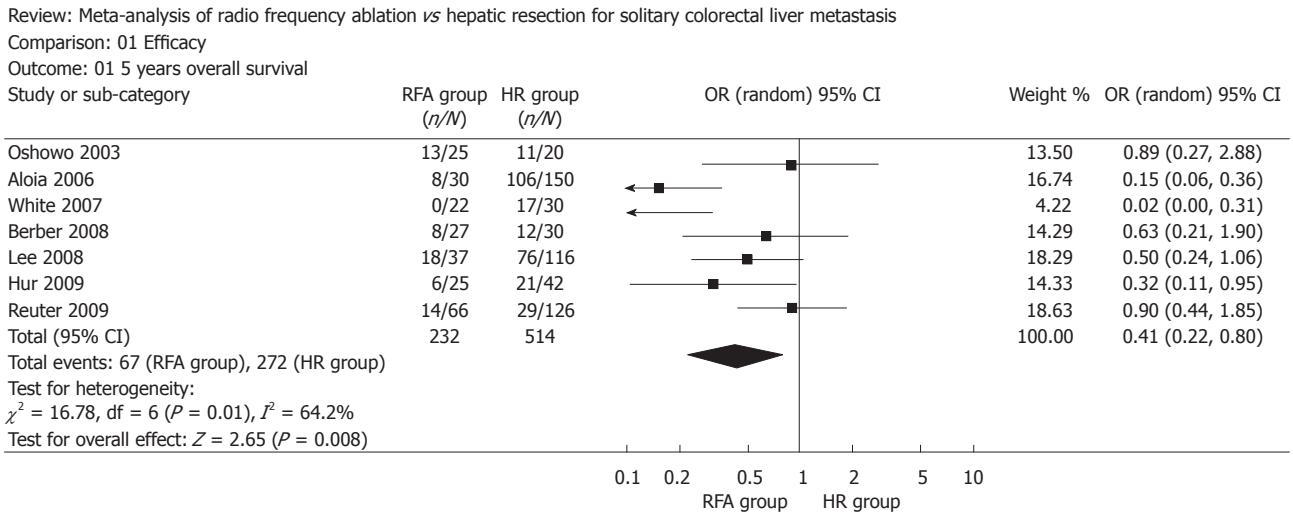


Figure 1 Results of the meta-analysis on 5 years overall survival. RFA: Radiofrequency ablation; HR: Hepatic resection; OR: Odds ratios; CI: Confidence intervals.

0.22-0.90, $P = 0.008$, $I^2 = 64.2\%$) (Figure 1).

Six trials investigated local intrahepatic recurrence[3,16-20]. Local recurrence was more frequently observed after RFA than after HR (OR: 4.89, 95% CI: 1.73-13.87, $P = 0.003$, $I^2 = 77.3\%$) (Figure 2).

Only two studies reported on 5 years disease-free survival. Aloia *et al*[16] reported that 5 years disease-free survival rates were higher after HR compared with RFA (50% *vs* 0%), whereas Lee *et al*[18] reported equivalent results between two groups (25.7% *vs* 30.1%). We did not perform an analysis because of the small number of trials included in the review.

Safety

There was no statistically significant difference in the postoperative morbidity (five trials reported this data[3,8,17,19,20], OR: 0.32, 95% CI: 0.07-1.52, $P = 0.15$, $I^2 = 75.6\%$) and mortality (all trials reported this data, OR: 0.58, 95% CI: 0.06-5.66, $P = 0.64$, $I^2 = 0\%$) between the two groups (Figures 3 and 4). There were no deaths reported in the RFA group, and 2 in the HR group.

DISCUSSION

This meta-analysis shows that the HR treatment group had better 5 years survival outcomes than the RFA treatment group for solitary CLM. The major contributing factor for this finding may be the higher local recurrence rate after RFA. In addition to being more likely to have a recurrence, RFA patients also recurred earlier than resection patients[3,20]. This could be due to incomplete ablation secondary to lesion size, heat sink effect, or the limitations of the modality[20]. Resection of the entire area of preexisting tumor is more oncologically sound than attempting thermal destruction of a frequently ill defined region in the liver[21]. This may explain the better outcomes following HR.

In a mouse xenograft model of CLM, von Breitenbuch *et al*[22] revealed that RFA led to an increased survival of residual neoplastic cells and significantly promoted the

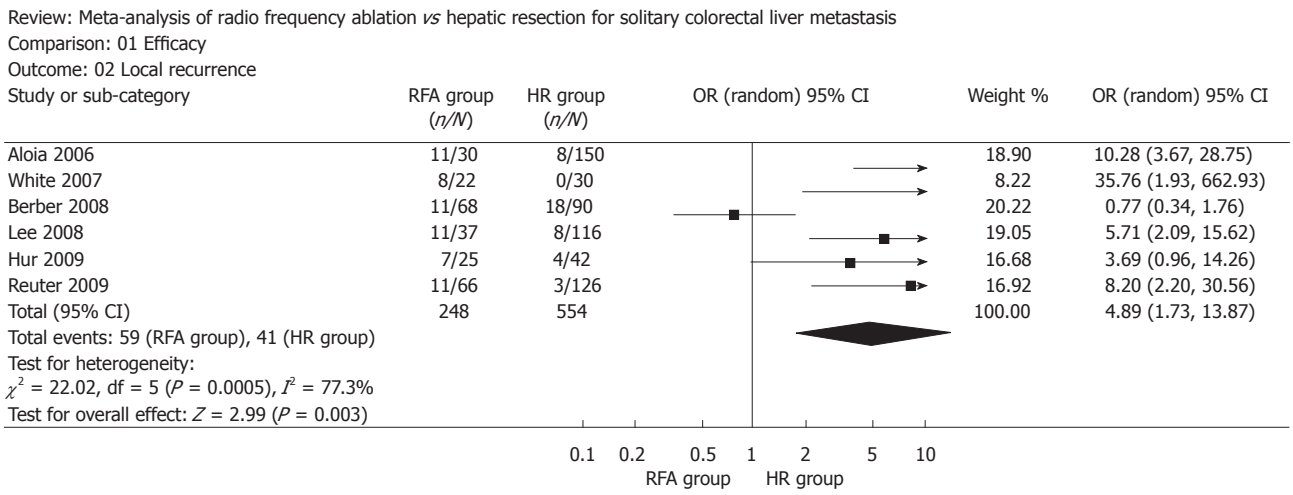


Figure 2 Results of the meta-analysis on local recurrence rate. RFA: Radiofrequency ablation; HR: Hepatic resection; OR: Odds ratios; CI: Confidence intervals.

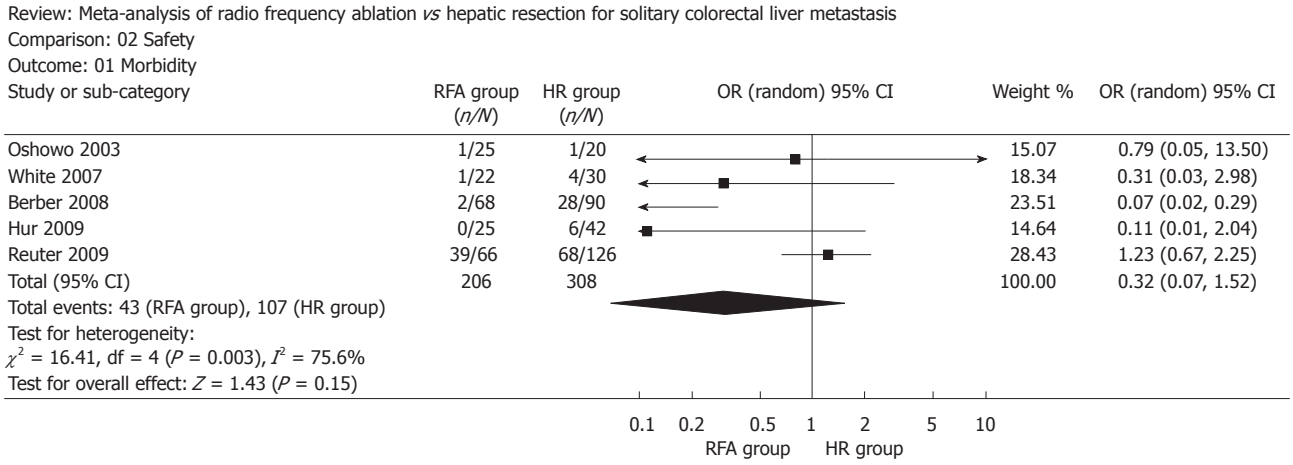


Figure 3 Results of the meta-analysis on postoperative morbidity. RFA: Radiofrequency ablation; HR: Hepatic resection; OR: Odds ratios; CI: Confidence intervals.

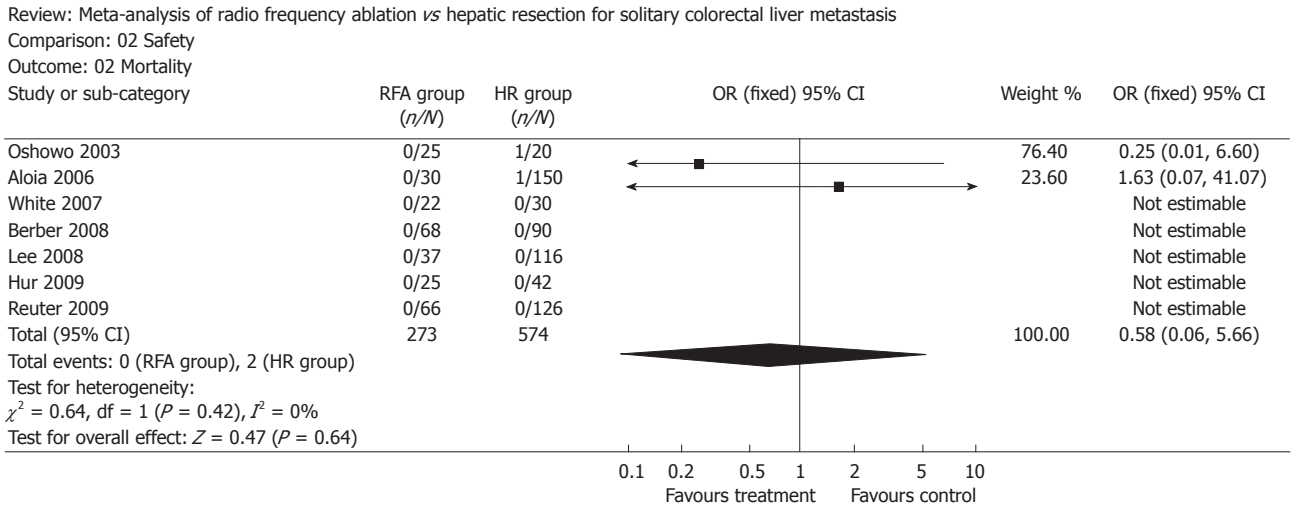


Figure 4 Results of the meta-analysis on postoperative mortality. RFA: Radiofrequency ablation; HR: Hepatic resection; OR: Odds ratios; CI: Confidence intervals.

proliferation of neoplastic cells. Recently, Nijkamp *et al*[23] found that RFA treatment resulted in a highly localized

hypoxia-driven acceleration of tumor growth occurring in the transition zone between necrosis induced by RFA and the normal liver tissue, and that the stimulated outgrowth of perilesional micrometastases is associated with profound and chronic microvascular disturbances, chronic tissue and tumor hypoxia, and stabilization of hypoxia-inducible factor (HIF)-1a and HIF-2a. These experimental findings may further explain the better outcome after RFA compared with HR in current study.

For liver metastases ≤ 3 cm, Mulier and colleagues found that local recurrence after RFA is extremely low in a recent review, and the authors proposed a randomized trial comparing resection and RFA for resectable CLM ≤ 3 cm is warranted[24]. However, in a study of 79 patients with solitary CLM ≤ 3 cm, RFA treatment resulted in a higher local recurrence rate than HR treatment (31% *vs* 3%, respectively). RFA was also associated with a marked decrease in the 5 years survival rate and the 5 years local recurrence-free rate compared with those of HR (18% *vs* 72% and 66% *vs* 97%, respectively)[16]. Similarly, another study of 60 patients showed that both time to recurrence after treatment of liver metastases and overall survival were significantly shorter, and marginal recurrence significantly more frequent, in the RFA group[15]. Although Hur *et al*[19] reported equivalent 5 years survival rates (56.1% *vs* 55.4%) and local recurrence-free survival rates (95.7% *vs* 85.6%) between HR and RFA groups in patients with tumors ≤ 3 cm, it must be noted that the limited number of patients (n = 38) in their study might have insufficient power to detect any differences.

In that review, Mulier *et al*[24] stated that the two randomized clinical trials[25,26] showed equivalent survival after percutaneous RFA and surgical resection for small HCC will encourage the use of RFA for resectable CLM. However, in one of the two studies, 19 of 90 patients (21%) who were randomized for RFA converted to HR[25]. More importantly, a recently published meta-analysis and a randomized clinical trial both found that HR was superior to RFA in the treatment of patients with small HCC with respect to survival and local control of the disease[27,28]. Thus, we agree with the idea proposed by Curley that "it is not yet time for a randomized clinical trial comparing resection with RFA for resectable CLM."

The results of this meta-analysis should be interpreted with caution for several reasons. First, all of data in the present study comes from nonrandomized studies, and the overall level of clinical evidence is low. Second, there is important heterogeneity between two groups, because it was not possible to match patients characteristics in all studies. We applied a random effect model to take between study variation into consideration. This does not necessarily rule out the effect of heterogeneity between studies, but one may expect a very limited influence. Finally, potential publication bias might be present due to the small number of trials included in the current study.

In summary, HR was superior to RFA in the treatment of patients with solitary CLM. RFA should be reserved for patients who are not optimal candidates for resection, rather than being used as a first-line therapeutic option. However, the findings have to be carefully interpreted due to the lower level of evidence.

COMMENTS

Background

Hepatic metastases are the commonest cause of morbidity and death of patients with colorectal cancer. Survival without treatment is very limited, with a median of 7.4 to 11 mo. Hepatic resection (HR) is the only chance of cure for patients with colorectal liver metastases (CLM) and 5 years survival rates after radical resection are about 27%-58%. Unfortunately, only up to 20% of patients are candidates for HR.

Research frontiers

Radiofrequency ablation (RFA) is an established effective nonsurgical ablative method for treatment of inoperable CLM, but its therapeutic efficacy for resectable CLM remains controversial, especially for solitary lesions.

Innovations and breakthroughs

This meta-analysis shows for the first time that HR was superior to RFA in the treatment of patients with solitary CLM with respect to survival and local control of the disease.

Applications

The results suggest that RFA should be reserved for patients with solitary CLM who are not optimal candidates for resection, rather than being used as a first-line therapeutic option.

Peer review

The article is a well written, well analysed one that is worth publishing.

REFERENCES

1 **El-Tawil AM**. Colorectal cancer and pollution. *World J Gastroenterol* 2010; **16**: 3475-3477
2 **Gillams AR**, Lees WR. Radiofrequency ablation of colorectal liver metastases. *Abdom Imaging* **2005; 30**: 419-426
3 **White RR**, Avital I, Sofocleous CT, Brown KT, Brody LA, Covey A, Getrajdman GI, Jarnagin WR, Dematteo RP, Fong Y, Blumgart LH, D'Angelica M. Rates and patterns of recurrence for percutaneous radiofrequency ablation and open wedge resection for solitary colorectal liver metastasis. *J Gastrointest Surg* 2007; **11**: 256-263
4 **Scheele J**, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* **1995; 19**: 59-71
5 **Bismuth H**, Adam R, Lévi F, Farabos C, Waechter F, Castaing D, Majno P, Engerran L. Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. *Ann Surg* 1996; **224**: 509-520; discussion 520-522
6 **Solbiati L**, Livraghi T, Goldberg SN, Ierace T, Meloni F, Dellanoce M, Cova L, Halpern EF, Gazelle GS. Percutaneous radio-frequency ablation of hepatic metastases from colorectal cancer: long-term results in 117 patients. *Radiology* 2001; **221**: 159-166
7 **Livraghi T**, Solbiati L, Meloni F, Ierace T, Goldberg SN, Gazelle GS. Percutaneous radiofrequency ablation of liver metastases in potential candidates for resection: the "test-of-time approach". *Cancer* 2003; **97**: 3027-3035
8 **Oshowo A**, Gillams A, Harrison E, Lees WR, Taylor I. Comparison of resection and radiofrequency ablation for treatment of solitary colorectal liver metastases. *Br J Surg* 2003; **90**: 1240-1243
9 **Aziz O**, Constantinides V, Tekkis PP, Athanasiou T, Purkayastha S, Paraskeva P, Darzi AW, Heriot AG. Laparoscopic versus open surgery for rectal cancer: a meta-analysis. *Ann Surg Oncol* 2006; **13**: 413-424
10 **Abdalla EK**, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases.

Ann Surg 2004; **239**: 818-825; discussion 825-827
11 **Otto G**, Düber C, Hoppe-Lotichius M, König J, Heise M, Pitton MB. Radiofrequency ablation as first-line treatment in patients with early colorectal liver metastases amenable to surgery. *Ann Surg* 2010; **251**: 796-803
12 **Gleisner AL**, Choti MA, Assumpcao L, Nathan H, Schulick RD, Pawlik TM. Colorectal liver metastases: recurrence and survival following hepatic resection, radiofrequency ablation, and combined resection-radiofrequency ablation. *Arch Surg* 2008; **143**: 1204-1212
13 **Leblanc F**, Fonck M, Brunet R, Becouarn Y, Mathoulin-Pélissier S, Evrard S. Comparison of hepatic recurrences after resection or intraoperative radiofrequency ablation indicated by size and topographical characteristics of the metastases. *Eur J Surg Oncol* 2008; **34**: 185-190
14 **Chow DH**, Sinn LH, Ng KK, Lam CM, Yuen J, Fan ST, Poon RT. Radiofrequency ablation for hepatocellular carcinoma and metastatic liver tumors: a comparative study. *J Surg Oncol* 2006; **94**: 565-571
15 **Park IJ**, Kim HC, Yu CS, Kim PN, Won HJ, Kim JC. Radiofrequency ablation for metachronous liver metastasis from colorectal cancer after curative surgery. *Ann Surg Oncol* 2008; **15**: 227-232
16 **Aloia TA**, Vauthey JN, Loyer EM, Ribero D, Pawlik TM, Wei SH, Curley SA, Zorzi D, Abdalla EK. Solitary colorectal liver metastasis: resection determines outcome. *Arch Surg* 2006; **141**: 460-466; discussion 466-467
17 **Berber E**, Tsinberg M, Tellioglu G, Simpfendorfer CH, Siperstein AE. Resection versus laparoscopic radiofrequency thermal ablation of solitary colorectal liver metastasis. *J Gastrointest Surg* 2008; **12**: 1967-1972
18 **Lee WS**, Yun SH, Chun HK, Lee WY, Kim SJ, Choi SH, Heo JS, Joh JW, Choi D, Kim SH, Rhim H, Lim HK. Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. *J Clin Gastroenterol* 2008; **42**: 945-949
19 **Hur H**, Ko YT, Min BS, Kim KS, Choi JS, Sohn SK, Cho CH, Ko HK, Lee JT, Kim NK. Comparative study of resection and radiofrequency ablation in the treatment of solitary colorectal liver metastases. *Am J Surg* 2009; **197**: 728-736
20 **Reuter NP**, Woodall CE, Scoggins CR, McMasters KM, Martin RC. Radiofrequency ablation vs. resection for hepatic colorectal metastasis: therapeutically equivalent? *J Gastrointest Surg* 2009; **13**: 486-491
21 **Curley SA**. Radiofrequency ablation versus resection for resectable colorectal liver metastases: time for a randomized trial? *Ann Surg Oncol* 2008; **15**: 11-13
22 **von Breitenbuch P**, Köhl G, Guba M, Geissler E, Jauch KW, Steinbauer M. Thermoablation of colorectal liver metastases promotes proliferation of residual intrahepatic neoplastic cells. *Surgery* 2005; **138**: 882-887
23 **Nijkamp MW**, van der Bilt JD, de Bruijn MT, Molenaar IQ, Voest EE, van Diest PJ, Kranenburg O, Borel Rinkes IH. Accelerated perinecrotic outgrowth of colorectal liver metastases following radiofrequency ablation is a hypoxia-driven phenomenon. *Ann Surg* 2009; **249**: 814-823
24 **Mulier S**, Ni Y, Jamart J, Michel L, Marchal G, Ruers T. Radiofrequency ablation versus resection for resectable colorectal liver metastases: time for a randomized trial? *Ann Surg Oncol* 2008; **15**: 144-157
25 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328
26 **Lü MD**, Kuang M, Liang LJ, Xie XY, Peng BG, Liu GJ, Li DM, Lai JM, Li SQ. [Surgical resection versus percutaneous thermal ablation for early-stage hepatocellular carcinoma: a randomized clinical trial]. *Zhonghua Yixue Zazhi* 2006; **86**: 801-805
27 **Zhou Y**, Zhao Y, Li B, Xu D, Yin Z, Xie F, Yang J. Meta-analysis of radiofrequency ablation versus hepatic resection for small hepatocellular carcinoma. *BMC Gastroenterol* 2010; **10**: 78
28 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912

S- Editor Tian L **L- Editor** O'Neill M **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i36.4149

World J Gastroenterol 2011 September 28; 17(36): 4149-4152
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CD133 and membrane microdomains: Old facets for future hypotheses

Christine A Fargeas, Jana Karbanová, József Jászai, Denis Corbeil

Christine A Fargeas, Jana Karbanová, József Jászai, Denis Corbeil, Tissue Engineering Laboratories (BIOTEC), Technische Universität Dresden, D-01307 Dresden, Germany
Author contributions: Fargeas CA, Karbanová J, Jászai J and Corbeil D contributed equally to this letter to the editor.
Supported by Deutsche Forschungsgemeinschaft (TRR83 No. 6; SFB655 B3; CO298/5-1)
Correspondence to: Denis Corbeil, PhD, Tissue Engineering Laboratories (BIOTEC), Technische Universität Dresden, Tatzberg 47-49, D-01307 Dresden,
Germany. corbeil@biotec.tu-dresden.de
Telephone: +49-351-46340118 Fax: +49-351-46340244
Received: April 20, 2011 Revised: June 16, 2011
Accepted: June 23, 2011
Published online: September 28, 2011

Abstract

Understanding all facets of membrane microdomains in normal and cancerous cells within the digestive tract is highly important, not only from a clinical point of view, but also in terms of our basic knowledge of cellular transformation. By studying the normal and cancer stem cell-associated molecule CD133 (prominin-1), novel aspects of the organization and dynamics of polarized epithelial cells have been revealed during the last decade. Its association with particular membrane microdomains is highly relevant in these contexts and might also offer new avenues in diagnosis and/or targeting of cancer stem cells.

Key words: AC133; Cancer; CD133; Membrane microdomains; Membrane vesicles; Prominin-1; Stem cell

Peer reviewer: Zoran Krivokapic, Professor, Dr., MD, FRCS, Institute for Digestive Disease, First Surgical Clinic, Clinical Center of Serbia, 6, Dr Koste Todorovica, Belgrade 11000, Serbia

Fargeas CA, Karbanová J, Jászai J, Corbeil D. CD133 and membrane microdomains: Old facets for future hypotheses. *World J Gastroenterol* 2011; 17(36): 4149-4152 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i36/4149.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i36.4149

TO THE EDITOR

We read with great interest a recent Editorial entitled "Multifaceted nature of membrane microdomains in colorectal cancer" by Jahn *et al*[1] published in issue 17 of the *World Journal of Gastroenterology* 2011 which proposes to describe the pioneering and recent studies on membrane microdomains (the so-called lipid rafts) and their potential roles in cancers. An important section dealing with prominin-1 (alias CD133), a cholesterol-binding glycoprotein often described as a stem and cancer stem cell marker, is unfortunately entirely based on a single publication released in 2009[2], thus leaving out valuable biochemical and morphological information concerning CD133 and membrane microdomains from earlier works. We fear that as such, it might lead to underestimation of the importance and complexity of such a molecular association and contribute to certain confusion, particularly with regard to the debated AC133 epitope of CD133 and its association with cancer. We propose to expose here earlier overlooked data regarding its expression in epithelial cells and summarize the current knowledge on its cell biology and association with distinctive membrane microdomains. We hope that this might enlighten current issues regarding the implication of CD133 in colorectal cancer, whether it is in metastases, or as a prognostic marker or as a cancer stem cell marker.

Actually, the demonstration of the presence of CD133 in Caco-2 cells and its association with membrane microdomains is much less recent than 2009, since it was more than a decade ago that we reported its presence

in this widely used human colon carcinoma-derived cell line[3]. The detection of CD133 by immunolabeling was originally documented by its particular epitope AC133 that appeared to be restricted to stem/progenitor cell populations but was also thought to be dependent on conformation and/or sensitive to changes in glycosylation[4]. This antigen was attractive in the context of stem/progenitor and cancer stem cells and has often been used to define them in numerous organ systems including the digestive tract, but at the same time controversy was generated on the implication of CD133 as a specific marker[5-10].

We have previously demonstrated in a key publication of 2000 using the Caco-2 cells as a model of enterocytic epithelial differentiation[3] together with a later study[11], that the AC133 epitope, but neither the CD133 transcript nor the CD133 protein, is down-regulated upon differentiation, with the result that only a minute sub-fraction of CD133 molecules will carry it[11]. We have therefore stressed several times in the literature that it is important to consider that AC133 antibody detects only a subpopulation of human prominin-1/CD133 glycoproteins carrying the AC133 epitope, and that consequently, AC133 antigen is not fully synonymous with CD133[11-13]. The importance of CD133 glycosylation states for the definition of cancer stem cells has been analyzed by Bindlingmaier and colleagues[14]. In the meantime, the *prominin-1* (*PROM1*) gene was shown to be transcriptionally active all along the gastrointestinal tract as CD133 mRNA is detectable by Northern blot[15], and several studies have demonstrated that in humans, as in mice, its protein is physiologically expressed in several differentiated epithelia[11,16-20]. Thus, the AC133 epitope might be simply down- or up-regulated during the process of differentiation or transformation, respectively[11]. The alteration of the general glycosylation pattern of intestinal cells might explain such a phenomenon[21]. Importantly, the lack of AC133 detection might additionally reflect its instability[22] or its differential accessibility[19] (see below). Of note, the proportion of CD133 molecules carrying (or not) the AC133 epitope in a given differentiated cell remains, however, unknown.

As proposed earlier[19] and pointed out in the Editorial of Jahn and colleagues, the molecular environment surrounding CD133 within the plasma membrane might influence the detection of certain epitopes (e.g., AC133 or those within putative ganglioside-binding sites[2]). To fully appreciate the importance of CD133, one should bear in mind that, at the subcellular level, CD133 selectively marks plasma membrane protrusions, e.g., microvilli and primary cilia, that are located in the apical domain of polarized epithelial cells including Caco-2 cells, and was therefore originally named prominin (from Latin, *prominere*)[3,16,23,24]. Within these protrusions, CD133 binds directly to plasma membrane cholesterol[25,26] and is incorporated into membrane microdomains that differ from those found in non-protruding areas of the plasma membrane, as demonstrated biochemically using mild detergents[25], and morphologically by co-localization with the ganglioside GM_1[27]. Such protein-lipid interactions appear essential to maintain the proper localization of CD133 in microvilli[25], and potentially its physiological function which yet remains elusive[28,29]. Thus, the direct binding of certain gangliosides to CD133[2,27] within the densely packed lipid microdomain might mask some CD133 epitope(s), particularly those in the vicinity of the membrane. Technically, they might be revealed, at least in part, using sensitive methods including harsh conditions for antigen retrieval as in the case of native tissues[8,19,30,31], upon cell-detachment as in the case of cell lines (e.g., Caco-2 cells)[32], or by chemical interference with membrane microdomain integrity[2].

Although tightly associated with plasma membrane, CD133 is nonetheless released into numerous physiological body fluids including urine, saliva, seminal fluids and cerebrospinal fluids in association with small membrane vesicles[33]. It is important to point out that such vesicles are budding from the tip of a microvillus or primary cilium by a molecular mechanism involving cholesterol-dependent membrane microdomains[26,34]. In other words, their release might be modulated by the cholesterol level (and possibly that of other lipids) within the plasma membrane. Interestingly, such release occurs solely during and after the differentiation of Caco-2 cells or, *in vivo*, of neural progenitor cells[33]. Based on the latter observation and the expression of CD133 (AC133 epitope in the case of humans) by numerous somatic stem cells, the concept of "stem cell-specific membrane microdomains" was postulated[33]. Given that membrane microdomains are implicated in several signaling cascades by allowing the formation of active transduction complexes[35], CD133-containing membrane microdomains might carry and/or functionally organize molecular determinants essential to maintain the stem cell and undifferentiated cell properties and their loss or disposal, e.g., *via* membrane vesicles, and could modify the status or even the fate of the cells[33,36]. Yet, these microdomains, given their dependence on cholesterol, seem to differ from those defined by Hakomori and co-workers in the glycosynapse concept, and which have been implicated in several biological phenomena related to tumorigenesis[37,38]. However, the coalescence of small CD133-lipid entities into the largest platform within the microvillar membranes might be dragged by carbohydrate moieties, as proposed earlier[13,25,28]. Thus, a certain interdependence of lipid rafts and glycosynapses *per se* might exist. Whether CD133 molecules carrying AC133 epitope are preferentially released upon differentiation remains to be determined. Collectively, numerous physiological and technical parameters might interfere with immuno-detection of certain CD133 epitopes, and importantly, the lack of their detection needs to be evaluated with some caution, and maybe alternative methods such as *in situ* hybridization should complement the investigation[18,39].

Clinically, in addition to its potential value as a biomarker in tissue diagnosis, the association of CD133/

lipid complexes with extracellular membrane vesicles might offer an alternative screening method for the detection of cancers associated with the digestive tract as demonstrated for central nervous system diseases[40]. Moreover, CD133 expression by cancer stem cells might contribute to outlining new prospects for more effective cancer therapy by targeting tumor-initiating cells.

REFERENCES

1 **Jahn KA**, Su Y, Braet F. Multifaceted nature of membrane microdomains in colorectal cancer. *World J Gastroenterol* 2011; **17**: 681-690
2 **Taïeb N**, Maresca M, Guo XJ, Garmy N, Fantini J, Yahi N. The first extracellular domain of the tumour stem cell marker CD133 contains an antigenic ganglioside-binding motif. *Cancer Lett* 2009; **278**: 164-173
3 **Corbeil D**, Röper K, Hellwig A, Tavian M, Miraglia S, Watt SM, Simmons PJ, Peault B, Buck DW, Huttner WB. The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J Biol Chem* 2000; **275**: 5512-5520
4 **Miraglia S**, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 1997; **90**: 5013-5021
5 **Ricci-Vitiani L**, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111-115
6 **O'Brien CA**, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110
7 **Shmelkov SV**, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St Clair R, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D, Rafii S. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest* 2008; **118**: 2111-2120
8 **Immervoll H**, Hoem D, Sakariassen PØ, Steffensen OJ, Molven A. Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas. *BMC Cancer* 2008; **8**: 48
9 **Chu P**, Clanton DJ, Snipas TS, Lee J, Mitchell E, Nguyen ML, Hare E, Peach RJ. Characterization of a subpopulation of colon cancer cells with stem cell-like properties. *Int J Cancer* 2009; **124**: 1312-1321
10 **Zhu L**, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT, Poppleton H, Zakharenko S, Ellison DW, Gilbertson RJ. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* 2009; **457**: 603-607
11 **Florek M**, Haase M, Marzesco AM, Freund D, Ehninger G, Huttner WB, Corbeil D. Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. *Cell Tissue Res* 2005; **319**: 15-26
12 **Fargeas CA**, Corbeil D, Huttner WB. AC133 antigen, CD133, prominin-1, prominin-2, etc.: prominin family gene products in need of a rational nomenclature. *Stem Cells* 2003; **21**: 506-508
13 **Fargeas CA,** Fonseca AV, Huttner WB, Corbeil D. Prominin-1 (CD133): from progenitor cells to human diseases. *Future Lipidol* 2006; **1**: 213-225
14 **Bidlingmaier S**, Zhu X, Liu B. The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. *J Mol Med* (Berl) 2008; **86**: 1025-1032
15 **Fargeas CA**, Florek M, Huttner WB, Corbeil D. Characterization of prominin-2, a new member of the prominin family of pentaspan membrane glycoproteins. *J Biol Chem* 2003; **278**: 8586-8596
16 **Weigmann A**, Corbeil D, Hellwig A, Huttner WB. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc Natl Acad Sci USA* 1997; **94**: 12425-12430
17 **Fargeas CA**, Joester A, Missol-Kolka E, Hellwig A, Huttner WB, Corbeil D. Identification of novel Prominin-1/CD133 splice variants with alternative C-termini and their expression in epididymis and testis. *J Cell Sci* 2004; **117**: 4301-4311
18 **Jászai J**, Janich P, Farkas LM, Fargeas CA, Huttner WB, Corbeil D. Differential expression of Prominin-1 (CD133) and Prominin-2 in major cephalic exocrine glands of adult mice. *Histochem Cell Biol* 2007; **128**: 409-419
19 **Karbanová J**, Missol-Kolka E, Fonseca AV, Lorra C, Janich P, Hollerová H, Jászai J, Ehrmann J, Kolár Z, Liebers C, Arl S, Subrtová D, Freund D, Mokry J, Huttner WB, Corbeil D. The stem cell marker CD133 (Prominin-1) is expressed in various human glandular epithelia. *J Histochem Cytochem* 2008; **56**: 977-993
20 **Missol-Kolka E**, Karbanová J, Janich P, Haase M, Fargeas CA, Huttner WB, Corbeil D. Prominin-1 (CD133) is not restricted to stem cells located in the basal compartment of murine and human prostate. *Prostate* 2011; **71**: 254-267
21 **Ogier-Denis E**, Codogno P, Chantret I, Trugnan G. The processing of asparagine-linked oligosaccharides in HT-29 cells is a function of their state of enterocytic differentiation. An accumulation of Man9,8-GlcNAc2-Asn species is indicative of an impaired N-glycan trimming in undifferentiated cells. *J Biol Chem* 1988; **263**: 6031-6037
22 **Zhou F**, Cui C, Ge Y, Chen H, Li Q, Yang Z, Wu G, Sun S, Chen K, Gu J, Jiang J, Wei Y. Alpha2,3-Sialylation regulates the stability of stem cell marker CD133. *J Biochem* 2010; **148**: 273-280
23 **Dubreuil V**, Marzesco AM, Corbeil D, Huttner WB, Wilsch-Bräuninger M. Midbody and primary cilium of neural progenitors release extracellular membrane particles enriched in the stem cell marker prominin-1. *J Cell Biol* 2007; **176**: 483-495
24 **Florek M**, Bauer N, Janich P, Wilsch-Braeuninger M, Fargeas CA, Marzesco AM, Ehninger G, Thiele C, Huttner WB, Corbeil D. Prominin-2 is a cholesterol-binding protein associated with apical and basolateral plasmalemmal protrusions in polarized epithelial cells and released into urine. *Cell Tissue Res* 2007; **328**: 31-47
25 **Röper K**, Corbeil D, Huttner WB. Retention of prominin in microvilli reveals distinct cholesterol-based lipid microdomains in the apical plasma membrane. *Nat Cell Biol* 2000; **2**: 582-592
26 **Marzesco AM**, Wilsch-Bräuninger M, Dubreuil V, Janich P, Langenfeld K, Thiele C, Huttner WB, Corbeil D. Release of extracellular membrane vesicles from microvilli of epithelial cells is enhanced by depleting membrane cholesterol. *FEBS Lett* 2009; **583**: 897-902
27 **Janich P**, Corbeil D. GM1 and GM3 gangliosides highlight distinct lipid microdomains within the apical domain of epithelial cells. *FEBS Lett* 2007; **581**: 1783-1787
28 **Corbeil D**, Röper K, Fargeas CA, Joester A, Huttner WB. Prominin: a story of cholesterol, plasma membrane protrusions and human pathology. *Traffic* 2001; **2**: 82-91
29 **Zacchigna S**, Oh H, Wilsch-Bräuninger M, Missol-Kolka E, Jászai J, Jansen S, Tanimoto N, Tonagel F, Seeliger M, Huttner WB, Corbeil D, Dewerchin M, Vinckier S, Moons L, Carmeliet P. Loss of the cholesterol-binding protein prominin-1/CD133 causes disk dysmorphogenesis and photoreceptor degeneration. *J Neurosci* 2009; **29**: 2297-2308
30 **Lardon J**, Corbeil D, Huttner WB, Ling Z, Bouwens L. Stem cell marker prominin-1/AC133 is expressed in duct cells of

the adult human pancreas. *Pancreas* 2008; **36**: e1-e6
31 **Kemper K**, Sprick MR, de Bree M, Scopelliti A, Vermeulen L, Hoek M, Zeilstra J, Pals ST, Mehmet H, Stassi G, Medema JP. The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation. *Cancer Res* 2010; **70**: 719-729
32 **Jaksch M**, Múnera J, Bajpai R, Terskikh A, Oshima RG. Cell cycle-dependent variation of a CD133 epitope in human embryonic stem cell, colon cancer, and melanoma cell lines. *Cancer Res* 2008; **68**: 7882-7886
33 **Marzesco AM**, Janich P, Wilsch-Bräuninger M, Dubreuil V, Langenfeld K, Corbeil D, Huttner WB. Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. *J Cell Sci* 2005; **118**: 2849-2858
34 **Corbeil D**, Marzesco AM, Fargeas CA, Huttner WB. Prominin-1: a distinct cholesterol-binding membrane protein and the organisation of the apical plasma membrane of epithelial cells. *Subcell Biochem* 2010; **51**: 399-423
35 **Simons K**, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; **1**: 31-39
36 **Bauer N**, Fonseca AV, Florek M, Freund D, Jászai J, Bornhäuser M, Fargeas CA, Corbeil D. New insights into the cell biology of hematopoietic progenitors by studying prominin-1 (CD133). *Cells Tissues Organs* 2008; **188**: 127-138
37 **Hakomori SI**. Structure and function of glycosphingolipids and sphingolipids: recollections and future trends. *Biochim Biophys Acta* 2008; **1780**: 325-346
38 **Regina Todeschini A**, Hakomori SI. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *Biochim Biophys Acta* 2008; **1780**: 421-433
39 **Jászai J**, Fargeas CA, Graupner S, Tanaka EM, Brand M, Huttner WB, Corbeil D. Distinct and conserved prominin-1/CD133-positive retinal cell populations identified across species. *PLoS One* 2011; **6**: e17590
40 **Huttner HB**, Janich P, Köhrmann M, Jászai J, Siebzehnrubl F, Blümcke I, Suttorp M, Gahr M, Kuhnt D, Nimsky C, Krex D, Schackert G, Löwenbrück K, Reichmann H, Jüttler E, Hacke W, Schellinger PD, Schwab S, Wilsch-Bräuninger M, Marzesco AM, Corbeil D. The stem cell marker prominin-1/CD133 on membrane particles in human cerebrospinal fluid offers novel approaches for studying central nervous system disease. *Stem Cells* 2008; **26**: 698-705

S- Editor Sun H **L- Editor** Logan S **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 September 28; 17(36): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Gloria González Aseguinolaza, BSc, MSc, PhD, Department of Gene Therapy in Hepatology, FIMA, CIMA University of Navarra, Navarra, Spain

Dario Conte, Professor, GI Unit - IRCCS Osp. Maggiore, Chair of Gastroenterology, Via F. Sforza, 35, Milano 20122, Italy

Bijan Eghtesad, Dr, Associate Professor, Department of General Surgery, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland OH 44195, United States

Dr. Alessandro Ferrero, MD, Department of Surgery, Mauritian Hospital, Largo Turati 62, 10128 Torino, Italy

Alfred Gangl, Professor, Department of Medicine 4, Medical University of Vienna, Allgemeines Krankenhaus, Waehringer Guertel 18-20, Vienna A-1090, Austria

Satoru Kakizaki, MD, PhD, Assistant Professor, Department of Medicine and Molecular Science, Gunma University, Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan

Yuyuan Li, Professor, Department of Gastroenterology, First Municipal People's Hospital of Guangzhou, 1 Panfu Road, Guangzhou 510180, Guangdong Province, China

Fabrizio Montecucco, MD, Assistant, Division of Cardiology, Department of Internal Medicine, University of Geneva, Avenue de la Roseraie 64, 1211 Geneva, Switzerland

Dr. Vance Matthews, PhD, BS, Cellular and Molecular Metabolism Laboratory, Baker University of Texas Medical Branch, IDI, PO Box 6492, St Kilda Road Central, VIC 8008, Melbourne, Australia

Noriko Nakajima, MD, PhD, Associate Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Nihon University School of Medicine, 1-8-13 Kandasurugadai Chiyoda-ku, Tokyo 101-8309, Japan

Christoph Reichel, Priv.-Doz., Dr., Head of the Gastroenterological Rehabilitation Center Bad Brückenau, Clinic Hartwald, German Pension Insurance Federal Office, Schlüchterner Str. 4, 97769 Bad Brückenau, Germany

Francisco Rodriguez-Frias, PhD, Proteins Hepatitis Molecular Genetics Unitat, Biochemistry Department, Vall d'Hebron Unicersitary Hospital, Barcelona 08035, Spain

Chanjuan Shi, MD, PhD, Assistant Professor, Department of Pathology, Vanderbilt University , 1161 21st Ave. So, MCN C-2318A, Nashville, TN 37232-2561, United States

Naoaki Sakata, MD, PhD, Division of Hepato-Biliary Pancreatic Surgery, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan

Phil Sutton, Associate Professor, Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, Melbourne, VIC 3010, Australia

Masahiro Tajika, MD, PhD, Department of Endoscopy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan

Yuk Him Tam, Dr., Department of Surgery, Prince of Wales Hospital, Shatin, Hong Kong, China

Tamara Vorobjova, Senior Researcher in Immunology, Department of Immunology, Institute of General and Molecular Pathology, University of Tartu, Ravila, 19, Tartu 51014, Estonia

Chunqing Zhang, Professor, Department of Gastroenterology, Shandong Provincial Hospital, Jinan 250021, Shandong Province, China

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 September 28; 17(36): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 4-5, 2011
13th Duesseldorf International Endoscopy Symposium, Duesseldorf, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011, Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
2nd International Congress on Abdominal Obesity, Buenos Aires, Brazil

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A whole-system strategic approach, Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine, Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-19, 2011
41. Kongress der Deutschen Gesellschaft für Endoskopie und Bildgebende Verfahren e.V., Munich, Germany

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform, Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 6-7, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 7-9, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 15-16, 2011
Falk Symposium 177, Endoscopy Live Berlin 2011 Intestinal Disease Meeting, Stauffenbergstr. 26, 10785 Berlin, Germany

April 18-22, 2011
Pediatric Emergency Medicine: Detection, Diagnosis and Developing Treatment Plans, Sarasota, FL 34234, United States

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 7-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course, Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV SPIGC, II ESYS, Napoli, Italy

June 14-16, 2011
International Scientific Conference on Probiotics and Prebiotics-IPC2011, Kosice, Slovakia

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 29-2, 2011
XI Congreso Interamericano de Pediatria "Monterrey 2011", Monterrey, Mexico

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
New Advances in Inflammatory Bowel Disease, La Jolla, CA 92093, United States

September 10-14, 2011
ICE 2011-International Congress of Endoscopy, Los Angeles Convention Center, 1201 South Figueroa Street Los Angeles, CA 90015, United States

September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
Cardiology & Gastroenterology | Tahiti 10 night CME Cruise, Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week, Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course, Washington, DC 20001, United States

November 11-12, 2011
Falk Symposium 180, IBD 2011: Progress and Future for Lifelong Management, ANA Interconti Hotel, 1-12-33 Akasaka, Minato-ku, Tokyo 107-0052, Japan

December 1-4, 2011
2011 Advances in Inflammatory Bowel Diseases/Crohn's & Colitis Foundation's Clinical & Research Conference, Hollywood, FL 34234, United States

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 September 28; 17(36): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a weekly, open-access (OA), peer-reviewed journal supported by an editorial board of 1144 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJG* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJG* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJG* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJG* is to report rapidly the most recent results in basic and clinical research on esophageal, gastrointestinal, liver, pancreas and biliary tract diseases, *Helicobacter pylori*, endoscopy and gastrointestinal surgery, including: gastroesophageal reflux disease, gastrointestinal bleeding, infection and tumors; gastric and duodenal disorders; intestinal inflammation, microflora and immunity; celiac disease, dyspepsia and nutrition; viral hepatitis, portal hypertension, liver fibrosis, liver cirrhosis, liver transplantation, and metabolic liver disease; molecular and cell biology; geriatric and pediatric gastroenterology; diagnosis and screening, imaging and advanced technology.

Columns

The columns in the issues of *WJG* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in gastroenterology; (9) Brief Article: To briefly report the novel and innovative findings in gastroenterology and hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice gastroenterology and hepatology.

Name of journal

World Journal of Gastroenterology

ISSN and EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Indexed and Abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifer, and Directory of Open Access Journals. ISI, Thomson Reuters, 2010 Impact Factor: 2.240 (35/71 Gastroenterology and Hepatology).

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission

System at: http://www.wjgnet.com/1007-9327office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece.

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…"; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be

used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in boldfaced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in boldfaced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixudiarrhoea. *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/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1007-9327/g_info_20100315220036.htm

Frontier: http://www.wjgnet.com/1007-9327/g_info_20100315220305.htm

Topic highlight: http://www.wjgnet.com/1007-9327/g_info_20100315220601.htm

Observation: http://www.wjgnet.com/1007-9327/g_info_20100312232427.htm

Guidelines for basic research: http://www.wjgnet.com/1007-9327/g_info_20100315220730.htm

Guidelines for clinical practice: http://www.wjgnet.com/1007-9327/g_info_20100315221301.htm

Review: http://www.wjgnet.com/1007-9327/g_info_20100315221554.htm

Original articles: http://www.wjgnet.com/1007-9327/g_info_20100315221814.htm

Brief articles: http://www.wjgnet.com/1007-9327/g_info_20100312231400.htm

Case report: http://www.wjgnet.com/1007-9327/g_info_20100315221946.htm

Letters to the editor: http://www.wjgnet.com/1007-9327/g_info_20100315222254.htm

Book reviews: http://www.wjgnet.com/1007-9327/g_info_20100312231947.htm

Guidelines: http://www.wjgnet.com/1007-9327/g_info_20100312232134.htm

RESUBMISSION OF THE REVISED MANUSCRIPTS

Please revise your article according to the revision policies of *WJG*. The revised version includes manuscript and high-resolution image figures. The author should re-submit the revised manuscript online, along with printed high-resolution color or black and white photos; Copyright transfer letter, and responses to the reviewers, and science news are sent to us *via* email.

Editorial Office

World Journal of Gastroenterology

Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,

Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315222607.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJG is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.