World Journal of Gastrointestinal Oncology

Online Submissions: http://www.wjgnet.com/1948-5204office
wjgo@wjgnet.com
doi:10.4251/wjgo.v2.i3.130

World J Gastrointest Oncol 2010 March 15; 2(3): 130-135
ISSN 1948-5204 (online)

EDITORIAL

Carcinogenesis and chemoprevention of biliary tract cancer in pancreaticobiliary maljunction

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Received: June 22, 2009 Revised: July 13, 2009
Accepted: July 20, 2009
Published online: March 15, 2010

Abstract

Pancreaticobiliary maljunction (PBM) is a high risk factor for biliary tract cancer. In PBM, since the pancreatic duct and bile duct converge outside the duodenal wall beyond the influence of the sphincter of Oddi, pancreatic juice and bile are constantly mixed, producing a variety of harmful substances. Because of this, the biliary mucosa is repeatedly damaged and repaired, which causes an acceleration of cell proliferative activity and multiple gene mutations. Histological changes such as hyperplasia, metaplasia, and dysplasia ultimately result in a high incidence of carcinogenesis. In a nationwide survey by the Japanese Study Group on PBM, coexisting biliary tract cancer was detected in 278 of the 1627 registered cases of PBM (17.1%). Of these cases, in those with dilatation of the extrahepatic bile duct, cancer was often detected not only in the gallbladder but also in the bile ducts. More than 90% of cancer cases without dilatation of the extrahepatic bile duct develop in the gallbladder. Standard treatment for PBM is a cholecystectomy and resection of the extrahepatic bile duct. However, cholecystectomy alone is performed at nearly half of institutions in Japan. Conversely, reports of carcinogenesis in the remnant bile duct or pancreas after diversion surgery are steadily increasing. One of the causes for this is believed to be an accumulation of gene mutations which were present before surgery. Anticancer drugs are ineffective in preventing such carcinogenesis following surgery, thus the postoperative administration of chemopreventive agents may be necessary.

Key words: Chemoprevention; Gallbladder cancer; Bile duct cancer; Carcinogenesis; Pancreaticobiliary maljunction

Peer reviewer: Hans Chung, MD, FRCPC, Sunnybrook Odette Cancer Centre, 2075 Bayview Avenue, T-Wing Toronto ON, M4N 3M5, Canada

Tsuchida A, Itoi T. Carcinogenesis and chemoprevention of biliary tract cancer in pancreaticobiliary maljunction. *World J Gastrointest Oncol* 2010; 2(3): 130-135 Available from: URL: http://www.wjgnet.com/1948-5204/full/v2/i3/130.htm DOI: http://dx.doi.org/10.4251/wjgo.v2.i3.130

INTRODUCTION

Pancreaticobiliary maljunction (PBM), namely an anomalous arrangement of the pancreaticobiliary duct or an abnormal junction of the pancreaticobiliary ductal system, is a high risk factor for biliary tract cancer[1]. In PBM, the main pancreatic duct and common bile duct anatomically converge outside the duodenal wall, causing a reciprocal reflux of pancreatic juice and bile, which produces carcinogenic substances such as activated pancreatic enzymes and secondary bile acid, resulting in repeated damage and repair of the biliary mucosa, which contributes to pro-inflammatory prostaglandins and various gene mutations. This in turn causes histological changes such as hyperplastic epithelium (hyperplasia), metaplastic epithelium (metaplasia), and dysplastic

epithelium (dysplasia), ultimately resulting in biliary carcinogenesis[2]. Accordingly, when PBM is diagnosed the standard treatment consists of cholecystectomy and resection of the dilated extrahepatic bile duct to prevent carcinogenesis[1]. However, for PBM without dilatation of the extrahepatic bile duct, cholecystectomy alone is often performed since the incidence of bile duct cancer is low in such cases. This course of treatment is still controversial[3,4]. While the risk of carcinogenesis is mitigated considerably through preventive standard surgeries such as these, reports of carcinogenesis in the remnant bile duct and pancreas following surgery have been on the rise in recent years[5]. One of the causes for this is believed to be an accumulation of gene mutations which were present prior to surgery. In this paper, we attempt to elucidate the various carcinogenic processes in PBM and its treatment options, as well as the prevention of postoperative carcinogenesis.

EPIDEMIOLOGY

PBM is frequently reported in Asia, particularly in Japan and Taiwan, and it is known as an Asian disease[6,7]. Coexisting PBM is found in nearly all cases of congenital bile duct dilatation, and is also found in Western countries[8]. Funabiki *et al*[9] have suggested that the number of cases diagnosed might rise if there was increased interest in the diagnostic criteria for PBM in Western countries. Hasumi *et al*[6] conducted a survey on the incidence of PBM and biliary tract cancer at 133 facilities in Japan, and showed that 414 of the 12 399 patients (3.3%) on whom hepatobiliary surgery was performed had PBM. They furthermore reported that 10.4% of the patients with gallbladder cancer (80/769) and 4.4% of those with bile duct cancer (32/735) had coexisting PBM. In a nationwide survey carried out by the Japanese Study Group on PBM over 10 years from 1990 to 1999, the aggregate number of 1627 PBM cases were examined in detail[10]. As a whole, biliary tract cancer was detected in 278 of the 1627 cases (17.1%). Of these, in the 1239 cases of PBM with dilatation of the extrahepatic bile duct, there were 131 cases (10.6%) with coexisting biliary tract cancer, which was located in the gallbladder in 85 cases, the bile duct in 44 cases, and of unknown origin in 2 cases. On the other hand, of the 388 cases of PBM without dilatation of the extrahepatic bile duct there were 147 cases (37.9%) with coexisting biliary tract cancer, which was located in the gallbladder in 137 cases and in the bile duct in 10 cases. The results show that "with dilated PBM there is often carcinogenesis in the bile duct besides in the gallbladder, and with undilated PBM there is carcinogenesis in the gallbladder more than 90% of the time." These results are believed to originate from the short exposure period to carcinogenic substances and their low concentration, which is due to the moderate degree of bile stasis within the bile duct with undilated PBM[11].

CARCINOGENIC PROCESS

Pathophysiology

A mixture of pancreatic juice and bile is constantly being produced with PBM, and when bacterial infections and an increase in intrapressure in either the pancreatic duct or the bile duct are also present, pancreatic enzymes easily become activated. All pancreatic enzymes are detected at extremely high levels within the bile of PBM patients[9]. Among the activated pancreatic enzymes, amylase and lipase have little damaging action on the biliary epithelium, but trypsin activates Ca^{2+} along with phospholipase A2. Among the pancreatic juices, phospholipase A2 has a particularly powerful destructive action on the pancreatic duct and biliary epithelium, and also converts the lecithin within the bile into lysolecithin and free fatty acids that have a strong damaging action on cell membranes[12]. Furthermore, bile acid also has tissue damaging action, and it has been posited that this promotes phospholipase A2 activity, especially when the damage from secondary bile acid itself is added in. However, Shimada *et al*[13] have suggested that secondary bile acid does not play a major role in PBM carcinogenesis. As these substances are harmful to tissue, the biliary mucosa suffers long-term damage, the cell cycle accelerates, and various changes to the epithelium and DNA damage occur. Most previous studies have shown that the proliferative activity of gallbladder mucosa with PBM was higher than that of gallbladder mucosa without PBM, regardless of whether or not cancer was present[14-16]. In addition, studies by Hanada *et al*[14] and ourselves[17], on gallbladder mucosa in PBM cases showed that there is a significant acceleration of cell proliferative activity, and the thickness of the membrane was thicker than that in cases without coexisting PBM. In addition, Tanno *et al*[18] and Tokiwa *et al*[19] reported a high incidence of hyperplastic changes in the membrane that were already present in infants, and that they possessed activity values that were largely equivalent to the cell proliferative activity in the gallbladder mucosa in adults. Since there is a possibility of this easily developing cancer occurring if factors promoting carcinogenesis are at work in this process, the PBM biliary epithelium which is constantly being exposed to harmful substances can be said to be in a precancerous state.

Pathological findings

While various histopathological findings, such as hyperplasia, metaplasia, and dysplasia, have been detected in gallbladder mucosa with PBM, the most characteristic change is hyperplasia[14]. Other than PBM, although hyperplasia in the gallbladder mucosa of cholelithiasis or noncancerous lesions for routine gallbladder cancer have been detected, these are localized and moderate in degree[20]. Conversely, hyperplasia is detected in almost all parts of the gallbladder mucosa with PBM. Metaplastic change is a serious pathological change related to the

development of gallbladder cancer without coexisting PBM[21]. However, PBM is characterized by the fact that a low frequency of metaplastic epithelium occurs in less than 10%, and metaplastic change is lower than in patients with cholelithiasis. The incidence of dysplasia in noncancerous epithelium of gallbladder cancer patients with PBM is more than double that of gallbladder cancer patients without PBM[14]. In a previous study by our group[22], a high incidence of hyperplastic change was detected in infancy, and although the incidence is lower from adolescence onwards than in infancy, it is still high. Conversely, metaplasia and dysplasia were rarely seen in infancy, and only detected from adolescence onwards. Furthermore, dysplasia was most often discovered in the mucosa surrounding gallbladder cancer. Thus, hyperplastic epithelium can be present from the early stages of infancy or at birth, whereas metaplasia and dysplasia appears with age. Although it is unclear whether hyperplastic epithelium itself is a precancerous state, this strongly suggests that a hyperplasia-dysplasia-carcinoma sequence exists in the PBM carcinogenic process[2,9].

Gene mutation

Analyses have been conducted on the various oncogenes, tumor suppressor genes, *etc*., in resected specimens of PBM patients. In previous studies, the incidence of K-*ras* mutation in gallbladder cancer of PBM patients was 33%-83%, which is higher than in non-PBM gallbladder cancer patients[14,22-25]. Furthermore, there is a high incidence of K-*ras* mutations in benign epithelium with PBM. Iwase *et al*[23] reported detecting K-*ras* mutations in 36% of cases with hyperplasia. Matsubara *et al*[25] reported mutations in 31.6% of inflammatory epithelium, and in 47.6% with both hyperplasia and metaplasia. We detected K-*ras* mutations in 64% of cases with hyperplasia, in 28% with metaplasia, and in 17% with dysplasia[22]. Furthermore, Tomishige *et al*[26] reported that K-*ras* mutations were detected in PBM patients one month after birth, which suggests that genetic mutation occurs at an early phase of life. Since K-*ras* mutations are detected in noncancerous epithelium and hyperplasia, these epithelia seem to be in a genetically precancerous state, and represent an early event in multistep carcinogenesis.

To detect p53 in PBM patients, the "detection of gene mutations using PCR-SSCP and the direct sequence method" and methods for viewing the "overexpression of the p53 protein using anti-p53 monoclonal antibodies" have been reported, but gene mutation analysis has almost never been performed. Hanada *et al*[27] analyzed exon 5-8 of p53 using PCR-SSCP and reported the detection of an abnormal band on exon 7, 8 in 3 of the 6 cases (50%) of stage I gallbladder cancer with coexisting PBM. Matsubara *et al*[25] detected p53 gene mutation in 34.8% of cases with an inflammatory epithelium, in 47.6% with both hyperplasia and metaplasia, and in 60% with cancer, stating that these were mainly exon 5, 6, 8. However, Nagai *et al*[28] reported that p53 gene mutation was not detected in cases of hyperplasia and dysplasia, but that the gene mutation was observed in 4 of 26 cases (16%) of cancer. It is still unclear whether p53 gene mutation is present in noncancerous epithelia. Conversely, with regard to the overexpression of the p53 protein, Hanada *et al*[27] stated that overexpression was observed in 4 of the same 6 cases (67%) of stage I gallbladder cancer mentioned above. Moreover, in our study and in those of other researchers, there was a 62%-100% positivity rate for cancer, but noncancerous lesions were all negative. However, Matsubara *et al*[25] stated that overexpression was observed in 8.3% of cases with an inflammatory epithelium, in 33.3% with both hyperplasia and metaplasia, and in 80% with cancer. Since their criteria for immunostaining counted anything stained as positive, even if the staining was negligible, it is thought that this resulted in higher positive rates than in other studies. These results clearly indicated that overexpression of the p53 protein was largely negative in benign epithelium in cases with PBM, but that gene mutations occur with a high frequency. Since the mutation of p53 is regarded as a late event in carcinogenesis within the adenoma-carcinoma sequence for cancer of the large intestine, it may also occur in relatively later stages of the carcinogenic process for PBM as well.

Microsatellite instability (MSI) is reflected in abnormalities of DNA repair genes, and is an important factor leading to carcinogenesis. Nagai *et al*[29] reported the detection of MSI in 16 of 23 cases (69.6%) of gallbladder mucosa in PBM patients. Of these, it was detected in 8 cases (50%) of mutations in the transforming growth factor type Ⅱ receptor, in 2 cases (12.5%) of mutations in the insulin-like growth factor type Ⅱ receptor, in 4 cases (25%) of LOH. In addition, Nagai *et al*[28] reported 0% MSI in PBM patients with hyperplasia, 57.1% with dysplasia, and 52% with cancer. This suggests that MSI is similar to *p53* mutations in that it comes into play as a late event in the carcinogenic process for PBM.

There have been additional studies on abnormalities in cancer related genes and cell cycle related factors involved in the carcinogenic process for PBM. However, since only a limited number of cases were analyzed, further investigation is required.

CHEMOPREVENTION

The standard treatment for PBM when there is coexisting cancer is to perform surgery according to the stage of the cancer, and to perform diversion surgery for the prevention of carcinogenesis when there is no coexisting cancer. However, cases of carcinogenesis in remnant bile duct and the pancreas have been steadily increasing, even when preventative diversion surgery has been performed[5]. Furthermore, a cholecystectomy alone is performed at nearly half of institutions in Japan for cases without dilatation of the extrahepatic bile duct[1], thus there is a slight possibility of carcinogenesis in the remnant bile duct. Since these cases did not have

coexisting cancer prior to surgery, the use of adjuvant therapy for cancer and likewise anticancer drugs would not be indicated for the prevention of postoperative carcinogenesis. Ordinary anticancer drugs inhibit the DNA synthesis of cancer cells, but are unable to suppress the growth of inflammatory lesions and precancerous lesions.

Recently, progress has been made in research to suppress carcinogenesis by using a variety of chemical agents. Non-steroidal anti-inflammatory drugs, which are COX-2 inhibitors, and VK2, which is a therapeutic agent for osteoporosis, are the most promising medicines among these agents. Animal experiments showed that COX-2 inhibitors suppress carcinogenesis[30] and that, epidemiologically, long-term users of aspirin showed a 40% decrease in mortality rate due to colon cancer compared to the natural control[31]. Furthermore, it was also revealed that COX-2 has a strong correlation on cell growth, carcinogenesis, invasion, and metastasis at the cellular level[32,33]. Similar results have been also reported for various tumors other than colorectal tumors, suggesting that COX-2 has potential for use in chemoprevention and is a target for treatment. At the same time, VK2 is not only used in the clinical treatment of osteoporosis and other ailments, but is known to exhibit anti-tumor effects *in vitro* and *in vivo*[34,35]. In clinical studies on malignant tumors, the administration of VK2 induced a decrease in the number of blastic cells in a patient with post-myelodysplastic syndrome (MDS) (AML)[36]. In addition, there is a similar report that mature neutrophils increased, and anemia and a decrease in blood platelets improved in a patient with MDS in which the ratio of blastic cells declined due to VK2 administration[37]. Furthermore, female patients with viral cirrhosis of the liver in the VK2-treated group had a significantly lower onset of hepatocellular carcinoma than the control group[38]. In addition, a significant suppressant effect on the incidence of relapse was detected in cases treated with VK2 among patients with hepatocellular carcinoma after curative treatment[39]. In a large number of clinical trials for patients with osteoporosis, neurological disease, or for postmenopausal women, no severe adverse events were reported with long-term VK2 treatment[40-43]. Thus, it may be safely used as a chemopreventive agent.

Using the Syrian golden hamster PBM carcinogenesis model developed by Tajima *et al*[44], we examined whether COX-2 inhibitors and VK2 could suppress carcinogenesis, and found that early stage gallbladder cancer appeared in approximately 30% of animals in the control group with the carcinogenic substance N-nitrosobis (2-oxopropyl) amin (BOP). In contrast, in the COX-2- and VK2-treated group, carcinogenesis was suppressed through the suppression of cell growth in the gallbladder mucosa, respectively[45]. Furthermore, compared to the control group, the incidence of dysplasia, a precancerous lesion, declined in the treated group, suggesting that both agents suppress the cell cycle in PBM gallbladder mucosa. Based on the results of these experiments, further studies on the clinical efficacy of potential chemopreventive agents for PBM are warranted.

CONCLUSION

PBM is a high risk factor for biliary tract cancer, and several patients reportedly had hyperplastic changes and gene abnormalities in the biliary mucosa at birth. Since the first steps have already been taken towards carcinogenesis at the fetal stage, preventive surgeries must be performed immediately once a diagnosis has been made. In addition, it is essential to reduce the risk of carcinogenesis by using chemoprevention in order to prevent postoperative carcinogenesis.

ACKNOWLEDGMENTS

The authors are indebted to Professor J. Patrick Barron of the International Medical Communications Center of Tokyo Medical University for his review of this manuscript.

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S- Editor Li LF **L- Editor** Webster JR **E- Editor** Yang C