Bilioenterostomy enhances biliary carcinogenesis in hamsters

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The aim of this study was to examine whether the type of bilioenterostomy enhances biliary carcinogenesis in the hamster model. Syrian hamsters were divided into the following groups: simple laparotomy (control group), cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDB group) and cholecystoileostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CIDB group). Following these procedures, all hamsters received N-nitrosobis(2-oxopropyl)amine. The diameter of the extrahepatic bile duct and plasma levels of cholecystokinin (CCK) were measured and the number of neoplastic lesions was counted microscopically. Proliferative effect of the procedures on the biliary epithelium was examined by proliferative cell nuclear antigen. In the CDDB group the extrahepatic bile duct was significantly dilated and carcinogenesis of the gall-bladder and extrahepatic bile duct was enhanced. In the CIDB group the CCK bioactivity was stimulated and intrahepatic biliary duct, but not gall bladder and extrahepatic bile duct, carcinogenesis was promoted more than that observed in the CDDB group. Proliferation of the biliary duct epithelium was enhanced in both the CDDB and CIDB groups. Cholecystoduodenostomy enhanced intra- and extrahepatic bile duct carcinoma, whereas cholecystoileostomy promoted only intrahepatic bile duct carcinoma. Some factors in the intestinal juice seem to play a role in the promotion of biliary tract carcinoma.

Introduction

Pancreaticobiliary maljunction (PBM*) and congenital choledochal dilatation are often associated with cancers of the gall bladder or extrahepatic bile duct, but not with the intrahepatic bile duct carcinoma (1-5). Whether intrahepatic bile duct carcinoma and the extrahepatic biliary carcinoma have the same etiology is obscure. The question was examined in a new model for the induction of biliary carcinoma with N-nitrosobis(2-oxopropyl)amine (BOP) with bilioenterostomy in the hamster (6). In this model all hamsters developed dilated common bile ducts comparable with human congenital choledochal dilatation. Exogenous cholecystokinin (CCK) has been shown to promote intrahepatic biliary carcinogenesis (7). However, it is still unclear whether the reflux of duodenal juice is an important factor for dilatation of the common bile duct and for biliary carcinogenesis. In the present study we examined the effects of duodenal or ileal juice reflux into the biliary tract by cholecystoduodenostomy or cholecystoileostomy respectively.

Materials and methods

Animals

Outbred 7-week-old female Syrian golden hamsters (SLC Inc., Shizuoka, Japan) were housed individually in plastic cages on sawdust bedding. They were kept at 24 ± 2°C and 50 ± 20% humidity with 12 h alternate light and dark, fed a CE-2 pelleted diet (fat 4.4% and protein 24.8%; Clea Japan Inc., Tokyo, Japan) and provided drinking water ad libitum. Animals were checked daily and weighed weekly during the experiment. All experiments were done following the Guidelines for Animal Experimentation of Nagasaki University.

Carcinogenesis after treatment with BOP

Animals were randomly divided into three groups: simple laparotomy (control group), cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct below the opening of the pancreatic duct (CDDB group) and cholecystoileostomy with dissection of the extrahepatic bile duct on the distal end of the common duct above (CIDB group) (6, Figure 1). Of the 110 operated hamsters, 92 tolerated the procedures up to the post-operative day 7. Of these, 30 hamsters were controls, 32 were in the CDDB group and 30 in the CIDB group. Eighteen of these hamsters died early of biliary peritonitis. Twenty seven animals (control, n = 10; CDDB, n = 10; CIDB, n = 7) that survived were killed on day 14 of the experiment for CCK bioassay (2 week study) and the remaining 65 hamsters (control, n = 20; CDDB, n = 22; CIDB, n = 23) were given BOP s.c. (Nakarai Tesque, Kyoto, Japan) at a dose of 10 mg/kg body wt in 0.9% NaCl solution weekly for 9 weeks. At post-operative week 16 all hamsters were sacrificed (16 week study).

All hamsters were fasted overnight, killed by exsanguination and the liver, duodenum, biliary system and pancreas were removed en bloc. The maximal diameter of the extrahepatic bile duct was measured as previously reported (6). The pancreas was spread out on a piece of paper to ensure a maximal transectional area for subsequent sectioning. Portions of the liver, an area of anastomosis including duodenum, the common bile duct and the duodenal segment of the pancreas were embedded en bloc and cut in step sections (5 sections/animal). Histological sections were stained with hematoxylin and eosin and were examined by light microscopy. The numbers of pancreaticobiliary neoplastic lesions by anatomical location were counted in the representative sections. Carcinoma was diagnosed on the basis of invasion (6).

Total bilirubin measurement, CCK bioassay and DNA synthesis of the biliary epithelium

At necropsy blood samples from the vena cava were collected in ice-chilled tubes containing heparin and were centrifuged (3000 r.p.m.) at 4°C for 10 min. Total bilirubin level was measured. Plasma was stored at -80°C until CCK was extracted and concentrated on SepPak C18 cartridges (Waters Ass., Milford, MA). Plasma CCK levels were determined by dispersed acini, as described elsewhere (8,9). Proliferative cell nuclear antigen (PCNA) was demonstrated in the specimens of the 16 week study with anti-PCNA/horseradish peroxidase (DAKO, Denmark) after household microwave treatment. Labeled nuclei in more than 1000 non-neoplastic epithelia of the intra- and extrahepatic bile ducts, the gall-bladder and the pancreatic ducts were counted in every 10 sections and the percentages of labeled nuclei (labeling index, LI) were determined.

Statistical analyses

The tumor incidence was analyzed by the χ² test with continuity correction. Student’s t-test was also used to compare the diameter of the common bile duct, total bilirubin level, CCK activity and PCNA labeling indices.

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Table I. Diameter of the extrahepatic bile duct and levels of serum total bilirubin

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 week study</th>
<th>16 week study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of hamsters</td>
<td>Diameter (mm)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>CDDB</td>
<td>10</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>CIDB</td>
<td>7</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Expressed as mean ± SE.

*P < 0.01 compared with the control

#P < 0.01 compared with CDDB

Results

Diameter of the extrahepatic bile duct and level of total bilirubin

The maximal diameter of the extrahepatic bile duct in both the CDDB and CIDB groups was significantly larger than in the control group (P < 0.01). On the other hand, the diameter of the duct in the CDDB group was greater than in the CIDB

Fig. 1. Operative procedure (A) Cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDB group). (B) Cholecystoileostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CIDB group). b. Bile duct, d. duodenum, g. gall-bladder, i. ileum, s. stomach, cd. cholecystoduodenostomy, ci. cholecystoileostomy, db. dissection of the common duct

Diameter of the extrahepatic bile duct and level of total bilirubin

The maximal diameter of the extrahepatic bile duct in both the CDDB and CIDB groups was significantly larger than in the control group (P < 0.01). On the other hand, the diameter of the duct in the CDDB group was greater than in the CIDB

Fig. 2. Histological sections of the biliary tract in the 16 week study (hematoxylin and eosin stain) (A) CDDB, papillary tumor (thick arrowhead) at the hepatic hilum with marked dilatation of the extrahepatic bile duct (B) CIDB, mild dilatation of the extrahepatic bile duct (thin arrowhead). b. Bile duct, d. duodenum, g. gall-bladder, i. ileum, p. pancreas, ci. cholecystoileostomy

Table II. Fasting bioassay of plasma CCK

<table>
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<th>Groups</th>
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<th>16 week study</th>
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<tbody>
<tr>
<td></td>
<td>No of hamsters</td>
<td>Plasma CCK (PM)</td>
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<td>Control</td>
<td>10</td>
<td>6.4 ± 0.6</td>
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<tr>
<td>CDDB</td>
<td>10</td>
<td>2.6 ± 0.3</td>
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<tr>
<td>CIDB</td>
<td>7</td>
<td>8.7 ± 0.9</td>
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</table>

Expressed by mean ± SE

*P < 0.01 compared with the control

#P < 0.01 compared with CDDB

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Table III. Incidences of carcinoma induced in the biliary system and pancreas of the hamster (16 week group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of hamsters</th>
<th>No. of hamsters with carcinoma</th>
<th>Intrahepatic bile duct</th>
<th>Extrahepatic bile duct</th>
<th>Gall-bladder</th>
<th>Pancreas</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>1 (5)</td>
<td>0</td>
<td>0</td>
<td>10 (50)</td>
<td></td>
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<tr>
<td>CDDB</td>
<td>22</td>
<td>8 (36)(^a)</td>
<td>6 (27)(^a)</td>
<td>10 (46)(^b)</td>
<td>16 (72)</td>
<td></td>
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<tr>
<td>CIDB</td>
<td>23</td>
<td>17 (74)(^bcd)</td>
<td>2 (9)</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
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</table>

Numbers in parentheses are per cent.
\(^aP < 0.05\) compared with the control.
\(^bP < 0.01\) compared with the control.
\(^cP < 0.05\) compared with CDDB.

Table IV. PCNA labelling indices (%)

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of hamsters</th>
<th>Intrahepatic bile duct epithelium</th>
<th>Extrahepatic bile duct epithelium</th>
<th>Gall-bladder epithelium</th>
<th>Pancreatic epithelium</th>
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<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7.4±2.8</td>
<td>0.3±0.1</td>
<td>0.4±0.3</td>
<td>0.2±0.1</td>
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<tr>
<td>CDDB</td>
<td>10</td>
<td>11.4±2.9</td>
<td>26.0±1.3(^c)</td>
<td>33.8±2.3(^c)</td>
<td>2.5±1.0</td>
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<tr>
<td>CIDB</td>
<td>10</td>
<td>29.7±4.4(^abc)</td>
<td>0.9±0.5(^b)</td>
<td>6.3±0.8(^bd)</td>
<td>4.8±2.8</td>
</tr>
</tbody>
</table>

Expressed by mean ± SE.
\(^aP < 0.01\) compared with the control.
\(^bP < 0.01\) compared with CDDB.
\(^cP < 0.01\) compared with the control.
\(^dP < 0.05\) compared with the control.

Group (\(P < 0.01\)). These differences were readily observed in the 2 week study (Table I, Figure 2). There was no jaundice in the 2-week study group, but in the 16-week study the total bilirubin level of the CDDB group was significantly higher than in the control and CIDB groups (Table 1).

Bioassay of CCK

In the 2 week study plasma CCK levels were significantly elevated in the CIDB group (\(P < 0.05\)) and were significantly decreased in the CDDB group (\(P < 0.01\)) compared with the control. However, the level of CCK was not changed in any groups of the 16 week study (Table II).

Carcinoma in the biliary system and the pancreas

No carcinoma was observed in any groups of the 2 week study. Table III summarizes the incidence of carcinoma in the intra- and extrahepatic bile ducts, the gall-bladder and the pancreas in the 16 week study. The intrahepatic bile duct carcinoma incidence was increased in the CDDB and CIDB groups compared with the control (\(P < 0.05\) and \(P < 0.01\) respectively) and significantly more animals bearing tumors were found in the CIDB than in the CDDB group (\(P < 0.01\)). A statistically significant increase in the incidence of carcinoma was also noted in both the extrahepatic bile duct and the gall-bladder of the CDDB group (\(P < 0.05\) and \(P < 0.01\) respectively compared with the control). No significant difference was noted in the incidence of pancreatic carcinoma. Histologically the intrahepatic bile duct lesions were tubular adenocarcinoma, the extrahepatic bile duct and gall-bladder tumors were papillary adenocarcinoma and pancreatic tumors were ductular adenocarcinoma.

PCNA labelling indices

The pattern of labeling is presented in Figure 3 and the LI of the biliary epithelium and the pancreatic ductal epithelia are summarized in Table IV. A significant increase in LI was found in the intrahepatic bile duct of CIDB (\(P < 0.01\) compared with the control and CDDB) and in the extrahepatic bile duct and the gall bladder of CDDB (\(P < 0.01\) compared with the control and CIDB). There was no significant difference in the LI of pancreatic ductal epithelium among the three groups.

Discussion

Patients with PBM are prone to gallbladder and extrahepatic bile duct carcinomas, but rarely to intrahepatic bile duct carcinomas (2–5). Therefore, the mechanism of carcinogenesis of the biliary tree seems to be different.

Tajima et al. (6) have shown a significant promotion of biliary carcinoma by BOP after CDDB. In this model the maximal diameter of the extrahepatic bile duct was significantly greater than that in controls. We have also found that in the CIDB group the extrahepatic bile duct was significantly dilated compared with the control, but was of lesser magnitude than in the CDDB group. This is analogous to the human situation, where patients with PBM without choledochal dilatation mainly develop gall-bladder cancer (10). The CDDB group, resembling PBM with choledochal dilatation, showed a significantly higher incidence of biliary carcinoma compared with the control. On the other hand, as in patients without choledochal dilatation, the CIDB group showed no significant rise in extrahepatic bile duct dilatation (P < 0.01). These differences were readily observed in the 2 week study (Table I, Figure 2). There was no jaundice in the 2-week study group, but in the 16-week study the total bilirubin level of the CDDB group was significantly higher than in the control and CIDB groups (Table 1).

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Accelerated cell proliferation in the biliary epithelium stained with anti-PCNA/horseradish peroxidase. (A) Intrahepatic bile duct of the CIDB group, note the labeling of most epithelial cells (original magnification × 200). (B) Gall-bladder of the CDDB group showing labeling of many nuclei (× 400). (C) Extrahepatic bile duct of the CDDB group with labeling of many cells, especially at the base of the epithelium (× 400).

Fig. 3. Accelerated cell proliferation in the biliary epithelium stained with anti-PCNA/horseradish peroxidase. (A) Intrahepatic bile duct of the CIDB group, note the labeling of most epithelial cells (original magnification × 200). (B) Gall-bladder of the CDDB group showing labeling of many nuclei (× 400). (C) Extrahepatic bile duct of the CDDB group with labeling of many cells, especially at the base of the epithelium (× 400).

and gall-bladder carcinogenesis compared with the control. These results suggest that resection of the extrahepatic bile duct in PBM patients with choledochal dilatation and prophylactic cholecystectomy in PBM patients without choledochal dilatation may have a therapeutic effect (10).

CDDDB enhanced the dilatation of the biliary tract more than that observed after CIDB. Because a significant dilatation of the extrahepatic bile duct without jaundice was observed in the CDDDB group of the 2 week study, it seems that some factor in the refluxed duodenal juice, rather than stenosis caused by bilioenterostomy, was responsible for the choledochal dilatation.

We demonstrated that exogenous CCK octapeptide promoted the carcinogenesis in intrahepatic bile duct carcinoma and loxiglumide (a CCK receptor antagonist) inhibited its effect in the CDDDB + BOP hamster model (7). In rats and humans drainage of pancreatic juice and bile from the duodenum and jejunum is known to stimulate endogenous CCK release from CCK-secreting cells (11,12). In the CDDDB group, which had pancreaticobiliary drainage, fasting CCK bioactivity was elevated significantly in the 2 week study, but not in the 16 week study. This finding is consistent with the possibility that endogenous CCK-secreting cells in the fasting state are exhausted over the 4 weeks of endogenous stimulation (13). In the post-prandial state plasma CCK levels in the CDDDB group must be higher than in the control and the CDDDB groups (13). The reason for lower CCK levels in the 2 week CDDDB group could be due to continuous pancreatic juice and bile flow through cholecystoduodenostomy into the duodenum. Also, the high but not significant CCK levels in the CDDDB group in the 16 week study could have been due to impaired pancreaticobiliary flow due to the gall-bladder cancer and extrahepatic bile duct carcinoma, as was evidenced by development of jaundice.

In the 16 week study intrahepatic bile duct carcinoma was mainly observed in the CIDDB group with stimulated endogenous CCK release by pancreaticobiliary juice from the first part of the small intestine. Oversecretion of endogenous CCK in BOP-treated hamsters, as well as pharmacological doses of exogenous CCK administration seem to promote intrahepatic bile duct carcinogenesis (7). On the other hand, carcinoma of the extrahepatic bile duct and the gall-bladder were significantly higher in the CDDDB group in which the common bile duct was dilated, even in the 2 week study. Therefore, it appears that factors that promote choledochal dilatation in hamsters also promote carcinoma of the gall-bladder and the extrahepatic bile duct, as seen in humans with PBM (2–5).

The non-tumorous epithelium of the intrahepatic bile duct in the CIDBB group, and the non-neoplastic epithelium of the extrahepatic bile duct and the gall-bladder in the CDDDB group were mainly labeled with PCNA, which labels the intranuclear protein engaged in the cell cycle which is essential for cellular DNA synthesis (14,15). The acceleration of DNA synthesis in the intrahepatic bile duct epithelium by endogenous CCK release or by inflammation induced by reflux of duodenal juice could provoke BOP carcinogenesis in the intrahepatic bile duct in the CIDB or CDDDB group (16,17). Hamsters with a long common bile duct rarely develop gall-bladder carcinoma or extrahepatic bile duct carcinoma after BOP treatment. Consequently, it could be assumed that a mixture of pancreatic juice, bile, and refluxed duodenal juice seem to be essential for inflammation of the common bile duct to occur. None of the procedures affected pancreatic cancer incidence, possibly because of the lower LI of the pancreatic ductal cells. Pancreatic carcinoma in hamsters is believed to be activated by BOP without the presence of pancreatitis (18).

In conclusion, the present study suggests that reflux of intestinal and pancreatic juice into the bile duct plays an
important role in the BOP carcinogenesis of the gall-bladder and extrahepatic bile duct and that this event also amplifies dilatation of the biliary duct.

Acknowledgement

This work was supported by Grant-in-Aid for General Scientific Research (C) 06671215.

References


Received on December 21, 1995; revised on March 19, 1996; accepted on March 26, 1996.