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), choledocoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDDB 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 cell nuclear antigen. In the CDDDB 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. The maximal diameter of the extrahepatic bile duct was measured as previously reported (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 CDDDB group and 30 in the CIDB group. Eighteen of these hamsters died early of biliary peritonitis. Twenty seven animals (control, n = 10; CDDDB, 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; CDDDB, 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).

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.

Carcinogenicity after treatment with BOP

Animals were randomly divided into three groups: simple laparotomy (control group), choledocoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct below the opening of the pancreatic duct (CDDDB group) and cholecystoileostomy with dissection of the extrahepatic bile duct on the distal end of the common duct as above (CIDB group) (6). Eighteen of these hamsters died early of biliary peritonitis. Twenty seven animals (control, n = 10; CDDDB, 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; CDDDB, 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).

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 choledocoduodenostomy or cholecystoileostomy respectively.

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 1. 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>0.2±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>
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</table>

Expressed as mean ± SE

"P < 0.01 compared with the control

"P < 0.01 compared with CDDB

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, d. duodenum, cd. cholecystoduodenostomy, ci. cholecystoileostomy, db. dissection of the common duct.

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 group.

Table II. Fasting bioassay of plasma CCK

<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>Plasma CCK (pM)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>6.4±0.6</td>
</tr>
<tr>
<td>CDDB</td>
<td>10</td>
<td>2.6±0.3</td>
</tr>
<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

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 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intrahepatic bile duct</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>1 (5)</td>
</tr>
<tr>
<td>CDDB</td>
<td>22</td>
<td>8 (36)(^a)</td>
</tr>
<tr>
<td>CIDB</td>
<td>23</td>
<td>17 (74)(^b,c)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are per cent.
\(^a P < 0.05\) compared with the control.
\(^b P < 0.01\) compared with the control.
\(^c P < 0.05\) 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>
</tr>
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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>
</tr>
<tr>
<td>CDDB</td>
<td>10</td>
<td>11.4±1.9</td>
<td>26.0±1.5(^c)</td>
<td>33.8±2.3(^c)</td>
<td>2.5±1.0</td>
</tr>
<tr>
<td>CIDB</td>
<td>10</td>
<td>29.7±4.4(^a,b)</td>
<td>0.9±0.5(^b)</td>
<td>6.3±0.8(^b,d)</td>
<td>4.8±2.8</td>
</tr>
</tbody>
</table>

Expressed by mean ± SE.
\(^a P < 0.01\) compared with the control.
\(^b P < 0.01\) compared with CDDB.
\(^c P < 0.01\) compared with the control.
\(^d P < 0.05\) compared with the control.

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.

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
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.
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 CDDDB group showing labeling of many nuclei (× 400) (C) Extrahepatic bile duct of the CDDDB 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.
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.

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References

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