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Induction of Extrahepatic Biliary Carcinoma by N-Nitrosobis(2-oxopropyl)amine in Hamsters Given Cholecystoduodenostomy with Dissection of the Common Duct

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The methods we used to produce a carcinoma in the extrahepatic bile duct and gallbladder in hamsters are described along with the characteristics of the induced tumors. Female Syrian golden hamsters were first subjected to cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDB) and were, 4 weeks later, treated with weekly subcutaneous injections of N-nitrosobis(2-oxopropyl)amine (BOP) at a dose of 10 mg/kg body weight for 9 weeks. The animals were killed at the 12th, 16th and 20th week after the initiation of BOP treatment. Extrahepatic bile duct carcinoma developed in 16%, 24% and 41% and gallbladder carcinoma occurred in 58%, 81% and 82% of the hamsters, respectively, at the corresponding times of killing. The incidences were significantly higher than those in sham-operated controls ($P<0.01$). The induced extrahepatic bile duct carcinomas were predominantly of the polypoid type and gallbladder carcinomas were of the papillary type in growth form, being morphologically similar to early stage biliary carcinoma in humans. Immunohistochemical staining using bromodeoxyuridine and anti-bromodeoxyuridine monoclonal antibody demonstrated that the CDDB procedure greatly accelerated the cell kinetic activity of the biliary epithelium, and this was considered to be a major factor promoting the development of biliary carcinomas in this hamster model. In conclusion, this new model provides a high incidence of tumor development at the extrahepatic biliary tract and is expected to be useful for clarifying the characteristics of this highly malignant tumor.

Key words: Chemical carcinogenesis — Extrahepatic biliary carcinoma — N-Nitrosobis(2-oxopropyl)amine — Cell kinetics — Syrian golden hamster

Biliary carcinoma is one of the most malignant carcinomas of the digestive system. To clarify the characteristics of biliary carcinoma, numerous investigators have attempted to produce carcinomas in the biliary tree of laboratory animals, including hamsters,[1-3] mice,[4] dogs,[5,6] cats,[6] and guinea pigs.[7] The results have been less than satisfactory, especially in inducing extrahepatic biliary carcinoma.

The incidence of extrahepatic biliary carcinoma in clinical observations of the choledochal cyst is very high.[8,9] Reflux of pancreatic juice into the biliary tract through the accompanying anomalous arrangement of the pancreaticobiliary ductal union is considered to be one factor promoting the development of biliary carcinoma.[10,11] It is also recognized that the malignant potential of the choledochal cyst is accelerated by enteric internal drainage.[8,9,12]

The present study was undertaken to produce an extrahepatic biliary carcinoma in hamsters. We carried out cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDB) in hamsters in such a way that the pancreatic juice and duodenal contents would enter the biliary tract. Administration of a chemical carcinogen followed.

We report herein our method for induction of carcinomas in the extrahepatic bile duct and gallbladder, as well as the histopathological characteristics of the induced tumors.

MATERIALS AND METHODS

Animals A total of 234 7-week-old female Syrian golden hamsters (Shizuoka Laboratory Animal Center Co. Ltd., Shizuoka) were housed three per plastic cage and kept under standard laboratory conditions in the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine. The animals were given a standard pellet diet and water *ad libitum* during the experiment. All experiments were done following the Guidelines for Animal Experimentation of Nagasaki University.

Surgical techniques The extrahepatic biliary tract in hamsters is composed of a gallbladder and extrahepatic bile duct. The latter consists of three bile duct segments: the hepatic ducts, common bile duct and common duct.[13] A schema of the completed surgical procedure of CDDB is illustrated in Fig. 1. Following anesthesia with sodium pentobarbital (50 mg/kg of body weight) an upper abdominal midline incision was made and the distal end of the common duct was doubly ligated with 6-0 TI·CRON

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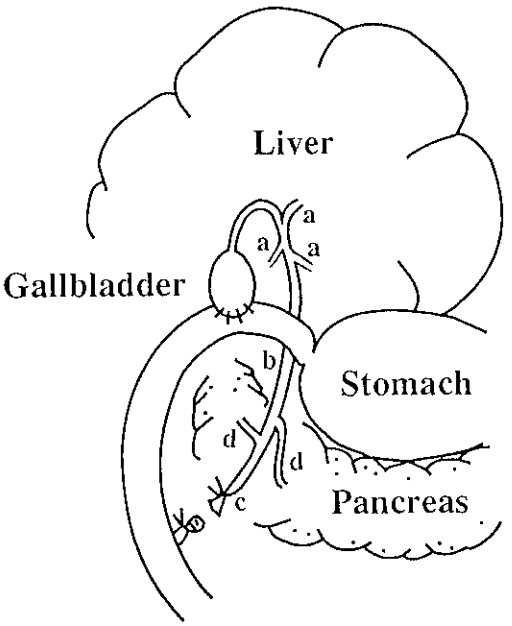

Fig. 1. Surgical procedure of cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct in the hamster. a, hepatic duct; b, common bile duct; c, common duct; d, pancreatic duct.

and dissected. A 5-mm-long incision was made in both the gallbladder fundus and the duodenal wall approximately 10 mm distal to the pyloric ring of the stomach, and cholecystoduodenostomy was then done using a continuous suture with 7-0 Nylon. The control hamsters underwent simple laparotomy (SL).

Carcinogenic studies Carcinogen: N-Nitrosobis(2-oxopropyl)amine (BOP) was synthesized and provided by Dr. Y. Mori, Laboratory of Radiochemistry, Gifu Pharmaceutical University, Gifu. It was administered in 0.9% NaCl solution at a concentration of 1.5 mg/ml.

Experimental protocol: Fig. 2 shows the experimental protocol. One hundred and seven hamsters underwent CDDB and the 74 survivors received weekly subcutaneous injections of BOP at a dose of 10 mg/kg body weight. BOP administration was started four weeks after the surgery and was continued for nine consecutive weeks. These animals were grouped into three according to the length of the observation periods, i.e., one group each was killed at the 12th (CDDB-1), 16th (CDDB-2) and 20th week (CDDB-3) after the initiation of BOP injection. Sixty hamsters which underwent SL received the same BOP treatment and were also grouped into three (SL-1, SL-2 and SL-3) with the same observation periods as used for the CDDB groups. Any animal which died during the observation period was excluded from analysis. At autopsy, the maximum external diameter of the

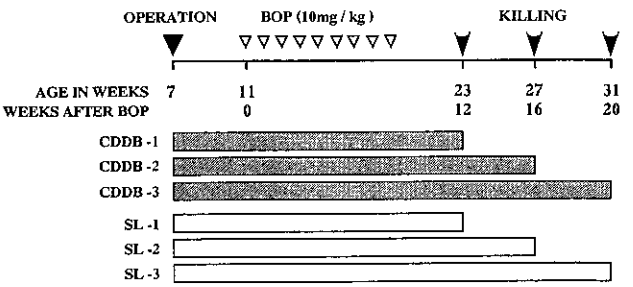

Fig. 2. Experimental protocol.

common bile duct was measured, using slide calipers. The extrahepatic bile duct and gallbladder, removed *en bloc* with the liver, pancreas and part of the duodenum, was fixed in 10% neutral formalin.

Histological examination: The formalin-fixed specimen was cut into six blocks and embedded in paraffin. One slice was taken from each block, i.e., one from the hepatic ducts, one from the common bile duct, one from the common duct, one from the gallbladder and five from the liver. Two slices of the pancreas were included in the above. All these slices were stained with hematoxylin and eosin (H & E). The number of histologically verified carcinomas was counted with particular attention to the location and growth pattern of the induced tumors. Carcinoma was diagnosed on the basis of disruption of the polarity of the epithelial cells and evidence of an invasive nature.

Cell kinetic studies Forty-two hamsters underwent CDDB and the survivors were killed at the 4th (Group 1), 8th (Group 2), 12th (Group 3), 16th (Group 4) and 20th week (Group 5) after the surgery, without BOP treatment. Twenty-five animals which underwent SL also received the same schedule of necropsy, being grouped as Group 6, 7, 8, 9 and 10, respectively. To label cells in the DNA synthesis phase, bromodeoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO), 50 mg/kg of body weight, was given by intraperitoneal injection to all hamsters 20 to 30 min before killing.

BrdU-labeled cells were detected in formalin-fixed tissue sections obtained from the hepatic ducts, common bile duct, common duct and gallbladder by indirect immunoperoxidase staining using an anti-BrdU monoclonal antibody. Details of this technique have been described elsewhere.[14, 15] In brief, the deparaffinized tissue sections were denatured with 2 *N* HCl and treated with 0.05% protease, type XXII (Sigma). These sections were covered with anti-BrdU monoclonal antibody (Becton Dickinson, Mountain View, CA), treated with biotinylated horse-anti-mouse immunoglobulin G antibody (Becton Dickinson), and developed in diaminobenzidine

tetrahydrochloride and H_2O_2 in Tris buffer. The slides were finally counterstained with Mayer's hematoxylin.

For determination of the BrdU labeling index (LI), the most BrdU-positive area was selected from several high-power microscopic fields and a count of labeled and unlabeled cells was made up to a minimum of 1000 serial cells in the selected area. The LI was calculated as the percentage of BrdU-labeled cells with respect to the total number of cells scored.

Statistical evaluation The incidence of tumor production was statistically analyzed using the chi-square test. Student's *t* test was also used to compare body weight, diameter of the common bile duct, number of tumors per animal and BrdU LI.

RESULTS

Carcinogenic studies Table I lists the number of hamsters examined, changes in body weight, and diameter of the common bile duct at autopsy. Approximately 30% of the hamsters in groups CDDB-1, 2 and 3 died of liver abscess and/or peritonitis a few days after the surgery, while in the SL groups there were no operative deaths. After BOP treatment, the mortality rate increased in each CDDB group. This was due to the hepato- or systemic toxicity of BOP in the earlier stages of the study and due to the advanced carcinomas of the liver and pancreas in later stages. Two hamsters in group SL-3 died of advanced pancreatic carcinoma.

At autopsy, almost all the hamsters in groups CDDB-1, 2 and 3 had a marked dilatation of the extrahepatic bile duct. The common bile duct caliber ranged from 2.6 to 11.9 mm in diameter, while in controls, a common bile duct dilatation of more than 3 mm in diameter was seen in only two hamsters in group SL-2 and two animals in group SL-3. There were statistically significant differences in the average diameter of the common bile duct between the CDDB groups and corresponding SL groups ($P<0.01$).

Extrahepatic bile duct carcinomas The incidence, location, gross shape and histology of extrahepatic bile duct carcinomas induced in the hamsters with CDDB and SL are shown in Table II. Carcinoma of the extrahepatic bile duct developed in 16% of hamsters in group CDDB-1, 24% in group CDDB-2 and 41% in group CDDB-3. In the SL groups, however, no hamster developed extrahepatic bile duct carcinoma, except for one in group SL-2. The difference was statistically significant between groups CDDB-3 and SL-3 ($P<0.01$).

A total of 24 carcinomas developed in 17 hamsters in the CDDB groups; multicentric primary carcinomas were found in one hamster in group CDDB-1 (triple cancer), one in group CDDB-2 (triple cancer) and three in group CDDB-3 (double cancer in all three). The common locations of these carcinomas were the dilated lumen of the hepatic ducts and common bile duct. The carcinomas were of a pedunculated or sessile polypoid type protruding into the lumen of the bile duct (16 lesions) and a superficial type spreading along the bile duct wall (eight lesions). Histologically, all the polypoid lesions were tubular adenocarcinoma (Fig. 3), while all the superficial types were papillary adenocarcinoma. Among the 24 carcinomas, 22 lesions were confined to the mucosal layer of the bile duct. In the SL groups, a superficial-type carcinoma developed in the hepatic duct in one animal, in which the common duct was obstructed by an advanced pancreatic carcinoma. In peri- and non-carcinomatous areas of the extrahepatic bile duct, marked metaplastic, hyperplastic and dysplastic changes were evident over the entire tract in hamsters in the

Table I. Changes in Number and Body Weight of Hamsters during the Experiment, and Diameter of the Common Bile Duct Examined at Autopsy

Group	Treatment	Experimental period[a] (wk)	Initial no. of hamsters	No. (%) of survivals at BOP initiation	No. (%) of hamsters killed	Average body weight (g)[b]			Average diameter of the common bile duct (mm) at autopsy[c]
						Initial	At BOP initiation	Final	
CDDB-1	CDDB+BOP	12	29	20 (69)	19 (66)	111±5	131±9[d]	167±23[d]	4.60±1.69[d]
CDDB-2	CDDB+BOP	16	36	25 (69)	21 (58)	110±5	128±11[e]	150±14[e]	5.50±1.37[e]
CDDB-3	CDDB+BOP	20	42	29 (69)	22 (52)	112±5	133±11[f]	143±19[f]	5.31±2.01[f]
SL-1	SL+BOP	12	20	20 (100)	20 (100)	111±8	150±7	185±15	0.71±0.14
SL-2	SL+BOP	16	20	20 (100)	20 (100)	110±7	147±10	187±17	0.95±0.86
SL-3	SL+BOP	20	20	20 (100)	18 (90)	110±5	153±8	178±20	1.26±1.63

a) Period from initial BOP treatment to killing (wk, weeks).
b, c) Mean±SD.
d) Significantly different from SL-1 ($P<0.01$).
e) Significantly different from SL-2 ($P<0.01$).
f) Significantly different from SL-3 ($P<0.01$).

Table II. Extrahepatic Bile Duct Carcinomas Induced in BOP-treated Hamsters after Cholecystoduodenostomy with Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	No. of hamsters killed	No. (%) of hamsters with carcinoma	Total no. of carcinomas	Tumor location[a]			Tumor shape[b]		Histology[c]	
				HD	CBD	CD	Poly	Super	Tub	Pap
CDDB-1	19	3 (16)	5	3	1	1	5	0	5	0
CDDB-2	21	5 (24)	7	5	2	0	4	3	4	3
CDDB-3	22	9 (41)[d]	12	7	3	2	7	5	7	5
SL-1	20	0	0	0	0	0	0	0	0	0
SL-2	20	1 (5)	1	1	0	0	0	1	0	1
SL-3	18	0	0	0	0	0	0	0	0	0

a) HD, hepatic ducts; CBD, common bile duct; CD, common duct.
b) Poly, polypoid type; Super, superficial type.
c) Tub, tubular adenocarcinoma; Pap, papillary adenocarcinoma.
d) Significantly different from SL-3 ($P<0.01$).

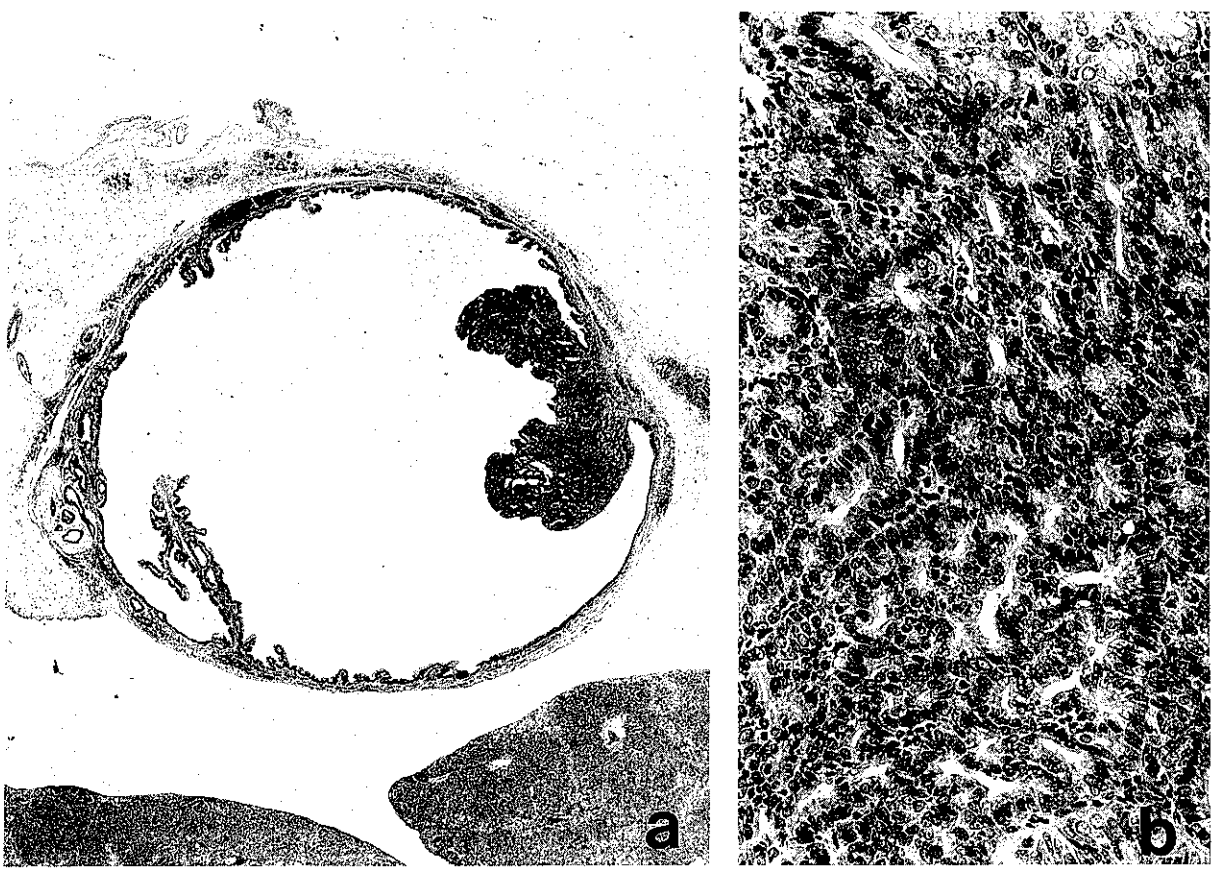

Fig. 3. a. A polypoid carcinoma arising in the common bile duct of a hamster in CDDB-2. The tumor protrudes into the lumen of the markedly dilated bile duct. (H & E, ×20). b. High magnification of the same tumor as in Fig. 3a, showing mild cellular pleomorphism and nuclear hyperchromasia with back-to-back glandular structures and loss of polarity, findings compatible with a diagnosis of well-differentiated tubular adenocarcinoma. (H & E, ×200).

CDDB groups, while in contrast, these changes were slight and confined to the common duct in hamsters in the SL groups.

Gallbladder carcinomas Table III shows the incidence and morphological features of gallbladder carcinomas induced in hamsters in each experimental group. Carci-

Table III. Gallbladder Carcinomas Induced in BOP-treated Hamsters after Cholecystoduodenostomy with Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	No. of hamsters killed	No. (%) of hamsters with:		Total no. of carcinomas	Tumor shape		Histology[a]	
		Carcinoma	Invasive carcinoma		Papillary	Nodular	Tub	Pap
CDDB-1	19	11 (58)[b]	4 (21)[c]	11	11	0	0	11
CDDB-2	21	17 (81)[d]	10 (48)[d]	17	16	1	1	16
CDDB-3	22	18 (82)[e]	10 (45)[f]	18	18	0	0	18
SL-1	20	0	0	0	0	0	0	0
SL-2	20	1 (5)	0	1	1	0	0	1
SL-3	18	2 (11)	1 (6)	2	2	0	0	2

a) Tub, tubular adenocarcinoma; Pap, papillary adenocarcinoma.
b) Significantly different from SL-1 ($P<0.01$).
c) Significantly different from SL-1 ($P<0.05$).
d) Significantly different from SL-2 ($P<0.01$).
e) Significantly different from SL-3 ($P<0.01$).
f) Significantly different from SL-3 ($P<0.05$).

Table IV. Carcinomas of the Liver and Pancreas Induced in BOP-treated Hamsters after Cholecystoduodenostomy with Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	No. of hamsters killed	Liver					Pancreas				
		No. (%) of hamsters with carcinoma	Average no. of carcinomas per animal	Histology[a]			No. (%) of hamsters with carcinoma	Average no. of carcinomas per animal	Histology[a]		
				Tub	Pap	Others			Tub	Pap	Others
CDDB-1	19	7 (37)[b]	1.47[c]	24	2	2	10 (53)	1.16	19	2	1
CDDB-2	21	16 (76)[d]	2.71[d]	50	4	3	17 (81)	2.00	35	3	4
CDDB-3	22	19 (86)[e]	5.14[e]	104	5	4	18 (82)	2.18	41	4	3
SL-1	20	0	0	0	0	0	9 (45)	0.60	10	1	1
SL-2	20	4 (20)	0.50	10	0	0	17 (85)	1.80	27	5	4
SL-3	18	10 (56)	2.11	36	1	1	16 (89)	2.33	35	4	3

a) Tub, tubular adenocarcinoma; Pap, papillary adenocarcinoma.
b) Significantly different from SL-1 ($P<0.01$).
c) Significantly different from SL-1 ($P<0.05$).
d) Significantly different from SL-2 ($P<0.01$).
e) Significantly different from SL-3 ($P<0.05$).

noma of the gallbladder developed in 58% of hamsters in group CDDB-1, 81% in group CDDB-2 and 82% in group CDDB-3, while only a few hamsters in the SL groups had gallbladder carcinoma. The differences were statistically significant between the CDDB groups and the corresponding SL groups ($P<0.01$). Approximately half the gallbladder carcinomas in the CDDB groups showed an invasive growth into the liver or the duodenum at the site of anastomosis to the gallbladder.

All but one of the gallbladder carcinomas was papillary in gross shape, and the tumors grew into the lumen of the gallbladder with marked papillary projections. Histologically, the lesions were papillary adenocarcinoma. The only exception was a tubular adenocarcinoma of nodular form observed in a hamster in group CDDB-2. Fig. 4a shows a representative case of gallbladder carcinoma invading the liver and anastomosed duodenum. The tumor exhibits a characteristic appearance of papillary adenocarcinoma and co-exists with an irregular glandular proliferation at the tip of the liver infiltration (Fig. 4b). Hyperplastic and dysplastic changes were marked in the peri- and non-carcinomatous areas of the gallbladder in hamsters in the CDDB groups, but metaplastic change was rarely evident.

Carcinomas in other organs Carcinogenicity in the liver and pancreas is summarized in Table IV. The liver in most hamsters in the CDDB groups showed a high degree of tumor development from the bile ductule (cholangiocarcinomas). The incidence of carcinoma and the average number of carcinomas per animal were statistically higher in the CDDB groups than in the corresponding SL groups.

Pancreatic carcinomas were induced frequently in hamsters in all experimental groups. The majority of

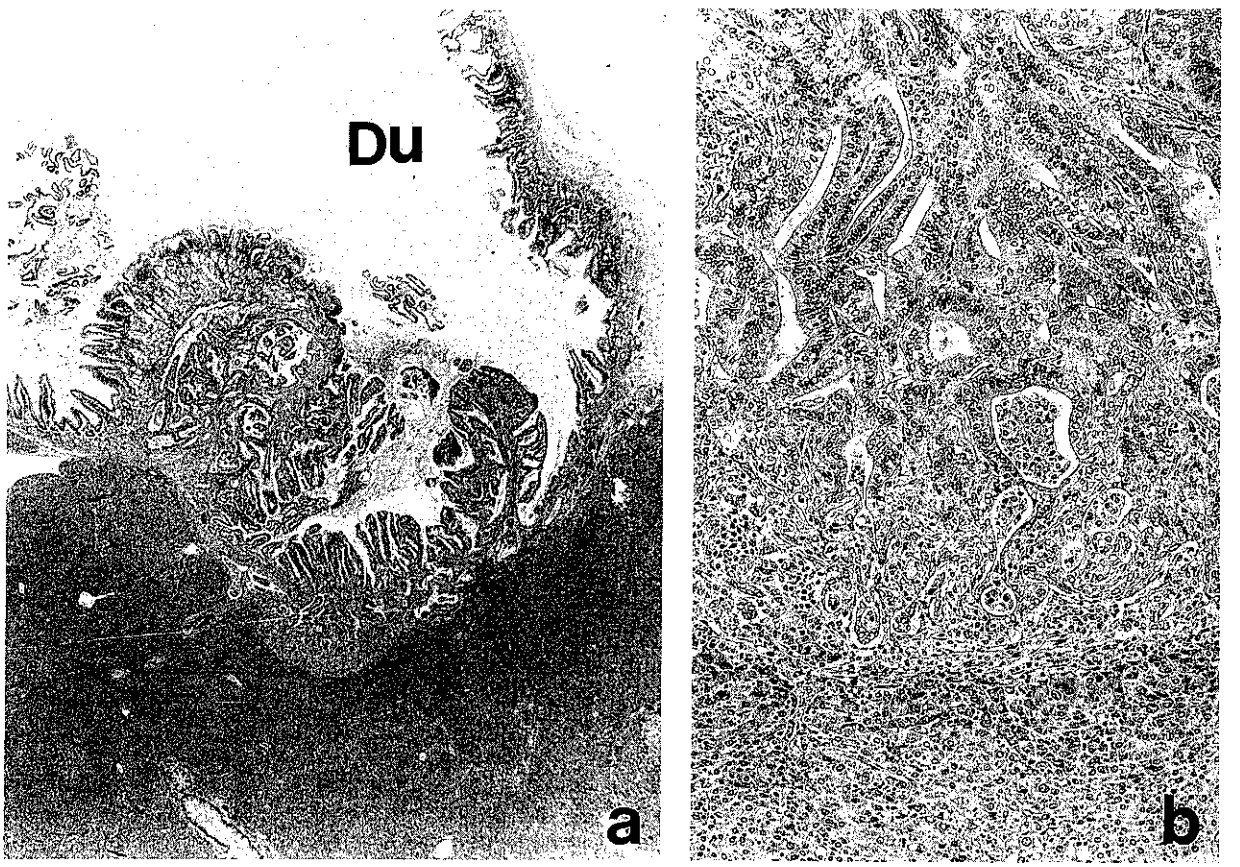

Fig. 4. a. An invasive gallbladder carcinoma in a hamster in CDDB-2. The tumor projects into the lumen of the gallbladder, with papillary configuration. There is evidence of invasion into the adjacent liver tissue and into the anastomosed duodenum (Du). (H & E, ×10). b. High magnification of the portion of liver invasion in the same tumor as in Fig. 4a. There is a marked papillary epithelial proliferation with thin connective tissue stalks (upper). Irregular glandular elements coexist at the tip of the invasion (middle). (H & E, ×100).

these tumors were ductular adenocarcinomas. Neither the incidence of carcinoma nor the average number of carcinomas per animal differed significantly among the corresponding groups.

Cell kinetic studies The BrdU LIs in the biliary epithelium in hamsters undergoing CDDB and SL are shown in Table V. The epithelium of the extrahepatic bile duct in hamsters with CDDB showed a significantly high LI of 4.78% in the hepatic ducts, 4.27% in the common bile duct and 4.13% in the common duct at the 4th week, compared with findings in the controls ($P<0.05$). The LIs in these three bile duct segments in CDDB hamsters gradually increased with time, and were significantly higher than those in controls at the 8th to the 20th week ($P<0.01$).

The gallbladder epithelium in CDDB-treated hamsters showed an extremely high LI of 8.57% at the 4th week. The epithelium reached a maximum LI of 15.71% at the 12th week and maintained conspicuously high LIs of 14.67% and 14.15% at the 16th and 20th week, respectively. In the controls, the gallbladder epithelium had a fairly low LI, from 0.59% to 1.49%. There was a statistically significant difference in the LI between the CDDB hamsters and controls at each time of killing ($P<0.01$).

DISCUSSION

Carcinomas of the biliary tract, including the extrahepatic bile duct and gallbladder, were induced frequently in the hamster model described herein. The Syrian golden hamster was used because the anatomical structure of its pancreaticobiliary ductal system is similar to that of humans,[13] and the bile acid composition and pancreatic juice components in this species also resemble those of humans.[16-19]

Table V. BrdU Labeling Indices in the Biliary Epithelium of Hamsters after Cholecystoduodenostomy with Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	Operative procedure	Experimental period[a] (wk)	Initial no. of hamsters	No. of hamsters killed	BrdU labeling index (%)[b]			
					Extrahepatic bile duct[c]			Gallbladder
					HD	CBD	CD	
1	CDDB	4	8	6	4.78±2.43[d]	4.27±2.29[d]	4.13±2.54[d]	8.57±3.95[e]
2	CDDB	8	9	6	6.49±1.75[f]	6.12±2.02[f]	6.11±1.71[f]	7.77±2.87[f]
3	CDDB	12	8	5	8.07±2.16[g]	6.29±1.14[g]	8.26±2.93[g]	15.71±2.67[g]
4	CDDB	16	8	6	9.47±4.35[h]	9.67±5.38[h]	6.36±1.64[h]	14.67±4.85[h]
5	CDDB	20	9	6	10.33±3.41[i]	9.88±4.16[i]	9.09±2.59[i]	14.15±5.20[i]
6	SL	4	5	5	0.63±0.36	0.52±0.21	0.75±0.47	0.76±0.32
7	SL	8	5	5	0.92±0.52	0.70±0.28	1.14±0.65	0.59±0.30
8	SL	12	5	5	1.12±0.69	0.90±0.41	1.34±0.89	1.33±1.25
9	SL	16	5	5	1.08±0.62	0.94±0.55	1.22±0.70	1.05±0.65
10	SL	20	5	5	1.36±1.01	1.57±1.31	1.15±0.69	1.49±1.20

a) Period from the operation to killing (wk, weeks).
b) Mean±SD.
c) HD, hepatic ducts; CBD, common bile duct; CD, common duct.
d) Significantly different from Group 6 ($P<0.05$).
e) Significantly different from Group 6 ($P<0.01$).
f) Significantly different from Group 7 ($P<0.01$).
g) Significantly different from Group 8 ($P<0.01$).
h) Significantly different from Group 9 ($P<0.01$).
i) Significantly different from Group 10 ($P<0.01$).

Extrahepatic bile duct carcinomas developed in the common bile duct and hepatic ducts as well as in the common duct in our hamster model. Pour *et al.*[20,21] reported the neoplastic response of the pancreaticobiliary system in hamsters after subcutaneous administration of BOP. They stated that there was no evidence of neoplastic growth in the common bile duct and hepatic ducts, but that the common duct showed a slight tendency toward malignancy. In 1987, Kamano *et al.*[22] reported the first successful induction of common duct carcinomas in cholecystectomized hamsters, by subcutaneous administration of BOP, but there was no evidence of tumor formation in the common bile duct and hepatic ducts.

In the present study, gallbladder carcinomas developed with a high incidence and with a short latency. In the CDDB hamsters, the occurrence of gallbladder carcinoma exceeded 80% at the 16th week (112 days). The reported incidence and latency of experimental gallbladder carcinomas in hamster models were 63% and 240 days,[1] 61% and 145–226 days,[3] and 68% and 56–154 days.[23]

The induced extrahepatic bile duct carcinomas were morphologically similar to those found in humans. One of the gross shapes of the induced carcinomas was a polypoid type protruding into the lumen, a characteristic shape of early carcinoma of the extrahepatic bile duct in humans.[24,25] The other gross shape of the induced carcinomas, i.e., the superficial type, is also frequently seen in human bile duct carcinoma, especially in the early stage of the disease.[25] Histologically, scirrhous adenocarcinoma is the most common advanced bile duct carcinoma in humans,[26] but the papillary and tubular adenocarcinomas recognized in the present study are also predominant types of human early bile duct carcinoma.[27,28]

The gallbladder carcinomas found in the present study resembled those in humans. All but one of the carcinomas had marked papillary projections into the lumen, one of the typical forms of growth in human gallbladder carcinoma.[29] Approximately half of the induced gallbladder carcinomas invaded the surrounding tissue, and papillary structures were usually accompanied by irregularly formed glandular elements in the invasive portion. A similar arrangement is often seen in cases of invasive gallbladder carcinoma in humans.[30]

Cells in the DNA synthesis phase are more susceptible to the tumorigenic effects of chemical carcinogens,[31,32] and DNA synthesizing cells can be identified by immunohistochemical staining methods using BrdU and anti-BrdU monoclonal antibody.[14,15,33] In the present study, an extremely high BrdU LI in the epithelium of both the extrahepatic bile duct and gallbladder was demonstrated in CDDB hamsters but not in SL hamsters. Carcinogenic effects of BOP on these replicating cells would be enhanced, leading to the high frequency of biliary carcinomas. The CDDB procedure itself may well be an important factor in our hamster model. BOP is also well recognized as a potent pancreatic carcinogen in

hamsters,[20,21] though the levels of BOP and/or its metabolites secreted into the bile after administration of BOP are reported to be significantly higher than those secreted in the pancreatic juice.[34] Therefore, the carcinogens conveyed with refluxing pancreatic juice into the biliary tract would not have a strong influence on the biliary carcinogenesis in the CDDB hamsters.

In conclusion, we have prepared a new hamster model for the induction of extrahepatic biliary carcinoma which is morphologically similar to that seen in humans. This tumor system will facilitate investigations on mechanisms of tumor development in the biliary tree, the biological behavior of this highly malignant tumor,[35,36] chemotherapeutic research and so on.

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