Journal of Surgical Research **136,** 106–111 (2006)
doi:10.1016/j.jss.2006.04.025

Bile-Reflux into the Pancreatic Ducts is Associated with the Development of Intraductal Papillary Carcinoma in Hamsters

Tomohiko Adachi, M.D.,[1] Yoshitsugu Tajima, M.D., Ph.D., Tamotsu Kuroki, M.D., Ph.D., Takehiro Mishima, M.D., Amane Kitasato, M.D., Kenzo Fukuda, M.D., Ph.D., Ryuji Tsutsumi, M.D., Ph.D., and Takashi Kanematsu, M.D., Ph.D.

Department of Transplantation and Digestive Surgery, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Submitted for publication February 20, 2006

Background. **Reflux of pancreatic juice into the biliary tract is a well-known risk factor for the development of biliary carcinoma. In this study, we investigated the significance of bile-reflux into the pancreatic ducts in pancreatic carcinogenesis, especially in the development of carcinoma in the main pancreatic duct in hamsters.**

Materials and methods. **Syrian hamsters were subjected to three different surgical procedures: cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (Model A); cholecystoduodenostomy along with a dissection of the common bile duct (Model B); or simple laparotomy (Model C). The animals then received weekly subcutaneous injections of *N*-nitrosobis(2-oxopropyl)amine (BOP), for 9 weeks, and were killed for pathological investigation at 16 weeks after the initial BOP administration.**

Results. **Pancreas carcinomas developed in 95, 88, and 90% of the Model A hamsters ($n = 22$), B ($n = 24$), and C ($n = 21$), respectively. The induced pancreatic tumors were histologically classified into four types: papillary; tubular; cystic adenocarcinoma; or intraductal carcinoma of the main pancreatic duct consisting of intraductal papillary carcinoma (IPC) and intraductal tubular carcinoma (ITC). The number and the incidence of IPCs induced in Model A hamsters were 24 lesions and 77% and were statistically higher than those in Model B (7 lesions and 29%) and C hamsters (7 lesions and 33%) ($P < 0.01$). Bile-reflux into the pancreatic ducts was clearly demonstrated in only hamsters of Model A by means of Indocyanine green injection via the portal vein. Proliferative cell nuclear antigen labeling indices of the epithelial cells in the main pancreatic duct in hamsters, with no BOP treatment, were 3.8, 0.8, and 1.1% in Models A ($n = 10$), B ($n = 10$), and C ($n = 10$), respectively, and the difference was statistically significant ($P < 0.01$).**

Conclusions. **Our findings suggest that bile-reflux into the pancreatic ducts is a significant factor predisposing to the development of IPC of the pancreas through an acceleration of epithelial cell kinetics of the main pancreatic duct.**

Key Words: **intraductal papillary carcinoma; main pancreatic duct; pancreas; bile reflux; pancreaticobiliary maljunction.**

INTRODUCTION

Pancreaticobiliary maljunction (PBM), which is defined as a congenital anomaly consisting of a union of the pancreatic and bile ducts that is located outside of the duodenal wall [1, 2], has recently been well recognized as a high-risk state for developing biliary carcinoma [3–5]. In this disorder, pancreatic juice flows into the biliary tracts because the pressure in the pancreatic ducts is higher than that in the biliary tree, and thus, proteolytic pancreatic enzymes and phospholipase A2 activated in the biliary tree subsequently stimulate the biliary epithelial cells, leading to biliary carcinogenesis [6, 7]. Two-way regurgitation, i.e., the reflux of bile up to the pancreatic ducts, can occur in this anomaly [8, 9] because the action of the sphincter of Oddie does not functionally affect the anomalous union. Therefore, various pathological conditions are induced even in the pancreas.

[1] To whom correspondence and reprint requests should be addressed at Department of Transplantation and Digestive Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail: adatomo@nifty.com.

0022-4804/06 $32.00

Acute and chronic pancreatitis, hyperamylasemia, and pancreas carcinoma are reported to be the most important pancreatic disorder associated with PBM [9–11]. Recently, some clinical reports have demonstrated the occurrence of intraductal papillary mucinous carcinoma (IPMC) in the main or large pancreatic ducts of patients with PBM. To our knowledge, at least 16 cases of pancreas carcinoma have been reported in patients with PBM [6, 12–22], and 5 (31%) of these tumors were noted to be IPMC histologically. Because IPMC accounts for only about 3% of all pancreas carcinomas [23, 24], this clinical evidence suggests a strong relation between IPMC and PBM, supposing a hypothesis that bile-reflux into the pancreatic ducts may be a risk factor for the development of IPMC.

We established a hamster model mimicking PBM [25], in which biliary carcinomas were frequently induced with chemicals, and the developmental process and morphobiological characteristics of the induced carcinomas were evaluated in our laboratory [25–29]. In the present study, we investigated the significance of bile-reflux into the pancreatic ducts in pancreatic carcinogenesis in hamsters, focusing on the development of carcinoma in the main pancreatic duct. The Syrian golden hamster was used because the anatomical structure of its pancreaticobiliary ductal system, the bile acid composition, and pancreatic juice components in this species are similar to those of humans [30–32].

MATERIALS AND METHODS

Animals

A total of 97 seven-week-old female Syrian golden hamsters (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used. The average weight of the hamsters at the time of initiation of the experiments was 100 g. The animals were housed one per cage with sawdust bedding under standard laboratory conditions in the Laboratory Animal Center for Biochemical Research at Nagasaki University Graduate School of Biomedical Sciences and were also given a standard pellet diet and water *ad libitum* during the experiment. All experiments were performed following the Guidelines for Animal Experimentation of Nagasaki University Graduate School of Biomedical Sciences.

Surgical Techniques

With the intention of manipulating the bile flow into the pancreatic ductal system, we prepared three surgical modifications in hamsters (Fig. 1). Following intraperitoneal administration of sodium pentobarbital (50 mg/kg body weight), the hamsters were subjected to a cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct [25], so that the bile would regurgitate into the pancreatic ducts (Model A). The animals also underwent a cholecystoduodenostomy along with dissection of the common bile duct, interrupting the connection between the pancreatic and biliary tracts (Model B). In this model, the bile never refluxed into the pancreatic ducts. The sham-operated controls were subjected to a simple laparotomy (Model C).

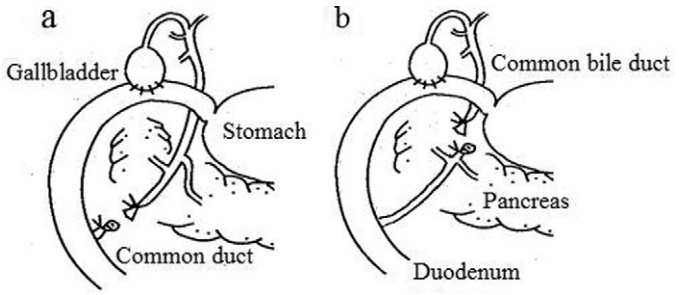

FIG. 1. Surgical procedures in hamsters. (a) Model A: cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct. (b) Model B: cholecystoduodenostomy with dissection of the common bile duct.

Bile-Reflux Studies

Bile-reflux into the pancreatic ducts was evaluated by means of an injection of Indocyanine green (ICG; Diagnogreen Inj. Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) via the portal vein. Four hamsters each from Models A, B, and C were subjected to relaparotomy 12 weeks after surgery and received a slow bolus injection of 1 mL ICG (2.5 mg/mL) via the portal vein for 1 min. The stream of ICG into the pancreaticobiliary ductal system was then observed macroscopically 30 min after the pigment injection.

Carcinogenic Studies

To investigate the effect of bile-reflux into the pancreatic ducts on pancreatic carcinogenesis, the hamsters received weekly subcutaneous injections of *N*-nitrosobis(2-oxopropyl)amine (BOP; Nakarai Chemical Co., Kyoto, Japan) at a dose of 10 mg/kg body weight for 9 consecutive weeks. The BOP administration was begun 4 weeks after surgery. At 16 weeks after the initial administration of BOP, the animals were killed for pathological investigation. During the autopsy, the pancreas was removed en bloc with the attached duodenum and fixed in 10% buffered formalin. The formalin-fixed tissue was then embedded in paraffin and processed routinely for hematoxylin and eosin staining. The number of hamsters examined were 22, 24, and 21 from Models A, B, and C, respectively. The lesions induced in the pancreas of the hamsters were classified by reference to the WHO classification of tumors of the hamster [33].

Cell Kinetic Studies

Proliferating cell nuclear antigen (PCNA) was used for evaluation of the epithelial cell kinetic activity of the main pancreatic duct. Pancreatic tissue sections obtained from 10 hamsters each without BOP treatment from Models A, B, and C were cut at 4 μm, mounted on glass slides coated with 5-aminoprophyltriethoxy saline, and dewaxed in xylene. The sections were treated with microwave heating for 5 min in PBS at 500 W. After blocking of endogenous peroxidase, the sections were incubated with mouse monoclonal antibodies against PCNA (clone-PC 10; DAKO, Kyoto, Japan) at a dilution of 1:100. The cell nuclei were counterstained with hematoxylin. The proportion of labeled nuclei (labeling index, LI) was determined by counting the labeled nuclei in >1000 epithelial cells of the main pancreatic duct.

The cell kinetic studies were also done for the tumors of the pancreas induced by BOP.

Statistical Analyses

The Mann-Whitney *U* and Kruskal–Wallis tests were used for statistical analyses. Differences at $P < 0.05$ were considered to be significant.