Journal of Surgical Research **143,** 270–275 (2007)
doi:10.1016/j.jss.2007.02.034

Long-Term Changes in Hepatobiliary Physiology After Roux-en-Y Hepaticojejunostomy

Johanna Laukkarinen, M.D., Ph.D.,*,†,[1] Pierce Chow, M.D., Ph.D.,*,‡ Juhani Sand, M.D., Ph.D.,† Päivi Kärkkäinen, M.D., Ph.D.,§ Sidney Yu, Ph.D.,‖ Satchithanantham Somanesan, B.Sc.,‖ Irene Kee, B.Sc.,* In-Chin Song, Dip.M.T.,* Teck-Hin Ng, Dip.M.T.,* and Isto Nordback, M.D., Ph.D.†

**Department of Experimental Surgery, Singapore General Hospital, Singapore, Singapore; †Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, Tampere, Finland; ‡Dept of General Surgery, Singapore General Hospital, Singapore, Singapore; §Department of Pathology, Helsinki University Hospital, Helsinki, Finland; ‖Department of Nuclear Medicine, Singapore General Hospital, Singapore, Singapore*

Submitted for publication August 1, 2006

Hepaticojejunostomy (HJ) is a common operation used to by-pass extrahepatic biliary obstructions and to establish biliary-enteric continuity after resections for benign and malignant diseases. Little is known about the effect of this procedure on hepatobiliary physiology. The aim of the present study was to investigate in a swine model the changes in biliary dynamics, bile composition, and hepatic histology induced by Roux-Y HJ. Twenty-four swine (57 (47 to 76) kg) underwent cholecystectomy, with HJ (Group I; *n* = 12) or without any biliodigestive anastomosis (Group II, *n* = 12), and were followed up for 6 or 12 mo by repeated weight scaling, blood, serum, and bile analysis, ^{99m}Technetium (Tc), diethyliminodiacetic acid (HIDA) dynamic biligraphy, and histological analysis. During follow-up, HJ was associated with less weight gain, colonization of the bile duct with aerobic bacteria *Escherichia coli* dominating (in 75% of the animals), a shortened hilum-intestine transit time but reduced liver clearance in dynamic biligraphy, and fibrous periportal changes in liver histology (in 50% of the animals). We conclude that during 1 y follow-up HJ with no anastomotic stricture formation is associated with improved extrahepatic bile drainage, but with ascending contamination of bile ducts with bacteria, which might be involved with the fibrous periportal changes in the liver resulting in diminished excretion of Tc-HIDA from the hepatocytes into the bile. The clinical significance of these changes, and the reduced weight gain observed is a topic of further investigations.

Key Words: **hepaticojejunostomy; cholecystectomy; bile flow; bile composition; cholescintigraphy.**

INTRODUCTION

Hepaticojejunostomy (HJ) is a common operation, not only to by-pass extrahepatic biliary obstructions, but also to establish biliary-enteric continuity after resections for benign and malignant diseases. In most of the patients, biliary reconstruction is considered to afford satisfactory long-term outcome with no symptoms or rise of the serum liver values. However, 7 to 38% of patients have been reported to develop anastomotic strictures, leading to the need for subsequent treatment with percutaneous transhepatic dilation, endoscopic dilation, or operative revision [1–4]. In the overwhelming majority of patients, HJ does not cause detectable major complications. It is known that the destruction of the sphincter of Oddi by endoscopic sphincterotomy results in ascending contamination of the initially sterile bile duct in rabbits [5] and in man [6], as well as in enhanced common bile duct drainage [7], but hepatic changes have not been reported. Despite the fact that HJ is a common operation, knowledge about the changes in hepatobiliary physiology after the operation is surprisingly limited.

The aim of the present study was to investigate in a swine model the changes in overall health and weight, biliary dynamics, bile composition and hepatic histology induced by Roux-Y HJ.

[1] To whom correspondence and reprint requests should be addressed at Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, P.O. Box 2000, 33521 Tampere, Finland. E-mail: johanna.laukkarinen@fimnet.fi.

0022-4804/07 $32.00

MATERIALS AND METHODS

Twenty-four Yorkshire swine [weight median 57 (range 47 to 76) kg] underwent cholecystectomy. In addition, half of the swine either underwent Roux-en-Y HJ (Group I; HJ; $n = 12$) or were left without any biliodigestive anastomosis (Group II; no HJ; $n = 12$). The animals were followed up for 6 or 12 mo by repeated blood, serum, and bile analysis, ^{99m}Technetium (Tc), diethyliminodiacetic acid (HIDA) dynamic biligraphy, and histological analysis of the liver and hepatic duct samples. The animals were fed with standard pig chow and were allowed free access to water and free movement in their cages.

For all of the experiments and operations, anesthesia was induced after 2-day fast by 5% halothane (Narcotan, Léčiva, Czech Republic) inhalation after ketamine (Ketalar; Pfizer, Hameln Pharmaceuticals GmbH, Hamlin, Germany) 100 mg/10 kg i.m. premedication, and maintained by 2% halothane inhalation vaporized with 100% oxygen after intubation with a cuffed endotracheal tube. In the induction of the anesthesia before the operations, the animals received amoxicillin (Amoxin; Merckle GmbH, Blaubeuren, Germany) 5 mg/10 kg i.v. All of the operations were performed by one surgeon (JL).

In all 24 animals, an upper midline laparotomy was performed, liver sampled, and gallbladder bile aspirated, and kept anaerobic on ice until analysis. The cystic duct was closed by clamping, the common bile duct cannulated with thin (24 gauge) polyethene tubing and 4 mL of bile duct (hepatic) bile collected. Cholecystectomy was performed, ligating cystic duct and artery with 3-0 polylactic acid sutures (Vicryl; Ethicon, Edinburg, United Kingdom). In Group I (HJ), bile duct was cut proximal to cystic duct and the distal end ligated. Jejunum was transected 15 cm distal to ligament Treitz, and an end-to-side HJ performed with interrupted 5-0 polytrimethylene carbonate (Maxon; Syneture, Norwalk, CT) sutures in one layer. The diameter of the bile duct anastomosis was measured by probing, whereafter the distal end of the transected jejunum was closed. An end-to-side entero-entero-anastomosis was made 50 cm distal to the HJ anastomosis in two layers with 4-0 polytrimethylene carbonate (Maxon) interrupted and continuous sutures. In Group II (no HJ), the diameter of the bile duct was estimated by external probes. In both Groups I and II, the abdominal cavity was washed with saline, and the abdomen was closed in two layers. Prior to extubation, ketorolac tromethamine (Toradol; Roche, Basel, Switzerland) 6 mg/10 kg i.m. was given for postoperative pain medication, and the daily i.m. injections were continued for the three following days.

Weight scaling, blood and serum analysis, and dynamic biligraphy were performed under general anesthesia at 3 mo in all 24 animals and repeated at the end of follow-up at 6 or 12 mo (12 animals, 6 in both groups at both time points). At that time, laparotomy was performed, bile was sampled for biochemical and microbiological analysis from the hepatic duct, and the inner diameter of the HJ-anastomosis (Group I) or bile duct (Group II) was measured by probing, after which hepatic duct and liver were sampled for histology. The animals were then euthanized by exsanguination.

In dynamic biligraphy, ^{99m}Tc HIDA (volume 1.5 mL, radioactivity 3 mCi) was injected into the cannulated ear vein, and the study performed by obtaining serial analog images for 90 min at 1-min intervals with a γ-camera (Starcam; GE Medical Systems, Huntley, IL). The organs or parts of the organs (liver, liver hilum, and duodenum) were determined within the region of interest (ROI). The registered counts per min per ROI were corrected by the Tc half-life and background radiation. Hepatic maximal uptake, hepatic clearance at 15, 30, 45, and 60 min, and the hilum-intestine transit time were determined.

Blood samples aspirated from the femoral vein were analyzed for full blood count and partly centrifuged to obtain serum. Serum samples were analyzed for sodium (S-Na; method: ion selective electrode), potassium (S-K; method: ion selective electrode), creatinine (S-Crea; method: Jaffe reaction), glucose (S-Gluc; method: enzymatic determination), and amylase (S-Amyl; method: kinetic, substrate ED-PNP-maltoheptoside); of the liver function tests, total bilirubin (S-Bil; method: diatzo reaction), direct bilirubin (S-Bil-Dir; method: diatzo reaction), bile acids (method: enzymatic, colorimetric), alanine aminotransferase (S-ALT; method: kinetic, according to the European Committee for Clinical Laboratory Standards (ECCLS) reference), alkaline phosphatase (ALP; method: kinetic, substrate p-nitro phenyl phosphate in AMP), glutamyltransferase (S-GT; method: kinetic, according to ECCLS reference), and lactate dehydrogenase (S-LDH; method: kinetic, according to Nordic reference) were studied. Total cholesterol (fS-T-Chol; method: enzymatic), HDL-cholesterol (fS-HDL-C; method: direct enzymatic), LDL-cholesterol (fS-LDL-C; method: Friedewald formula), Triglycerides (fS-TG; method: enzymatic), total protein (S-Prot; method: photometric), and albumin (S-Alb; method: immunoturbidimetric) were also determined.

Aliquots of bile from the gallbladder and from the hepatic bile duct were frozen for subsequent analysis of bile acids and phospholipids (method: end point enzymatic assay kit; Wako Corp, Osaka, Japan). Determination of the cholesterol and bilirubin concentrations, pH (method: test strips by QA Supplies; Norfolk, VA) and the culture for aerobic and anaerobic bacteria was done immediately following the retrieval of bile.

Analysis of histology was performed blinded for the group from the formalin-fixed, paraffin-embedded liver (stained with hematoxylin and eosin, Masson-Goldner's trichrome and Gomori's reticulin) and hepatic duct (stained with hematoxylin and eosin) specimens by a specialized pathologist. Semiquantitative analysis of liver histology included grading of (1) periportal and/or bridging necrosis, (2) intralobular degeneration and focal necrosis, (3) portal inflammation, and (4) fibrosis according to the histological activity index (HAI; the Knodell score) [8], and (5) centrilobular and periportal cholestasis according to Desmet [9]. In bile duct histology, atrophy, mucosal inflammation, submucosal vascularization, submucosal fibrosis and scar formation were all analyzed semiquantitatively on a scale none, mild, and marked.

The data are shown as mean and SEM or as median and range, as indicated. Student's t test, χ^2-test, Mann-Whitney t-test, and General linear model variance analysis were used to calculate the statistical significance of the differences. Differences of $P \leq 0.05$ were considered statistically significant.

The study was conducted in accordance with the Helsinki Declaration for Scientific Experimentation on Animals. This study was approved by the Institutional Animal Care and Use Committee of the Singapore General Hospital and all animal experiments were carried out at the premises of the Department of Experimental Surgery, Singapore General Hospital.

RESULTS

Preoperatively, the two groups were comparable in terms of weight, bile duct diameter, dynamic biligraphy determinations (liver maximum, hilum-intestine time, liver clearance), and blood, serum, bile duct bile, and gallbladder bile concentrations. All of the animals remained healthy and gained weight during the follow-up period. There was no difference in the weight of animals between the two groups at 3 mo [60 (48 to 64) *versus* 64 (58 to 72) kg, respectively], but the weight of the Group I (HJ) animals was lower compared with the Group II (no HJ) animals at 6 mo [72 (58 to 77) kg *versus* 90 (86 to 100) kg; $P = 0.01$] and at 12 mo [90 (87 to 120) kg *versus* 145 (128 to 150) kg; $P = 0.003$], respectively. Blood and serum determinations at 3, 6, and 12 mo did not differ from the preoperative concentrations in Groups I and II or between the two groups.