

Evaluation of Hepatobiliary Excretion and Enterobiliary Reflux in Rats with Biliary Obstruction Submitted to Bilioduodenal or Biliojejunal Anastomosis

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Abstract Bilioduodenal and biliojejunal anastomoses are effective for the treatment of biliary obstruction. The objective of this study was to compare the effects of these anastomoses on hepatobiliary excretion and enterobiliary reflux. Enterobiliary reflux and biliary excretion were evaluated respectively after oral administration of technetium (^{99m}Tc) in combination with sodium phytate and intravenous infusion of ^{99m}Tc with diisopropyl-iminodiacetic acid. Enterobiliary reflux occurred to an equal degree in the bilioduodenal and biliojejunal groups. Maximum hepatic activity time (T_{\max}) and radiotracer clearance half-time ($T^{1/2}$) were similar in both groups. However, when compared with that found for the sham-operated group, T_{\max} and $T^{1/2}$ were higher in the biliojejunal group ($P = 0.02$ and $P = 0.01$, respectively). Histopathological analysis showed marked reduction in ductal proliferation in both groups. These data undermine the theoretical advantages attributed to biliojejunal anastomosis and further the understanding of the pathophysiology of cholangitis that occurs even with patent anastomosis.

Keywords Enterobiliary reflux · Hepatobiliary excretion · Biliary obstruction · Bilioduodenal and biliojejunal anastomoses · Scintigraphic study

Introduction

Bilioduodenal (BD) and biliojejunal (BJ) anastomoses are procedures frequently employed in the treatment of biliary obstruction (BO) [1]. The choice of the anastomoses modality used depends on the nature of the affection, the experience of the surgeon, and the age and general condition of the patient [2, 3]. Cholangitis occurs in about 22% of patients submitted to biliodigestive anastomosis. Although this frequently occurs secondary to narrowing of the anastomosis, it may also be caused by enterobiliary reflux or bacterial proliferation [4]. The pathogenesis of such cholangitis has not been fully defined, and it can occur even when the anastomosis is wide and pervious [5]. Enterobiliary reflux has been considered to be a causal factor of cholangitis occurring after a BD anastomosis without documented stenosis. A BJ anastomosis is performed to keep food residues away from the bile duct and, theoretically, to prevent enterobiliary reflux and cholangitis. It represents an alternative for patients with BO and presents a favorable prognosis [6]. However, the onset of motor disorders in the jejunal loop may favor stasis, bacterial proliferation, and cholangitis [7].

In view of the fact that following biliointestinal anastomosis the anatomical and functional barriers existing between the bile ducts and the intestine are lost [8], the objective of our study was to compare the effects of BD and BJ anastomosis on hepatobiliary excretion and enterobiliary reflux in rats with extrahepatic BO.

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Materials and methods

The use of laboratory animals followed the Council for International Organization for Medical Sciences ethical code for animal experimentation. A total of 72 adult male Wistar rats, weighing 250–350 g, were used. Of those, 54 were submitted to a biliary obstruction, and the remaining rats were used as the sham-operated group (SO). After 15 days of biliary obstruction, the animals were divided into three groups of 18 rats each: BD, BJ, and BO. Biochemical blood workup and liver biopsy were taken from each animal of the SO and BO groups and repeated at 90 days after the biliodigestive anastomosis procedures. (Fig. 1).

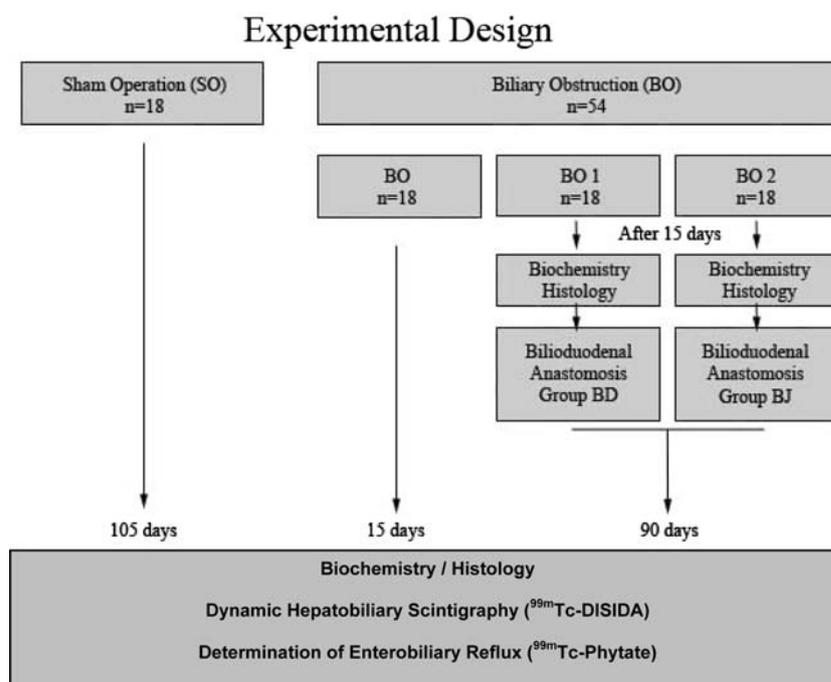
Scintigraphic study of enterobiliary reflux (15 min and 4 h after administration of the radiotracer) and biliary excretion over a 30-min period were carried out in three subgroups of six animals each within each of the studied groups (SO, BO, BD, and BJ). Surgical procedures were performed under sulfuric ether anesthesia. The animals were ventrally shaved and swabbed with an alcohol solution and 2% iodine, followed by a 50-mm midline incision in the abdominal wall starting below the sternum. For biliary obstruction, traction was exerted on the duodenal loop downward and to the left, straightening the bile duct, which was dissected free over its upper portion between the division into hepatic ducts and the juxtapancreatic middle segment of the duct. About 3 mm above the insertion of the major pancreatic duct, the dissected bile duct was ligated with five knots (5/0 Prolene blue monofilament suture, Ethicon, Inc.), and the suture was wound around the

hepatic duct to 5 mm below the hepatic duct junction, where an additional ligature was performed again with five knots [9]. BD anastomoses were performed in a side-to-side manner after a 1-cm incision in the bile duct and in the duodenum, and BJ anastomoses were performed in an end-to-side manner, with the exclusion of a 15-cm loop. At the same procedure, a liver biopsy was performed and amounts of total bilirubin, conjugated bilirubin, unconjugated bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were determined.

At 90 days after the biliodigestive anastomosis procedures, the animals were submitted to the same biochemical blood workups scintigraphic study of the enterobiliary reflux and biliary excretion. After that, the animals were sacrificed, and a second liver biopsy was taken from each animal.

Enterobiliary reflux was quantified using a Digitrac 75 Orbiter gamma camera with a low-energy all-purpose collimator (Siemens, Hoffman Estates, IL, USA) in the static mode. The animals were manually immobilized for the introduction of a flexible 6-Fr catheter through the mouth into the stomach. The catheter was used to administer 3.7 MBq of technetium (^{99m}Tc) in combination with 20 mg sodium phytate diluted in 2 ml of 0.9% saline solution. The catheter was then removed. At 30 s before the end of each predetermined period of surgical evaluation (15 min and 4 h after administration of the radiotracer), the animals were anesthetized with sulfuric ether and submitted to laparotomy. The liver and bile duct (as a unit), as well as the stomach, small intestine, large bowel, and the excluded Roux-en-Y loop were isolated,

Fig. 1 Experimental design. *SO* sham operation, *BO* biliary obstruction, *BD* bilioduodenal anastomosis, *BJ* biliojejunal anastomosis, ^{99m}Tc -DISIDA technetium- ^{99m}Tc diisopropyl-iminodiacetic acid, ^{99m}Tc -Phytate technetium- ^{99m}Tc phytate



and their ends were tied with 4–0 cotton sutures. The tying of the jejunum and duodenum near the anastomosis to the bile duct was performed during the first period of surgical evaluation.

The organs were individually placed in glove fingers and submitted to a radioactivity count by quantitation of the gamma-ray emission (kilocount/minute). Background radioactivity was subtracted from the value obtained. The sum of the values for each of the organs was considered to be 100%, and the radioactivity emitted by the liver/bile duct, the stomach, and the intestinal segments was expressed as a percentage of that total. To study hepatic excretion, biliary flow, and the presence of the radiotracer in the small intestine, a scintillation camera (DST; Sopha Medical Vision International Buc Cedex, France) with a low-energy, all-purpose collimator was used in the dynamic mode with the radiotracer technetium-^{99m} diisopropyl-iminodiacetic acid (DISIDA).

The animals were anesthetized with intraperitoneal thiopental, the internal jugular vein was dissected, and a fine umbilical catheter (3.6 Fr × 41 cm) was introduced for injection of the radiotracer. In the subsequent dynamic study, frames of the heart, liver, and small intestine were obtained every 15 s over a 30-min period. The maximum hepatic activity time (T_{max}), the half-time for hepatic clearance of the radiotracer ($T^{1/2}$), and the time to appearance of radioactive bile in the small intestine were measured. Biochemical blood workups were then performed, and the animals were submitted to a second liver biopsy via laparotomy. Liver samples were immediately fixed in 10% buffered formalin for posterior embedding in paraffin. Sections 5- μ m thick were stained with hematoxylin and eosin (H&E) and examined under light microscopy. The slides were independently evaluated by two pathologists who were blinded to their identification. The parameters studied included fibrosis, inflammatory infiltrate, and ductal proliferation. Histological alterations and their effects on liver architecture were classified as absent, mild, moderate, or severe.

Statistical analysis

The nonparametric Kruskal–Wallis test, followed by the Dunn test, was used for comparison of the various independent groups. Means of two independent groups were compared using the Mann–Whitney test. The Wilcoxon test was used for analysis of related samples. The level of significance was set at 5% in all analyses.

Results

Blood biochemistry

Biliary-obstructed animals presented significantly higher total bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels than did control animals ($P < 0.0001$). Following biliodigestive anastomosis, biochemical values dropped at comparable rates in the two study groups ($P = 0.62$), and those values were not significantly different from the values observed for the control group ($P = 0.21$) (Table 1).

Liver histology

Semiquantitative microscopic analysis

In the BO group, there was severe bile-duct proliferation, mild fibrosis, and mild to moderate mixed inflammatory infiltrate in the liver portal spaces (Fig. 2B). After biliodigestive anastomosis (BD and BJ groups), there were no significant changes in the inflammatory infiltrate or fibrosis, although there was a marked reduction in bile-duct proliferation in most animals ($P = 0.001$), as shown in Figs. 2C and D. Moreover, there were no histopathological differences between the BD and the BJ groups (Table 2). Fatty degeneration, abscess formation, or hepatocyte necroses were not found in this series.

Table 1 Biochemical values detected in the sham operation, biliary obstruction, bilioduodenal anastomosis, and biliojejunal anastomosis groups

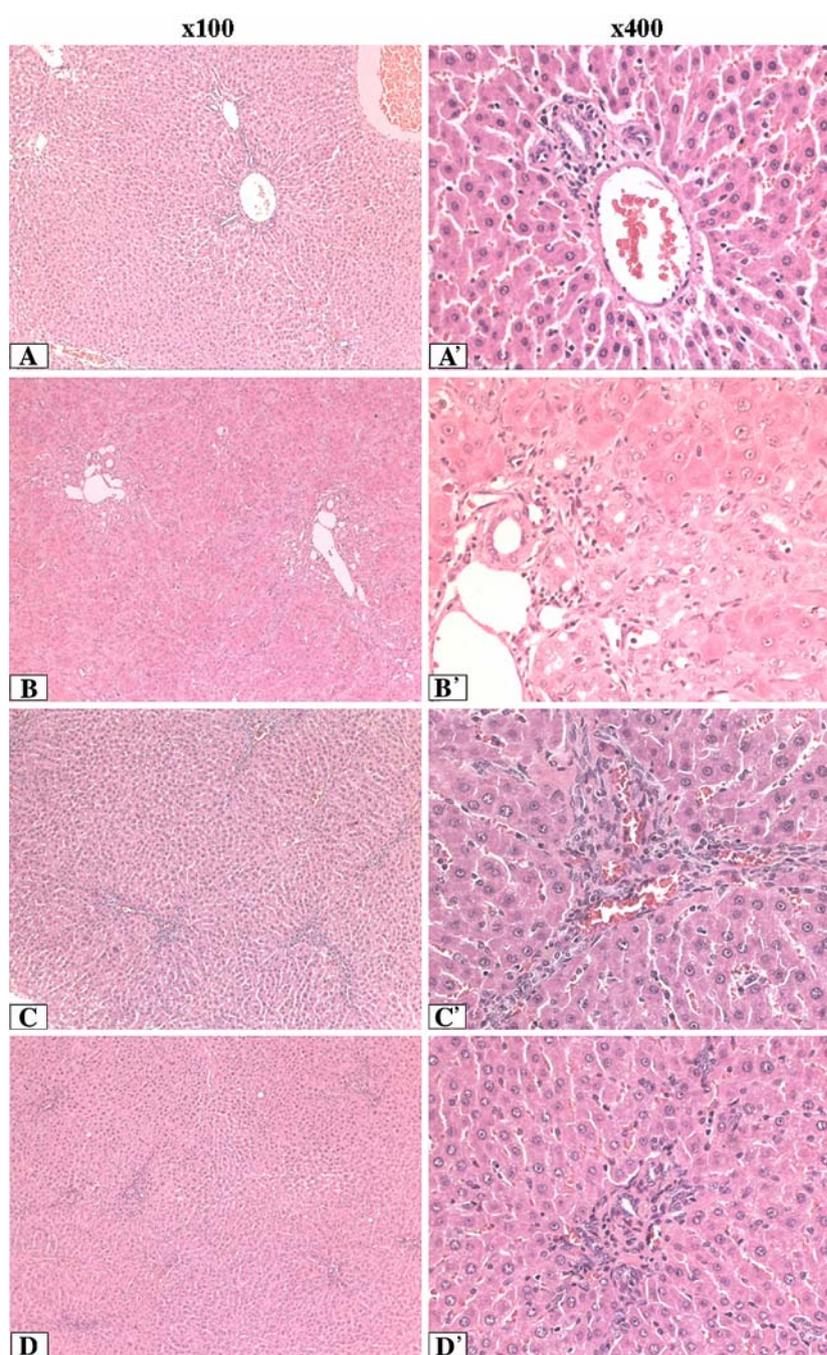
Group	TB	AP	ALT	AST
SO	0.23 ± 0.07	51.55 ± 8.95	49 ± 20.58	159.05 ± 43.81
BO	7.11 ± 1.86	294.94 ± 121.97	103.38 ± 39.25	435.16 ± 180.03
BD	0.33 ± 0.15	61.33 ± 50.74	75.55 ± 25.44	138.16 ± 56.32
BJ	0.35 ± 0.14	53.16 ± 23.33	64.22 ± 21.31	178.55 ± 56.25

Results presented as means ± standard deviations

TB total bilirubin, AP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, SO sham operation, BO biliary obstruction, BD bilioduodenal anastomosis, BJ biliojejunal anastomosis

SO vs. BO $P = 0.0001$

Fig. 2 Histopathological features of the liver biopsies [hematoxylin and eosin (H&E)]. **A & A'** sham group (controls); note the usual hepatic architecture. **B & B'** biliary obstruction group (*BO*); observe conspicuous biliary-ductal proliferation and moderate inflammatory cell infiltration with mild fibrosis at the portal space. **C & C'** bilioduodenal (*BD*) anastomosis group. **D & D'** biliojejunal (*BJ*) anastomosis group; observe similar histological aspects between these groups—discrete biliary-ductal proliferation associated with mild inflammatory cell infiltration and portal fibrosis; compare the degree of ductal proliferation with the *BO* group (**B & B'**). *Right column* ($\times 100$); *left column* ($\times 400$)



Static scintigraphic evaluation

Enterobiliary reflux

As can be seen in Fig. 3, enterobiliary reflux occurred to an equal degree in both anastomosis modalities at both time points evaluated at 15 min [5.55% for *BD* anastomosis and 0.34% for *BJ* anastomosis ($P = 0.13$)] and at 4 h [2.18% for *BD* anastomosis and 1.53% for *BJ* anastomosis ($P = 0.81$)].

Dynamic scintigraphic evaluation

Maximum hepatic activity time

Mean T_{\max} values were 106.16 and 790 s, respectively, for *SO* and *BO* animals ($P = 0.002$). After *BD* and *BJ* anastomosis, the respective values were equivalent (186.16 and 219.83 s). However, when compared with that found for *SO* animals, T_{\max} was higher in *BJ*-anastomosed animals ($P = 0.025$) (Fig. 4).

Table 2 Semiquantitative evaluation of the fibrosis, ductal proliferation, and inflammatory infiltrate in the sham operation, biliary obstruction, bilioduodenal anastomosis, and biliojejunal anastomosis groups

Histopathological Aspects	Grade	SO (n)	BO1 (n)	BD (n)	BO2 (n)	BJ (n)
Ductal proliferation*	Absent	18	–	1	–	2
	Mild	–	–	6	–	9
	Moderate	–	3	9	5	5
	Severe	–	15	2	13	2
Fibrosis	Absent	18	8	3	1	5
	Mild	–	10	11	16	9
	Moderate	–	–	4	1	4
	Severe	–	–	–	–	–
Inflammatory infiltrate	Absent	18	–	–	–	–
	Mild	–	9	6	16	11
	Moderate	–	7	10	2	6
	Severe	–	2	2	–	1

SO sham operation, BO1 biliary obstruction before bilioduodenal anastomosis, BD bilioduodenal anastomosis, BO2 biliary obstruction before biliojejunal anastomosis, BJ biliojejunal anastomosis

*SO vs. BD ($P < 0.001$), BO1 vs. BD ($P = 0.0001$), SO vs. BJ ($P < 0.001$), BO2 vs. BJ ($P = 0.0001$), SO vs. BD ($P < 0.01$), SO vs. BJ ($P < 0.01$)

Fig. 3 Radioactivity emitted by the liver/bile duct expressed as a percentage of the total radioactivity emitted by the digestive tract and liver/bile duct together in the groups studied after introduction of technetium-^{99m} phytate into the stomach through an orogastric catheter. SO sham operation, BO biliary obstruction, BD bilioduodenal anastomosis, BJ biliojejunal anastomosis

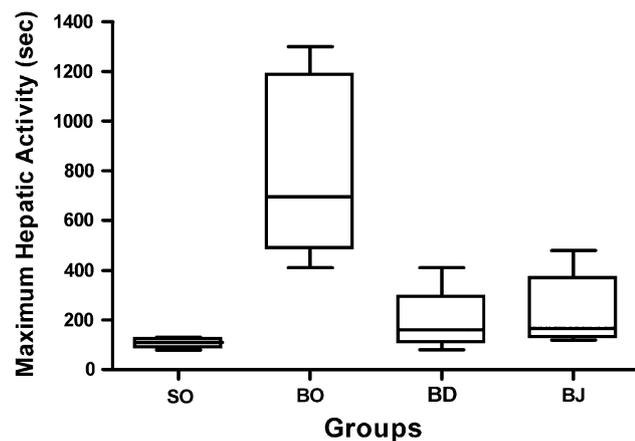
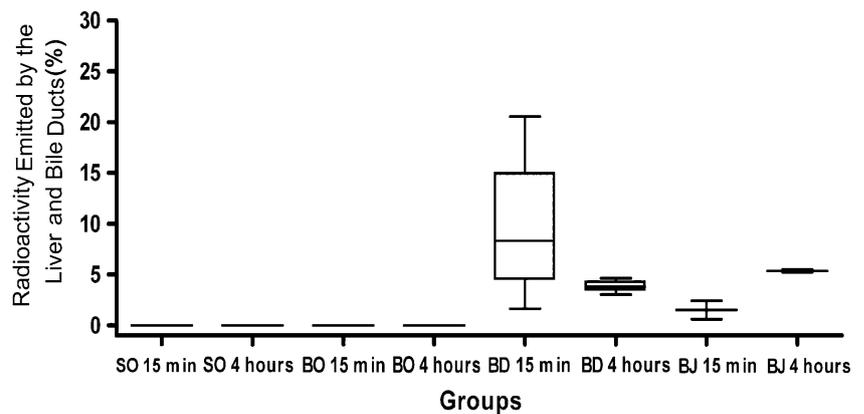


Fig. 4 Maximum hepatic activity, expressed in seconds, obtained by intravenous injection of technetium-^{99m} diisopropyl-iminodiacetic acid. SO sham operation, BO biliary obstruction, BD bilioduodenal anastomosis, BJ biliojejunal anastomosis. SO vs. BO $P = 0.002$, SO vs. BJ $P = 0.025$, BO vs. BD $P = 0.005$, BO vs. BJ $P = 0.005$

Half-time for hepatic clearance of the radiotracer

Mean $T^{1/2}$ values were 266.66, 446.66, and 551.66 s for the SO, BD, and BJ groups, respectively. There was no difference between the BD and BJ groups. However, when compared with the SO group, BJ-anastomosed animals presented slower hepatic clearance ($P = 0.017$) (Fig. 5).

Excretion time of the radiotracer into the small intestine

Excretion time of the radiotracer into the small intestine, expressed in seconds, was 105, 171.66, and 166.66, respectively, for the SO, BD, and BJ groups, with difference between SO and the biliodigestive anastomosis groups ($P = 0.005$) There was no differences between the BD and BJ groups. The radiotracer was not found in the small intestine of the BO group (Fig. 6).

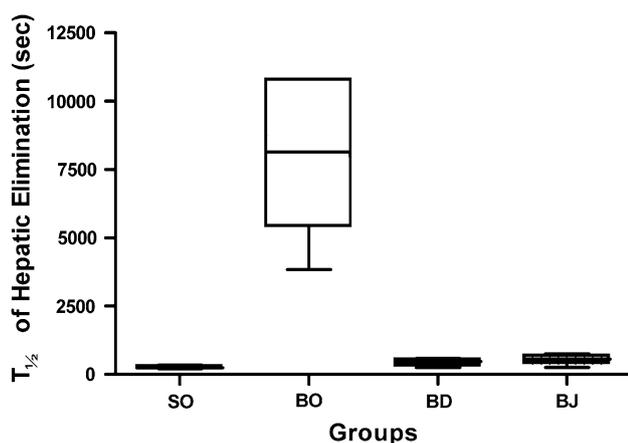


Fig. 5 Half-time for hepatic clearance of the radiotracer ($T^{1/2}$), expressed in seconds, obtained by intravenous injection of technetium- 99m diisopropyl-iminodiacetic acid. *SO* sham operation, *BO* biliary obstruction, *BD* bilioduodenal anastomosis, *BJ* biliojejunal anastomosis. *SO* vs. *BO* $P = 0.002$, *SO* vs. *BJ* $P = 0.017$. *BO* vs. *BD* $P = 0.002$, *BO* vs. *BJ* $P = 0.002$

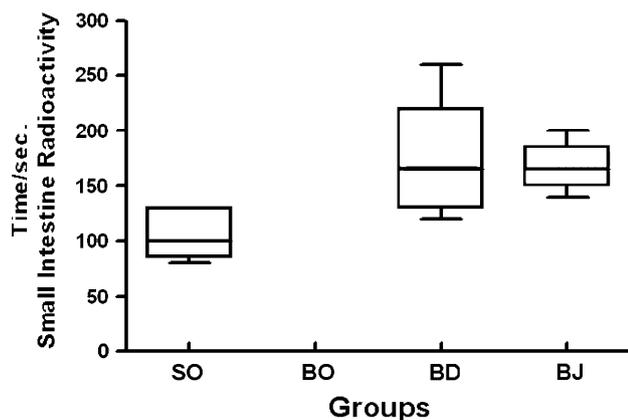


Fig. 6 Time of appearance of the radiotracer into the small bowel, expressed in seconds, obtained by intravenous injection of technetium- 99m diisopropyl-iminodiacetic acid. *SO* sham operation, *BO* biliary obstruction, *BD* bilioduodenal anastomosis, *BJ* biliojejunal anastomosis. *SO* vs. *BD* $P = 0.005$, *SO* vs. *BJ* $P = 0.005$

Discussion

This study design has not been described previously. It was developed to evaluate hepatobiliary and digestive disorders in rats with extrahepatic BO and submitted to biliary decompression with the duodenum and the jejunum. In clinical practice, selection of the modality of biliary anastomosis used in cases of extrahepatic BO depends more on the experience of the surgeon than on the pathogenesis of the disease to be treated [3]. BD and BJ anastomoses are frequently employed in extrahepatic BO; however, even when the anastomoses are pervious, episodes of cholangitis may occur due to enterobiliary reflux [6] or due to stasis in the Roux-en-Y excluded loop [7, 10, 11].

In humans, the length of the small intestine is approximately 260 cm. The standard 40 cm employed in the Roux-en-Y procedure is 15% of this length. As the length of the small intestine in rats is approximately 100 cm [12], 15% of the total length would correspond to 15 cm, the length that was used in this study.

Contact between enteric content and the bile duct is a common occurrence after BD anastomosis and endoscopic biliary drainage [13], but it has also been reported after BJ anastomosis in rats with chronic extrahepatic BO, in which it was accompanied by the appearance of bezoars in the anastomoses [14]. In addition, after biliary drainage, even with ample and pervious anastomoses or in the absence of cholestasis, mixed inflammation, as well as bacterial contamination of the bile duct, can occur in the portal spaces of the liver [14]. Therefore, evaluation of the effects of BD and BJ anastomosis on hepatobiliary transport and enterobiliary reflux is justified.

Scintigraphic methods are frequently used in the evaluation of gastrointestinal transit and hepatocellular function. Such methods have the sensitivity and specificity needed to obtain anatomical and functional information about the liver and bile duct, even in the presence of elevated serum bilirubin levels [15]. Dynamic hepatic scintigraphy evaluates hepatocellular function and bile duct permeability by means of radiotracers that are taken up by the hepatocytes, secreted through the bile duct, and finally excreted into the small intestine. Gastrointestinal transit and enterobiliary reflux can be studied by means of removing the organs and taking static readings of the radiation emitted by the organs and their contents [11, 16, 17].

Recanalization of the hepatic duct after single ligation or ligation and transection of the common bile duct constitutes a frequent cause of failure in experimental biliary obstruction [18]. The results obtained in this study indicate that our method effectively reduces the chance of recanalization of the hepatic duct. The biochemical markers of hepatic cholestasis and hepatic-duct proliferation were significantly elevated in the BO group. In addition, these animals did not excrete the radiotracer into the small intestine, a fact that ensured the efficiency of the experimental model of BO used in our study.

After BD and BJ anastomosis, anastomosis permeability was demonstrated by normalization of total, conjugated, and unconjugated bilirubin, as well as alkaline phosphatase, and by the appearance of the radiotracer in the small intestine.

In clinical practice, after BJ anastomosis, 10–15% of patients present symptoms of blind-loop syndrome (cholestasis and cholangitis). These symptoms may be due to mechanical obstructions, such as stenosis of the biliodigestive anastomosis, or recurrent lithiasis. However, in cases in which these complications have been ruled out, a

functional obstacle might be responsible for the onset of cholangitis [8]. Transection of the small intestine and reanastomosis involve the loss of the duodenal pacemaker control in the excluded Roux-en-Y loop, together with disturbance of the postprandial contractile pattern. The excluded loop does not transmit the distal propagation of the migratory motor complex and is influenced by ectopic pacemakers with peristaltic waves of lower frequency and retrograde activity [19, 20, 21]. These motor disorders of the excluded loop favor bile stasis and cholangitis. Among the proposed alternatives are uncut BJ anastomosis, which preserves the continuity of the intestinal wall and separates the enteric content of the bile duct by occlusion of the jejunal lumen [7, 11], and BJ anastomosis with duodenojejunal anastomoses [2, 22], which has the objective of decompressing the excluded loop with stasis.

In our study, enterobiliary reflux was present at an equal degree in both anastomosis groups. Theoretically, enterobiliary reflux should be more marked in BD anastomosis, mainly during the early postoperative period, due to the obligatory passage of food through the anastomosis. However, if this biological phenomenon does occur, it appears to be transitory. Enterobiliary reflux was detected by direct observation of food in the bile ducts of rats submitted to BD and BJ anastomosis, in which 5-cm lengths of the excluded loop were used [15]. Nevertheless, it has been reported that rats with BO submitted to BJ anastomosis with a 2.5-cm Y loop present no enterobiliary reflux [23]. Similarly, enterobiliary reflux was not observed when standard BJ anastomosis was compared with BJ anastomosis without transection of the jejunal loop [11]. Enterobiliary reflux and the presence of motor dysfunction in the excluded Roux-en-Y loop, together with stasis in the bile duct, favor bacterial proliferation and explain the delay in hepatobiliary excretion observed in our study, as well as the higher incidence of cholangitis detected in humans [1, 2, 22].

Upon qualitative histological analysis, the BO group presented intense hepatic-ductal proliferation with fibrosis and moderate mixed inflammatory infiltration. At approximately 90 days after biliodigestive anastomosis, ductal proliferation regressed; the fibrosis and inflammatory infiltrate, although still present, were classified as mild and moderate, respectively; and there was no fatty degeneration or hepatocyte necrosis. There was no difference between the BD and BJ anastomosis groups.

These results differ from those of studies in which rats without previous BO were submitted to standard or uncut BJ anastomosis and evaluated after 28 [11] or 180 days [7]. In those studies, intense hepatic fibrosis with abscesses, focal fatty degeneration, hepatocyte necrosis, and ductal proliferation were detected, especially after standard BJ anastomosis. Findings of such intense histopathological changes are not in agreement with our data or with the data

of other studies [13, 23] and might be explained by substenosis of the anastomosis, underscoring the need for comparisons of the technical aspects and methods used for evaluating cholestasis.

In summary, our results indicate that the two decompression modalities are equivalent regarding recovery from cholestasis and liver damage; both induce enterobiliary reflux with similar intensity, and hepatobiliary excretion occurs to an equal degree. These data undermine the theoretical advantages so far attributed to BJ anastomosis and further the understanding of the pathophysiology of cholangitis that occurs even with patent anastomosis. These observations, together with other clinical and experimental data, offer insights for objective selection of biliary decompression procedures and open possibilities for the proposal and evaluation of alternative methods to minimize the occurrence of enterobiliary reflux, biliary stasis, and cholangitis.

At the moment, BD anastomosis is an easily executed technique. Our study, and others [2, 22], have increased the knowledge of this procedure in relation to hepatobiliary flow and enterobiliary reflux, showing that, whenever feasible, it is a viable surgical option for the treatment of BO, particularly in the era of laparoscopy.

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