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Influence of Biliary Drainage on the Repair of Hepatic Lesions in Biliary Fibrosis

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Background. **Bilioduodenal (BD) and biliojejunal (BJ) derivation induce enterobiliary reflux and bile stasis. Decompression of the excluded loop of the Roux-en-Y (BJD) was proposed to minimize these effects. The aim of this study was to compare the influence of these three modalities of biliary bypass on hepatic lesion repair in rats with secondary biliary fibrosis.**

Materials and Methods. **Rats with 15 d of biliary obstruction underwent BD, BJ, and BJD drainage and were compared with a group submitted to simulated operation (SO) and biliary obstruction (CBO). The serum values of total and fractional bilirubin, alkaline phosphatase (ALP), and aminotransferases (AST and ALT), as well as hepatobiliointestinal excretion determined with ^{99m}Tc-Disida, were used for comparison. In addition, we used morphometric analyses to estimate the mass of the hepatocytes, bile ducts, and liver fibrosis. We also counted hepatic stellate cells (SC).**

Results. **For each of the three modalities of biliary drainage, there were significant reductions in bilirubin, AST, ALP, and the number of SCs. The recovery of the estimated mass of all histologic components occurred only after BJ and BJD; in the BD group, the estimated hepatocyte mass was reduced compared with the SO group. The residual hepatic radioactivity of ^{99m}Tc-Disida was greater in the BJD group than in the SO group.**

Conclusions. **The interposition of the jejunal loop between the biliary tree and the intestine may slow hepatobiliary clearance of radioactivity, even though it provides the resolution of cholestasis and is effective in recovering from hepatic lesions.**

Key Words: **obstructive jaundice; liver fibrosis; choledochostomy; choledochoduodenostomy; choledochojejunostomy; liver regeneration; radionuclide imaging; cholangitis; portal hypertension; hepatic stellate cells.**

INTRODUCTION

Clinical [1–3] and experimental [4–7] records show that effective biliary anastomosis reverses hepatic changes resulting from chronic biliary obstruction. In contrast, bilioduodenal (BD) and biliojejunal (BJ) anastomosis induce enterobiliary reflux and stasis in the excluded jejunal loop [7–10].

Rats with secondary biliary fibrosis that received BD showed improvement of cholestasis and anatomopathologic liver alterations; however residual hepatic fibrosis and portal hypertension remained [11]. Other studies conducted under similar hepatic lesion conditions reported recovery of hepatic excretion, histologic liver changes and portal pressure levels after BJ [5].

Comparative studies of BD and BJ that evaluated the liver and the bile ducts reported some enterobiliary reflux and contamination of the biliary duct in both modalities of derivation but with greater intensity after BD [8]. In scintigraphic analyses of hepatobiliointestinal flow, BJ was associated with residual hepatic stasis [7, 10] but showed better recovery of the hepatic lesions [8].

In contrast, the motility changes of the excluded loop of the Roux-en-Y promoted biliary stasis, bacterial

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growth, cholangitis, and hepatic fibrosis [8, 10, 12]. The biliojejunal Roux-en-Y derivation with excluded jejunal loop decompression *via* latero-lateral anastomosis with the duodenum (BJD) is an alternative treatment that may minimize the adverse effects of stasis in BJ and ensure the benefits of BD [10, 13].

Thus, the current belief that the results of biliary obstruction treatment depend more on the underlying disease and the patency of the anastomosis than upon the modality of biliary bypass is not absolute. The frequent indications of BD due to technical ease, especially regarding videolaparoscopic access, should be cautiously taken into account when considering the treatment of biliary obstruction [14–16].

In this context, the changes in the liver and bile ducts resulting from biliodigestive anastomoses should be the subject of experimental study. The objective of this study was to evaluate the effects of BD, BJ, and BJD on anatomopathologic liver lesions and on the hepatobilio-intestinal flow in rats with secondary biliary fibrosis.

MATERIALS AND METHODS

The study was approved by the Ethics and Animal Experimentation Committee of the Faculty of Medicine of Ribeirão Preto at the University of São Paulo (FMRP-USP). We used 137 adult male albino Wistar rats with initial weights ranging from 222 to 346 g. The surviving animals were divided into two groups as described below.

Distribution of the Animals into Groups and Subgroups (Fig. 1)

- Simulated operation group (SO): $n = 8$ animals. The animals in this group underwent a simulated operation involving traction of the duodenum and careful handling of the bile duct to avoid trauma. After 2 mo, we measured hepatobiliary excretion by scintigraphy with ^{99m}Tc-Disida. We also performed splenectomy, blood sampling for biochemical tests, and total hepatectomy for histologic analyses of the left lower lobe fragment.
- Biliary obstruction group (BO): $n = 33$ animals. The BO animals had two weeks of biliary obstruction and were divided into four subgroups: CBO (8 animals), BDpre (9 animals), BJpre (8 animals), and BJDpre (8 animals).

The CBO subgroup underwent the same evaluation as the SO group. After 2 wk of biliary obstruction, the animals in subgroups BDpre, BJpre, and BJDpre underwent blood sampling for biochemical evaluation and removal of a fragment of the left lower lobe of the liver for anatomopathologic study. Following these procedures, the animals received bilioduodenal, biliojejunal Roux-en-Y anastomosis, or biliojejunal Roux-en-Y anastomosis with decompression of the excluded jejunal loop by anastomosis with the duodenum. These groups were then labeled as BD, BJ, and BJD, respectively. After 2 mo of biliary drainage, these groups underwent the same evaluation as the SO group.

Surgical Technique

After 12 h of fasting, each animal was weighed, cleaned, anesthetized, positioned in the dorsal decubitus position, and submitted to trichotomy of the anterior abdominal wall. Antisepsis was performed using polyvinyl pyrrolidone iodine (dermoiodine; J. P. Indústria Farmacêutica SA Ribeirão Preto). The abdominal cavity was accessed by median laparotomy in the upper abdomen, with lateral retraction of the edges of the incision with delicate retractors. The peritoneal cavity was kept moist with saline solution to prevent drying of the viscera.

Anesthesia

For the anesthetic, we used a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg) with intramuscular administration [17]. We used intraperitoneally administered thiopental (20 mg/kg) to study hepatobilio-intestinal flow [18].

Biliary Obstruction

After bile duct isolation, a ligature was placed about 5 mm from the confluence of the lobar ducts with 5-0 prolene thread (prolene blue monofilament suture; Ethicon, Inc., São José dos Campos (São Paulo), Brazil). This first ligature was followed by a second, which was placed 3 mm above the biliopancreatic junction and the transection of the bile duct between the ligatures [19].

Biliodigestive Anastomosis (Fig. 2)

All anastomoses were made using single-layer continuous sutures with Vicryl 6-0 (polyglycolic acid) thread.

- BD group: Anastomosis between the dilated bile duct opened transversally and the duodenum opened longitudinally, for an extent of 1 cm, in a latero-lateral manner.
- BJ Group: Latero-lateral anastomosis with the jejunal loop sectioned about 5 cm from the duodenojejunal angle, followed by a terminolateral jejuno-jejunal anastomosis 15 cm from the hepatojejunal anastomosis.
- BJD Group: Latero-lateral biliodigestive anastomosis with the jejunal loop sectioned about 5 cm from the duodenojejunal angle. Latero-lateral anastomosis was then performed between the excluded loop of the Roux-en-Y and the first portion of the duodenum 5 cm away from the biliodigestive anastomosis. Jejuno-jejunal anastomosis was performed 15 cm from the hepatojejunal anastomosis.

The abdominal wall was closed in the same way in all animals. The peritoneum and aponeurosis were sutured *en bloc* with a 4.0 nylon monofilament continuous suture; the skin was closed in the same manner.

Biochemical Analysis

We measured the serum levels of total bilirubin, direct bilirubin, indirect bilirubin [20], alkaline phosphatase [21], alanine aminotransferase, and aspartate aminotransferase [22].

Histologic Analyses of the Liver

The liver fragments were fixed in 10% buffered formalin for 24 h, followed by progressive dehydration. For morphometric analyses, 4 μm slices were stained with Masson's trichrome stain. For the identification of stellate cells, serial slices of 3 μm thickness underwent immunohistochemical reactions with the monoclonal anti-desmin lyophilized rat antibody (IgG1, NCL-DES-DER11; Novocastra Laboratories Ltd., Newcastle, UK) according to the manufacturer's specifications.

For the morphometric analyses, we captured images using an Axiophot microscope (Carl Zeiss, Hallbergmoos, Germany) coupled to a digital camera (Sony Minato, Tokyo, Japan). The images were transferred to a computer *via* card capture by Frame Grabber (Carl Zeiss) in the standard RGB, with 648 $\times$ 474 lines for analysis using

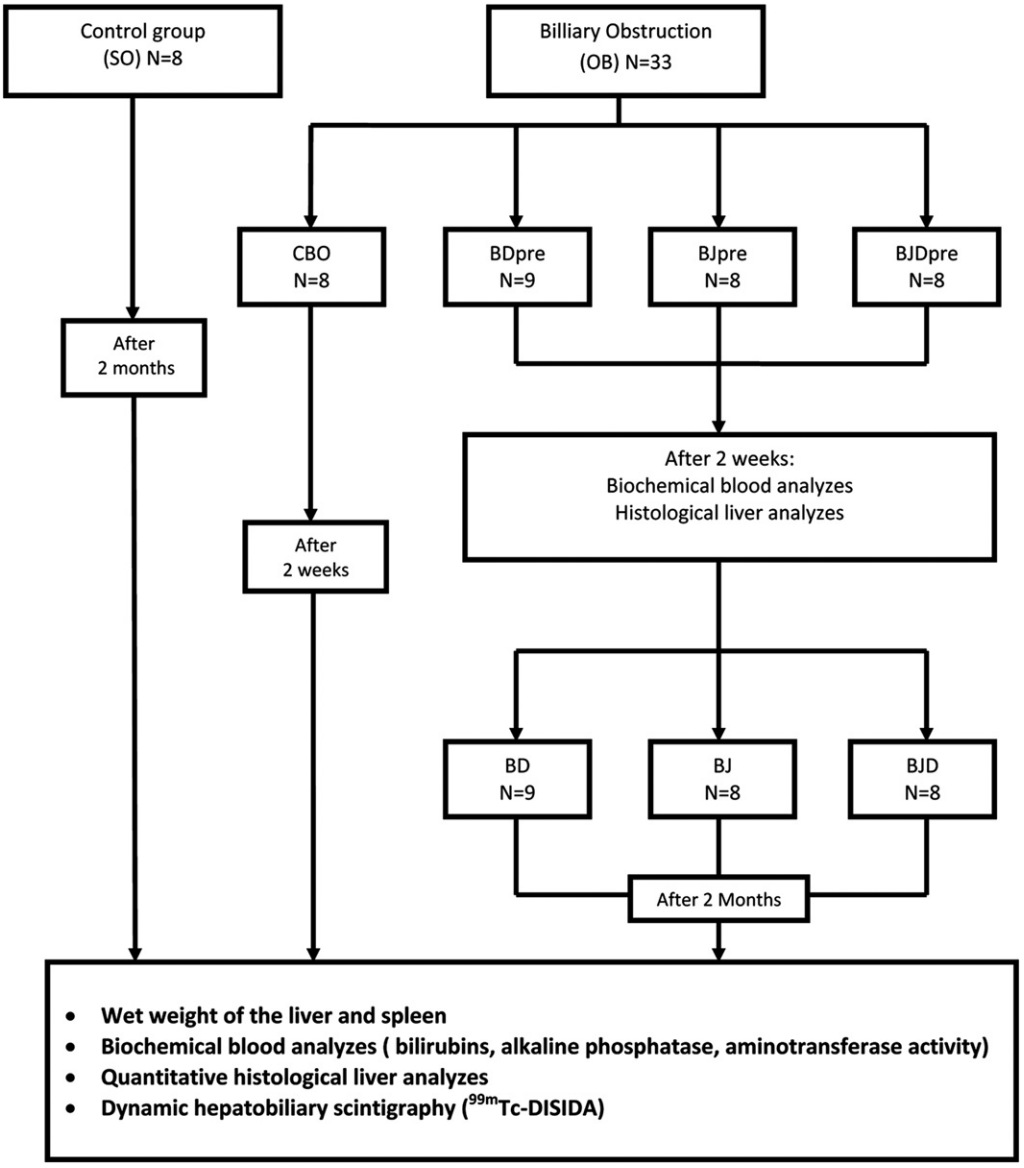

FIG. 1. Experimental design.

the graphics software KS400, ver. 2.0; Kontron Elektronik GmbH [23]. Three different structures were identified: hepatocytes, which were represented by structures colored red; fibrosis, which was represented by structures colored blue; and bile ducts, which were manually marked. We used a feature called "threshold", which allows analyses of the three channels in the RGB color spectrum. We measured the areas of the selected components and the fractions of hepatocyte volume, fibrosis, bile ducts, and other histologic elements using an automatic process. The absolute weights of the different components were calculated as the product of the volume fraction and liver weight, expressed in g/kg of body weight [5].

Morphometric analysis of the hepatic stellate cells was performed in a similar fashion with the same equipment described above. Thus, using an optical framer, 30 fields were selected at random from each slide at 256× magnification. The hepatic metabolic zone 1 area was chosen for counting the SCs using the software KS 400. The results are given as the sum of the number of SCs in each of 30 fields obtained from each slide.

Quantification of the Excretory Hepatic Function and Verification of the Permeability of the Biliary Tract with ^{99m} Tc Disida

A dose of 185 MBq (5 mCi) of the radioisotope was combined with 10 mg of Disida (N 2.6 α acetanilina diisopropyl iminodiacetic acid) and 0.3 mg of stannous chloride dihydrate in a lyophilized form under high vacuum (DISIDA-^{99m}Tc IPEN São Paulo). To study hepatobiliary excretion, we used a gamma camera (SOPHA DST collimator leap; Buc Cedex, France) in a dynamic mode.

Prior to this procedure, the rats were separated and fasted for 24 h; they also did not receive liquid for the 12 h before the procedure. After intraperitoneal anesthesia with thiopental, the animals underwent dissection of the internal jugular vein and a navel catheter (3.6 Fr × 41 cm Becton-Dickinson) was used for injection of the radiotracer. The dynamic study was immediately initiated, with the blood phase flow measured for 1 min (recorded every 2 s) and the functional phase for 30 min (recorded every 15 s) for the heart, liver, bile ducts,

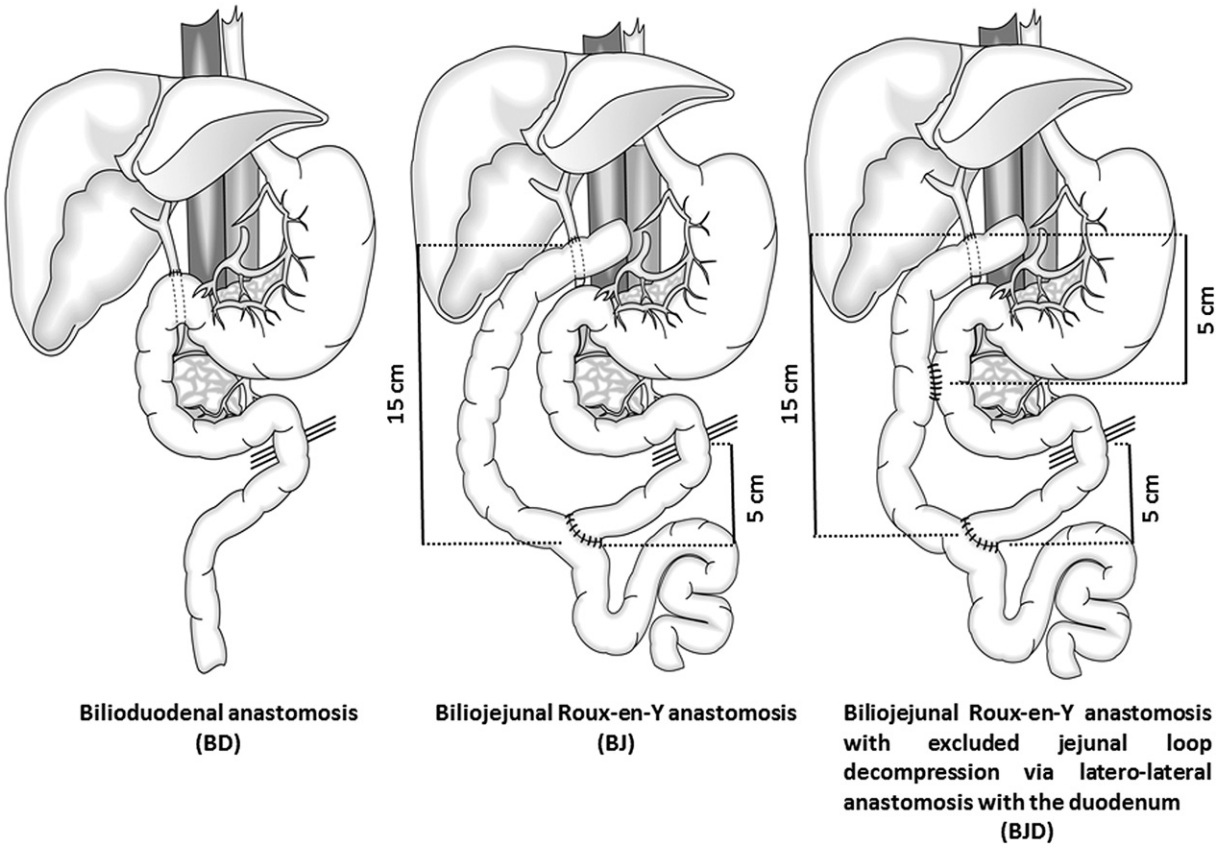

FIG. 2. Representation of the three modalities of biliodigestive bypass.

and small intestine. We assessed the time of maximum hepatic activity (TMax) and the time at which half of the radiopharmaceutical had been excreted by the liver parenchyma (T½). The 20 min/max ratio was calculated using the activity/time curve. We analyzed static images to ascertain the time at which radiotracer appeared in the small intestine (T intestine).

Statistical Analysis

A mixed-effects [24, 25] model in the PROC MIXED function of SAS software, ver. 9 (SAS Inc., Cary, NC) was used to analyze the dependent variables. We analyzed the independent variables via the ANOVA model using the PROC GLM function of SAS software, ver. 9 [26]. To compare the groups that underwent dynamic hepatobiliary scintigraphy, we generated Kaplan-Meyer graphs and adjusted them to the proportional hazards model proposed by Cox (1984) using LIFETEST and PHREG functions of SAS 9.0 [27]. The level of significance was set at 1%.

RESULTS

Blood Chemistry Analyses

The total bilirubin, AST, and ALP fractions in the CBO, BDpre, BJpre, and BJDpre groups were significantly higher than those in the SO group. After BD, BJ, and BJD, there was a significant reduction in the total bilirubin, AST, and ALP fractions compared with animals in the BDpre, BJpre, BJDpre, and CBO groups. ALT values were significantly reduced after BD and BJ compared with the CBO group. The results are presented in Table 1.

Estimated Mass of the Liver and Quantitative Microscopic Histologic Component Analyses

After biliary obstruction, there was a significant increase in the estimated mass of the liver and spleen. Following each of the three biliary bypass procedures, we saw a significant reduction of these values such that the values became equivalent to those in the SO group. The animals in the CBO group showed a significant increase over the animals in the SO group in the estimated mass of hepatocytes, fibrosis, and ducts. Among rats treated with one of the three biliary drainage procedures, there was a significant reduction in the estimated mass of the hepatocytes, fibrosis, ducts, and other histologic elements relative to the CBO group. The estimated mass of these histologic components did not differ between the treated groups and the SO group. The estimated hepatocyte mass was significantly lower only in the BD group compared with the SO group. The results are presented in Table 2.

TABLE 1

Mean Biochemical Values of Total Bilirubin (TB mg/dL), Direct bilirubin (DB mg/dL), Indirect Bilirubin (IB mg/dL), Alkaline Phosphatase (ALP U/mL), Alanine Aminotransferase (ALT U/mL), and Aspartate Aminotransferase (AST U/mL) Found in Groups BDpre, BD, BJpre, BJ, BJDpre, BJD, CBO, and SO, with Their Respective Standard Deviations

Groups	TB	DB	IB	ALP	ALT	AST
SO	0.41 ± 0.06	0.09 ± 0.03	0.32 ± 0.06	59.63 ± 6.93	39.5 ± 9.4	123.63 ± 42.4
CBO	8.76 ± 1.47	5.99 ± 1.08	2.78 ± 0.54	206.13 ± 38.79	127.5 ± 86.27	561.63 ± 156.9
BDpre	6.17 ± 1.22	3.83 ± 1.19	2.33 ± 0.46	301.22 ± 109.36	81.11 ± 34.84	383.78 ± 114.74
BD	0.41 ± 0.09	0.13 ± 0.1	0.28 ± 0.08	24.22 ± 3.23	53.33 ± 49.15	159.89 ± 90.7
BJpre	6.86 ± 1.2	4.64 ± 0.78	2.22 ± 0.74	193.63 ± 99.54	60.13 ± 22.6	348.13 ± 79.38
BJ	0.45 ± 0.09	0.11 ± 0.04	0.34 ± 0.09	28.25 ± 15.4	43 ± 25.19	189.38 ± 38.65
BJDpre	6.74 ± 1.26	3.9 ± 1.1	2.84 ± 1.57	207 ± 126.79	56.88 ± 10.83	589.13 ± 176.03
BJD	0.56 ± 0.23	0.14 ± 0.05	0.43 ± 0.23	26.25 ± 10.39	101.5 ± 78.81	190.5 ± 86.18
	*1	*1	*2	*3	*4	*5

Significant differences ($P < 0.01$) among groups: *1 = SO *versus* (CBO, BDpre, BJpre, BJDpre), CBO *versus* (SO, BDpre, BJpre, BJDpre, BD, BJ, BJD), BDpre *versus* BD, BJpre *versus* BJ, BJDpre *versus* BJD;*2 = SO *versus* (CBO, BDpre, BJpre, BJDpre), CBO *versus* (SO, BD, BJ, BJD), BDpre *versus* BD, BJpre *versus* BJ, BJDpre *versus* BJD; *3 = SO *versus* (CBO, BDpre, BJpre, BJDpre), CBO *versus* (SO, BD, BJ, BJD, BDpre), BDpre *versus* BD, BJpre *versus* BJ, BJDpre *versus* BJD, BDpre *versus* BJpre, BDpre *versus* BJDpre; *4 = SO *versus* CBO, CBO *versus* BJDpre, BD *versus* CBO, BJ *versus* CBO; *5 = SO *versus* (CBO, BDpre, BJpre, BJDpre), CBO *versus* (SO, BDpre, BJpre, BD, BJ, BJD), BDpre *versus* BD, BJpre *versus* BJ, BJDpre *versus* BJD, BDpre *versus* BJDpre, BJpre *versus* BJDpre.

Analysis of Histologic Stellate Cells

There was a significant increase in the number of stellate cells after 15 d of biliary obstruction (SO: 15.13 ± 10.56; CBO: 110 ± 30.83; BDpre: 152.22 ± 36.56; BJpre: 149 ± 36.02; BJDpre: 147.75 ± 59.47). Following treatment with one of the three modalities of biliodigestive anastomosis, the number of hepatic stellate cells was significantly reduced (BD: 41.78 ± 13.12; BJ: 51.38 ± 11.06; BJD: 38 ± 10.53) to values similar to the SO group (Figs. 3 and 4).

Analysis of Dynamic Hepatobiliary Scintigraphy with ^{99m}Tc-Disida

The radiotracer elimination occurred rapidly in the SO, BD, BJ, BJD groups, with no significant differences in TMax and $T^{1/2}$ between any of the groups. The animals of the CBO group experienced no excretion of radioactive substance into the small intestine.

The radioactive element appeared in the small intestine significantly later in the operated groups than in the SO group, but there was no difference between the BD, BJ, and BJD groups. After the biliodigestive bypass, there was a biliary flow delay, but the excretory function was not compromised. In the BJD group, the R20/Max was higher than in the SO group. The results are presented in Table 3 and the analyses of all results are presented in Table 4.

DISCUSSION

Most experimental studies evaluating the effects of biliary drainage on cholestasis and anatomopathologic lesions of chronic extrahepatic biliary obstruction in rat livers have employed one form of anastomosis,

TABLE 2

Effects of Biliary Obstruction (CBO) and of the Derivations (BD, BJ, and BJD) Compared with the Control (SO) on Relative Masses of the Liver and Spleen (g/kg) as well as the estimated mass (g/kg) of Histologic Elements of the Liver (Mean ± Standard Deviation)

Groups	SO	CBO	BD	BJ	BJD	
Hepatic mass	30.02 ± 2.38	64.8 ± 9.38	30.15 ± 3.81	29.87 ± 3.36	29.89 ± 2.85	*1
Splenic mass	2.95 ± 0.4	6.09 ± 0.65	2.63 ± 0.37	3.06 ± 0.9	3.13 ± 0.68	*1
Hepatocytes	20.52 ± 2.04	32.33 ± 4.2	16.67 ± 2.06	18.77 ± 1.52	18.4 ± 2.12	*2
Fibrosis	0.47 ± 0.18	7.19 ± 2.25	1.47 ± 0.34	1.43 ± 0.48	1.34 ± 0.52	*1
Bile ducts	0.02 ± 0.02	5.38 ± 2.48	0.07 ± 0.08	0.34 ± 0.33	0.21 ± 0.18	*1
Other histologic elements	9.02 ± 1.14	19.9 ± 3.89	11.93 ± 2.23	9.34 ± 1.33	9.94 ± 1.28	*1

$P < 0.01$ (significant difference) for group comparison: *1 = CBO *versus* (SO, BD, BJ, BJD); *2 = CBO *versus* (SO, BD, BJ, BJD) and SO *versus* BD.

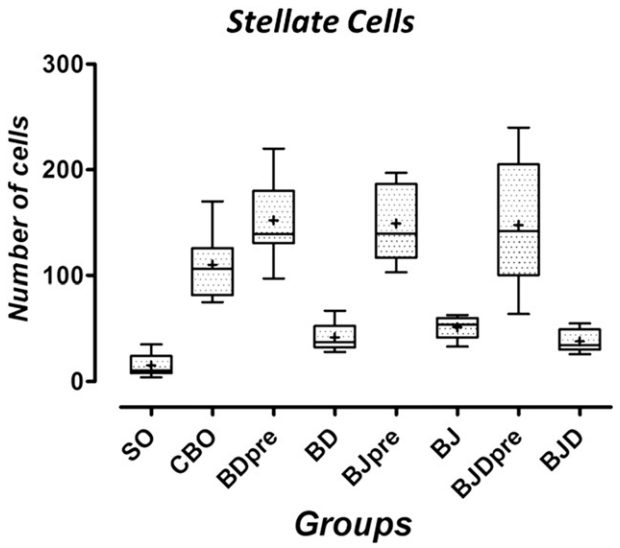

FIG. 3. Representation of the number of hepatic stellate cells in rats with biliary obstruction that underwent (CBO, BDpre, BJpre, and BJDpre), bilioduodenal anastomosis (BD), biliojejunal anastomosis (BJ), and biliojejunal anastomosis with decompression of the excluded loop (BJD), and in the control group (SO). (Median) + (Mean) significant differences ($P < 0.01$) between the groups: SO *versus* (CBO, BDpre, BJpre, BJDpre), CBO *versus* (SO, BD, BJ, BJD, BDpre), BDpre *versus* BD, BJpre *versus* BJ, BJDpre *versus* BJD.

either with the duodenum [3, 4, 11] or with the jejunum [5, 6, 28]. Only a few experimental studies have compared the effects of the two modalities of biliary bypass on the recovery of liver lesions [7, 8, 29]. These studies have demonstrated that both BD and BJ promote the resolution of cholestasis and the partial repair of anatomopathologic lesions of the liver, with recovery of the distribution of hepatocytes and regression of ductal proliferation, fibrosis, and hepatosplenomegaly.

Some of the recorded adverse effects of biliodigestive anastomosis include the occurrence of enterobiliary reflux, contamination of the biliary duct, and increased inflammation in the portal spaces, especially after BD [8, 29–31]. The few studies assessing hepatobiliointestinal flow by scintigraphy with radioactive isotopes show stasis in the excluded Roux-en-Y loop and a higher incidence of cholangitis [7, 10, 32].

Thus, enterobiliary reflux and stasis occur with the modalities of biliodigestive shunt that are most often performed, and may be associated with the residual anatomopathologic changes of the liver after effective biliary bypass. Therefore, studies should be performed to assess the comparative effects of different modalities of bilioenteric shunt to help select the most appropriate biliary bypass technique, especially in the era of video laparoscopy, where BD is frequently used [7].

Decompression of the excluded jejunal loop through a duodenojejunal anastomosis after BJ was proposed to minimize the enterobiliary reflux and stasis in the excluded Roux-en-Y loop [10]. We conducted the present study to identify the possible benefits of this modality of biliary derivation for reducing contact between the enteric content and the bile duct and for reducing stasis in the excluded Roux-en-Y loop.

In this study, the method used to cause bile duct [19] obstruction ensured adequate dilation of the biliary tree in most cases, permitting bilioenteric anastomosis and the utilization of 85.3% of the animals. The postoperative mortality was high (BD: 30.7%, BJ: 63%, and BJD: 75%) and was associated with the duration of surgery and the levels of cholestasis, as the surviving animals had levels of total and direct bilirubin that were lower than those of the CBO group. The elevated and varied coefficients of mortality, especially in the early postoperative period, may have been related to different degrees of hepatocellular lesions caused by biliary obstruction [8], the anesthetic agents utilized, and the phenomena of ischemia and reperfusion of the liver after biliary decompression [30, 33].

In this study, the alterations resulting from biliary obstruction were well characterized. Such obstruction was associated with an increase in canalicular enzymes, an absence of radioactive substance excreted into the small intestine, hepatosplenomegaly, a reduction in the proportional volume of hepatocytes, an intense proliferation of bile ducts, and liver fibrosis. These changes characterize secondary biliary fibrosis with portal hypertension.

The quantitative evaluation of the observed hepatic lesions by morphometric analyses of histologic components showed similarities between the CBO, BDpre, BJpre, and BJDpre groups (Table 5). In this analysis, we observed that hepatomegaly was due to increased hepatocyte mass and fibrosis (7.19 ± 2.25 g/kg) that prevailed over duct mass (5.38 ± 2.48 g/kg).

After the three modalities of biliodigestive drainage, there was a significant reduction in the serum levels of TB and its fractions, as well as those of AST and ALP, reaching values similar to those observed in the SO group. There was no significant elevation of ALT in the groups undergoing biliary bypass compared with the SO group. This finding, coupled with the absence of recorded hepatocellular necrosis in similar experimental models, strengthens the role of biliary obstruction as the cause of the described lesions [5, 8, 34].

Our examination of hepatobiliary excretion showed that bilioenteric anastomoses were patent after the three modalities of biliary shunt. Hepatobilio-intestinal flow recovered, as shown by the normalization of the parameters of the dynamic hepatobiliary scintigraphy (TMax, T½) and the appearance of radiotracer in the small intestine. Although there was a roughly 40-s delay in the appearance of radiotracer in the small intestine of rats submitted to biliary bypass, the recovery of TMax and

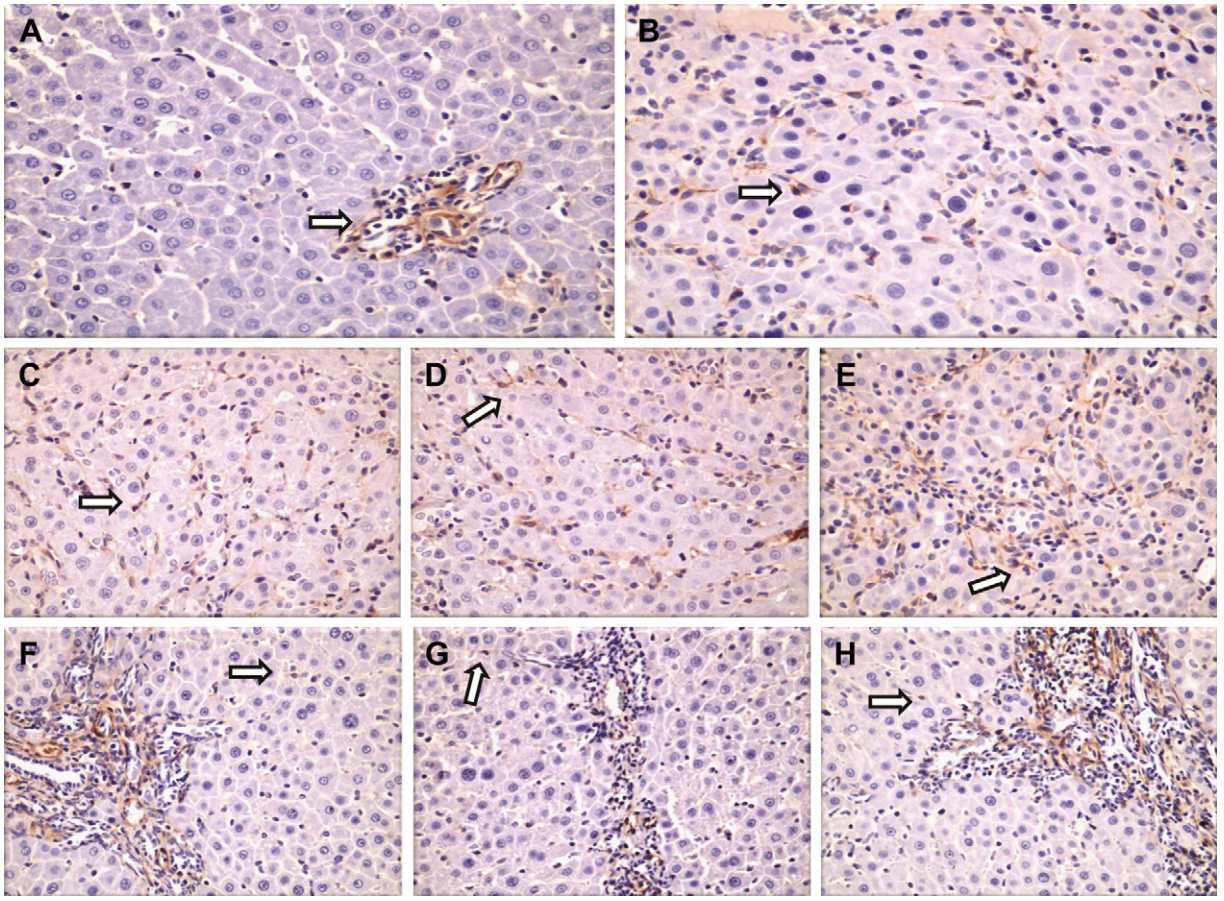

FIG. 4. Slide obtained from histologic sections of the liver with 400× magnification; arrows indicate hepatic stellate cells stained by anti-desmin antibody with significant reduction after the three modalities of biliary shunt. Groups: SO (A); CBO (B); BDpre (C); BJpre (D); BJDpre (E), BD (F), BJ (G), and BJD (H).

T½ shows that there was no impairment of excretory hepatic function; these results contrast with those of other studies [7, 10, 32].

The R20/Max often used to evaluate renal excretory function was calculated, taking the liver as a region of interest in which to identify possible residual cholestasis [35, 36]. In this study, after BJD complete recovery of R20/Max (BJD: 0.21 ± 0.05 *versus* CO: 0.12 ± 0.01) did not occur, despite the recovery of TMax and T½ and visual evidence of the radiotracer in the small intestine at times similar to those for BD and BJ. This disparity may result from disorders of the migratory motor complexes [9, 37, 38] that generate stasis of the excluded jejunal loop and have already been

TABLE 3

Time Values for Hepatic Elimination of Half of the Radiotracer (T½), Maximum Hepatic Activity (TMax), Expressed in Minutes, Time of Appearance of Radioisotope in the Small Intestine (T Intestine), Expressed in Seconds, Obtained After Injection of ^{99m}Tc-DISIDA Intravenously in Different Groups (Mean ± Standard Deviation)

Groups	SO	CBO	BD	BJ	BJD	
T intestine	93.75 ± 6.94	NE	145 ± 16.77	131.25 ± 10.61	151.13 ± 29.95	*1
TMax	1.5 ± 0	8.51 ± 4.48	2.03 ± 0.32	2.13 ± 0.38	2.19 ± 0.26	*2
T½	4.31 ± 0.42	127.42 ± 86.43	6.03 ± 0.92	6.66 ± 1.45	6.94 ± 1.09	*2
R20/Max	0.12 ± 0.01	0.91 ± 0.07	0.17 ± 0.02	0.16 ± 0.06	0.21 ± 0.05	*3

NE = no excretion of radiotracer during the studied period.

$P < 0.01$ (significant difference) among groups: *1 = CBO *versus* (SO, BD, BJ, BJD); SO *versus* (BD, BJ, BJD); *2 = CBO *versus* (SO, BD, BJ, BJD);*3 = CBO *versus* (SO, BD, BJ, BJD), SO *versus* BJD.

TABLE 4

Evolution of Cholestasis, Liver Lesions, and Hepatobiliointestinal Flow After Three Modalities of Biliary Bypass

Groups	Anatomo-pathologic liver recovery	Biochemical markers of cholestasis	T intestine	Relative biliary stasis by Disida	Recovery of the number of stellate cells
BD	P	C	P	C	C
BJ	C	C	P	C	C
BJD	C	C	P	P	C

C = complete recovery after biliary bypass; P = partial recovery after biliary bypass; biochemical markers of cholestasis = TB, DB, ALP.

demonstrated in previous studies conducted in our laboratory [7], and possibly the greater presence of enterobiliary reflux.

The interposition of a jejunal loop of insufficient size between the duodenum and the bile duct may facilitate enterobiliary reflux and residual stasis after BJD. One clinical evaluation found that the use of an intermediary loop of 20 cm in patients undergoing BJD was not accompanied by enterobiliary reflux [39]. In this study, we used an interposed jejunal loop of 5 cm, which corresponds to a length of 13 cm in humans [40]. The decision to use this loop length was based on the technical ease of performing the anastomosis, considering the distance between the dilated bile duct and duodenum, as well as the findings of a previous study where no differences were found in the recovery of hepatic lesions or the induction of reflux between BJ with Roux loops of 5 or 10 cm [29]. However, duodenojejunal anastomosis performed 5 cm from the biliary anastomosis could allow backflow of the enteric content into the biliary duct and cause transitory obstruction with food debris in the bile duct [41, 42].

This study did not quantitatively evaluate the enterobiliary reflux, but it is likely that with BD, such reflux will be more intense than with BJ and BJD; this would explain the partial recovery of the hepatic lesions, as has been demonstrated previously [8]. The absence of bile flow obstruction and residual cholestatic as assessed by scintigraphic and biochemical modalities suggest that stasis due to enterobiliary reflux occurs transiently and intermittently with low pressure in the bile ducts [7].

Several mechanisms may contribute to the development of portal hypertension in chronic extrahepatic cholestasis [43]. The deposition of collagen in the Disse's space and edema of the hepatocytes can lead to distortions, with sinusoidal narrowing and increased hepatic vascular resistance [44]. Hyperplasia of the hepatic stellate cells can, by itself or by contraction of the dendritic processes, lead to retraction of the sinusoidal bed and increase the vascular resistance of the liver [45]. The hepatic stellate cells are among the main sources of fibrotic deposition, especially of type I collagen, in the extracellular matrix [46].

Animals with 2 wk of biliary obstruction had a significant increase in stellate cells, in addition to their increases in fibrosis and bile duct mass. Each of the three modalities of biliary bypass promoted a similar reduction in the number of stellate cells, which was accompanied by a diminution of the fibrosis and bile duct mass in addition to the recovery of the estimated splenic mass. In contrast, some studies have emphasized a delayed regression of portal hypertension, indicating a mismatch between histologic recovery and portal flow repair [5, 6, 47]. The reason for this delay is not fully understood, but it may be due to the persistence of hepatic stellate cell proliferation [48]. The present results, as well as those of other studies, reinforce the association between the apoptosis of stellate cells and the resolution of hepatic fibrosis [49, 50, 51].

Biliary obstruction increased the estimated mass of the hepatocytes, bile ducts, fibrosis, and other histologic components (represented mainly by inflammatory cells). The two modalities of biliary bypass that separated the enteric content from bile duct (BJ and BJD) resulted in complete recovery of all histologic components to values similar to those of the SO group. In the BD group, however, despite some lessening of the mass of the fibrosis, ducts, and other histologic elements, the mass recovery of hepatocytes was incomplete relative to the SO group.

This incomplete anatomopathologic recovery in BD may be related to the presence of enterobiliary reflux, contamination of the bile duct and maintenance of inflammation by episodes of cholangitis. This phenomenon has been experimentally demonstrated by culturing bile and directly inspecting the anastomosis. Food debris and fur were observed to occupy the anastomoses, the bile duct, and the afferent loop of the BJ, sometimes including the formation of trichobezoar in the bile ducts of rats subjected to bilioduodenal anastomosis [8].

TABLE 5

Volume Fractions of Hepatocytes, Fibrosis, Bile Ducts, and Other Histologic Elements (Mean ± SD) for Groups Underwent Biliary Obstruction

Groups	CBO%	BDpre%	BJpre%	BJDpre%	
Hepatocytes	50 ± 4	41 ± 6	46 ± 3	45 ± 6	*1
Fibrosis	11 ± 2	12 ± 4	11 ± 4	12 ± 4	
Bile Ducts	8 ± 3	6 ± 3	7 ± 4	6 ± 1	
Other histologic elements	31 ± 4	41 ± 1	35 ± 5	31 ± 14	*2

$P < 0.01$ (significant difference) among groups: *1 = CBO *versus* BDpre; *2 = CBO *versus* BDpre, BDpre *versus* BJDpre.

Studies of hepatobilio-intestinal flow and enterobiliary reflux after biliary derivation are scarce. There are quantitative clinical records of excluded Roux-en-Y loop stasis [10, 32], and experimental records of hepatobilio-intestinal flow evaluated by scintigraphy in our laboratory confirm those earlier clinical findings [7]. Furthermore, quantitative assessment of the reflux by means of labeled substances may be more complex and does not represent what has been shown in clinical and experimental findings.

In summary, the biliary obstruction technique that was used in this study produced hepatic changes consistent with chronic extrahepatic cholestasis and secondary biliary fibrosis. The three modalities of biliodigestive anastomosis employed reversed biliary cholestasis, chronic hepatic lesions, and splenomegaly, but BJ had the best performance in the maintenance of hepatic excretory function and in the repair from liver lesions resulting from chronic biliary obstruction. Thus, technical alternatives that minimize the possible adverse effects related to bilio-intestinal anastomosis should be employed to ensure the repair of alterations resulting from biliary obstruction and protect the liver against additional lesions associated with the biliary bypass.

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