ORIGINAL RESEARCH

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A new model of reversible obstructive jaundice using rapidly absorbable suture materials

Abstract

Purpose: Reversible obstructive jaundice models have some limiting features, including the need for a second anaesthesia, re-laparotomy and surgical intervention after common bile duct ligation. The present study investigates the feasibility of a new application that can eliminate these limitations. Rapidly absorbable suture materials were used for ligation; therefore, spontaneous biliary decompression was anticipated by the self release of these rapidly degrading materials.

Methods: Common bile ducts in Wistar Albino rats were ligated with silk, polyglytone 6211, or irradiated polyglactine 910 (n=7 for each group). Rats were grouped according to both the suture materials and the experiments termination date: 5 days (sham, silk5, polyglytone5, polyglactine5) and 21 days (silk21, polyglytone21, polyglactine21) after the ligation. Biochemical and morphologic changes of liver were assessed.

Results: The group polyglactine21 showed significantly lower mean ALT, AST, GGT, total and direct bilirubin values when compared with the group polyglactine5 (p=0.004-0.037). Morphologic changes did not correlate with the biochemical amelioration. In the group polyglytone21, not only the biochemical but also the morphologic changes significantly ameliorated when compared with the group polyglytone5 (p=0.003-0.043). No procedure associated mortality was observed.

Conclusion: Common bile duct ligation with polyglytone offers a new reversible model for prolonged obstructive jaundice which abolishes the need for relaparotomy and a second surgical intervention and significantly reduces mortality.

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Obstructive jaundice (OJ) is a common health problem that has pathophysiological effects on several organs. Numerous clinical and experimental studies have been conducted on the mechanisms and control of damage to organs [1, 2]. In order to form an experimental OJ model, the common bile duct (CBD) is ligated, usually to form an irreversible OJ; however, with this experimental model is not possible to observe reversal of liver damage. To investigate the liver regeneration process, a reversible obstructive jaundice (ROJ) model would be necessary. Moreover, the ROJ model imitates jaundice due to bile stone obstruction. A limited number of studies on the ROJ model are presented in the literature; however, each of these models has distinct limitations [3-8]. For example, a second laparotomy and a new surgical intervention are required for the biliary decompression model, and high mortality is observed.

The present study investigates the feasibility of a new ROJ model that would eliminate these limitations. In this model, rapidly absorbable suture materials are used to ligate the CBDL and spontaneous biliary decompression is anticipated, following the self-release of these rapidly degrading suture materials.

Methods

For this study, approval was obtained from the University Animal Research Local Ethics Committee (protocol no: 2009/16). The experiments and care of the animals were undertaken in the Experimental Research Center of the university. Wistar Albino rats weighing between 250-300 grams were used. The animals were fed and taken care of in accordance with the national regulations and guidelines. Likewise, the experiments were conducted following the same features and institutional standards.

Experimental design

With exception of the sham group, CBDs were ligated with silk, polyglytone 6211 (PGL), and irradiated polyglactine 910 (IrPG). Sutures (6-0 PGL and 8-0 IrPG) were the thinnest suture materials commercially available in order to minimize the tissue reaction and to accelerate the degradation of the suture materials. The experiments were grouped as follows: the animals terminated on the fifth day of the CBDL (sham, Silk5, PGL5 and IrPG5 groups) and the animals terminated on twenty-first of the CBDL (Silk21, PGL21 and IrPG21 groups). There were five rats in the sham group and seven in each of the other groups.

Characteristics of the Suture Materials

PGL is a synthetic, monofilament and rapidly absorbable material that causes minimal tissue reaction [9]. It loses 50%-80% of its tensile strength within 2 weeks and its total tensile strength within 3 weeks [10,11]. Although they do not constitute significant factors in the present model, bacteria and pH also cause this suture material to lose its tensile strength. Degradation is completed through hydrolysis within 8 weeks [11].

The second rapidly absorbable suture used, polyglactine 910, is a synthetic multifilament material. The inflammatory response to polyglactine 910 in soft tissue is less severe than that of catgut, silk or polypropylene [12]. The degradation of normal polyglactine 910 is through hydrolysis and the process is accelerated in acidic and basic environments [13,14]. Normal polyglactine 910, loses approximately 50% of its tensile strength within 2 weeks and 93% within 3 weeks [15]. The polyglactine 910 used in the present study is irradiated polyglactine 910 and is different from normal polyglactine 910 in that the gamma radiation shortens the hydrolysis period even more, so that when applied intraorally it degrades after 14 days [16].

IrPG causes minimal reaction in tissue. The filaments in this suture material start decomposing at the end of the first week and its braiding disintegrates completely by the end of the second week [17].

Operative procedure

Rats were anaesthetized before the operation by intramuscular administration of 40 mg/kg ketamine HCl and 10 mg/kg xylazine HCl. The anterior abdominal wall was disinfected by applying 10% povidone iodine standardized solution. Laparotomy was performed with an upper midline incision of 3 cm in length. The CBD was exposed by gently pulling the duodenum to the front and downward. In all the rats, atraumatic needles were passed through the posterior of the CBD without dissection. With exception of the sham group, the relevant suture materials were knotted three times in all groups. Next, the abdomen was closed with an interrupted 3-0 silk suture. On the day of the termination, animals were prepared according to the surgical procedure described above and sacrificed by withdrawing blood.

Biochemical evaluation

Serum levels of, ALT, AST, GGT, ALP, total and direct bilirubin levels were measured.

Table 1. Mean serum biochemical values

	ALT (U/L)	ALP (U/L)	T bili (μmol/L)	D bili (μmol/L)
Sham	56.4 (5.66)	93 (13.23)	1.74 (0)	1.71 (0)
Silk5	165 (16.61)	263.52 (29.04)	222.81 (18.63)	171.51 (13.68)
Silk21	89.83 (15.63)	273.2 (25.92)	73.70 (33.34) [§]	54.54 (25.30) [§]
PGL5	179.57 (22.75)	272.85 (17.50)	232.73 (40.86)	178.18 (30.43)
PGL21	94.16 (9.35) [†]	261 (7.92)	4.96 (1.88) [†]	3.24 (1.02) [†]
IrPG5	182.16 (29.64)	272 (28.75)	257.69 (18.12)	193.91 (13.33)
IrPG21	94.33 (5.99) [‡]	195.66 (23.36)	17.61 (13.85) [‡]	13.16 (10.60) [‡]

Values in () represent (±SEM). §, †, and ‡ represent $p < 0.05$ between Silk21 and Silk5, between PGL21 and PGL5, and between IrPG21 and IrPG5, respectively

Table 2. Mean HAI and category scores

	Total HAI	Category I	Category II	Category III	Category IV	Bile duct pro.
Sham	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Silk5	8.71 (1.20)	2.00 (0.48)	2.42 (0.36)	2.14 (0.40)	2.14 (0.40)	2.71 (0.28)
Silk21	9.66 (2.57)	3.83 (1.47)	0.83 (0.47) [*]	2.50 (0.50)	2.50 (0.50)	1.83 (0.40)
PGL5	12.14 (1.10)	4.42 (0.57) [*]	1.71 (0.47)	3.00 (0.37)	3.00 (0)	2.71 (0.18)
PGL21	6.16 (1.44) [§]	2.33 (0.66) [§]	0.50 (0.22) [§]	1.66 (0.42) [§]	1.66 (0.42) ^{§†}	1.16 (0.16) [§]
IrPG5	12.33 (0.61)	4.16 (0.40) [*]	1.66 (0.42)	3.50 (0.22) [*]	3.00 (0)	2.33 (0.21)
IrPG21	11.33 (1.97)	4.50 (1.36)	1.16 (0.60)	2.83 (0.40)	3.00 (0)	1.66 (0.42)

Values in () represent (±SEM). Categories I, II, III, and IV are the components of total HAI. Category I: Periportal ± bridging necrosis. Category II: Intralobular degeneration and focal necrosis. Category III: Portal inflammation. Category IV: Fibrosis. Bile duct pro.: Bile duct proliferation. *, §, and † represent $p < 0.05$ between the marked groups and Silk5, between PGL21 and PGL5, and between PGL21 and IrPG21, respectively

Histopathological evaluation

The liver tissue and the ligated section of CBD were examined histopathologically. After fixation in 10% formalin solution, the tissues were embedded in paraffin and sections of 5 μm thickness were prepared. Sections were stained with hematoxylin-eosin, and evaluated under a light microscope. The "Histological Activity Index" was used to evaluate liver tissue damage [18].

Statistical analysis

For data analysis, SPSS 17 for Windows (SPSS Inc., Chicago, Illinois, USA) was used. Statistical significance was set as $p < 0.05$. When the Kruskal-Wallis test revealed significant dif-

ferences between the groups, the Mann-Whitney U test was used to compare pairs of groups.

Results

One rat from the group Silk21 died due to intraabdominal infection. Two rats from the groups PGL21 and IrPG21 were excluded due to wounds on the back and feet and intraabdominal abscess. In the Silk21, IrPG21 and PGL21 groups, 4, 3, and 1 rats respectively were observed to be jaundiced. In the 5 day groups, the CBD of the rats had dilated up to 5 mm. In the 21 day groups, diameters of the CBDs were between 3 and 20 mm. This dilatation was measured to be highest in the silk suture group and lowest in the group PGL21.

FIGURE 1. Morphologic changes of the liver by the 5th day post-surgery: A: Moderate portal inflammation and portal fibrosis, B: Moderate portoportal bridging necrosis, portal fibrosis, portal inflammation and parenchymal lytic necrosis (hematoxylin-eosin x200). Morphologic changes in the PGL21 group C: Mild bile duct proliferation, D: Mild portal inflammation, bile duct proliferation, and central vein dilatation (hematoxylin-eosin x100)

OJ progressed in all the 5 day groups. Severity of the biochemical and the morphologic changes were similar among these groups. OJ also progressed in the 21 day groups but was not as severe as in the 5 day groups. However, mean AST, GGT values and focal necrosis scores were found to be close to those of the sham group. The biochemical results are presented in Table 1.

The 5 day groups and the 21 day groups were compared, matching the same suture materials. Mean total and direct bili-

rubin values in the group Silk21 were lower than the group Silk5 ($p \leq 0.007$); other parameters were similar. The group IrPG21 also showed significantly lower means of ALT, AST, GGT, and total and direct bilirubin values compared with the group IrPG5 ($p = 0.037-0.004$). Morphologic changes did not correlate with the biochemical amelioration. The histopathological evaluation of the liver samples is presented in Table 2.

In the 5 day groups, intralobular degeneration and focal necrosis were mild while periportal inflammation, periportal $\pm$ bridging necrosis, portal fibrosis were moderate in severity (Figure 1A, B). Cirrhosis did not develop in any of the rats. Among the 21 day groups, morphologic changes were significant in the PGL21 group. Compared to the PGL5 group, the HAI score was found to be lower in the PGL21 group ($p=0.010$). Severity of periportal inflammation, periportal $\pm$ bridging necrosis and intralobular degeneration and focal necrosis showed a significant decrease in the PGL21 group compared with the PGL5 group ($p=0.043$, $p=0.033$ and $p=0.027$ respectively). Moreover intralobular degeneration and focal necrosis were not detected in some rats in the PGL21 group. Portal fibrosis regressed only in the PGL21 group ($p=0.013$). Bile duct proliferation was moderate in the Silk21 and IrPG21 groups while it significantly regressed in the PGL21 group compared with PGL5 group ($p=0.002$) (Figure 1C, D). Liver injury progressed to cirrhosis in two rats of the Silk21 and IrPG21 groups.

The most important finding of this study is that in the group PGL21, not only the biochemical but also the morphologic changes significantly ameliorated compared to the group PGL5 ($p=0.043-0.003$). Biochemical and morphologic changes were not in evidence among the 21st day groups. In the ligated area of the CBD, inflammatory cells and fibrosis were observed; however, there was no significant difference among the CBDL groups for these parameters. No necrosis was detected at the ligation site.

Discussion

An experimental model is expected to be adaptable to the clinic, to be easy to implement, to be effective and to have a low mortality. CBDL is a suitable model to form OJ, a disease which causes permanent obstruction. In order to observe the liver's morphologic and functional damage reversal processes, there are only a limited number of ROJ models. In the existing models, a second anaesthesia, relaparotomy and other surgical procedures are necessary after CBDL, which raises mortality to up to 50% [3-8].

Kirkland et al., reported to achieve biliary drainage again by opening the mini clips that they had placed on the CBD [4]. Likewise, Yu et al. proposed a similar application that they called a mini-occluder [5]. On the other hand, Oruç et al. ligated a longitudinally cut vessel cannula covering the CBD, and then achieved biliary decompression by cutting the knot over the cannula [8]. The studies conducted by Kirkland and Oruç reported a decrease in the serum ALP and total bilirubin levels without mentioning liver enzymes. Kirkland et al. re-

ported that the bile duct proliferation and periportal fibrosis dramatically regressed in the liver tissue; however, the efficiency of the model was as low as 37%. Oruç et al. observed regressions in only three (portal and central venous dilatation, and parenchymal focal necrosis) of the seven histopathological parameters after the decompression. Although technically easy to apply, these methods require relaparotomy and surgical procedures. Moreover, the high mortality percentages cannot be ignored.

In two different studies, both Posner et al. and Roggin et al. reported that they suspended the CBD by a vessel loop to the abdominal wall [6,19]. After a five-day ligation, following a second anaesthesia, the loop was located, cut and withdrawn, and thus biliary decompression was achieved. Both researchers reported biochemical and morphologic recovery of the liver; however, in the study by Roggin, the mortality rate was not mentioned [19]. Due to the increased probability of the CBD injury during surgical procedures and withdrawal of the vessel loop, the frequency of the mortality can be expected to increase.

Rodriguez-Garay et al. ligated the CBD by a cannula alongside and that after 7-10 days they removed the cannula by pulling it off the knots to achieve decompression [20]. In this manner, they demonstrated the possibility of forming a reversible, mild CBD stenosis. In the study, biliary decompression was observed for a short time period of an hour and reversal of liver damage was not presented.

Aronson et al. achieved biliary decompression through choledochojunostomy [7]. On the other hand, Li et al. achieved the external and internal drainage of the bile using a modified method of placing a catheter into the CBD. Although they reported a functional and morphologic recovery of the liver, they did not present a statistical analysis of the histopathological evaluation [3]. Using Li's method, relaparotomy and serious surgical procedures are also required to achieve decompression.

In the present study, to provide a new model, two different rapidly absorbable suture materials were used for the CBDL. The sutures were expected to degrade rapidly, resulting in spontaneous opening of the CBD and recovery of the OJ. The CBDs were degraded effectively with all of the suture materials used in the study, which is demonstrated by the biochemical and the morphologic assessments. The CBD dilatation and liver damage were less in the groups that were ligated with the rapidly absorbable sutures compared with the group ligated with silk suture; however, no significant biochemical and morphologic differences were observed among these groups. The major finding of this study is that the group PGL21 showed

significantly better biochemical and morphologic results compared with the group PGL5. Nevertheless, the liver regeneration was still significantly different from the sham group. This is probably due to the limited time left for the liver regeneration after the self release of the PGL. Moreover, the speed of the regression of the inflammation and the fibrotic process in the CBDL area may play a role. Inflammation and the fibrotic process occur around the CBD and chronic liver damage progresses through fibrosis after 7 days of CBDL [21,22]. Therefore, the reversibility of the anatomic bile drainage and liver function recovery might be insufficient.

There are studies reporting that after 14 days of jaundice, when decompression is achieved through choledochojejunostomy, the collagen deposits accumulated around the portal area, the proliferated bile ducts and the apoptosis all decrease by the 7th day [23]. Abdel-Aziz et al. also observed that all the morphologic changes caused by a 14-day prolonged jaundice, including the periportal changes, reversed within 2-3 weeks' time after the biliary decompression [24]. Likewise, Aronson et al. reported the regression of the liver parenchyma damage (except for collagen deposition) following the decompression, after more than 3 weeks of prolonged jaundice [7].

It has been suggested that at least 7 days must elapse after the decompression for the liver damage to regress [23]. It appears that the use of biliodigestive anastomosis results in a faster and more effective recovery of the liver parenchyma than the anatomic drainage [7,8]. The most important reason for this is probably that biliodigestive anastomosis results in more effective biliary decompression. Regression of the liver damage in the presence of inflammation and fibrotic process around the CBD appears to be a new subject for further studies on anatomic decompression models.

In conclusion, the group PGL21 significantly showed better biochemical and morphologic results compared with the group PGL5. As a limitation, the termination point of the experiment needs to be set up at a period longer than 21 days in order to leave time for the complete tensile strength loss and self release of the PGL suture that leads to more significant liver regeneration.

CBDL with PGL offers a new, reversible model for prolonged, obstructive jaundice that eliminates the need for re-laparotomy and extra surgical procedures and significantly reduces mortality.

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