Original Article

Postoperative anastomotic bile duct stricture is affected by the experience of surgeons and the choice of surgical procedures but not the timing of repair after obstructive bile duct injury

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Abstract: Bile duct injury (BDI) is one of the most severe complications of biliary operation. This study is to investigate the correlation between the timing of bile duct repair and anastomotic bile duct stricture. Transverse BDI models were constructed in 60 dogs that were divided randomly into BDI₁₀, BDI₁₅, BDI₂₀, and BDI₃₀ groups according to days of injury (5, 10, 15, 20, and 30 days). The morphological and histological changes of anastomotic stoma of hepaticojejunostomy (HJ) were observed after bile duct reconstruction. TGF-β1, α-SMA, and collagen of anastomotic stoma were detected. After HJ, the concentration of direct bilirubin decreased significantly, dropping to 50% after one week, and returning to normal levels after three weeks. The anastomotic diameter shrunk from 1.5 cm to 0.6 cm without significant difference. At 3 months and 6 months after HJ, the expression of TGF-β1 in the anastomotic tissue in BDI₁₀ group was higher than that in BDI₁₅, BDI₂₀, and BDI₃₀ groups. However, no significant differences were observed (F = 1.282, P > 0.05 at 3 months; F = 1.308, P > 0.05 at 6 months). Similarly, the expression of α-SMA and collagen did not vary significantly. For obstructive BDI, repairing time is not a relevant factor for postoperative anastomotic stenosis, but surgeons and operation methods are the key factors. For patients with BDI, hospitals should focus on the experience of surgeons and the choice of operation methods in order to achieve a good long-term effect.

Keywords: Iatrogenic bile duct injury, surgery, anastomotic stenosis, hepaticojejunostomy

Introduction

Iatrogenic bile duct injury (BDI) that mostly occurs in laparoscopic cholecystectomy (LC) is one of the most severe complications of biliary operation. In China, laparoscopic technique is developing rapidly in primary hospitals that have bad medical environments. As a result, the incidence of iatrogenic bile duct injury increases, with transverse injury of the bile duct accounting for most of the damages. Bile duct injury not only brings significant morbidity to patients, but also has negative impacts on doctors and the society, because every case of biliary injury can give rise to a medical lawsuit [1]. Therefore, it is important to study how to achieve good BDI reconstruction and long-term outcomes. The timing of repair, the pattern of operation, and the experience of surgeons are main factors for a good reconstruction of bile duct injury [2]. Currently, the optimal timing of repair (especially for transverse injury of bile duct) is a controversial factor, especially for injuries that are initially unrecognized [3]. The main reason for the controversy is that the relationship between the timing of repair and anastomotic bile duct strictures is not clear. Our previous experiments demonstrated that 10-20 days after transverse injury of bile duct is the best timing for repair according to surgical difficulty and the compensation of the liver [4], but its influence on the anastomotic bile duct stric-
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The nature of anastomotic stricture is the hyperplasia of scar. The healing process of anastomotic stoma in the hepaticojejunostomy (HJ) is a scar healing process, in which scar-related factors such as transforming growth factor (TGF)-β1, collagen fiber and α-smooth muscle actin (SMA), play important roles. In this study, scar-related factors of anastomotic bile duct stoma are observed in different timing after HJ to explore the relevance between anastomotic bile duct stricture and the timing of repair, in order to provide an experimental basis for the future treatment of bile duct injury.

Materials and methods

Animals

Sixty mongrel dogs weighing between 12 and 15 kg, regardless of gender, were obtained from the Experimental Animal Center of Anhui Medical University. The dogs were breeding at 22-25°C in 40% to 60% humidity with fresh water and food. All experimental protocols for animal studies were approved by the Animal Experimentation Committee of Anhui Medical University, following the guidelines established by the National Science Council of China.

Dogs with BDI were randomly divided into five groups of 12 dogs according to days of injury (5, 10, 15, 20, and 30 days) and named as BDI_5, BDI_10, BDI_15, BDI_20, and BDI_30 groups. The data of BDI_0 group (control group) were obtained from the dogs of BDI_5 group before injury.

After 12 h fasting and 4 h deprivation of water, the dogs were anesthetized with intraperitoneal injection of 2.5% thiopental (25 mg/kg). After the dogs were prepared with povidone iodine and draped with sterile towels, subcostal incision was performed. The cystic duct and the distal end of the common bile duct were ligated and the diameter of common bile duct was measured. Then, the abdomen was closed layer by layer. To prevent infection, penicillin (800,000 units) was administered via intramuscular injection twice a day for consecutive 3 days.

Sample collection

Before the operation, and one week, two weeks and three weeks after both operations, blood samples were collected for the detection of liver function using direct bilirubin (Beyotime, China) on automatic biochemical analyzer (Hitachi, Japan) according to manufacturer’s instructions.

Tissue sections of anastomotic stoma were collected at different times. The observation time was one, three, four and six months after HJ in each group.

Morphological and histological observation

At the time of one, three, four and six months after HJ, three dogs in each group underwent euthanasia, and their abdomens were opened for the observation of the anastomotic stoma between bile duct and jejunum. The diameter of anastomotic stoma was measured using a ruler. The change of histology of the anastomotic stoma was observed using immunohistochemistry.

Immunohistochemistry

Streptavidin-peroxidase (SP) method was performed on the Ventana NexES automated stainer according to the manufacturer’s protocol. Primary antibodies of anti-TGF-β1, α-SMA and collagen fiber (Santa Cruz, USA) were used at 1:50. SP kits were purchased from Zymed, Co. (USA). Immunohistochemistry was examined and images were taken using OLYMPUS BX51 microscope (Tokyo, Japan) at 1:100. The scoring was independently assessed by two pathologists. VIDAS image analysis (OPTON, Co., Germany) was performed to measure TGF-β1 and α-SMA expression. Positive cells were stained with claybank, and a quantitative method was used to evaluate the number of positive cells. Masson’s trichrome staining and Mass image analysis (OPTON, Co., Germany) were used for the detection of collagen fiber.
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Figure 1. Changes of the levels of direct bilirubin (DBIL) after HJ. Blood samples were collected from dogs with BDI before the operation, and one, two and three weeks after the operation. Histograms represent averages of at least 3 measurements of DBIL concentration. Data are expressed as means ± SD. *, P < 0.05 (Fisher’s exact test) compared with values before HJ at each time point.

Anastomotic tissues from each group were stained and 6 fields were selected for imaging using light microscopy, where collagen staining was shown in blue green. The optical density of all fields was measured for comparison.

Statistical analysis

All statistical analyses were performed using SPSS11.0 software. Pearson’s χ² test or Fisher’s exact test was used to analyze the differences in the comparison of rates. The results of experiments were presented as means ± SD and differences between various groups were assessed using ANOVA or Dunnett t-test. P < 0.05 was considered statistically significant.

Results

HJ reduces the blood level of direct bilirubin in dogs with BDI

To measure the levels of direct bilirubin in dogs, blood samples were collected before the operation, and one, two and three weeks after the operation. The data showed that the levels of bilirubin in dogs before HJ did not continue to rise as the duration of BDI prolonged, but there was a plateau between day 5 and day 20 after bile duct obstruction (60.03 ± 1.97 vs. 41.58 ± 1.30 mmol/L). After operative biliary drainage, direct bilirubin was decreased significantly in each group dropping to 50% after one week, and to normal level after three weeks (Figure 1). These data suggested that HJ reduced the blood level of direct bilirubin in dogs with BDI for various durations.

Diameter of anastomotic stoma is reduced after biliary reconstruction

To evaluate changes of anastomotic diameter, the anastomotic stoma was observed during HJ and one, three, four and six months after HJ. The anastomotic diameter was controlled at about 1.5 cm during the operation of HJ. The diameter of anastomotic stoma was about 0.620 ± 0.132 cm after one month, 0.602 ± 0.085 cm after three months, 0.635 ± 0.109 cm after four months, and 0.628 ± 0.093 cm after six months, without statistical difference between groups after the operation of reconstruction (Figure 2). These data indicated that the diameter of anastomotic stoma caused by HJ was reduced after biliary reconstruction.

Expression of α-SMA and collagen in anastomotic tissues 3 months after HJ was not different in dogs with different durations of BDI, but the expression of TGF-β in dogs with 5 days of BDI was the highest in all groups

To detect and measure the expression of scar-related factors such as TGF-β1, α-SMA and collagen 3 months after bile duct reconstruction, microscopic inspection and SP were used. All anastomotic tissues showed expression of TGF-β1, α-SMA and collagen, where TGF-β1 and α-SMA were stained into brown color, and collagen was stained into green color. Three months after HJ, the expression of TGF-β in anastomotic tissues of BDI group (0.665 ± 0.024) was significantly higher than that of
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Figure 2. Anastomotic diameter after biliary reconstruction. A. Image of anastomotic diameter during HJ. B. Image of anastomotic diameter 6 months after HJ. C. Anastomotic diameter at one, three, four and six months after HJ. Histograms represent averages of at least 3 measurements of the anastomotic diameter. Data are expressed as means ± SD.

other groups (F = 29.578, P < 0.05). The expression of TGF-ß in BDI_{10} (0.469 ± 0.075), BDI_{15} (0.434 ± 0.018), BDI_{20} (0.432 ± 0.028), and BDI_{30} (0.413 ± 0.035) was not significantly different from each other (F = 1.282, P > 0.05). In addition, no significant difference was observed between the expression of α-SMA in BDI_{5} (0.330 ± 0.029), BDI_{10} (0.328 ± 0.047), BDI_{15} (0.440 ± 0.016), BDI_{20} (0.332 ± 0.034), and BDI_{30} (0.368 ± 0.058) (F = 0.523, P > 0.05). Similarly, the expression of collagen in BDI_{5} (0.277 ± 0.025), BDI_{10} (0.289 ± 0.003), BDI_{15} (0.221 ± 0.020), BDI_{20} (0.257 ± 0.030), and BDI_{30} (0.274 ± 0.038) was not significantly different from each other (F = 2.120, P > 0.05) (Figure 3). These data indicated that the expression of α-SMA and collagen in anastomotic tissues 3 months after HJ was not different in dogs with different durations of BDI, but the expression of TGF-ß in dogs with 5 days of BDI was the highest in all groups.

Expression of α-SMA and collagen in anastomotic tissues 6 months after HJ was not different in dogs with different durations of BDI, but the expression of TGF-ß in dogs with 5 days of BDI was the highest in all groups.

To detect and measure the expression of scar-related factors such as TGF-ß, α-SMA and col-
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Expression of scar-related factors after 3 months

- TGF-β1
- α-SMA
- Collagen

* Significant difference
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Figure 3. Expression of TGF-β1, α-SMA and collagen in anastomotic tissues 3 months after HJ in dogs with 5, 10, 15, 20, and 30 days of BDI. A. Immunohistochemical microscopy. B. Quantification of expression of TGF-β1, α-SMA and collagen. Data points represent averages of at least 3 measurements of protein expression. Data are expressed as means ± SD. *, P < 0.05 compared with values of other time points.

In our experiments, the concentration of direct bilirubin one week after HJ was decreased by 50% compared with that before HJ. Three weeks after HJ, the concentration of direct bilirubin was close to normal. These data suggested that biliary reconstructive surgery was successful. In addition, the morphology of anastomotic stoma observed at different follow-up times showed that there were certain anastomotic contractures from a diameter of 1.5 cm to 0.6 cm one month after the operation. Follow-ups from 1 to 6 months of each group showed that the diameter of anastomotic stoma was maintained at about 0.6 cm, and there was no statistical difference between groups. Therefore, we believe that the timing of repair had no direct impact on the anastomotic bile duct stricture in at least six months after reconstructive surgery.

Scar healing process depends on the expression of scar-related factors, such as TGF-β1, α-SMA, or collagen. TGF-β1 is an important signaling transducer that plays an important role in the interaction between cells and extracellular matrix. Abnormal expression of TGF-β1 could lead to regulatory disorder among inflammatory cells, repair cells and collagen metabolism, and result in the extension of tissue-healing.
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Expression of scar-related factors after 6 months

- TGF-β1
- α-SMA
- Collagen

* indicates significant difference.
process, deposition of extracellular matrix and scar contracture. Myofibroblast is a kind of atypical fibroblast with the features of both fibroblasts and smooth muscle cells on ultrastructures. Researches in recent years elucidated the correlation between myofibroblast and scar contracture [12], and showed that the contraction of myofibroblast is related to α-SMA, which is an important label of myofibroblast [13]. According to the results of the present study, TGF-β1 was the only factor that had difference between groups. The expression of TGF-β1 in BDI_5 group was higher than that in BDI_10 and BDI_30 groups. By contrast, the expression of α-SMA and collagen showed no significant differences between groups. Therefore, in our experiments, anastomotic stenosis after HJ showed no connection with the timing of repair after BDI.

In conclusion, for obstructive BDI, repairing time is not a relevant factor for postoperative anastomotic stenosis, and surgeons and operation methods are the key factors affecting anastomotic stenosis. Therefore, for patients with BDI, hospitals should focus on the experience of surgeons and the choice of operation methods in order to achieve a good long-term effect.

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Disclosure of conflict of interest

None.

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