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Experiences with Surgical Reconstruction of Hepatic Jejunal Anastomosis in Rats

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1. Abstract

1.1. Objectives

Based on establishing the animal model of hepatic jejunal anastomosis, the degree of anastomosis solidity, histological characteristics, postoperative complications were investigated after hepatic jejunal anastomosis.

1.2. Methods

SPF SD rats were randomly divided into 4 groups (6 in each group). Liver function was detected at the 1st, 7th, and 14th days after surgery. The volume of bleeding, infection, anastomotic leakage, and the bursting pressure was measured in the 7th day and 14th day after operation. Histological examination was performed to assess the pathological morphological changes of the anastomosis.

1.3. Results

All animals survived well and there were no abnormalities in appetite and activity. There was no significant difference in AST and ALT. Complications such as infection, bleeding, anastomotic leakage, and intestinal obstruction were not observed. The anastomotic healing was good. Histological examination showed that the surface of the intestinal mucosa villi epithelium was intact, and the intestinal wall serous layer was adhered to the hepatic lobule. Lymphocyte infiltration, granulation tissue and many new capillary blood vessels can be observed.

1.4. Conclusion

Hepatic jejunal anastomosis has sufficient healing fastness, which could be used as the new biliary reconstruction method is used in clinic to make up for the past deficiencies.

2. Introduction

It is relatively difficult to reconstruct the bile duct with a small diameter and multiple bile ducts after hilar cholangiocarcinoma [1, 2]. Even if the bile duct reconstruction can be completed, the risk of complications is higher, such as biliary-enteric anastomosis stenosis, bile fistula, even unable to implement biliary reconstruction [3-5]. Therefore, how to better complete the reconstruction of the biliary tract after the resection of the lesion has begun to attract scholars' attention. In our present study, the healing method of liver tissue jejunal anastomosis were investigated, which might offer the clinical application value of hilar cholangiocarcinoma.

3. Materials and Methods

3.1. Animals

Twenty-four specific pathogen free (SPF) Sprague-Dawley (SD) rats (aged 9-11 weeks) were obtained from XXX. All rats were housed individually in polycarbonate cages in in an animal facility with appropriate temperature (22 ± 2°C) and humidity (50 ± 10%) with a 12 h light/dark cycle. All rats had free access to bedding, enrichment food, and water throughout the study. Rats were randomly divided into 4 groups, each with 6 animals, including the preoperative group (without surgery), the postoperative day 1, the 7th day and the 14th day group. All animal experiments are carried out in accordance with in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Biomedical Ethics Committee for Animal Use and Protection of XXX.

3.2. Surgery

Rats was in the supine position with the limbs fixed on the operating table. Skin routinely is disinfected and draped after anesthesia through pentobarbital sodium (20 mg/ml, 60 mg/kg, i.p.). Free the liver and jejunum after splitting the abdominal cavity. Cut off the proximal jejunum at the end of the duodenum and the part of mesangium. Sterilize the distal intestine and close the stump. A side incision in the jejunum at 2 cm from the stump was made to connect liver tissues with 8-0 absorbable thread (Figure 1). After the operation, close the abdomen layer by layer. After the surgery, rats were recovered with penicillin administration every day for a week, preventing infection and cannula blocking.

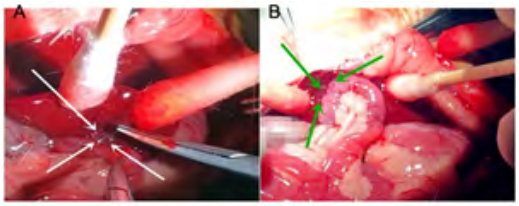

Figure 1: Representative images of hepatic jejunal anastomosis at the operation time.

3.3. Hepatic Function Assay

Blood samples were collected at day 1, the 7th day, the 14th day after surgery and before surgery and centrifuged. The supernatant was collected for hepatic function tests thought the levels of aspartate transaminase (ALT) and alanine aminotransferase (AST) by an automatic biochemical analyzer (Mindray-120, Guangzhou, China) in accordance with the instructions.

3.4. Bursting Pressure Measurement

Bursting pressure was measured as previously described [6]. In brief, a silk thread was used to ligate the liver tissue at the distal end of the jejunal anastomosis. The proximal end was ligated using a No. 8 urinary catheter. Normal saline was injected into the abdominal cavity and the burst pressure was determined with sphygmomanometer (Figure 2).

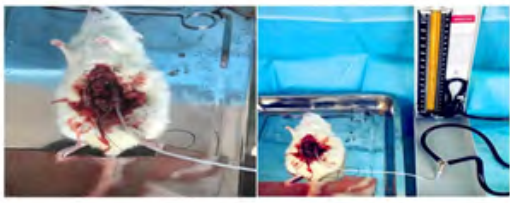

Figure 2: Representative images of measure of burst pressure.

3.5. Histological Analysis

Paraffin-embedded tissue sections were routinely deparaffinized, baked, dewaxed, and hydrated. Hematoxylin-eosin (H&E) staining was performed according to standard protocols. The stained sections were examined under a light microscope (Olympus, DP26, Japan) by two pathologists, who were blinded to the treatment.

3.6. Statistical Analysis

All statistical analyses were performed using SPSS 21.0 software (SPSS, Chicago, IL, United States). Student's t-test was used to analyze the differences between two groups, while a one-way ANOVA and Bonferroni correction were used to assess multiple groups. Values of $p < 0.05$ were considered statistically significant.

4. Results

4.1. Operative Assessment

All surgeries were successfully performed and all rats survived to the scheduled date after the operation. There was no abnormality in appetite and activity, and no other symptoms were observed. None of complications were observed, such as abdominal cavity infection, bleeding, and intestinal obstruction, when all experimental animals were sacrificed at the 7th and 14th day after operation (Figure 3). We also measured the alteration of burst pressure and no significant difference were observed.

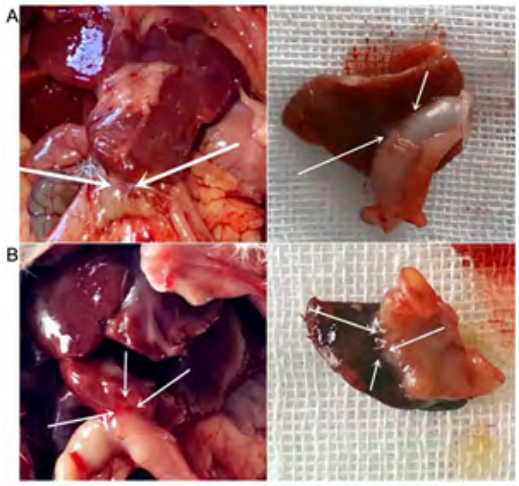

Figure 3: Representative images of surgical reconstruction of hepatic jejunal anastomosis at the 7th day and the 14th day after operation.

4.2. Liver Function

Liver function was assessed using blood from the inferior vena cava and the results showed that there was no significant difference in the ALT and AST level at the 7th and 14th day after the operation, even a little increase at the first day after the operation (Figure 4). These data showed that surgical reconstruction of hepatic jejunal anastomosis did not affect liver function.

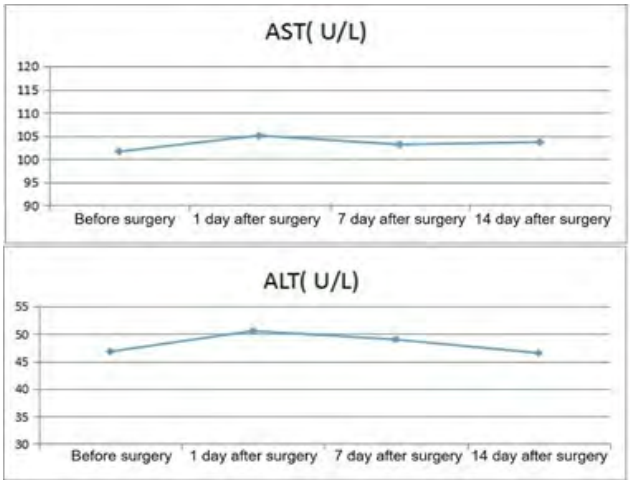

Figure 4: The alteration of AST and ALT at the indicated time.

4.3. Pathological Changes

To assess the wound of hepatic jejunal anastomosis, H&E stain was performed. The hepatic jejunum anastomosis healed well under the microscope (Figure 5). The lamina propria lymphatic vessels, capillaries, small blood vessels, and intestinal mucosal villi epithelium were observed in the liver tissue and jejunum anastomosis (Figure 5). In most areas, the adhesion of intestinal wall tissue and liver tissue with inflammatory cell infiltration, vascular congestion and regional lymphocyte infiltration can also be observed. A great number of new capillaries were observed, indicating hepatic jejunal anastomosis could effectively construct hilar cholangiocarcinoma. Histological analysis of liver samples was evaluated inflammatory markers.

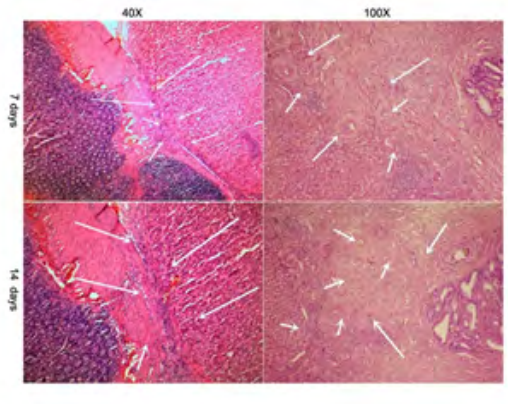

Figure 5: Representative microphotographs of anastomosis at the 7th day and the 14th day after operation.

5. Discussion

In our present study, a novel surgical procedure for hilar cholangiocarcinoma was explored in a rat model and assessed the potential possibility for clinical application. In this experiment, mechanical and histological methods were used to evaluate the degree of healing of liver tissue and jejunum anastomosis. Our results suggested that surgery of hepatic jejunal anastomosis effectively broaden the indications involving hilar surgery and improved the quality of life of patients. Mechanical strength is an important index for studying intestinal healing ability, and anastomotic burst pressure is one of the important indexes for evaluating mechanical strength [6]. The burst pressure is mainly used to evaluate the healing ability of the intestinal tissue at present. The significance of anastomotic burst pressure is that the greater the burst pressure, the better the healing of the bowel [7-10]. In our present study, the liver tissue jejunostomy had healed 7 days after the operation and there was no significant difference in the burst pressure of the liver tissue jejunostomy at the 7th and 14th days after the operation.

Intestinal anastomosis healing is an inflammatory process to wounds and foreign bodies (such as suture reaction), which can be roughly divided into three stages[11, 12]: inflammation (0 to 5 days): the local tissue of the broken end of the intestinal tube after the intestinal anastomosis has different degrees of hemorrhage and necrosis after about 15 mins to 30 mins, accompanying with inflammatory cell infiltration[13, 14]; Second, fibrosis (2 to 21 days): the anastomotic tissues develop from the serosal layer, muscle layer and mucosa at 7th days after the operation; Third, maturity (21 days to 2 years): fibrous connective tissue would replace inflammatory cells about 2-3 weeks[15] . In our present study, H&E staining showed that the liver tissue jejunostomy had healed 7 days after the operation without any surgical complications, including infection, anastomotic stenosis, anastomotic leakage, and intestinal obstruction. The anastomosis between liver and jejunum is as far as possible from the peritoneum to avoid the anastomotic stenosis caused by compression in the early postoperative period. There was no stenosis in the anastomotic jejunal loop at the 7th day and the 14th day after operation by histological analyz

6. Conclusion

The anastomosis after hepatic jejunostomy is well healed, with fewer postoperative complications, which helps to broaden the indications involving hilar surgery, increase the success rate of radical or palliative resection of hilar cholangiocarcinoma, and improve the quality of life of patients. Patients who have not benefited from surgery in the past can benefit from surgery, which can be used as a new type of biliary reconstruction method to make up for past deficiencies.

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