A Simple Method for Bile Duct Anastomosis and Interval Bile Collection in the Liver-Transplanted Rat

. TH. RAT. TECN.

HUA-SHENG XU, M.D., LYNN K. ROSENLOF, M.D., J. BAYNE SELBY, M.D.,* AND R. SCOTT JONES, M.D.

Departments of Surgery and *Radiology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Submitted for publication August 6, 1991

A mini T-tube is introduced for the bile duct anastomosis of rat liver transplantation as well as interval bile collection. The validity of the T-tube was evaluated in 14 liver-transplanted rats and compared to 14 rats using traditional stent for bile duct anastomosis. Changes of biliary tree after the T-tube anastomosis were examined by T-tube cholangiography on sample rats at 4 days and at 4 months after liver grafting. Additionally, bile volumes and rates of bile salt secretion were compared in the continuously flowing cannula and the chronic T-tube fistula in normal rats. The results show that the mini T-tube facilitates bile duct anastomosis and study of bile secretion after liver transplantation in rats without increase in surgical difficulty or interference of biliary enterohepatic circulation. © 1992 Academic Press, Inc.

Since Kamada and Calne [1] introduced the cuff technique, rat liver transplantation has become a popular animal model for study of cold preservation, rejection, and metabolism in the transplanted liver. Bile duct anastomosis in the liver-transplanted rat is commonly performed by using a polyethylene tube as a stent to reconstruct common bile duct and effectively restore the biliary enterohepatic circulation [2-4]. However, traditional stents do not allow intermittent bile sampling. Our laboratory has recently made a mini T-tube to modify the stent for the bile duct anastomosis and periodic access to the biliary tree for study of bile secretion in the liver-transplanted rat. The T-tube is simple to make, and its use for reconnection of common bile ducts does not increase surgical difficulty during rat liver transplantation.

MATERIALS AND METHODS

The short arm of the T-tube was made using 2 cm of PE-90 Intramedic polyethylene tubing and the long arm was made using 12 cm of PE-50 Intramedic polyethylene

0022-4804/92 \$4.00 Copyright © 1992 by Academic Press, Inc. All rights of reproduction in any form reserved. tubing (Clay Adams, Becton Dickinson & Co., Parsippany, NJ). The PE-50 tube was inserted through two holes notched with a scalpel on the opposite sides of the PE-90 tube with the PE-50 tube extending 0.5-1 cm (Fig. 1A). The joint of two tubes was glued using DUCO Cement (Devcon Corp., Wood Dale, IL). After the glue dried, a 23-gauge needle was used through one end of the PE-90 tube (short arm) to punch holes in the PE-50 tube and link the flow between two connected tubes (Fig. 1B). The short end of the PE-50 tube was melted with heat and sealed with the same glue (Fig. 1C). Leakage and obstruction were checked by injecting water through the long arm. The extra length of the PE-90 tube was cut and left at 2 mm for each short arm.

Orthotopic liver transplantation was performed in 14 male Sprague-Dawley rats weighing 250-300 g as described by Kamada and Calne [1] with alteration of the common bile duct anastomosis. The proximal and distal segments of the common bile ducts in the donor and the recipient rats were cannulated with 5-mm Tygon tubes (ID 0.43 mm, OD 0.94 mm) or PE-50 polyethylene tubes and secured with 4-O silk, respectively. After the donor liver was revascularized, the Tygon tubes in the donor and recipient common bile ducts were inserted into each short arm of the T-tube. The ligatures securing the Tygon tubes were tied to the long arm to prevent the tubes from dislodging (Fig. 2). The long arm was tunneled subcutaneously to the back of the rat's neck, exteriorized via a small incision through the skin, and secured with a suture. The free end of the long arm was sealed with a cap made of a short segment of PE-90 polyethylene tubing with one end closed. No restraining apparatus was required and the rat had normal activity. During bile collection, the experimental rat was put into a Bollman cage and the external arm was extended by 20 cm of PE-10 polyethylene tubing filled with normal saline. The free end of the PE-10 tube was kept slightly lower than the rat body and bile flow was drained by siphoning.

T-tube cholangiography was performed using renograffin-60 (Squibb) contrast material in one rat 4 days

F- 056



FIG. 1. Construction of the T-tube.

and one rat 4 months after liver transplantation. Spot films with magnification were obtained using a standard fluoroscopy table.

Bile volumes and total bile salt secretion rates were measured in eight male Sprague-Dawley rats with the T-tube chronic bile fistula for 1 week and in eight control rats with continuously flowing cannula on the day of surgery to evaluate the validity of the T-tube for bile sampling. The surgical procedure was carried out under ether anesthesia. In the experimental rats, the common bile duct was anastomosed with a T-tube as described above but without liver transplantation. The rats were housed in separate cages with free access to food and water. Bile samples from the T-tube were collected 2 hr per day at 9 AM for 1 week. In the control group, the proximal common bile duct was cannulated with a 20 cm of PE-50 polyethylene tubing and the distal duct was ligated. The bile samples were collected for 2 hr following the recovery from anesthesia. The control rats were sacrificed using halothane after the first bile sampling. The concentration of total bile salts was measured by a modification of the 3 α -hydroxysteroid dehydrogenase technique with sodium tauroocholate as the standard (Sigma Chemical Co., St. Louis, MO). Bile volume was measured using a 1.0-ml glass pipet with 0.01 ml graduation. Bile salt output per hour was calculated from the average value of the first 2 hr bile collection. Daily bile volumes and bile salt outputs drained from T-tubes were compared with the data from continuously flowing cannulas (single PE-50 tubing) during the first 2 hr of bile drainage in the control rats. Statistical analysis was performed using Student's t test.

RESULTS

Fourteen rats underwent liver transplantation with T-tube bile fistula. One rat died on Day 4 and postmortem examination demonstrated the T-tube kinked with the bile duct. Three rats died within 1 month related to the bile obstruction. Autopsy revealed the proximal common bile duct was dilated and filled with sludge. Four rats temporarily had yellow urine during the second or third week and survived. No late biliary obstruction caused mortality after 1 month in this group. In comparison, using the traditional stent for the bile duct anastomosis, 2 out of 14 liver-transplanted rats died with bile sludge obstruction within 1 month and 3 rats had yellow urine for several days during the same period and survived.

The films of T-tube cholangiography showed normal filling of the common bile duct and hepatic bile ducts with good flow into the duodenum in one rat (Fig. 3). The second cholangiogram showed a dilated common bile duct after 4 months, but no obstruction.

Upon simple inspection, the bile flow and bile salt secretion rates in rats with T-tubes did not appear different from control rats with continuously flowing cannula (Fig. 4 and Table 1). No T-tube was dislodged during the study.

DISCUSSION

Liver transplantation in rats has been successfully developed for research since late 1970s [1]. Thereafter, some modified methods of the liver revascularization were introduced, such as the use of the cuff technique for



FIG. 2. The position of the T-tube in the liver transplanted rat. (a) Long arm of the T-tube. (b) Two short arms. (c) Common bile duct. (d) Tygon tube cannulated in the common bile duct. (e) Glue sealing the end of PE-50 tube and fixing the joint.



FIG. 3. T-tube cholangiogram of a liver-transplanted rat on Day 4. Arrow points at two short arms of the T-tube. The hepatic and common bile ducts were normally filled with contrast-medium and had adequate flow into the deodenum without signs of obstruction.

three vascular anastomoses [3, 5] and the hepatic arterial reconstruction [6, 7]. Initially, the common bile duct was reconstructed as described by Kamada and Calne by telescoping a polyethylene tube secured in the donor bile duct into a larger diameter tube in the recipient bile duct. The technique was later modified by using a single tube as stent to connect both ends of the bile ducts. With improvement of surgical technique, 1-week survival rates in the liver-transplanted rats were reported from 77 to 95.3% [1, 3, 6, 8] and a variety of information from experimental liver transplantation was produced.

Since rat bile ducts are small and friable especially after liver transplantation and a subsequently compromised blood supply, anastomosis of the donor and recipient's common bile ducts remains a major barrier to progress in study of bile secretion in the postoperative liver-transplanted rats. Initially, methods of "the extracorporeal bile duct" with 14 cm of bile flow path developed by Weis *et al.* [9] and a long "bile duct T-cannula" with 7.5 cm of each arm developed by Klauda *et al.* [10]



FIG. 4. Daily change of bile volumes (cycles) and bile salt outputs (triangles) drained by the T-tube in normal rats are compared with the bile volume (solid cycle) and the bile salt output (solid triangle) from continuously flowing cannula in the control rats on Day 1.

for bile collection in normal intact rats were employed for rat liver transplantation in this lab. Those methods drastically increased the length of bile flow path and resulted in a high incidence of bile obstruction and bile leakage. A Y-tube made of multiple sizes of polyethylene tubing was then designed and applied in this lab to reduce the length of the bile flow path between the liver and duodenum [11]. This made bile study in the liver transplanted rat possible, but the bile flow in the anastomosed bile duct still had to go through a 3- to 5-cm connected tube and tube disconnection occasionally occurred. The simplified mini T-tube described above is easy to make and small enough to be employed between two ends of the donor and recipient's common ducts without increasing the path of bile flow after anastomosis and interrupting the bile enterohepatic circulation. Punching the holes in the PE-50 tube via PE-90 after gluing the joint of two tubes and melting extra length of PE-50 tubing effectively prevents the tube from internal obstruction by the glue and dislodgement of the arms of

TABLE 1

Bile Volumes and Bile Salt Outputs

	Bile volumes (ml/100 g/hr)	Bile salt outputs (µmole/100 g/hr)
Control group	0.34 ± 0.02	14.4 ± 1.7
T-tube group		
Day 1	0.32 ± 0.03	13.0 ± 2.5
Day 2	0.33 ± 0.04	15.1 ± 2.4
Day 3	0.34 ± 0.05	16.5 ± 2.9
Dav 4	0.36 ± 0.04	16.6 ± 2.2
Dav 4	0.36 ± 0.03	17.9 ± 2.4
Dav 6	0.35 ± 0.05	17.7 ± 2.2
Day 7	0.37 ± 0.04	17.7 ± 2.5

Note. Values are expressed as mean \pm SEM. 100 g = 100 g body weight.

the T-tube. The results of the animal study show that the T-tube used for bile duct reconstruction and bile sampling in the rats after liver transplantation is safe and satisfactory. High-quality T-tube cholangiography can be performed easily using standard contrast material.

This study also indicates comparable bile flow rates or bile salt outputs between daily bile drainage from the T-tube and the bile collection from continuously flowing cannula in normal rats. The results may be explained by noting that the smaller inner diameter of the mini-Ttube prevents air reflux into the bile duct and an intact sphincter of Oddi function maintains a normal low pressure in the bile duct, which makes the bile siphoning drainage effective. The results demonstrate that the technique is not only useful for the bile duct anastomosis and studies of bile secretion and composition in the liver transplanted rat, but also applicable to any experimental model requiring periodic access to the biliary tree while maintaining the enterohepatic circulation.

REFERENCES

- Kamada, N., and Calne, R. Y. Orthotopic liver transplantation in the rat. Transplantation 28: 47, 1979.
- 2. Zimmermann, F. A., Butcher, G. W., Davies, H. S., Brons, G., Kamada, N., and Turel, O. Technique for orthotopic liver trans-

plantation in the rat and some studies of the immunologic responses to fully allogeneic liver grafts. *Transplant. Proc.* **11**: 571, 1979.

- 3. Miyata, M., Fischer, J. H., Fuhs, M., Isselhard, W., and Kasai, Y. A simple method for orthotopic liver transplantation in the rat. *Transplantation* 30: 335, 1980.
- 4. Howden, B., Jablonski, P., Grossman, H., and Marshall, V. C. The importance of the hepatic artery in rat liver transplantation. *Transplantation* 47: 428, 1989.
- 5. Tsuchimoto, S., Kusumoto, K., Nakajima, Y., et al. Orthotopic rat liver transplantation in the rat: A simplified technique using the cuff method for suprahepatic vena cava anastomosis. Transplantation 45: 1153, 1988.
- Hasuike, Y., Monden, M., Valdivia, L., et al. A simple method for orthotopic liver transplantation with arterial reconstruction in rats. Transplantation 45: 830, 1988.
- Steffen, R., Ferguson, D. M., and Krom, R. A. F. A new method for orthotopic rat liver transplantation with arterial cuff anastomosis to the recipient common hepatic artery. *Transplantation* 48: 166, 1989.
- Kamada, N., and Calne, R. Y. A surgical experience with five hundred thirty liver transplants in the rat. Surgery 93: 64, 1983.
- 9. Weis, E. E., and Barth, C. A. The extracorporeal bile duct: A new model for determination of bile flow and bile composition in the intact rat. J. Lipid Res. 19: 856, 1978.
- Klauda, H. C., McGovern, R. F., and Quackenbush, F. W. Use of a bile duct T-cannula as a new technique for studying bile acid turnover in the rat. *Lipids* 8: 459, 1973.
- 11. Xu, H. S., Wang, D. J., Pace, R. C., and Jones, R. S. Beneficial effects of prewarming the donor liver for rat liver transplantation. *Amer. J. Surg.*, in press.