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Improved Techniques for Orthotopic Liver Transplantation in Pigs: A preliminary study

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ABSTRACT

Orthotopic liver transplantations were performed on 12 pigs between November, 1986 and February, 1987. Survivals and causes of death were assessed according to the type of surgical procedure employed. Six operations were carried out according to the original procedure of Terblanche et al, whereas for other six animals, the transplantation was conducted by our modified procedure in which cannulation was made into the splenic vein without splenectomy at the veno-venous (v-v) bypass and a diaphragmatic cuff was used for the anastomosis of suprahepatic vena cava. 1/6 animals with original procedure and 5/6 with our modified procedure survived for more than 7 days. The total ischemic time was significantly short in the group subjected to the modified procedure. The major cause of death in the group on which the original procedure was conducted was gastric ulceration, accounting for 67% of the mortality, whereas in the modified procedure group, the mortality rate was reduced significantly to 17%. This operative technique has provided excellent survival rates in our transplanted animals.

Surgical techniques for orthotopic liver transplantation in the pigs have been established by the pioneering work of Calne et al[2], and Terblanche et al[7]. However, several investigators[1,3,4,8] reported that technical failures such as intraperitoneal hemorrhage from the anastomosis of suprahepatic vena cava and gastric ulcer with massive gastrointestinal hemorrhage are the major causes of death in the pigs receiving liver grafts. This preliminary study describe our technical modifications with special reference to prevention of bleeding or kinking of anastomosis of suprahepatic vena cava and the occurrence of gastric ulceration in orthotopic liver transplantations.

MATERIALS AND METHODS

Animals.

The average weight of the pigs used was 20 kg. Donor and recipient animals were littermates obtained from same farm. No immunosuppressive drugs were administered in this study. The animals were deprived of food for 24 hr preoperatively.

Anesthesia.

Following intramuscular premedication with ketamine hydrochlorid (15 mg/kg, Parke-Daris, Zürich, Switzerland), all operations were conducted under general anesthesia. The animals were ventilated via a cuffed endotracheal tube. Arterial pressure, heart rate and ECG were monitored and blood gas analysis was made.

Donor operation.

The surgical techniques for donor hepatectomy were essentially the same as those of Terblanche et al[7]. Our modified procedure consisted of trimming the excess supradiaphragmatic portion of vena cava and preparation of the diaphragmatic muscle cuff. The procedure is conducted as follows: A midline abdominal inci-

sion is made from the mid sternum to the pubis. First, the common bile duct is ligated and divided as caundly as possible. The liver is then mobilized by dissection until the donor liver is connected only to its four major vascular structures: the infra and suprahepatic cavae, the portal vein and the hepatic artery. The hepatic artery is dissected down to its origin from the celiac artery. Once immobilization of the liver and dissection of its vascular structures has been completed, the pig is intravenously administered 1 mg of heparin per kg of body weight. After the hepatic artery has been clamped, a cannula is inserted into the portal vein followed by core cooling of the liver with 1–2 l of Ringer's lactate (4°C). The suprahepatic vena cava situated close to the heart is opened via incision of the diaphragma. After this procedure has been carried out, the liver soon takes on a pallid color. Immediately after core cooling, exanguination of the pig occurs by connecting the iliac vein to a blood collection bag. The blood collected is transfused into the recipient pig. The aorta is then mobilized from the aortic bifuration to a point above the celiac axis and its branches are divided without ligation. The liver is then removed by transecting the abdominal aorta, the portal vein and vena cava above and below the liver, leaving enough vessel lengths to permit anastomosis.

The excised liver graft is placed in a sterile stainless steel basin containing Euro-Collin's solution (Green Cross Corp., Osaka, Japan) at 4°C. Following the ligation of aortic branches, other holes or untied branches of the vascular attachments are carefully ligated by bench surgery. Finally, the excess supradiaphragmatic portion of the vena cava is excised leaving approximately 2–3 mm of the supradiaphragmaic vena cava. The cuff of the diaphragmatic muscle is then trimmed leaving a 2–3 mm cuff on the vena cava (Fig. 1).

Veno-venous (v-v) bypass.

During the anhepatic phase, a pump-assisted portal- and inferior vena cava- to superior vena cava bypass system as illustrated in Fig. 2 is instituted. A v-v bypass consists of 5 mm of silicon tubes inserted in the splenic, left iliac and left external jugular veins. The splenic and iliac cannulae are connected to a roller pump - Sarns RUA14 (Sarns Inc., Ann Arbor, Mich.), by which blood is delivered to the external jugular vein at a flow rates of 25–30 ml/min/kg. For cannulation into the splenic vein, two different

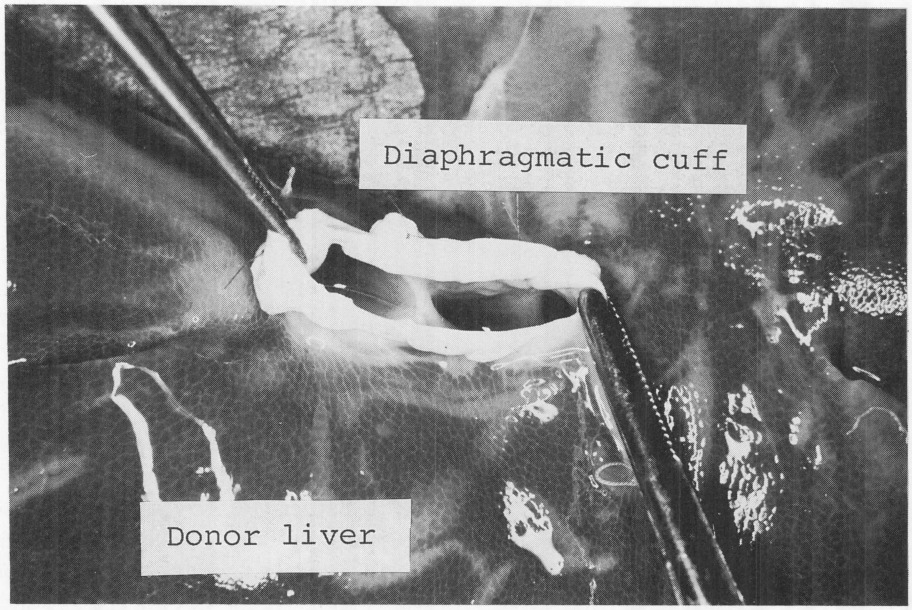

Fig. 1. Trimming of the excess portion of the suprahepatic vena cava and diaphragmatic muscle in the donor liver (diaphragmatic cuff). This was done in the first part of our modified procedure.

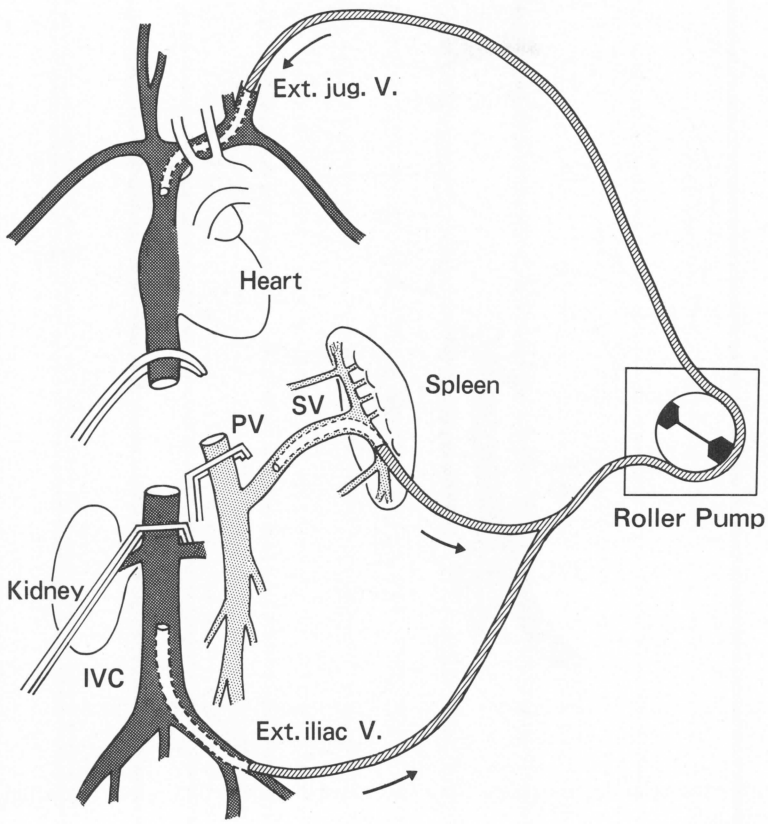

Fig. 2. Method used for pump-assisted veno-venous bypass during anhepatic phase. Shunting tube for decompression of portal blood is inserted into the splenic vein via its branch without splenectomy. This method was conducted in the second part of our modified procedure.

methods were conducted in this study. In the first 6 operations, cannulation into splenic vein was done following splenectomy according to the method of Terblanche et al[7]. In the remaining six operations, the shunting tube was inserted into the splenic vein via its branch while preserving the spleen (Fig. 2).

Orthotopic liver transplantation.

Following completion of recipient hepatectomy and the hemodynamic state undergoing v-v bypass becomes steady, the prepared donor liver is placed in the right subdiaphragmatic space. Vascular anastomosis of the graft starts with the suprahepatic vena cava, using a running suture of 5—0 Surgilene (Davis and Geck, Pearl River, N.Y). Portal vein anastomosis is then performed with running 6—0 Surgiline suture. The final knot is not tied securely at the portal vein anastomosis so as to avoid purse-stringing of the anastomosis on refilling. This procedure is called the "growth factor", and was originally introduced in human liver transplantation by Starzl et al[6]. At this point, the portal vein clamp is removed and flushed of approximately 50 ml of blood from the infrahepatic vena cava. After clamping the infrahepatic vena cava, the suprahepatic cava clamp is removed, allowing perfusion of the liver with portal blood. The infrahepatic vena cava anastomosis is done with 5—0 Surgilene. After discontinuing the bypass and bleeding from the anastomosis has been stopped, hepatic arterial flow is reconstructed by an end-to-side aorta-to-aorta anastomosis with running 6—0 Surgilene suture. Biliary reconstruction is done by cholecystoduodenostomy using an inner layer of 4—0 Dexon (Davis and Geck, Pearl River, N.Y) suture and an external layer of silk. The completed orthotopic liver transplantation is shown in Fig. 3. After bleeding has been stopped and the abdominal cavity

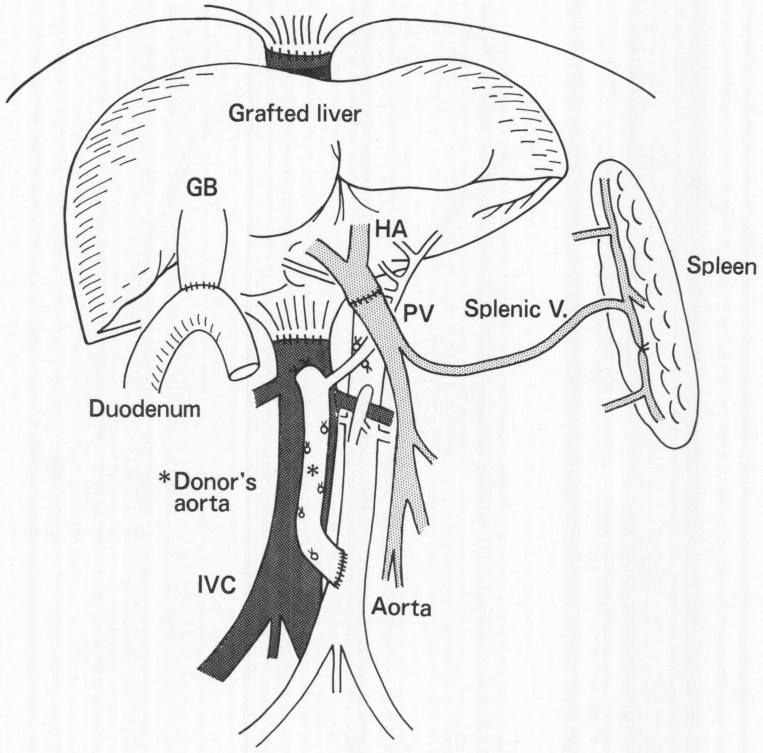

Fig. 3. Orthotopic liver transplantation in pigs. Note that hepatic arterial flow is reconstructed by an end-to-side aorta-to-aorta anastomosis.

has been rinsed with warm saline, the abdominal wall is closed in two layers.

Comparative study.

The 12 consecutive orthotopic liver transplantations in the pigs were performed between November, 1986 and February, 1987. Two different surgical procedures were used in the operations. In the first 6 operations, *original procedure* described by Terblanche et al[7], in which a bypass catheter is inserted directly into the cut end of the splenic vein following splenectomy and in which no diaphragmatic cuff is prepared, was used. *Our modified procedure*, in which a bypass catheter is inserted into the splenic vein via its branch without splenectomy and in which a diaphragmatic cuff is made, was conducted on the other six animals. Survival times, causes of death, recipient operation times, total ischemic times and v-v bypass times of the 6 pigs subjected to the modified procedure were compared with those of the other animals on which the original procedure was conducted. Postmorten examination was performed in each case.

Statistics

The data obtained from the operations were analysed by the student's t test.

RESULTS

Survivals and causes of death.

Table 1 shows the results of each group of animals to which no immunosuppressive drugs were administered. For the original procedure group, the mean operation time, total ischemic time and v-v bypass time were 237 ± 41 min, 192 ± 57 min and 66 ± 11 min, respectively. For the other group, the mean operation time, total ischemic time and v-v bypass time were 253 ± 34 min, 115 ± 25 min and 69 ± 7 min, respectively. Total ischemic time in the latter group was significantly less ($p<0.05$) that in the former.

The mean survival time was 4.4 days in the original procedure group and 22.8 days in the modified procedure group. The percentage of 7-days survival was 33% in the former and 83% in the latter. Thus, the survival rate of the pigs receiving orthotopic liver transplantation was

Table 1. Survivals and causes of death in orthotopic liver transplantation in the pigs (Nov.86-Feb.87)

Pig NO	Recipient operation times (min)	Total ischemic times[b] (min)	V-V bypass times[c] (min)	Survival (days)	Causes of death
Original procedure[a]					
1	175	198	67 (54[d])	8 hr	Intraperitoneal hemorrhage
2	245	191	70 (54)	6 hr	Intraperitoneal hemorrhage
3	280	193	62 (49)	11	Perforated gastric ulcer
4	200	287	64 (50)	2	Bleeding gastric ulcer
5	255	176	51 (35)	8	Bleeding gastric ulcer
6	270	107	86 (72)	5	Bleeding gastric ulcer
	237 ± 41[e]	192 ± 57*	66 ± 11 (52 ± 11)	4.4 + 4.3	
Modified procedure[a]					
7	240	102	62 (50)	13	Malnutrition
8	250	140	78 (55)	74[f]	Alive with good function
9	315	98	60 (35)	14	Perforated gastric ulcer
10	245	100	73 (62)	4	Non-functioning liver
11	260	156	77 (56)	18	Thrombus in the heart
12	210	98	69 (48)	28[f]	Alive with fair function
	253 ± 34	115 ± 25*	69 ± 7 (51 ± 9)	22.8 ± 22.3	

[a] See MATERIALS AND METHODS. [b] Duration between donor's portal vein clamping and the reflow of recipient' portal blood. [c] Total V-V bypass times. [d] Duration of portal vein bypass.
[e] mean + SD. [f] At the time of writing (March, 14, 87), pigs 8 and 12 were alive with good or fair liver function. * significant ($p<0.05$)

remarkably improved in the group on which the modified procedure was carried out.

There was a great difference between the two groups with respect to the cause of death. The major cause of death in the original procedure group was gastric ulceration, accounting for 67% of the mortality. The mortality rate of gastric ulceration was reduced significantly to 17% by the modified procedure. In the former, 2 pigs died of intraperitoneal hemorrhage from the posterior wall of suprahepatic vena caval anastomosis which could not be controlled during the operation. The other four pigs died of perforated or bleeding gastric ulcer with massive gastrointestinal hemorrhage. These gastric ulcers were always situated at the esophagogastric junction of the stomach (pars esophagea). Lesions at this site were either erosion of the whole area, or shallow cleft-like ulcers such as shown in Fig. 4. It was frequently found in pigs dying from bleeding gastric ulcer that a wide band of mucosal and submucosal hemorrhage was present along the greater curve of the stomach. This was also noted by Terblanche et al[8].

In the modified procedure group, no early death occurred within 48 hr, such as by intraperitoneal hemorrhage. Pig 7 suffured from severe anorexia and weight loss and died 13 days after transplantation. No gastric ulcer or rejection could be detected in these animals. Pig 9 died of peritonitis due to perforated gastric ulcer which occurred at the pars esophagea of the stomach 14 days following transplantation. Necropsy revealed that the splenic vessels of pig 9 were thrombosed and the spleen showed macroscopically what appeared to be an infarction. Pig 10 suffering from rapidly increasing jaundice died 4 days after transplantation and was of particular importance. At necropsy the grafted liver was hard and its cut surface of the liver mottled with massive bile plugs in the intrahepatic bile ducts. Pig 11 died of massive thrombus in the heart and superior vena cava due to the residual catheter inserted in the superior vena cava for intravenous alimentation. At the time this paper was being written, pigs 8 and 12 were alive with good liver function on 74 and 28 days following transplantation. Throughout this study, all postmorten findings showed the

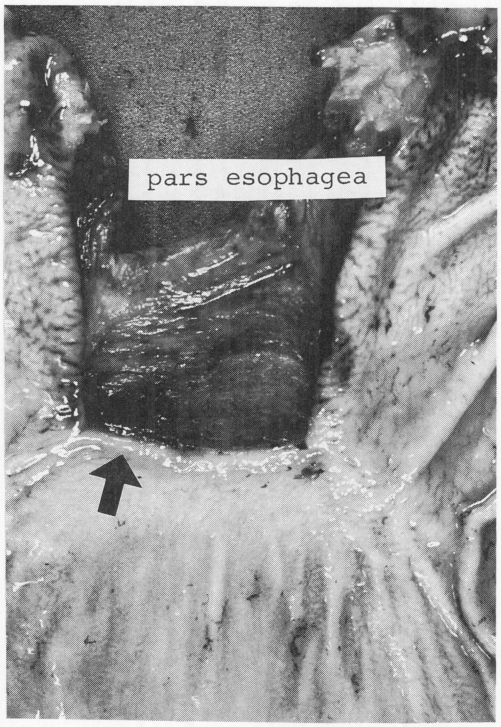

Fig. 4. A shallow cleft-like ulcer (⬅) at the esophagogastric junction of the stomach (Pig 5).

sites of vascular and biliary anastomosis to be patent and there to be no apparent evidence of rejection in the grafted livers.

DISCUSSION

The survival rate of orthotopic liver transplantation in the pigs improved in the latter part of the series through the authors' experience and better techniques. We are convinced that portal decompression from a branch of the splenic vein without splenectomy in the v-v bypass to prevent gastric ulceration and use of a diaphragmatic cuff to strengthen the anastomosis of the suprahepatic vena cava, are the major factors responsible for the improved survival rate.

One major problem in pig liver transplantation has been the occurrence of gastric ulceration postoperatively[1,3,4,8]. Terblanche et al[8], report ulceration in the pig to occur in the pars esophagea of the stomach and responsible for 44 percent of the deaths of pigs which survived beyond 36 hr. Recently, Meijer et al[4] reported that a 30% mortality rate due to gastric ulcer complications is observed in pigs surviving more than three days. In our earlier experiment in which splenectomy for v-v bypass was performed essentially the same as by Terblanche et al[7], sixty-seven percent of pigs after receiving liver grafts died from massive gastrointestinal hemorrhage due to ulceration in the pars esophagea of the stomach. In contrast, the mortality rate of the gastric ulcer was only 17% by our modified methods in which no splenectomy was performed. Based on the present results and those of Terblanche et al[8], interference with the blood supply to the stomach on removals of the spleen and liver may possibly be the cause of gastric ulceration and preservation of the spleen apparently prevents high incidence of gastric ulceration. The Cape Town group[3] demonstrated that direct bile-duct to bile-duct anastomosis reduces the incidence of gastric ulceration as well as biliary stasis and cholangitis. Although cholecystoduodenostomy was used for biliary reconstruction throughout the present study, low motality due to gastric ulceration was observed in our modified procedure group.

It is well recognized that unlike dogs, pigs more closely resemble man in apparently having no hepatic vein sphincters and thus show no hepatic outflow block following liver transplantation[5]. Despite the advantages of the swine model for liver transplantation, kinking or bleeding at the anastomosis of suprahepatic vena cava is a fatal complication apparent within 24 hr after transplantation[1,8]. In general, the excess suprahepatic vena cava of a donor liver is thought to be a major cause of kinking or bleeding at this anastomosis. Consequently technical modification made available 2—3 mm of supra hepatic vena cava and a cuff of diaphragmatic muscle to avoid the kinking or bleeding at the suprahepatic vena caval anastomosis. With the addition of these modifications and the performance of a technically precise operation, we have been able to achieve 7-days survival rates in 83% in our transplanted pigs.

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