Original Article

A New Porcine Model of Autologous Living-donor Liver Transplantation

Nobuyuki Hojo, Toshimitsu Ishibashi, Toshihiko Yasuda, Yasunaru Sakuma, Taketo Fujiwara, Yoshikazu Yasuda, Hideo Nagai

Abstract

Background: Living-donor liver transplantation（LDLT）requires greater surgical experience, higher technical skills, and a more complete understanding of the hepatobiliary system than whole-liver transplantation from brain-dead donors. The usefulness of allograft models of LDLT is limited by problems related to techniques and immunosuppression. Experimental LDLT without rejection requires animal models of autologous transplantation. Porcine models are more anatomically similar to human LDLT than are other experimental models, but successful outcomes of porcine LDLT are very difficult to obtain. We evaluated a new porcine model of autologous LDLT that simulated human surgery in terms of procedural characteristics and technical difficulty. ***Methods***: Fifteen pigs were used, each serving as donor and recipient. The left lobe of the liver was removed and used as the graft. Hepatic and portal venoplasty of the liver graft was performed. The right lobe was excised. The opening of the right medial hepatic vein was enlarged by cutting into the suprahepatic inferior vena cava to facilitate anastomosis. The hepatic vein, portal vein, hepatic artery, and bile duct were reconstructed. ***Results***: Nine of the 15 pigs survived for the target period of 7 or 14 days after surgery. Four of the other 6 pigs died within 4 days after surgery because of technical failures, which might have been avoided by improved surgical skills. ***Conclusions***: The present study revealed that the LDLT model using one pig simultaneously as donor and recipient was technically possible.

（***Keywords***: Living-donor liver transplantation, Autologous transplantation, Porcine model, Surgical techniques）

Introduction

Liver transplantation has been developed as a curative treatment for end-stage chronic liver diseases and severe acute liver failure. However, a critical shortage of donor livers remains a serious problem throughout the world. In Japan and other Asian countries, transplantation of organs from brain-dead donors is uncommon, even though humans meeting the criteria for brain death are medically and legally considered dead. Consequently, living-donor liver transplantation（LDLT）has received considerable at-

Department of Surgery Jichi Medical University School of Medicine

tention, especially in Asian countries.

LDLT requires greater surgical experience, higher technical skills, and a more complete understanding of the hepatobiliary system than does liver transplantation from brain-dead donors[1]. In general, even if surgical procedures have been established clinically, experimental models are required for technical evaluation, as well as refinement of procedures. Ethically, surgical techniques should be tested and developed in laboratories, not in operating theaters by inexperienced surgeons.

Welch first performed experimental liver transplantation in dogs in 1955[2]. Subsequently, liver transplantation has been extensively done in dogs and pigs[3-9], but most experimental models were designed for total-liver replacement. Successful experimental LDLT with long-term survival was first reported in 1974 by Mizumoto et al, who performed the procedure in dogs[10]. This allograft model had several problems related to techniques and immunosuppression. During the next 30 years, LDLT was performed in different animal models including pigs[11] and monkeys[12], as well as in humans. Both clinically and experimentally, LDLT has been associated with problems such as complicated techniques and difficulty in controlling rejection. Improved animal models are thus required to explore optimal conditions for LDLT in humans and to clarify potential pathophysiological problems, such as small graft size and time-dependent ischemic damage to the liver. Experimental LDLT without concern about rejection can be achieved only by autologous transplantation. Experimental studies of autologous LDLT were performed by Kasai et al in 1997[13] and by Chung et al in 2002[14], both of whom used dogs. Porcine models are more anatomically similar to human LDLT than canine models that can be accomplished with relative ease. We therefore evaluated a new porcine model of autologous LDLT that simulated human surgery in terms of procedural characteristics and technical difficulty.

Materials and Methods

I. Animals

The experimental animals were 15 Landrace large white Duroc (LWD) pigs of both sexes, weighing 17.0-22.5 kg. Each pig served as both the donor and recipient. One LWD pig, weighing 10.0-17.5 kg, was used per operation. Blood for transfusion (400-800 ml) was collected, and the subhepatic inferior vena cava (IVC) and the portal vein were harvested for vessel grafting.

II. Anesthesia

The animals were fasted on the day before the experiment. After intramuscular injection of 25 mg/kg pentobarbital and 0.015 mg/kg atropine sulfate, anesthesia was induced by intravenous injection of 4 mg/kg propofol and 1 mg/kg ketamine. The trachea was intubated, and anesthesia was maintained by infusing 8 mg/kg/h propofol and 2 mg/kg/h ketamine. No volatile anesthetics or muscle relaxants were used.

III. Surgical procedures

The following surgical procedures were developed after pilot studies in 15 pigs, most of which died during or soon after surgery.

A. Donor surgery

First, donor surgery was performed.

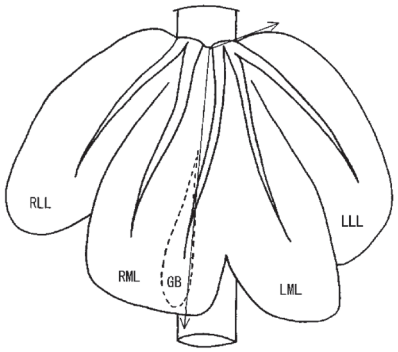

Figure 1. Incision line for left hepatic graft procurement. RLL: right lateral lobe, RML: right medial lobe, LML: left medial lobe, LLL: left lateral lobe, GB: gallbladder.

1. Laparotomy was performed through a reverse T-shaped incision. The portal pedicle was dissected to isolate the left hepatic artery and left portal branch. The sustentacular ligament of the left hepatic lobe was divided. 2. The incision line for the right medial lobe was established on a plane connecting the IVC to the left lateral margin of the gallbladder bed (Fig. 1). The hepatic parenchyma was divided by the forceps fracture method. 3. The left hilar plate, including the left hepatic duct, was divided. 4. The left caudate branch of the portal vein was divided. 5. The left hepatic artery and the left portal branch were divided. 6. The left and middle hepatic veins were divided, and the left half of the liver was removed for grafting. This procedure is similar to left hepatic lobe grafting in humans. 7. The stumps of the left and middle hepatic veins were sutured.

B. Bench surgery

Immediately after excision, the left hepatic lobe was immersed in ice-cold lactated Ringer's solution and perfused with 500 ml of lactated Ringer's solution at 4℃ and 1,000 ml of histidine-tryptophan-ketoglutarate (HTK) solution at 4℃ on the back table. The 8-shaped stumps of the left and middle hepatic veins were shaped into elliptical holes and sutured with strips of IVC 5-10 mm in length, harvested from another pig as vessel grafts (Fig. 2). The left portal branch was sutured with a portal vein graft.

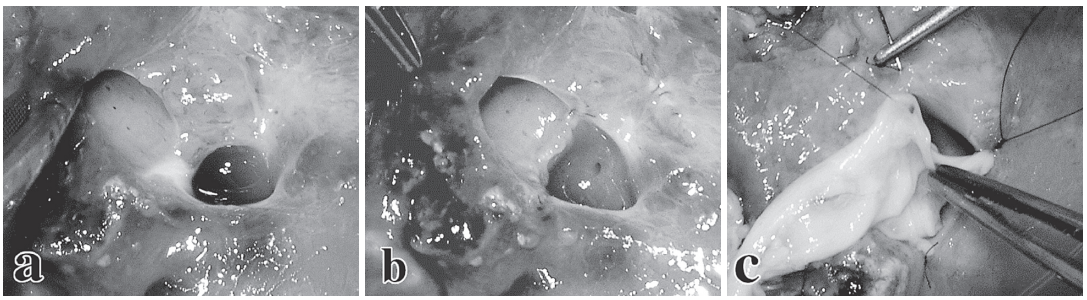

Figure 2. Hepatic venoplasty of the graft. The 8-shaped left and middle hepatic vein stumps (a) are shaped into an elliptical orifice (b), which is sutured with an IVC vessel graft harvested from another pig (c).

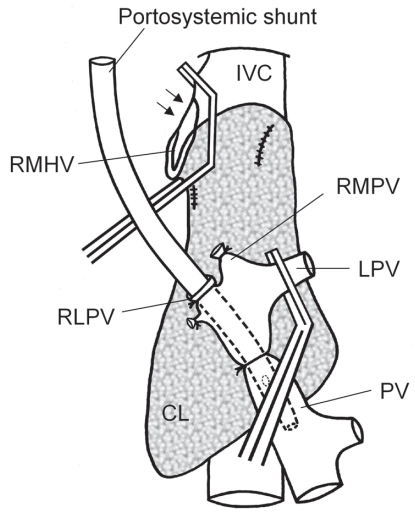

Figure 3. Portosystemic shunt and hepatic venoplasty of the recipient. CL: caudate lobe, IVC: inferior vena cava, PV: portal vein, LPV: left portal branch, RLPV: right lateral portal branch, RMPV: right medial portal branch, RMHV: right medial hepatic vein, Arrows: incision of the suprahepatic IVC.

C. Recipient surgery

After excision of the left hepatic lobe, recipient surgery was performed.

1. The portal pedicles in the right medial, lateral, and caudate lobes were exposed, and those in the medial and caudate lobes were ligated and divided. A portosystemic shunt (passive bypass) was created from the portal trunk to the left internal jugular vein via the right lateral portal branch, using a heparin-coated bypass tube (Anthron® VVT type, Toray, Tokyo, Japan) (Fig. 3). 2. The right lateral lobe was excised. 3. The right medial hepatic vein was grasped with vascular forceps to side-clamp the suprahepatic IVC, and the right medial lobe was excised. The entire liver, excluding the caudate lobe, was removed. 4. The opening of the right medial hepatic vein was enlarged by cutting to the suprahepatic IVC to facilitate anastomosis (Fig. 3). 5. After the completion of perfusion and angioplasty, the liver graft was put in place. 6. The vascular interposition graft attached to the left and middle hepatic veins of the liver graft was anastomosed to the right medial hepatic vein with continuous 6-0 polypropylene sutures (Prolene®, Ethicon, Somerville, NJ, USA). On completion of this procedure, lactated Ringer's solution was perfused through the portal vein of the liver graft to wash out the HTK solution from the graft. 7. The vascular interposition graft attached to the portal vein of the liver graft was anastomosed to the left portal branch with continuous 6-0 polypropylene sutures. 8. The portosystemic shunt was removed, and the liver graft was reperfused. 9. The hepatic artery of the liver graft was anastomosed to the resection stump of the left hepatic artery with interrupted 9-0 nylon sutures under microscope. 10. Biliary reconstruction was performed by duct-to-duct anastomosis with continuous 7-0 polypropylene sutures. 11. A Penrose drain was placed around the anastomosed hepatic duct, and the abdomen was closed.

Table 1　Operative data, survival, cause of death, and complications.

No.	Body weight (kg)	Operation time (min)	Weight of graft (g) (Graft-weight ratio [%])	Survival (days)	Liver weight at autopsy (g)	Cause of death	Complications
1	17.5	658	320 (1.8)	7	575	Euthanasia	Bile leakage
2	21.0	885	370 (1.7)	7	680	Euthanasia	Abnormal bile flow, hepatic artery thrombosis, gastric ulcer
3	20.0	677	380 (1.9)	7	610	Euthanasia	Bile leakage
4	18.0	640	320 (1.8)	7	550	Euthanasia	None
5	20.0	623	370 (1.9)	7	600	Euthanasia	Bile leakage, hepatic artery thrombosis, pleural effusion
6	17.5	773	300 (1.7)	2	−	Blood loss (catheter accident)	−
7	19.0	556	300 (1.6)	11	510	Gastrointestinal bleeding	Bile leakage
8	20.5	846	390 (1.9)	0	−	Excessive hemorrhage during surgery	Incompatible transfusion?
9	20.0	693	410 (2.1)	14	710	Euthanasia	Bile leakage
10	20.0	763	350 (1.8)	14	550	Euthanasia	Bile leakage
11	19.0	581*	350 (1.8)	0	−	Acidosis	−
12	19.0	705	370 (1.9)	0	−	Unknown	Malignant hyperthermia?
13	22.5	711	490 (2.2)	3	−	Ileus	−
14	17.0	661	300 (1.8)	14	580	Euthanasia	None
15	21.5	708	386 (1.8)	14	650	Euthanasia	Bile leakage

* : died during surgery

IV. Postoperative care

After extubation, the animals were allowed free access to water and food. To prevent infection and postoperative peptic ulcer, 500 mg x 2/day flomoxef sodium (Shionogi, Osaka, Japan) and 10 mg/day of lansoprazole (Takeda, Osaka, Japan) were administered for 5 days each. No immunosuppressants or anticoagulants were given. Target survival was 7 days in the first 5 animals because of the relatively poor general condition after surgery in pilot experiments. Since the postoperative course was uneventful in the first 5 animals, target survival was extended to 14 days in the next 10 animals.

V . Biochemical examination and autopsy

Blood samples were collected at the following times: before surgery; after excision of the left hepatic lobe; before reconstruction of the portal vein; 5 min, 30 min, and 2, 4, and 6 hours after reperfusion; and on days 1 to 7, 10, and 14 after surgery if the animals were alive at the respective time points. Antithrombin-III (AT-III), prothrombin time (PT), albumin (Alb), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T.B.) were measured.

At the time of autopsy, the transplanted liver was removed, weighed, and examined histologically.

Results (Table 1)

The mean surgery time was 707 ± 87 min. The mean graft-recipient body weight ratio was 1.8 ± 0.1%.

The first 5 animals survived for 7 days after surgery and were euthanized. Of the remaining 10 animals (target survival, 14 days) 4 survived for 14 days, 1 for 11 days, 1 for 3 days, and 1 for 2 days; 2 died immediately after surgery; and 1 died during surgery. The 4 animals that survived for 14 days were euthanized. The causes of death in the 6 animals that died before day 14 were gastrointestinal hemorrhage in 1 animal, an intravascular catheter accident in 1, technical mismanagement in 3, and unknown in 1. Of the 3 animals that died from technical mismanagement, one died of ileus caused by a bundle of gauze left in the abdominal cavity, another died of major hemorrhage during excision of the liver, and the last died intraoperatively from severe acidosis after the second session of reperfusion, caused by mismanagement of the portal vein anastomosis.

Postoperative complications were bile leakage in 8 animals, hepatic artery thrombosis in 2, gastrointestinal hemorrhage in 1, pleural effusion in 1, and gastric ulcer in 1.

The weight of the transplanted liver increased by 71.6 ± 10.3 % (on day 7, n = 5) in the animals that survived for initial target period of 7 days and increased by 73.0 ± 15.1% (on day 14, n = 4) in the animals who survived for 14 days.

The AT-III, PT, AST, ALT, ALP, and Alb levels started to recover about 2 days after surgery and were within normal ranges 7 or 14 days after surgery. The T.B. level fluctuated slightly, but remained low (Fig. 4).

No constriction was found near the anastomoses of the hepatic vein, portal vein, or bile duct in the removed liver grafts. One of the 2 animals with hepatic artery thrombosis had necrosis of the bile duct on the grafted side. Regeneration of hepatocytes was microscopically confirmed in the transplanted liver 7 or 14 days after surgery. No ischemic, congestive, or cholestatic changes were noted. There was only very mild lymphocytic infiltration around the allogenic interposition vessel grafts, suggested to be mild signs of rejection.

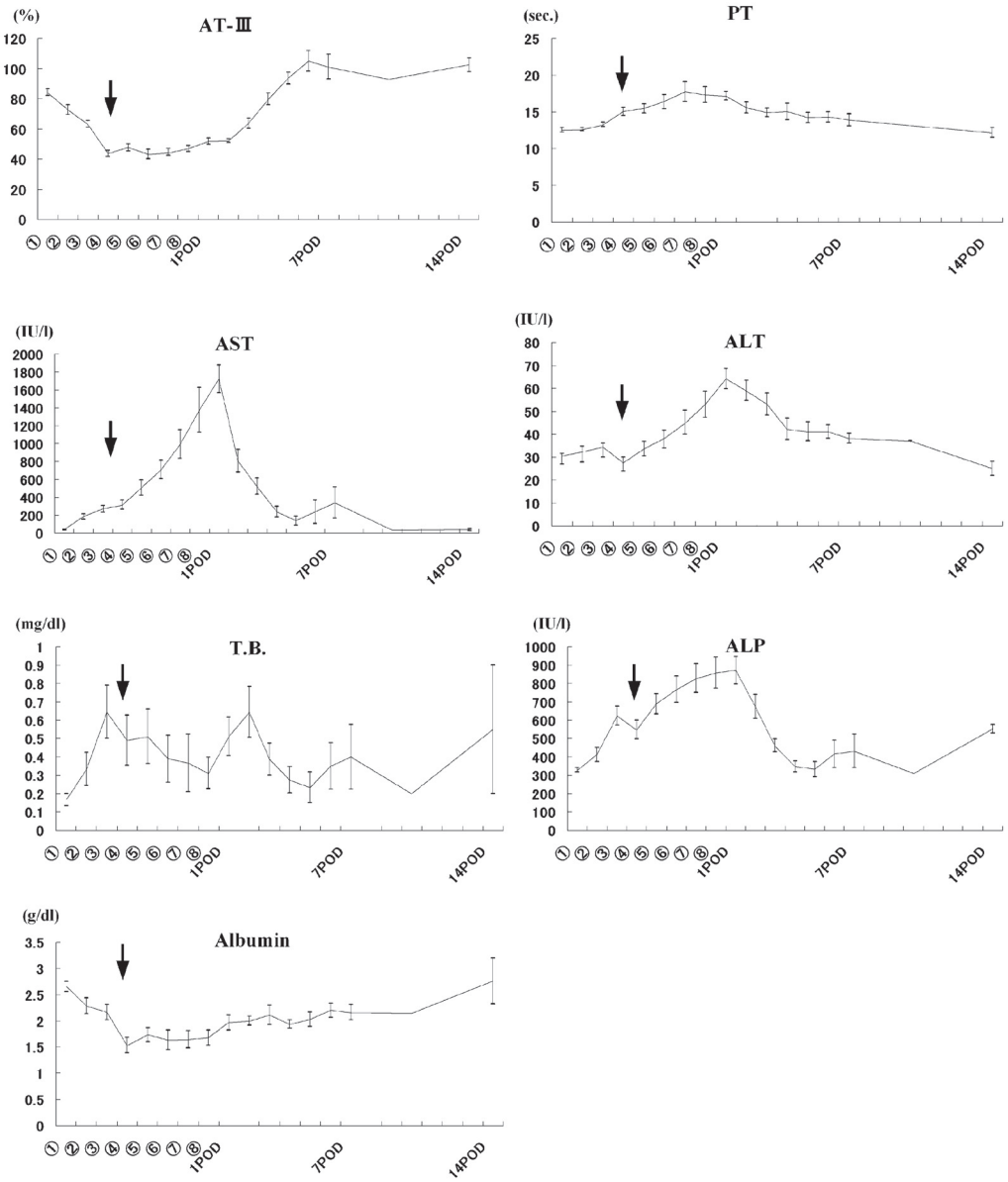

Figure 4. Biochemical markers before, during, and after autologous liver transplantation. AT-III: antithrombin-III, PT: prothrombin time, AST: aspartate aminotransferase, ALT: alanine aminotransferase, T.B.: total bilirubin, ALP: alkaline phosphatase, POD: Days after surgery, arrows: reperfusion.

① Before surgery, ② after removal of the liver graft, ③ before reconstruction of the portal vein, ④ 5 min after reperfusion, ⑤ 30 min after reperfusion, ⑥ 2 hours after reperfusion, ⑦ 4 hours after reperfusion, ⑧ 6 hours after reperfusion

Discussion

Our model of autologous liver transplantation has several important advantages, such as the absence of rejection and effects of immunosuppressants. Surgical techniques for LDLT can thus be directly and objectively evaluated.

Of the 6 pigs that died within 11 days after surgery, 3 died from surgical mismanagement and 1 died from postoperative mismanagement (blood loss from a disconnected intravascular catheter). These pigs probably would have survived much longer if perioperative management had been adequate. In the other animals, biochemical variables improved rapidly, and the histological findings and weight gain of the transplanted liver indicated that the transplants were functioning well and regenerating.

Porcine LDLT is superior to other animal methods because of anatomic similarities between pigs and humans. However, the usefulness of porcine models of LDLT has been limited by several problems, including difficulty in *in situ* excision of the hepatic parenchyma due to the risk of hemorrhage, the inability to perform hepatic vein-hepatic vein anastomosis because of the high fragility of the hepatic vein wall, difficulty in performing anastomoses of the portal vein and hepatic vein due to their proximity, and the need for complicated portosystemic bypasses[15]. Previously, Katoh et al[11] reported good results with a porcine model of allogenic LDLT, but did not describe the details of surgery. Kawarasaki et al[12] reported that LDLT in monkeys has several limitations, such as the availability of animals and unstable long-term results.

In our model, we have incorporated various technical modifications that can be or are being used for human LDLT. The incision line of the hepatic parenchyma is intentionally established apart from the division lines between lobes. Hemorrhage is reduced by precisely dividing the hepatic parenchyma by the forceps fracture method and by carefully ligating and excising the middle hepatic vein and left caudate branch of the portal vein.

An interposition graft can be applied to the hepatic vein in the liver graft to reinforce the venous wall and suture line. On the recipient side, an orifice large and strong enough to permit anastomosis is created by incising the wall of the hepatic vein to the suprahepatic IVC while side-clamping the IVC (Fig. 3), as is often done in human LDLT to prevent outflow block. Furthermore, a vessel graft is placed in the portal vein of the liver graft to facilitate anastomosis without tension. Excision of the entire recipient liver, excluding the caudal lobe, is done by separately excising the right and left liver lobes without IVC clamping or bypass. A portosystemic shunt is efficiently created during the anhepatic period by using a heparin-coated tube to establish a single passive bypass from the portal trunk to the internal jugular vein, without the use of a Biopump. By inserting a tube in the right lateral branch of the portal vein, portal vein anastomosis can be completed without disturbing blood flow during the anhepatic period. This procedure is similar to the technique developed by Kawasaki et al for creating a temporary right portal vein-IVC shunt[16].

Bile leakage occurred in 8 of the 10 animals that survived for 7 days or longer. Problems such as fragility of the porcine bile duct wall and decreased arterial blood flow in both the graft and recipient bile duct walls remain to be solved; these problems also occur in human LDLT when duct-to-duct anastomosis is used for biliary reconstruction[17, 18].

Our results fell short of our goals for postoperative morbidity and mortality. Some of potential limitations were poor control of hemorrhage during hepatic division and the lack of foolproof techniques for

procedures such as bile duct-to-duct anastomosis.

The present study revealed that the LDLT model using one pig simultaneously as donor and recipient was technically possible. We are planning of increasing the experience with this experiment so as to assess the usefulness of our autologous porcine model for training and evaluation and of surgical techniques for human LDLT.

References

1）Imamura H, Makuuchi M, Sakamoto Y et al. : Anatomical keys and pitfalls in living donor liver transplantation. J Hepatobiliary Pancreat Surg 7: 380-394, 2000.
2）Welch CS: A note on transplantation of the whole liver in dogs. Transpl Bull 2: 54-55, 1955.
3）Starzl TE, Kaupp HA Jr, Brock DR et al. : Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 111: 733-743, 1960.
4）Calne RY, Yoffa DE, White HJO et al. : A technique of orthotopic liver transplants in the pig. Br J Surg 55: 203-206, 1968.
5）Terblanche J, Peacock JH, Bowes J et al. : The technique of orthotopic liver homotransplantation in the pig. J Surg Res 8: 151-160, 1968.
6）Jones RS, Irving IM, Sells RA et al. : Hepatic transplantation in the neonatal pig. Br J Anaesth 45: 1116-1120, 1973.
7）Woodle ES, Hunter GC, Eisele P et al. : Orthotopic porcine liver transplantation: Operative technique. J Surg Res 39: 483-488, 1985.
8）Tanaka K, Nishimura A, Ogata S et al. : A simple method for orthotopic liver transplantation in pigs －A new cuff technique using expanded polytetrafluoroethylene vascular grafts for portal and vena caval anastomosis. Transplantation 58; 1139-1142, 1994.
9）Minor T, Saad S, Nagelschmidt M et al. : Successful transplantation of porcine livers after warm ischemic insult in situ and cold preservation including postconditioning with gaseous oxygen. Transplantation 65: 1262-1264, 1998.
10）Mizumoto R, Yokota T, Ohsawa J et al. : Survival of both donor and recipient in partial hepatic transplantation of dog. Jpn J Surg 4: 130-136 1974.
11）Katoh H, Ohkohchi N, Hirano T et al. : Viability of partial liver graft from living donor in pigs. Tohoku J Exp Med 175: 179-184, 1994.
12）Kawarasaki H, Iwanaka T, Tsuchida Y et al. : Partial liver transplantation from a living donor: Experimental research and clinical experience. J Pediatr Surg 29: 518-522, 1994.
13）Kasai S, Sawa M, Yamamoto T et al. : Orthotopic transplantation of a partial hepatic autograft in dogs. Surg Today 27: 1035-1039, 1997.
14）Chung KY, Kim MS, Lee JH et al. : Live donor partial liver harvest for canine liver transplantation: Development of an innovative technique for surgical training and research. Surg Today 32: 711-715, 2002.
15）Yassin YM, Eita MS, El-Mirghani M: Animal model for reduced left liver lobe transplantation. Transplant Proc 24: 1989-1991, 1992.
16）Kawasaki S, Hashikura Y, Matsunami H et al. : Temporary shunt between right portal vein and vena

cava in living related liver transplantation. J Am Coll Surg 183: 74-76, 1996.
17) Azoulay D, Marin-Hargreaves G, Castaing D et al. : Duct-to-duct biliary anastomosis in living related liver transplantation: The Paul Brousse technique. Arch surg 136: 1197-1200, 2001.
18) Kawachi S. Shimazu M, Wakabayashi G et al. : Biliary complications in adult living donor liver transplantation with duct-to-duct hepaticocholedochostomy or Roux-en-Y hepatichojejunostomy biliary reconstruction. Surgery 132: 48-56, 2002.

ブタを用いた新しい生体肝移植モデル

北條　宣幸　石橋　敏光　安田　寿彦
佐久間康成　藤原　岳人　安田　是和
永井　秀雄

要　約

（背景）ブタの肝臓は他の実験動物に比べ解剖学的により人間の肝臓に似ているが，肝静脈が脆弱である等の理由からブタの生体肝移植は大変困難とされる。ブタを用いた新しい生体肝移植モデルを考案した。（方法）１頭のブタをドナーとレシピエントの両方に使用した。肝左葉をグラフトとして摘出し，肝静脈，門脈に各々血管グラフトを縫合した。残った肝右葉を摘出した後，十分な強度と口径をもった吻合口を得るため右内側肝静脈を肝上部下大静脈まで切り込んだ。各脈管を再建した。（結果）15頭中９頭は目標とした７日または14日の生存が得られた。残る６頭中５頭は４日以内に死亡したが，うち４頭は手術の技術的ミスが死因で，いずれも技術向上により改善できるものであった。（結論）本実験により，１頭のブタをドナーとレシピエントに用いた生体肝移植手術は技術的に可能であることが明らかとなった。

自治医科大学外科学講座　消化器外科