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BRIEF COMMUNICATIONS

SIMPLIFIED TECHNIQUE OF ORTHOTOPIC LIVER TRANSPLANTATION IN PIGS[1]

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Background. Pig models have become common in transplantation immunological research. However, in pigs, clamping of the venous splanchnic system during orthotopic liver transplantation (OLT) is responsible for high morbidity and mortality rates; therefore, the use of venovenous bypass (VVB) is advocated. Because venous bypass can also cause specific complications, a simplified method for OLT in pigs has been developed and evaluated in terms of morbidity and mortality.

Methods. Twenty-three OLTs were performed between pairs of inbred miniature swine. Donor and recipient pairs (weighing 20–35 kg) were selected at 3–6 months of age. In the donor, the portal vein, infrahepatic vena cava, and suprahepatic vena cava were dissected, whereas the hepatic artery was preserved in continuity with the coeliac trunk and the abdominal aorta up to the iliac bifurcation. In situ cold perfusion was then performed. The recipient was prepared simultaneously by another surgical team. After total hepatectomy and complete portal and caval clamping, the suprahepatic vena cava and portal vein were sutured; VVB was not used. After completion of both venous sutures, the liver graft was reperfused. The infrahepatic vena cava was then anastomosed and unclamped. The donor aorta conduit was implanted end-to-side to the recipient infrarenal aorta, and the biliary reconstruction consisted of a cholecystojejunostomy with a Roux-Y loop.

Results. Twenty of 23 (87%) animals survived more than 1 week (7–483 days). The mean anhepatic time was 29.6±4.12 min. Although severe hypotension was noted during the anhepatic phase, the hemodynamic status rapidly recovered and stabilized after graft reperfusion.

Conclusion. Simplified technique without VVB is appropriate for successfully achieving OLT in pigs.

Venovenous active or passive bypass (VVB) has been believed to be essential for successful orthotopic liver transplantation (OLT) in pigs for more than 30 years (*1, 2*). Complete vascular clamping during the anhepatic phase, in fact, generates severe hypotension, acidosis, and hyperkalemia, which may cause the death of the animal. VVB has therefore been used in both experimental and clinical development of OLT technique. However, VVB in pigs is cumbersome and often complicates the procedure and may cause coagulation, metabolic disorders, and gas embolism (*3*). To avoid iatrogenic complications, a simplified OLT technique without VVB has been evaluated in a pig model. Although successful OLT might be achieved without bypass, the OLT technique must be simple, safe, reproducible, and adapted to the follow-up expected in the experimental setting: when short-term survival is needed, implantation that includes portal venous anastomosis using the cuff method (*4*) may be appropriate; however, when long-term survival is necessary, the OLT technique must take into consideration the rapid growth of the pig, thereby avoiding the cuff procedure which might narrow vascular anastomoses.

SLA^{dd} and SLA^{cc} NIH miniature breeder swine were generously donated by D.H. Sachs (Massachusetts General Hospital, Boston, MA) (*5*). SLA^{dd} and SLA^{cd} animals were bred in our facilities at Université Catholique of Louvain (Mr. E. Collignon, Centre A, Marbaix, LLN, Belgium). Donor and recipient pigs were selected at 3–6 months of age from the colony. Homozygous SLA^{dd} (class $I^{d/d}$, class $II^{d/d}$) pigs received OLT from heterozygous SLA^{cd} (class $I^{c/d}$, class $II^{c/d}$) donors.

Anesthesia was induced by Zoletil (Virbac, Carros, France) intramuscularly at a dose of 6 mg/kg. After intubation, inhalation anesthesia was maintained by enflurane (0–1.5%), nitrous oxide, and oxygen. For donor and recipient operations, we used a midline laparotomy. In the donor operation, portal vein and infrahepatic vena cava (IHVC) were dissected out. The hepatic artery was kept in continuity with the coe-

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liac trunk and abdominal aorta up to the iliac bifurcation. In situ liver perfusion (with either University of Wisconsin or Ringer's lactate solutions) was achieved through both portal vein and aorta. The harvested graft was immersed in a basin filled with cold perfusion solution at 4°C. During the donor preparation, another surgical team performed the recipient operation. The recipient animal was anesthetized, intubated, and a jugular catheter was positioned for daily blood sampling and intravenous infusion. A second catheter was placed in the carotid artery to monitor blood pressure during the procedure. The recipient liver was freed and all hepatic vessels were interrupted. The implantation site for the aortic conduit was prepared on the infrarenal aorta. During recipient operation, 500 ml of polygeline solution (Haemaccel; Hoechst, Germany) was infused starting just before and during the anhepatic phase. Baseline infusion consisted of one additional liter of Haemaccel during the whole procedure to maintain a stable hemodynamic condition. To take out the liver, we sequentially clamped the IHVC, portal vein, and suprahepatic vena cava (SHVC). The graft was then positioned in the right hepatic fossa, and for anastomosis of the portal veins and SHVC, running sutures of Prolene 6/0 (Ethicon, Johnson and Johnson, Dilbeek, Belgium) and Prolene 4/0 were used, respectively. After completion of these two anastomoses, the graft was reperfused, and the liver was flushed out, using the IHCV as a vent (to wash out the preservation solution and waste metabolites such as acids and potassium). Just after reperfusion, 5 mEq/10 kg of body weight of sodium bicarbonate solution were administered to correct metabolic acidosis. A 5/0 running Prolene suture was used for the IHVC anastomosis. The donor aorta conduit was finally implanted end-to-side to the recipient infrarenal aorta (with running Prolene 6/0 sutures). The biliary reconstruction consisted of a cholecystojejunostomy with a Roux-Y loop; for this anastomosis, PDS II 4/0 (Ethicon) running sutures were used.

Heparin, dopamine, or other vasoconstrictive drugs were not administered throughout the operation. The entire operative time for recipient was around 4 hr. The mean anhepatic time was 29.6±4.12 (SD) min. Hemodynamic status during the anhepatic phase changed dramatically, as previously reported (*6*). The mean systolic arterial blood pressure of 122±2.4 mmHg (range between 112 and 130) before portal vein clamping dropped to a mean of 51±1.5 mmHg (range between 46 and 57) immediately after total venous clamping (Fig. 1). During the anhepatic phase, the arterial blood pressure remained stable under rapid fluid infusion. After unclamping of portal vein and SHVC, the systolic arterial blood pressure rose immediately to a mean of 93±2.1 mmHg (range 89–101). At time of abdominal closure, the blood pressure became stable and returned to a value representing 82.8% of the initial blood pressure. Despite severe hypotension during the anhepatic phase, all recipients woke up on the operating table soon after cessation of anesthesia, and all were extubated within 1 hr. Antibiotics (Amoxicillin; Clamoxyl L.A., 15 mg/kg/day, Pfizer, Rixensart, Belgium) were given for 1 week after operation, and intravenous fluid (glucose 5%) was maintained for 2 days (2 L/24 hr).

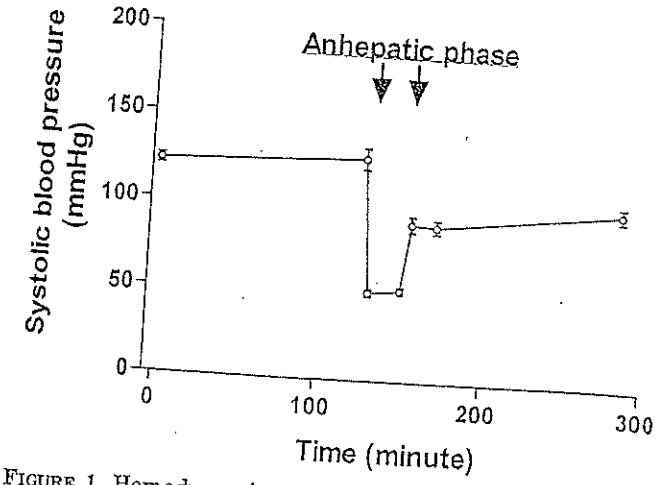

FIGURE 1. Hemodynamic status in the recipients (n=8) during operation. Systolic arterial blood pressure dropped after clamping of portal vein and vena cava (50–60% reduction of the preoperative arterial blood pressure). After allograft reperfusion, systolic arterial blood pressure recovered to 76.3% of the preoperative blood pressure. Data are expressed as mean±SEM.

Twenty of 23 (87%) animals survived for more than 1 week. Three animals died perioperatively: one animal did not recover from anesthesia, probably due to brain death; one animal died 6 hr after surgery due to extended splanchnic thrombosis, and one died on day 1 from pulmonary embolism. Twenty animals enrolled in two different groups in which treatment included no immunosuppression or tacrolimus (Fujisawa GmbH, Osaka, Japan) for 12 days, survived more than 1 week. In the untreated group, the animals survived up to rejection for 7, 16, 19, 21, 23, 37, and 45 days. In this group, one pig died at day 7 due to hepatic failure caused by rejection, but autopsy also revealed hepatic arterial thrombosis. In the tacrolimus-treated group, animals survived for 10, 14, 24, 27, 27, 44, 49, and 79 days and eventually died from intercurrent causes such as sepsis, embolism, post-transplant lymphoproliferative disorder, aerophagia, or intussusception. The other surviving pigs are doing well (at 182, 224, 440, 476, and 483 days). At time of death or sacrifice, sites of anastomosis were carefully observed, and all were patent.

The level of serum aspartate aminotransferase peaked on postoperative day (POD) 1, but went to normal level on POD4 (Fig. 2). The level of serum total bilirubin remained normal during the first postoperative week in Tacrolimus-treated animals, whereas it increased in untreated animals after POD3 in process of acute rejection.

Prior to use of the presently described method, five animals underwent portal vein and IHVC anastomoses by the cuff method previously described in dogs (*6*). Short polyethylene cuff (1 cm long and 7–12 mm in diameter) were designed for this purpose by BELDICO, MARCHE, Belgium. The portal vein and IHVC were anastomosed by using a cuff, and the flow was restored. Although attractive, this method did not shorten significantly the anhepatic time (around 25–30 min). On the other hand, this technique was inappropriate for long-term survival evaluation. Indeed, the cuff rapidly provoked narrowing of the portal venous anastomosis after 2 months, due to the rapid growth of the pigs (Fig. 3).

There are few reports in the literature about the OLT technique in pigs (*1, 2, 4, 8*). Recently, Flye et al. (*7*) reported pig OLT in NIH miniature swine and used a passive splenic and vena cava-to-jugular vein shunt to prevent severe hypo-

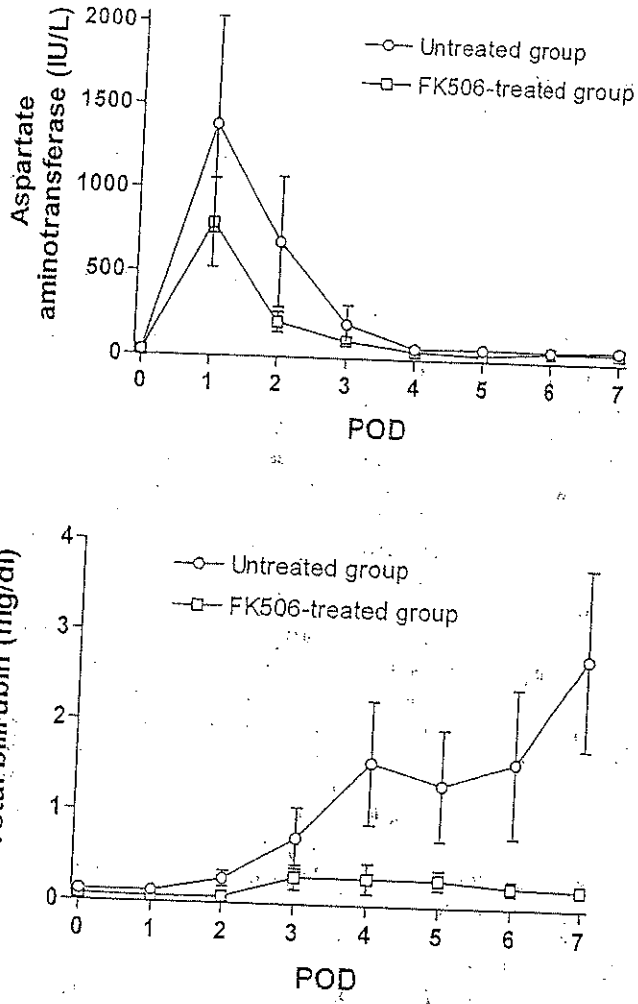

FIGURE 2. Changes in serum aspartate aminotransferase and total bilirubin during the first week post-OLT. Aminotransferase, peaking on POD 1 in both groups, returned to normal values within 4 POD. Total bilirubin level increased from POD 3 onward, due to rejection in the untreated group, whereas it remained normal in the FK506-treated group. Data are expressed as mean±SEM.

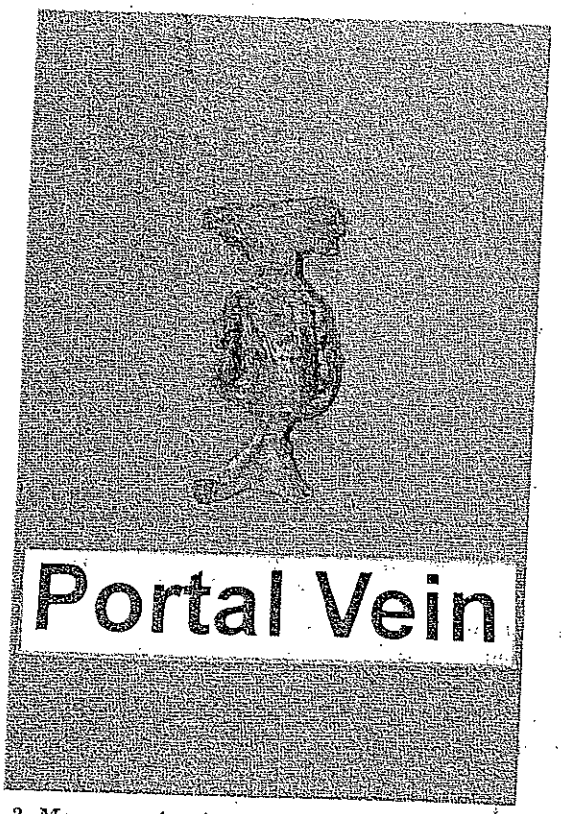

FIGURE 3. Macroscopic view of portal venous cuff showing severe anastomotic stenosis at 2 months post-OLT. This pig gained more than one-third of its weight during those 2 months (from 25 to 35 kg).

tension during the anhepatic phase. The rate of early survival in this report was 74%. Although VVB is useful for preventing stress and hypotension caused by portal and caval occlusion, it also causes specific complications such as bleeding, gas embolism, coagulation disorder, thrombosis, and metabolic disorders (*3*). The key for experimental animal models is the reproducibility and the low morbidity. Surgical technique in large animals must be as simple and as short as possible, inasmuch as there is no intensive care unit that can insure complicated postoperative care. This study showed that, in pigs, an anhepatic phase of 30 min can be tolerated without high morbidity. To even shorten the time of cross-clamping, the cuff method seems interesting, especially in experimental settings that do not need long follow-up. Successful canine OLT without bypass use and by utilizing cuffs for venous anastomoses has been reported (*6*). The reported mean time of anhepatic phase was 9.7 min. However, dogs are much more resistant to this procedure, and for ethical reasons, will not be available in the future for experimental settings. In addition, the cuff method proposed in dogs by Monden et al. (*6*) is not applicable in pigs at the level of the SHVC, rendering the cuff system useful only for portal and IHVC anastomoses. Because the completion of the SHVC anastomosis requires around 14 min, the anhepatic time is not significantly shortened, even by using cuffed portal vein anastomosis.

Goto (*8*) reported porcine OLT without the use of VVB by adopting a temporary aortic occlusion technique to prevent severe hypotension during the anhepatic phase. Comparable 24-hr survival (72%) was reported as well, in groups with or without VVB. Although such technique is attractive, we demonstrated in the present study that anhepatic phase without VVB could be easily tolerated during a 30-min period, which is usually sufficient to perform the two venous sutures preceding graft revascularization.

In conclusion, OLT might be easily achieved in pig models without the use of VVB. In the case of short-term follow-up (hours up to some days), the portal venous anastomosis might be achieved by using a cuff system, but in the case of long-term follow-up, running sutures are favored to overcome the rapid growth of pigs and subsequent portal vein narrowing. A simplified procedure of OLT without VVB is therefore proposed for use in studies of long-term immunological response to transplantation in pigs.

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VITAMIN E INHIBITS RENAL mRNA EXPRESSION OF COX II, HO I, TGFβ, AND OSTEOPONTIN IN THE RAT MODEL OF CYCLOSPORINE NEPHROTOXICITY

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Background. In a rat model of cyclosporine (CsA) nephrotoxicity, vitamin E preserves renal function and reduces free radicals, vasoconstrictive thromboxanes, and tubulointerstitial fibrosis. We examined the effect of vitamin E on tubule gene expression in this model.

Methods. In two of three groups, rats were treated with either CsA, or CsA plus vitamin E, whereas the control group received vehicles. We pooled purified tubules or whole kidney tissue in a novel manner to represent each treatment group, harvested RNA, and performed rigorously controlled qualitative reverse transcription-polymerase chain reaction.

Results. Cyclooxygenase (COX) I mRNA was detectable in control animals, was increased by CsA, but was unchanged by vitamin E. COX II mRNA was detected in controls, was inhibited in the CsA group, and was further inhibited with vitamin E. Hemeoxygenase I and TGF-β and osteopontin mRNA were increased in the CsA-treated group and were inhibited by vitamin E.

Conclusions. Our data support the involvement of free radicals, COX pathways, and pro-fibrotic genes in cyclosporine nephrotoxicity and suggest that the salutary effect of vitamin E involves the suppression of some of these genes.

Cyclosporine A (CsA) is a potent and selective immunosuppressive agent, and its introduction has permitted a steady increase in kidney and other organ transplantation. However, its clinical use has not been fully realized because of its frequent and at times severe renal toxicity. Several studies have been undertaken to understand the mechanism of this unwanted side effect (*1, 2*). Two main components of CsA nephrotoxicity have been recognized: (1) an acute and reversible renal vasoconstriction leading to acute reductions in renal blood flow and glomerular filtration rate, and (2) a chronic and irreversible tubulointerstitial fibrosis leading to chronic renal failure. Mediators and mechanisms postulated to be involved include thromboxanes and prostaglandins, fibrogenic growth factors including TGFβ as well as reactive oxygen species (ROS) (*2–4*). CsA-induced renal production of vasoconstrictive thromboxanes may result from lipid hydroperoxidation and/or induction of cyclooxygenase. The histologic findings in CsA nephrotoxicity are primarily those of tubulointerstitial fibrosis. Recent reports suggest a role of renal expression of fibrogenic growth factors such as TGFβ and extracellular matrix proteins including osteopontin, collagens, and fibronectin (*3–5*).

Regarding the possible role of cyclooxygenase in the production of vasoconstrictive prostanoids, the effects of CsA on tubule expression of either isoform of cyclooxygenase (COX) is not clear. Non-specific COX inhibitors have been shown to worsen CsA-induced nephrotoxicity (*6*). Differential up-regulation of COX II has therapeutic implication in CsA-nephrotoxicity, because specific COX II inhibitors are now available and could inhibit renal vasoconstrictor production without inhibiting the vasodilatory prostanoids elsewhere downstream of COX I.

We previously hypothesized that the antioxidant vitamin E suppresses CsA-induced lipid hydroperoxides and renal production of thromboxanes (*1*). In an earlier study, we found that administration of vitamin E suppressed tubulointerstitial fibrosis and limited renal dysfunction when coadministered with CsA in the rat model of CsA-induced nephrotoxicity. There was essentially no glomerular damage. Increase in urinary metabolites of prostanoid vasoconstrictors was also associated with CsA nephrotoxicity and similarly was reduced with vitamin E. In this report we have further explored the mechanism of CsA-induced tubulointerstitial in-

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