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Graft transection and warm perfusion in situ in canine partial orthotopic liver transplantation

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Abstract. Liver transplantation is now proven therapy for various forms of end-stage liver disease in children; however, the problem of donor liver shortage remains. To investigate the feasibility of graft procurement from living, genetically related adult donors without injury to either donor or recipient, partial orthotopic liver transplantation (PLT) using a graft transected and warm perfused in situ was evaluated in beagles; the viability of the graft was assessed in terms of energy metabolism, including blood ketone body ratio (KBR), as well as of recipient survival. PLT was performed in two groups with venovenous bypass. The left half of the donor liver was transected in situ, flush perfused with 2 l lactated Ringer's solution (4°C in group A, 20°C in group B), and immediately implanted into the recipient, who was totally hepatectomized, care having been taken to leave the inferior vena cava intact. Four of seven dogs survived for 5 days or longer (longest, 8 days) in group A and six of eight dogs (longest, 20 days) in group B. Causes of death were gastrointestinal bleeding, intussusception, or infection but not graft dysfunction. In both groups the KBR decreased significantly during the anhepatic period, recovered rapidly to the pre-anhepatic level after revascularization, and was maintained within a normal range thereafter. No significant differences in the time course of changes in KBR were seen between the two groups. These results suggest that a warm-perfused graft does not have a poorer viability than a cold-perfused one, that the concept of PLT with a graft transected and warm perfused in situ is feasible, and that it may be the solution to the problem of donor liver shortage.

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Liver transplantation (LT) is now proven therapy for irreversible forms of liver disease in children, including biliary atresia and congenital metabolic disorders, but the problem of donor liver shortage remains. One solution may be to graft part of the liver from living, genetically related adult donors. LT in children, especially in infants, using grafts from living donors presents special problems that differ from those in adults receiving grafts from brain-death donors. Since infants are especially prone to hypothermia and since the donors are living, every conceivable step must be taken to avoid injury to either donor or recipient, whether it be hypothermic damage due to cold flushing or damage to other organs due to grossly performed harvesting. Recently, successful reduced-size liver grafting has been reported for pediatric LT [3, 7]. However, little seems to be known about partial orthotopic liver transplantation (PLT) using grafts obtained by transection in situ, or about the feasibility or details of the harvesting technique. In addition, there has been no reliable method for assessing graft viability other than recipient survival. On the other hand, in a study of the influence of rapid cooling on graft viability, Otto et al. have reported that cold flushing causes endothelial damage, resulting in microcirculatory disturbance at reperfusion [16]. In this study, the feasibility of graft harvesting in situ was investigated and the viability of warm-perfused allografts was compared with that of

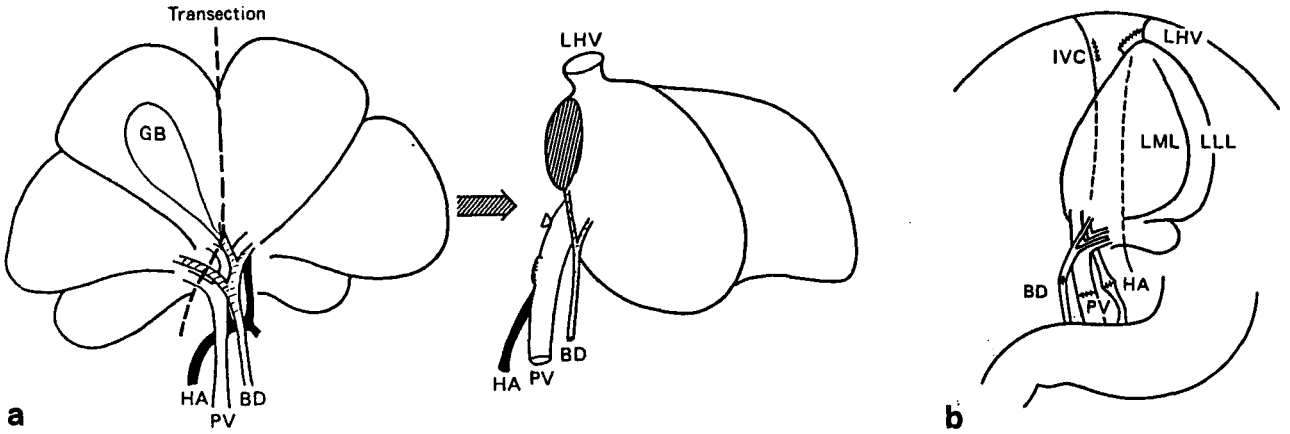

Fig. 1 a, b. Graft harvesting and transplantation. LML, LLL, and papillary process were isolated along with PV and HA. The graft was implanted with three-vessel anastomosis including end-to-end anastomosis of LHV between the donor and recipient and reconstruction of the PV and PD. *BD* Bile duct, *GB* gallbladder, *HA* hepatic artery, *LLL* left lateral lobe, *LML* left medial lobe, *PV* portal vein

cold-perfused ones in canine PLT using ketone body ratio, pyruvate, and lactate, as well as recipient survival, as indices of energy metabolism.

Materials and methods

Animals and surgical procedures

PLT was performed in beagles using the left halves of donor livers, which were transected and flushed in situ. Subjects were allowed no access to food, only to water, for 24 h prior to surgery. The operations were carried out with the animals under general anesthesia, using endotracheal intubation and mechanical ventilation with an O_2-air mixture. Anesthesia was induced by intravenous administration of ketamine 5 mg/kg body weight, with additional 1 mg/kg doses supplied as needed. Muscle relaxation was obtained by intravenous administration of pancronium bromide 0.1 mg/kg as needed. Arterial pressure and central venous pressure were monitored continuously via catheters located in the carotid artery and jugular vein. Donor and recipient operations were performed keeping the ischemic time of the graft as short as possible.

In donors, after mobilization of the whole liver, the left medial lobe, left lateral lobe, and papillary process were transected from the right half of the liver with good portal and arterial blood supply. All the vessels on the transected surface were elaborately ligated and sprayed with fibrin glue. After systemic heparinization with 3000 U of sodium heparin, the graft was isolated along with the trunks of the portal vein and hepatic artery. The graft was immediately flushed through the portal vein under hydrostatic pressure at 80 cm H_2O (Fig. 1 a). The dogs were divided into two groups. In group A ($n = 7$) the grafts were perfused with 2 l cold (4° C) lactated Ringer's solution. In group B ($n = 8$) the grafts were perfused with 2 l warm (20° C) lactated Ringer's solution. The donor was killed just after procurement and the blood was collected for transfusion. The transplantations were performed alternately in two groups.

In the recipients, the suprahepatic vena cava, infrahepatic vena cava, portal vein, and hepatic artery were clamped after systemic heparinization with 3000 U heparin and the application of a venovenous bypass with a flow rate of more than 60 ml/kg per min. Total hepatectomy was performed, special care being taken to leave the inferior vena cava (IVC) intact, closing all venous orifices draining into it. Only the orifice of the left hepatic vein was left open and utilized for anastomosis. Anastomoses were carried out in the following order (Fig. 1 b).

1. End-to-end anastomosis between the left hepatic vein of the donor and the left hepatic vein orifice of the recipient's IVC using 6-0 polypropyrene (Prolene).

2. End-to-end anastomosis of the portal vein using 7-0 polybutester (Novafil). At this stage, the graft was revascularized through the portal vein and to the hepatic vein.

3. End-to-end anastomosis of the hepatic artery using branch patches made from the gastroduodenal artery of the recipient and the splenic artery of the donor and 7-0 polybutester.

4. End-to-end anastomosis of the bile duct using a bioabsorbable polylactic acid splint. The polylactic acid splint is a tube 10 mm long and 1.6 mm in diameter, composed of D, L-lactic acid and glycolate copolymer synthesized according to a process devised by Jamshidi et al. [9]. It has been shown that the copolymer dissolves in about 4 months' time in vivo.

About 1054 ± 88 ml (1071 ± 126 ml in group A and 1000 ± 127 ml in group B) of canine acid citrate dextrose blood obtained from the donor and other dogs was transfused to maintain adequate blood pressure. During the entire procedure, glucose was infused at a constant rate (100 mg/kg per h initially, 0.5 mg/kg per h during the anhepatic period, and 200 mg/kg per h after revascularization of the allograft). Cyclosporin A (Sandimmune, Sandoz, Basel, Switzerland) was used in an intravenous infusion of 1 mg/kg per day in all cases and subcutaneous injection of 2 mg/kg per day in a few cases after the removal of the infusion catheter.

Sampling and assessment

Blood samples were taken for the duration of the experiments via a catheter inserted into the carotid artery. Analysis of blood gases,

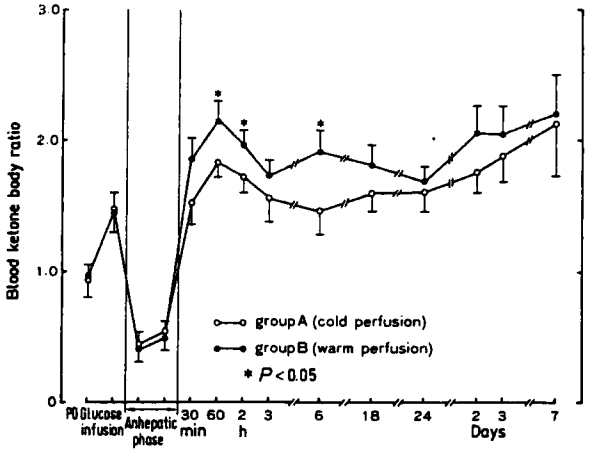

Fig. 2. Time course of changes in arterial blood ketone body ratio during and after partial orthotopic liver transplantation using either warm-perfused or cold-perfused graft. * Significant difference between group A and group B, *PO* preoperative, *PC* preclamp

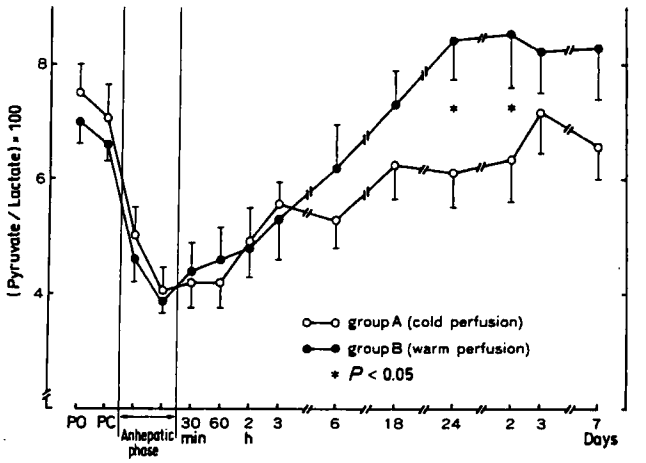

Fig. 3. Time course of changes in pyruvate/lactate ratio in arterial blood during and after partial orthotopic liver transplantation using either warm-perfused or cold-perfused graft. * Significant difference between group A and group B, *PO* preoperative, *PC* preclamp

hematocrit, and electrolytes was performed during and after surgery. Arterial blood ketone bodies (acetoacetate and 3-hydroxybutyrate) [13, 22], pyruvate [4] and lactate [5] were measured enzymatically. The enzyme used, 3-hydroxybutyrate dehydrogenase, was obtained from Sanwa Chemical (Nagoya, Japan) [6]. Other enzymes were purchased from Boehringer (Mannheim, FRG). When recipients died, autopsies were performed in all cases and specimens were taken for histological examination from the liver, lung, kidney, gastrointestinal tract, and other organs showing macroscopic abnormalities.

Values were expressed as mean ± SEM. Statistical significance between the means was determined using the Student's *t*-test. Values less than 0.05 were considered significant.

Results

Seven PLTs were performed in group A and eight in group B. None of the recipients were lost during surgery. The mean duration of the procedure, with specification of the individual phase, is given in Table 1. There were no significant differences in any value between the two groups. The time course of changes in blood gas, hematocrit, and electrolytes showed no significant difference between groups during and after surgery. Four of seven dogs survived for 5 days or longer in group A, the longest survival being 8 days. Six of eight dogs survived for 5 days or longer in group B, the longest survival being 20 days (Table 2). Most deaths in the two groups were due to gastrointestinal tract bleeding, intussusception, subphrenic abscess, or cardiac thromboembolism, but not to graft dysfunction. Macroscopic and microscopic investigations at autopsy showed no evidence of graft failure such as heterogeneous perfusion, hepatocellular necrosis, or

Table 1. Materials and mean duration of the procedure. Values are expressed as mean ± SEM; none of the differences is statistically significant

Group	A ($n=7$)	B ($n=8$)
Body weight (kg)	13.3 ± 0.3/	13.0 ± 0.6/
donor/recipient	11.4 ± 0.5	11.8 ± 0.5
Operating time (min)	324 ± 22	305 ± 20
Anhepatic time (min)	110 ± 2.5	100 ± 4.2
Total ischemic time (min)[a]	93 ± 6.2	80 ± 2.0
Warm ischemic time (min)[b]	48 ± 1.2	51 ± 2.4

[a] Duration from vascular clamp in donor to revascularization in recipient

[b] Duration from start of hepatic vein anastomosis to revascularization after portal vein anastomosis

Table 2. Survival and cause of death in partial orthotopic liver transplantation using cold- or warm-perfused allografts

No.	Duration of survival (days)	Cause of death
Group A (cold-perfused allografts)		
1.	2	Gastrointestinal bleeding
2.	4	Cardiac thromboembolism
3.	5	Cardiac thromboembolism
4.	4	Neurological sequelae
5.	7	Subphrenic abscess, peritonitis
6.	8	Intussusception
7.	7	Intestinal thromboembolism
Group B (warm-perfused allografts)		
1.	2	Pneumothorax, respiratory failure
2.	7	Intussusception
3.	7	Subphrenic abscess, peritonitis
4.	7	Cardiac thromboembolism
5.	10	Subphrenic abscess, peritonitis
6.	4	Gastrointestinal tract bleeding
7.	20	Peritonitis, dehydration
8.	7	Intussusception

Table 3. Changes in ketone body ratio and pyruvate and lactate levels in groups A and B; pyruvate/lactate level in arterial blood during and after orthotopic partial liver transplantation. Mean ± SEM. * Significant difference between groups A and B, $P < 0.05$. KBR, Ketone body ratio (acetoacetate/3-hydroxybutyrate), (P/L) × 100, pyruvate/lactate × 100

	Pre-operative	Preclamp	Clamp, 10 min	Pre-declamp	Time after revascularization								
					30 min	60 min	2 h	3 h	6 h	18 h	24 h	2 days	3 days[a]	7 days[b]
KBR (A, $n=7$)	0.94 ± 0.11	1.45 ± 0.10	0.44 ± 0.08	0.50 ± 0.08	1.53 ± 0.14	1.83 ± 0.10	1.72 ± 0.11	1.56 ± 0.17	1.46 ± 0.17	1.60 ± 0.14	1.61 ± 0.11	1.76 ± 0.13	1.89 ± 0.20	2.15 ± 0.34
KBR (B, $n=8$)	0.95 ± 0.07	1.47 ± 0.13	0.41 ± 0.09	0.55 ± 0.07	1.86 ± 0.14	2.17 ± 0.14*	1.98 ± 0.07*	1.73 ± 0.09	1.91 ± 0.16*	1.81 ± 0.13	1.68 ± 0.13	2.06 ± 0.20	2.04 ± 0.21	2.21 ± 0.38
Pyruvate (A)[c]	0.19 ± 0.02	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.24 ± 0.01	0.25 ± 0.02	0.24 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.03
Pyruvate (B)[c]	0.17 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.18 ± 0.02	0.16 ± 0.01*	0.16 ± 0.01*	0.15 ± 0.02	0.14 ± 0.02
Lactate (A)[c]	2.46 ± 0.36	3.57 ± 0.55	4.11 ± 0.53	5.45 ± 0.44	6.08 ± 0.70	5.73 ± 0.58	5.56 ± 0.58	4.74 ± 0.57	3.83 ± 0.37	3.09 ± 0.36	2.57 ± 0.33	2.41 ± 0.32	1.76 ± 0.28	1.96 ± 0.48
Lactate (B)[c]	2.68 ± 0.29	3.82 ± 0.11	5.16 ± 0.41	5.80 ± 0.43	5.76 ± 0.45	5.42 ± 0.56	5.07 ± 0.48	4.65 ± 0.46	4.01 ± 0.60	2.98 ± 0.43	1.95 ± 0.28	1.79 ± 0.31	1.83 ± 0.37	1.94 ± 0.46
(P/L) × 100 (A)	7.49 ± 0.48	7.12 ± 0.59	5.01 ± 0.46	4.12 ± 0.43	4.21 ± 0.43	4.20 ± 0.45	4.85 ± 0.47	5.55 ± 0.66	5.21 ± 0.45	6.25 ± 0.62	6.11 ± 0.76	6.32 ± 0.28	7.22 ± 0.79	6.40 ± 0.56
(P/L) × 100 (B)	6.95 ± 0.36	6.57 ± 0.28	4.60 ± 0.40	3.78 ± 0.20	4.37 ± 0.50	4.60 ± 0.58	4.68 ± 0.56	5.30 ± 0.42	6.20 ± 0.78	7.32 ± 0.61	8.54 ± 0.64*	8.66 ± 0.86*	8.26 ± 0.72	8.30 ± 0.81

[a] At 3 days (A) $n=6$, (B) $n=7$
[b] At 7 days (A) $n=3$, (B) $n=6$
[c] μmol/ml

infarction. No infiltration of mononuclear cells in the portal area was observed.

Table 3 shows the time course of changes in arterial blood ketone body ratio (KBR) and the concentrations of pyruvate and lactate in arterial blood. In both groups, the KBR decreased significantly during the anhepatic period ($P<0.01$, compared with preanhepatic level) and increased rapidly after revascularization of the allografts. It returned to the preanhepatic level within 30 min after revascularization. The KBR values of group B were higher than those of group A at most observed points, and statistical significance was obtained particularly at 1, 2, and 6 h after revascularization (Fig. 2). Concentrations of pyruvate and lactate increased progressively during the anhepatic period, reached their maximum within 1 h after revascularization, and then gradually returned to the preoperative level at 6 h postoperation. The pyruvate/lactate (P/L) ratio decreased after the beginning of the anhepatic phase and remained significantly low for 3 h (compared with preclamp level, $P<0.05$). While no significant difference was observed in the time course of changes of pyruvate and lactate, the pyruvate concentration tended to be higher in group B than in group A, and the lactate concentration remained higher in group A than in group B after revascularization. As a result, the P/L ratio appears to be lower in group A than in group B (Fig. 3).

Discussion

Bax et al. have reported on orthotopic nonauxiliary partial liver transplantation in canines using the left two lobes flushed as a whole, followed by transection ex situ [2]. They showed that such a procedure was feasible with 60% of a liver to maintain graft viability. Clinically, the technique of graft implantation with preservation of the IVC has recently been used successfully in children [18, 19]. A study by Mackenzie et al. concerning the tolerance of the canine liver to warm ischemia demonstrated that warm liver ischemia extended to 1 h in dogs was compatible with normal liver regeneration after hepatectomy under temporary splanchnic decompression [12]. We have also reported that the energy charge of canine livers after 60 min of warm ischemia recovered to the normal range with an enhancement of mitochondrial phosphorylative activity [11]. These facts lead us to propose that donor grafts consisting of only half the liver could cope with metabolic loads and maintain the recipient's life even after 1 h of warm ischemia. Clinically, 1 h is too short a time to transport a graft from where it is harvested to where it would be im-

planted, but it is long enough if the living donor and the recipient are lying side by side in the operating room. Vascular anastomoses would also be accomplished within this time. Warm perfusion would, moreover, avoid hypothermic damage in both the recipient and the donor when the graft is harvested in situ from a living donor. In addition, it is important for the pediatric surgeon to avoid hypothermia during and after surgery since children are markedly susceptible to it and it may cause deterioration of cardiovascular and coagulation function [1, 10].

From the viewpoint of graft viability, cold flush perfusion and the rewarming process after revascularization might be potentially harmful [8]. An investigation done by Otto et al. using electron microscopy showed that severe endothelial damage to the sinusoids was incurred due to cold (2° C) Ringer's flush perfusion [16]. They noted that the damage was aggravated after revascularization, resulting in microcirculatory disturbance and liver parenchymal cell impairment. An ultrastructural study of rat liver ischemia by Myagkaya et al. revealed that the difference in sensitivity to ischemia between sinusoidal wall cells and hepatocytes is more marked under cold ischemia [15]. Our investigations into the effects of hypothermia on the adenine nucleotide level in hepatectomized rabbits indicate that ATP-utilizing reactions were markedly accelerated by rewarming to such a degree that the ATP-generating sequences, even if enhanced, were unable to provide sufficient energy for the elevated ATP demand, resulting in an acute energy crisis [21].

We also reported that the metabolic viability of allografts after liver transplantation is mitochondrial in origin, that the normalization of the mitochondrial redox state is essential for the deranged metabolic state of allografts, and that the application of KBR, which reflects the mitochondrial redox potential, may provide rapid and accurate information on the cellular viability of allografts [17, 20]. Moreover, we reported that the rapid recovery of KBR is a prerequisite for the normalization of accumulated pyruvate, lactate, and amino acids [14]. A comparison of the results of the present study with those of previous studies, i.e., the changes in KBR, pyruvate, lactate, and P/L ratio, which showed quite similar patterns, proves that graft viability was satisfactorily maintained without any lethal consequences by the present method of transplantation, although the survival duration was less than expected due to complications other than those of hepatic origin, as confirmed by macroscopic and microscopic findings during autopsy. Therefore, the values obtained in the present study may be considered indicative of the effects of perfusion temperature on graft viability. The difference in the changes in KBR, pyruvate, lactate, and P/L ratio between the two groups after revascularization might be affected by cold perfusion and/or the rewarming process.

Although it cannot be determined whether warm perfusion is clinically applicable until more stable and prolonged survival of the recipients is established, it may be concluded from the results, in terms of energy metabolism as well as of survival, that warm-perfused grafts are no less viable than cold-perfused ones, and that the concept of PLT using grafts transected and warm perfused in situ from a living donor is feasible and may be a potential solution to the problem of donor liver shortage.

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