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The Experimental Study on the Temporary Portal Vein Arterialization in the Canine Liver Transplantation: Preliminary Report

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Summary

To evaluate the feasibility of temporary portal vein arterialization (PVA) in orthotopic partial liver transplantation (PLT), we performed 5 canine PLTs with PVA assessing the changes in arterial ketone body ratio (AKBR) as an index of hepatic energy status, and measuring portal pressure and flow. After anastomosis of hepatic vein, the graft liver was revascularized with arterial blood shunted from the external iliac artery to the hepatic side of the portal vein. By using this technique, both anhepatic period of the recipient and ischemic time, especially warm ischemic time , of the allograft were markedly shortened (31.0±4.5 min: Mean±SEM). Four out of 5 recipients survived for at least 5 days (13 days in average). The AKBR was restored immediately after PVA and showed almost the same values as those at preclamping and after completion of anastomoses of both portal vein and hepatic artery. No significant difference in portal venous pressure was observed between during PVA and after vascular reconstruction. Portal blood flow during PVA was about one fourth of the total hepatic blood flow at preclamping. These results suggest that PVA can be used as an alternative procedure in PLT.

Introduction

Portal vein arterialization (PVA) shunting arterial blood to the intrahepatic portal vein (PV) has been used as a therapeutic modality to ameliorate the adverse metabolic effects of portosystemic decompression[1,2,11]. Despite its efficacy demonstrated by many experimental and clinical studies, this procedure was discountinued by reason for the severe liver damage caused by excessive arterial perfusion of the portal tree[10,28]. Once assigned to oblivion, PVA has been retrieved due to microsurgical techniques that enable us to use a small artery for pressure-adapted low flow reperfusion without causing histological disruption of the hepatocytes[3,15].

Key words: Portal vein arterialization, Arterial ketone body ratio, Portal venous pressure, Hepatic blood flow,Liver transplantation.
索引語：門脈動脈化，血中ケトン体比，門脈圧，肝血流量，肝移植.
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Recently, Sheil et al. proposed the use of temporary PVA as a graft revascularization technique in orthotopic liver transplantation[18]. From the viewpoint of hepatic energy metabolism, we also found the beneficial effect of temporary PV-A as the revascularization technique after canine normothermic hepatic ischemia on hepatic energy status using arterial ketone body ratio (AKBR: acetoacetate/β-hydroxybutyrate)[25]. AKBR which reflects the hepatic mitochondrial redox state, has been shown to provide a rapid and accurate information with respect to graft viability[5,14,21].

We have, therefore, applied this techinique to various hepatic surgical procedures, such as radical block resection for hilar bile duct cancer invading vessels. Differing from the whole liver replacement, however, the feasibility of PVA in PLT, which is characterized by reduced size of graft and by the outflow tract through hepatic vein anastomosis, has yet to be investigated. In the present study, we applied the temporary PVA technique to canine partial liver transplantation (PLT) in dogs, and investigated its efficacy by measurement of AKBR as well as pressure and flow study.

Materials and Methods

Animals; Ten adult male beagles, weighing 9–10 kg, were subjected to 5 PLTs. All animals were purchased from *Japan Clea Company*, and were not allowed access to food other than water 24 hr prior to surgery.

Anesthesia; The operation was performed under general intubational anesthesia and mechanical ventilation with O_2-air mixture. Analgesia was maintained by initial intravenous administration of ketamine hydrochloride (5 mg/kg of body weight) and by supplemental dosages (1 mg/kg) as needed. Muscular relaxation was obtained by intravenous administration of pancronium bromide (0.1 mg/kg) as needed. The external jugular vein was cannulated for the intravenous continual infusion of lactate Ringer's solution (30 ml/kg/hr) and 5% glucose solution (8 mg/kg/hr). The carotid artery was cannulated for monitoring the systemic blood pressure (BP) and for sampling blood. Blood sugar (BS) and arterial oxygen tension (PaO_2) were kept at the levels of BS $>$ 150 mg/dl and PaO_2 $>$ 150 mmHg to exclude the possible effects of fasting[12] and hypoxia[23] on AKBR. Direct monitoring of portal venous pressure (PVP) and sampling blood to measure the oxygen tension were done via a catheter inserted into the gastroduodenal vein. Portal venous flow (PVF), hepatic arterial flow (HAF) and portal blood flow during PVA were measured with an ultrasonic range-gated pulsed Doppler flowmeter (CRYSTAL BIOTECH, Holliston, MA, USA)[6]. The flow probes were placed tightly around the portal vein (7–8 mm in diameter), hepatic artery (3 mm in diameter), and right external iliac artery just porximal to the shunt tube (4–5 mm in diameter).

Donor operation; Portal branches, hepatic arteries, and bile ducts to the right lateral, right medial lobes and caudate process were ligated and divided in the hepatic hilum. Hepatic parenchyma was transected along the interlobar plane between the right medial and left medial lobes. The graft liver, comprised of the left medial (LML), left lateral lobes (LLL) and papillary process, was removed[27] and flushed in situ with 1 liter of cold lactated Ringer's solution containing heparin (1000 unit/L) through the portal trunk. Vascular preparations were performed on a back table, keeping the graft cool by additional perfusion with 0.5 liter of the same lactated Ringer's solution. The left hepatic vein was prepared for anastomosis by taking a small cuff from the wall of the IVC.

Recipient preparation; Caval and portal decompression were performed with a centrifugal blood pump (Bio Medicus, Inc., Eden Parairie, Minnesota, USA) at a flow rate of more than 60

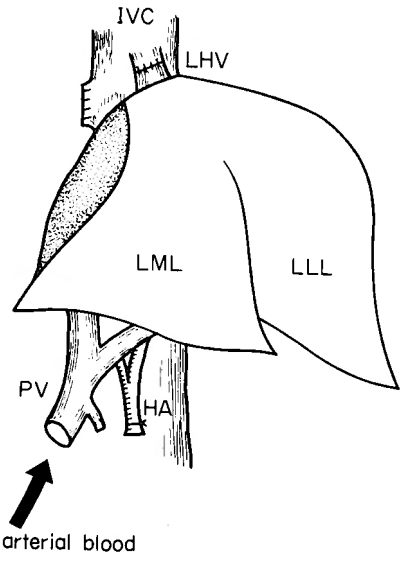

Fig. 1 Schematic drawing of liver transplantation with portal vein arterialization technique. (HA) hepatic artery; (IVC) inferior vena cava; (LHV) left hepatic vein; (LLL) left lateral lobe; (LML) left medial lobe; (PV) portal vein.

ml/min/kg. The inferior vena cava was decompressed through a proximal left femoral vein, and the splanchnic bed was drained through a splenic vein. The spleen was resected. Withdrawn venous blood was returned through the left external jugular vein. Then, total hepatectomy was performed accoring to the method described by Starzl[19] with special care as not to injure the IVC and closed all venous orifices draining into the IVC except for the orifice of left hepatic vein (LHV) which was left open and utilized for anastomosis to graft. Prior to removal of the recipient liver, but before vascular interruption, a heparin-coated tube (anthron bypass tube, No. VSS-460, Toray Co. Ltd., Tokyo, Japan; outer diameter, 4 mm; inner diameter, 3 mm; length, 60 cm) was inserted into the recipient external iliac artery via the femoral artery.

Transplantation; As shown in Fig. 1, the graft liver was put in orthotopically and the vascular reconstruction was performed as the following order: (1) Anastomosis between the LHV of donor and the LHV orifice of the recipient IVC. (2) Graft revascularization with PVA by insertion of the other end of the indwelling iliac arterial shunt tube to the hepatic side of PV (3) Anastomosis of the hepatic artery (HA). (4) Clamping of the iliac arterial shunt tube. (5) Anastomosis of the PV using. (6) Removal of the veno-venous bypass. (7) Removal of the heparin-coate tube and repair of the right femoral artery using. (8) Anastomosis of the bile duct.

Postoperative follow-up; Blood samples were taken for the measurement of AKBR throughout the operation, especially focusing the changes on the periods before, during, and after PVA. Arterial ketone bodies (acetoacetate and β-hydroxybutyrate) were enzymatically by KETOREX Kit (Sanwa Chemical, Nagoya, Japan) and KETO-340, a semi-automatic spectrophotometer designed for the measurement of ketone bodies (Ihara Electric Co., Kasugai, Japan)[13,24].

Results are represented as mean±SEM. Statistical significance was determined by paired Student's t test and p values less than 0.05 were considered to be significant.

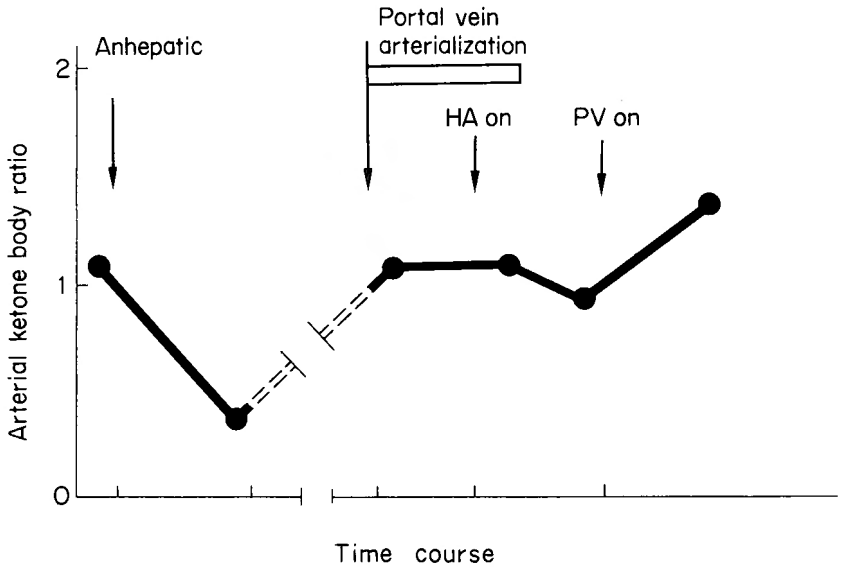

Fig. 2 Changes in arterial ketone body ratio during liver transplantation, focusing on the period of portal vein arterialization. Values represent mean of 5 animals.

Results

The weight of the graft liver was 156±10 g which was estimated to be about half the total weight of the donor liver (300±15 g) and recipient liver (295±16 g). The total operation time was 310±25 min, the anhepatic period was 85.0±10.0 min, and the total ischemic time of the graft, from the clamping of hepatic circulation in the donor until revascularization of portal blood flow by PVA in the recipient, was 74.5±12.0 min. The warm ischemic time of graft, from putting in the recipient's abdominal cavity until revascularization by arterial blood into the PV, was 31.0±4.5 min. The duration of PVA was 38.5±4.5 min.

AKBR decreased significantly during the anhepatic phase compared with preclamping state and rapidly restored immediately after PVA. The AKBR during PVA showed almost the same values as those at preclamping of the donor, and AKBR maintained higher levels during PVA plus HA inflow, and PV plus HA inflow (post-reconstruction) states. Moreover, this value of PVA was significantly higher than that of anhepatic state and that of only hepatic arterial inflow state (Fig. 2).

Concerning portal vein pressure (PVP), total hepatic blood flow (THBF=PVF+HAF) and oxygen tension in portal blood, PVP during PVA was 21.5±1.5 cmH_2O which was significantly ($p<0.05$) higher than that at preclamping (15.4±0.6 cmH_2O), and almost the same as the post-reconstructive value (21.0±1.0 cmH_2O). The preclamping PVF, HAF, and THBF were, 27.1±2.4 ml/kg/min, 4.5±0.8 ml/kg/min, and 31.7±2.8 ml/kg/min, respectively. During PVA, arterial blood flow into the PV was 8.5±0.7 ml/kg/min which was significantly lower than both the preclamping and post-reconstructive THBFs. PaO_2 of portal blood during PVA was 165.1±12.0

Table 1. Changes in portal vein pressure, total hepatic blood flow and portal vein blood oxygen tension at preclamping and during arterialized and post-reconstructive periods

		preclamping		arterialized	post-reconstructive	
portal vein pressure (cm H_2O)		15.4±0.6[a]		21.5±1.5[b]	21.0±1.0	
total hepatic blood flow (ml/kg/min)	PVF	31.7±2.8	27.1±2.4	8.5±0.7[b]	24.7±2.2	19.1±2.0
	HAF		4.5±0.8			5.5±0.9
portal vein oxygen tension (mm Hg)		92.5±11.0		165.1±12.0[b]	87.2±10.1	

PVF: portal venous flow, HAF: hepatic arterial flow

a) mean ±SEM

b) $p<0.05$, as compared with preclamping and/or post-reconstructive values

mmHg which was significantly higher than the preclamping (92.5±11.0 mmHg) and post-reconstructive (87.2±10.1 mmHg) levels (Table. 1). During PLT procedure, systemic blood pressure was maintained between 110 and 180 mmHg. Four out of 5 recipients survived for at least 5 days (13.0±3.5 days; mean±SEM), and another animal died of outflow block on the first postoperative day.

Discussion

Temporary PVA technique in liver transplantation was first introduced to minimize both the ischemic time for the allograft and the anhepatic period for the recipient, as well as to release the operators from the pressure of time constraints[18]. It might well be that the ultimate utility of this technique lies not in orthotopic liver transplantation but in PLT. In PLT for children, reconstruction of the hepatic vein and PV are the most difficult and time consuming parts of the whole procedure [27] that might exceed certain limits of the warm ischemic time. For success of liver transplantation, the warm ischemic time must not exceed these limits and should be as short as possible. Shotening the warm ischemic time of the graft by using PVA is, therefore, reasonable for maintaining the graft viability in good condition in PLT.

The present report describes the significant decrease in both anhepatic period and ischemic time of the graft as compared with previous reports[22,27], which are almost the same in method except for PVA. Especially noteworthy is that the warm ischemic time was only 31 minutes, about a half of the limit of warm ischemia time in animals[8,9] and humans[7].

Another advantage is the rapid recovery of the hepatic energy status of the graft. We have shown that normalization of the mitochondrial redox state is essential for the recovery of the reduced graft metabolism and that AKBR provides real-time information about these changes[5,21]. And immediate standing up and maintaining high level of AKBR after revascularization of graft is an evidence of securing excellent viability of the graft. According to the AKBR in this study, revascularization by PVA had the same beneficial effect on the restoration of the hepatic mitochondrial redox state as that of both PV and HA, which is in accordance with a previous report[25]. This is further supported by the significantly high level of AKBR during PVA compared with that of HA flow alone.

Possible explanations for the ameliorative effects of PVA are summarized as follows: (1) the significantly high levels of PaO_2 in portal blood during PVA contribute to the sufficient supply of ox-

ygen needed in electron transport along the respiratory chain in the mitochondria. Furthermore, PVA is reported to improve liver regeneration after hepatectomy because of its high concentration of oxygen[4]. (2) the significantly reduced warm ischemic time improves graft viability. It is reported that the negative correlation between the duration of warm ischemic period and the rate of AKBR recovery in PLT, and was suggested that graft viability depends on the warm ischemic time[22]. (3) the shunted arterial blood flow into the portal system maintains about one fourth of the preclamping THBF. According to a preliminary experiment using AKBR[26], the minimum blood flow needed to preserve the viability of the liver during PVA was only about 10% of the preclamping THBF. It is also reported that the safety limit of portal blood flow during PV-Art existed between 10% and 25% of THBF by measuring the tissue adenine nucleotides of the liver[17].

On the other hand, the main disadvantage of PVA is the risk of damage to the hepatocytes and portal vein branches caused by connecting the high pressure arterial system to the intrahepatic portal vein[1,2,10,11,28]. However, as long as the pressure and the inflow are maintained at or below the normal level, they have no adverse effect on hepatic function and architecture[3,15,18,25]. A significant increase in PVP during PVA was observed as compared to preclamping value in this study. This increase was considered not to be the effect of PVA but due to the slightly stenotic LHV anastomosis or reduced liver mass, since no significant difference in PVP was observed between PVA and post-reconstruction. This interpretation is supported by the previous report[25] in which PVP during PVA showed almost the same value at preclamping in the absence of LHV anastomosis or liver mass reduction. The aplication of the PVA may bring more successful result in liver transplantation[20].

In conclusion, the PVA technique in PLT is an effective and safe method for maintaining the functional reserve of the graft liver. This technique could be used as an alternative procedure in PLT clinically, especially when obtaining a graft from a living donor.

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和文抄録

肝移植における門脈動脈化に関する実験的研究
——Preliminaly report——

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イヌ5頭に，一時的な門脈動脈化の手技を導入した部分肝移植を行い，動脈血中ケトン体比の変動，門脈圧，肝血流量の変化を測定して，肝移植における門脈動脈化手技導入の意義について検討した．肝静脈吻合後に，外腸骨動脈に挿入したチューブで門脈本幹に動脈血で血行再開通を画り，この手技によって，レシピエントの無肝期と肝阻血時間，とくに温阻血時間を平均31分と短縮することができた．レシピエントの生存は，5頭中4頭において最低5日間，平均13日が得られた．無肝期に低下した動脈血中ケトン体比は門脈に動脈血流入開始後，速かに上昇し，ほぼ肝血流遮断前の値を示し，門脈，肝動脈両者の血行再建完成後も良好なその値を維持した．門脈動脈化中の門脈圧は，血行再建終了後と差はなかった．動脈化中の門脈血流量は肝血行遮断前の全肝血流量のほぼ4分の1であった．門脈の動脈化は，肝移植施行に際し導入しうる，有意義な手技と考えられる．