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BRIEF REPORT

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The beneficial effect of prostacyclin analogue (OP2507) on rat liver transplantation subjected to an extended anhepatic phase

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Abstract We investigated the effect of a prostacyclin analogue (OP2507) on PVG (RT1^c) recipients subjected to an extended anhepatic phase (AH) and transplanted orthotopically with PVG livers. All of the animals that underwent orthotopic liver transplantation (OLT) with a 20-min AH survived for 1 week with or without OP2507 (OP) treatment (10/10, 100 %). When the AH was lengthened to 45 min, the 1-week survival rate of recipients was poor in OP-untreated groups (1/10, 10 %). Treatment of the recipient with OP2507, 0.15 μg/kg per minute for 30 min, prior to the 45-min AH substantially improved 1-week survival (5/6, 83.3 %, $P < 0.05$). The serum TNF-α level at day 1 in OP-treated animals that underwent OLT with a 45-min AH was significantly lower than that in animals with 45-min AH OLT without OP treatment. We conclude that OP2507 treatment has potential usefulness as a perioperative treatment when the AH is extended during OLT.

Key words Liver transplantation, rat prostacyclin · Prostacyclin, liver transplantation, rat · TNF-α, liver transplantation, rat · Anhepatic phase, prostacyclin

Introduction

Even when a fresh and viable donor liver is used for transplantation, every liver is damaged to some extent during or after a prolonged anhepatic phase (AH). Such damage cannot be evaluated precisely in the clinical setting since all post-transplant complications are physiologically or pharmacologically manipulated. It has previously been demonstrated that in rat orthotopic liver transplantation (OLT) using the cuff technique, it is easy to assess the influence of the AH on the liver graft [5, 11] and that the maximum AH tolerated by rats is 26 min [1, 7, 9]. We, along with others, have also reported that prostacyclin (PGI_2) and its analogues are cytoprotective in various types of liver injuries following liver transplantation [3, 4, 14, 17, 19]. Therefore, in the present study, we have investigated the effect of treatment with a PGI_2 analogue (OP2507) on an extended AH during OLT and how this is related to post-transplant graft survival and the serum TNF-α level.

Materials and methods

Male PVG (RT1^c) rats supplied by the Animal Resources Center (Perth, Australia) weighing 250–300 g were used as donors and recipients. Rats were cared for according to the guidelines set by the National Health and Medical Research Council (Australia) and they had free access to food and water before and after surgery. All surgical procedures were carried out under ether anesthesia in the early morning [7]. Prior to donor hepatectomy, the animals were treated with 200 units of the anticoagulant heparin intravenously, and the liver grafts were flushed with 10 ml of chilled Ringer's solution. Following cuff formation, donor livers were stored prior to transplantation in 50 ml of Ringer's solution at 4 °C.

In the recipient operation, the procedures were divided into the following three stages. In stage 1, before the anhepatic phase (AH), following the initial incision, the recipient's native liver was mobilized, and after clamping the suprahepatic vena cava (SVC),

infrahepatic vena cava (IVC), and portal triads, the liver was removed. In stage 2, during AH, the SVC was anastomosed using 7-0 nylon suture, followed cuff anastomosis of the portal vein (PV) and the IVC, respectively. Finally, in stage 3, after AH, reperfusion of the liver graft was followed by anastomosis of the bile duct and closure of the skin; the hepatic artery was not reconstructed.

The AH is defined as the period of time between clamping and declamping of the PV. In the present study, we fixed the time of each stage in all recipient operations. Stage 1 was 30 min, stage 2 was either 20 min (safe AH) or 45 min (lethal AH), and stage 3 was 5 min. At the beginning of all operations, the cannulation and subsequent continuous infusion of OP2507 or Ringer's solution were carried out via the penile vein. OP2507 (OP; [15-cis-(4-propylcyclohexyl)-16,17,18,19,20-pentanor-9-deoxy-9α, 6-nitrilo-PGF1 methyl ester]) was dissolved in Ringer's lactate at 1 μg/ml. At the end of the operation, an intramuscular antibiotic (cefamandol sodium, 100 mg/kg) was given.

The animals were divided into six experimental groups as follows:

Group 1: No transplantation, OP (0.15 μg/kg per minute) treatment for 30 min
Group 2: AH 20 min, no treatment
Group 3: AH 20 min, OP (0.15 μg/kg per minute) treatment for stage 1 alone
Group 4: AH 45 min, no treatment
Group 5: AH 45 min, OP (0.15 μg/kg per minute) treatment for stage 1 alone
Group 6: AH 45 min, Ringer's solution for stage 1

In groups 4–6, during the first 25 min of 45-min AH, the recipient operation was ceased temporarily and the donor liver was stored in 50 ml of Ringer's solution at 4 °C in the refrigerator. The effect of this additional 25-min cold preservation time on graft survival could be ignored because rat livers stored at 4 °C for up to 4 h in Ringer's solution were all viable on transplantation if the AH was within 20 min (unpublished observations). During the remaining 20 min of the 45-min AH, the donor liver was transplanted into the recipient according to the stage 2 procedure. These procedures allowed all donor grafts to be subjected to the same conditions (e.g., warm ischemia or rewarming time) across every group of OLT, regardless of whether the AH was fixed at 20 min or 45 min.

In each group, 200-μl serum samples were taken from the tail veins of surviving rats on days 1 and 7 following OLT. The 1-week survival rate and the serum level of TNF-α were assessed in each of the groups. TNF-α was measured using an enzyme immunoassay (ELISA) according to the manufacturer's instructions (Pharmingen, San Diego, Calif., USA). Purified rat anti-mouse TNF-α monoclonal antibody and biotin rabbit anti-mouse TNF-α (Pharmingen) were used as primary and secondary antibodies, respectively.

All data are expressed as mean ± SEM. Fischer's exact test was used to determine significant differences in the survival rates, and an unpaired Student's t-test was used for assessing TNF-α. P values less than 0.05 in a two-sided fashion were considered significant.

Results

Using our established OLT techniques, OLT can be routinely performed with an anhepatic time of less than 20 min. In groups 2 and 3, 100 % (5/5) of the animals that underwent OLT with 20-min AH survived for 1 week with or without OP treatment. When the AH was extended to 45 min, the 1-week survival rate of recipients was poor in control groups 4 (0/5) and 6 (1/5; Table 1). The cause of death in nine rats that died within 3 days of transplantation was hepatic failure, as determined by histology. To determine the optimal dose or timing of OP treatment, several trials were initially performed. Continuous infusion of various doses (0.15–1.0 μg/kg) of OP, given from the beginning to the end of the recipient operation, induced hypotension after reperfusion, resulting in a poor survival rate (data not shown). The treatment with OP (0.15 μg/kg per minute) prior to the AH substantially improved 1-week survival (group 5, 5/6, 83.3 %; Fischer's exact test $P < 0.05$; Table 1). The cause of death in one animal was hepatic failure.

Two 20-min AH groups with or without OP treatment (groups 2 and 3) had no significant difference in the serum level of TNF-α on the 1st and 7th postoperative days (Table 2). The serum TNF-α level at day 1 in groups 5 (OP treatment, 77.8 ± 15.9) was significantly lower than that in groups 4 and 6 (no OP treatment, 129.7 ± 21.5 and 112 ± 9.9, respectively, $P < 0.05$).

Table 1 The effect of OP2507 treatment on 1-week survival after OLT with a 20-min or 45-min anhepatic phase

Groups	Duration of anhepatic phase (min)	Treatment prior to anhepatic phase	1-week survival [%]
1	–	OP (0.15 μg/kg per min), 30 min	5/5 (100)
2	20	None	5/5 (100)
3	20	OP (0.15 μg/kg per min), 30 min	5/5 (100)
4	45	None	0/5 (0)
5	45	OP (0.15 μg/kg per min), 30 min	5/6 (83.3)*
6	45	Continuous infusion of same volume of Ringer's solution as group 3, 30 min	1/5 (20)

* $P < 0.05$ vs group 4 or 6

Table 2 Peripheral blood level of TNF-α (mean ± SEM; pg/ml)

Groups	Before OLT	Day 1 after OLT	Day 7 after OLT
1	11.2 ± 2.8 ($n = 5$)	9.5 ± 4.5 ($n = 5$)	9.9 ± 5.3 ($n = 5$)
2	10.8 ± 3.1 ($n = 5$)	17.5 ± 6.1 ($n = 5$)	18.1 ± 7.0 ($n = 5$)
3	12.2 ± 4.5 ($n = 5$)	19.2 ± 7.1 ($n = 5$)	16.4 ± 9.0 ($n = 5$)
4	10.6 ± 5.0 ($n = 5$)	129.7 ± 21.5 ($n = 3$)	–
5	11.5 ± 4.4 ($n = 6$)	77.8 ± 15.9 ($n = 6$)*	22.2 ± 12.2 ($n = 5$)
6	9.9 ± 3.7 ($n = 5$)	112 ± 9.9 ($n = 3$)	23.9 ($n = 1$)

* $P < 0.05$ vs group 4 or 6

Discussion

In rat liver transplantation, intestinal congestion as a consequence of portal vein clamping during the AH greatly reduces graft survival when the AH lasts longer than 26 min during OLT [1, 7, 9]. Mesenteric venous congestion during the AH accelerates transmigration of endotoxins from the intestinal tract into the portal blood, thereby contributing to endotoxemia after OLT with or without the presence of a portovenous shunt [20]. Therefore, we hypothesize that an extended AH will enhance ischemia/reperfusion injury during OLT by activating neutrophils or macrophages to produce elastase [6], superoxide ions [10], or proinflammatory cytokines such as TNF-α and IL-6 [16].

In the present study, the experimental procedures in each group were identical except for the duration of the AH, which enabled us to evaluate the effect of prostacyclin treatment on liver injury related to the extended AH. OP2507 is a stable prostacyclin analogue with a variety of interesting biological properties, including vasodilation, antiplatelet aggregration, antileukocyte adherence, and cytoprotective effects [12, 13, 18]. OP treatment prior to the AH improved the survival rate of rats that underwent OLT with an extended (lethal 45-min) AH. This was associated with a decreased level of TNF-α. Tumor necrosis factor has been suggested to play an important role in the pathogenesis of several types of liver injuries following liver transplantation [2, 15, 16]. It seems likely from our results that OP treatment prior to the AH may stabilize the gut barrier function and inhibit the subsequent activation of the hepatic Kupffer cells, which have the highest capacity for TNF-α production.

In conclusion, OP2507 treatment is potentially useful as a perioperative treatment during OLT with an extended AH when administered at an appropriate dosage and with proper timing.

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References

1. Delriviere L, Kamada N, Kobayashi E, Enosawa S, Goto S (1994) Portosystemic shunt for orthotopic liver transplantation in the rat. J Surg Res 56: 457–460
2. Goto M, Takei Y, Kawano S, Tsuji S, Fukui H, Fushimi H, Nishimura Y, Kashiwagi T, Fusamoto H, Kamada T (1992) Tumor necrosis factor and endotoxin in the pathogenesis of liver and pulmonary injuries after orthotopic liver transplantation. Hepatology 16: 487–493
3. Goto S, Kim YI, Kodama Y, Kawano K, Yasunaga F, Takeyama M, Kobayashi M (1991) The beneficial effect of a stable prostacylin analogue (OP-4143) on rat liver preserved for twenty four hours with lactobionate solution. Transplantation 52: 926–928
4. Goto S, Kim YI, Kodama Y, Kai T, Kawano K, Delriviere L, Lynch SV, Kamada N, Kobayashi M (1993) The effect of a prostaglandin I_2 analogue (OP41483) on energy metabolism in liver preservation and its relation to lipid peroxidative reperfusion injury in rats. Cryobiology 30: 459–465
5. Goto S, Kamada N, Moore T, Ware F, Lord R, Kobayashi E, Kim YI (1994) The influence of intestinal congestion on survival of preserved liver grafts in rat liver transplantation. Transplantation 58: 974–977
6. Himmelreich G, Jochum M, Bechstein WO, Machleidt W, Neuhaus P, Slama KJ, Riess H (1994) Mediators of leukocyte activation play a role in disseminated intravascular coagulation during orthotopic liver transplantation. Transplantation 57: 354–358
7. Kamada N, Calne RY (1983) A surgical experience with five-hundred-thirty liver transplants in the rat. Surgery 93: 64–69
8. Kim YI, Kai T, Kitano S, Ishi T, Tatsuma T, Kamada N, Sugimachi K (1994) Hepatoprotection by a PG12 analogue in complete warm ischemia of the pig liver. Transplantation 58: 875–879
9. Lee S, Charter AC, Orloff MJ (1983) Simplified technique for orthotopic liver transplantation in the rat. Am J Surg 130: 38–42
10. Liu P, Vonderfecht SL, Fisher MA, McGuire GM, Jaeschke H (1994) Priming of phagocytes for reactive oxygen production during hepatic ischemia-reperfusion potentiates the susceptibility for endotoxin induced liver injury. Circ Shock 43: 9–17
11. Marzi I, Knee J, Menger MD, Harbauer G, Burheren V (1991) Hepatic microcirculatory disturbances due to portal vein clamping in the orthotopic rat liver transplantation. Transplantation 52: 432–435
12. Masuda Y, Ochi Y, Ochi Yo, Karasawa T, Hatano N, Kadokawa T, Okegawa T (1986) Protective effect of a new prostacyclin analogue OP-2507 against cerebral anoxia and edema in experimental animals. Eur J Pharmacol 123: 335–344
13. Masuda Y, Yasuba M, Zushi K, Ochi Y, Kadokawa T, Okegawa T (1988) Effect of OP-2507, a stable prostacyclin analogue on cerebral ischemia induced by unilateral ligation of common carotid artery in gerbils. Ant Int de Pharmacodyn The 294: 125–133
14. Monden M, Fortner JG (1982) Twenty-four and 48-hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 196: 38–42
15. Sankary H, Chong A, Foster P, Brown E, Williams J (1995) Improved viability of hepatic allografts from fasted donors is associated with decreased peripheral TNF activity. J Surg Res 58: 337–33
16. Steininger R, Roth E, Fugger R, Winkler S, Langle F, Grunberger T, Gotzinger P, Sautner T, Muhlbacher F (1994) Transhepatic metabolism of TNF-α, Il-6 and endotoxin in the early hepatic reperfusion period after human liver transplantation. Transplantation 58: 179–182

17. Tamaki T, Okouchi A, Kozaki M, Kawamura A, Uchino J, Pegg DE (1988) Hypothermic preservation of the rat liver assessed by orthotopic transplantation. III. Improved functional recovery with isotonic citrate solution an a stable prostacyclin analogue. Transplantation 46: 626–628

18. Terawaki T, Takakura T, Iguchi S, Wakitani K, Kira H, Okegawa T, Kawasaki A, Masuda Y (1988) Effect of a prostacyclin analog OP-2507 on acute ischemic cerebral edema in cats. Eur J Pharmacol 152: 63–70

19. Toledo-Pereya JH (1984) Role of prostaglandins (PG12) in improving the survival of ischemically damaged liver allografts. Trans Am Soc Artif Intern Organs 30: 390–394

20. Yokoyama I, Todo S, Miyata T, Selby R, Tzakis AG, Starzl TE (1989) Endotoxemia and human liver transplantation. Transplant Proc 21: 3833–3841

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