Transplant Int (1992) 5 [Suppl 1]: S366–S369

TRANSPLANT
International


# Efficacy of $PGI_2$ analog in preventing ischemia reperfusion damage of liver grafts from living donors

**S. Goto[1], Y. I. Kim, K. Kawano, T. Kai and M. Kobayashi**

[1] Department of Surgery I, Oita Medical College, Oita, Japan

**Abstract.** In living-related transplantation, warm ischemia/reperfusion damage (IRD) of liver grafts is inevitable during harvesting. In this study, we investigated the effects of prostacyclin ($PGI_2$) on IRD of liver grafts in the rat liver transplant model. Donor rats underwent 30-min warm ischemia of part of the liver (right lateral and medial lobes). After 10 min of reflow, the ischemic partial livers were flushed with Ringer's lactate and immediately transplanted into untreated recipients. Donor animals were divided into two groups: group I received vehicle, and group II received $PGI_2$ analog OP-41483 (OP, 500 ng/kg per min, i.v.) during the donor operation. One-week survival was studied and cellular adenine nucleotide levels of donor livers were assayed by high-performance liquid chromatography (HPLC). Donor treatment with $PGI_2$ analog group II significantly improved 1-week survival (86%), in comparison with the controls group I (25%). The levels of total adenine nucleotides (TAN, μmol/g dry wt) of the grafts just before implantation were well maintained by $PGI_2$ treatment (12.22), as compared with the controls (10.36). In summary, $PGI_2$ treatment of the donor maintained high energy metabolism of the liver graft after IRD and improved the survival of recipients after transplantation. Our study suggested that $PGI_2$ treatment of donors improves viability in liver grafts from living donors thus and increases graft availability for transplantation.

**Key words:** Living related transplantation – Warm ischemia/reperfusion damage – Prostacyclin

Recently, the shortage of hepatic grafts for children has prompted the use of reduced-sized grafts from adults and the use of living-related liver donors. These complicated donor-harvesting procedures result in an increased incidence of warm ischemia/reperfusion damage (IRD) to liver grafts and a decrease in graft viability. It is of utmost importance that living-donor hepatectomy is performed safely. Besides, one of the major reasons for retransplantation is the primary nonfunction of a graft resulting from warm IRD. Therefore, the alleviation of warm IRD during donor harvesting would be of benefit in liver transplantation.

Prostacyclin ($PGI_2$) and its analogs have several physiological actions such as antiplatelet aggregation [1], vasodilation [2] and cytoprotection [3], and our group as well as others have shown that they are protective in warm ischemia [4] or cold preservation of the liver [5–7]. The aim of this study was to investigate the effects of a stable $PGI_2$ analog (OP-41483) on graft viability after IRD, in particular in a setting of living-related transplantation.

## Materials and methods

*Transplantation procedure.* Male Wistar rats, weighing 200–300 g were used as donors and recipients. In a setting of IRD during harvesting of liver grafts from living donors, a temporary normothermic IRD was induced by methods described previously by our group [8]. Briefly, the left portal vein and hepatic artery of the donor rats were occluded with a microvessel clip 15 min after the abdomen was opened. After 30 min of liver ischemia, the vascular clip was released and the right and caudate lobes were excised, leaving behind only the ischemic left lateral and median lobes. After 10 min of reflow, the donor left lateral and median lobes were flushed with Ringer's lactate and immediately transplanted into untreated recipients without arterialization (Fig. 1). Details of the technique of liver removal and orthotopic liver transplantation have been described previously [9]. To estimate technical standards, results from eight fresh reduced-sized-liver transplants were added.

*Experimental protocol.* Donor rats were divided into two groups: group I received vehicle, and group II received $PGI_2$ analog (OP-41483,500 ng/kg per min, i.v.) continuously from soon after the abdomen was opened to the removal of the donor graft. OP-41483 (OP) [15-cyclopentyl-w-pentanor-5(E)-carbacyclin; Ono Pharmaceutical, Osaka, Japan] was dissolved in Ringer's lactate at 2.5 μg/ml. Fifteen rat liver transplants were performed in both groups for the preliminary 1-week survival study. Donor liver tissue specimens (50 mg–100 mg) were taken after opening the abdomen, after 30 min ischemia and after harvesting (just before implantation) for the determination of adenine nucleotide tissue concentrations.

*Offprint requests to:* Shigeru Goto, M. D., Department of Surgery 1, Oita Medical College, Oita, 879-56, Japan

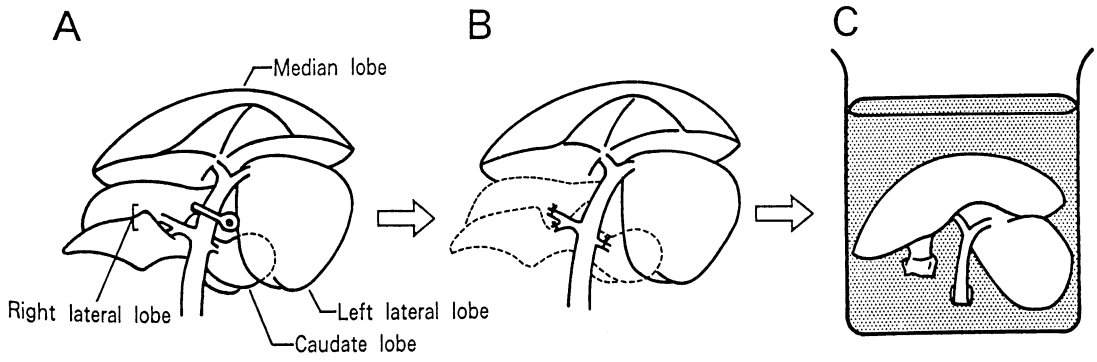


**Fig. 1.** A Schematic diagram for the induction of warm IRD liver during donor operation. Afferent vessels were occluded by a small clip. **B** After 30 min, the clip was released and right lateral and caudate lobes were removed. **C** After 10 min reflow, the reduced liver was flushed with 10 ml of cold Ringer's lactate and stored in 50 ml Ringer's lactate at 4 °C prior to implantation

*Measurement of adenine nucleotides.* The concentrations of adenine nucleotides in donor livers were measured by a modification of the technique described by Kamiike et al. [10]. Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were measured by high-performance liquid chromatography (HPLC) on an anion exchanger column, DEAE-2SW (4.6 × 250 mm, Toyo Soda Manufacturing), equilibrated with 0.38 M phosphate buffer, pH 6.56. The energy charge was calculated according to the formula proposed by Atkinson [11]; energy charge (EC) = (ATP + 0.5 ADP)/(ATP + ADP + AMP). Total adenine nucleotides (TAN) levels were calculated by the following formula: TAN = ATP + ADP + AMP.

*Statistical analysis.* Fisher's exact test and Student's *t*-test were utilized for determining significant differences in the survival rate and in the ATP and cyclic nucleotide levels respectively.

## Results

One-week survival rates for the two groups of animals are shown in Table 1. In group I, 25% of rats (2/8) survived; four animals died of liver failure within 24 h of transplantation and two rats died between the 1st and 3rd days. In contrast, $PGI_2$ treatment of the donor (group II) markedly improved 1-week survival (86%, 6/7), which was statistically significantly higher than that of the control group I (Fisher's exact test, $P < 0.01$). The 1-week survival of eight animals grafted with 70% of fresh liver was 100% (8/8), which indicated that the technical problems were completely overcome.

Figures 2–5 show the changes in adenine nucleotide concentrations in the livers biopsied at the end of the 30 min ischemia or just before implantation. The cellular ATP levels in the livers of both groups showed a marked decrease at the end of the 30 min ischemia, and returned to 60–70% of the pre-ischemia levels after donor harvesting (after 30 min ischemia and 10 min reflow) (Fig. 2). At the end of the 30 min ischemia, ATP [1.76 (SD = 0.58) μmol/g dry wt] in $PGI_2$-treated livers in group I was significantly higher than that of the control group [0.98 (0.20) μmol/g dry wt]. However, there was no significant difference in the ATP levels of liver grafts in the two groups just before implantation. On the other hand, the ADP levels in the livers decreased gradually after ischemia and returned gradually to normal after reperfusion without a significant difference between the two groups (Fig. 3). A significant increase in AMP [6.82 (0.86) μmol/g dry wt] was observed in $PGI_2$-treated livers at the end of the 30 min ischemia, as compared with that [5.44 (0.44) μmol/g dry wt] of the control group (Fig. 4). But, there was no significant difference in AMP levels of liver grafts in the two groups before implantation.

In contrast, the TAN levels in $PGI_2$-treated liver were 11.55 (1.44) μmol/g dry wt at the end of 30 min ischemia, and 12.22 (1.29) μmol/g dry wt before implantation, which were significantly higher than those of the control group 8.85 (0.36) μmol/g dry wt and 10.36 (0.90) μmol/g dry wt respectively (Fig. 5). No significant differences were observed in the energy charge (EC) of the two groups at the end of ischemia or after donor harvesting.

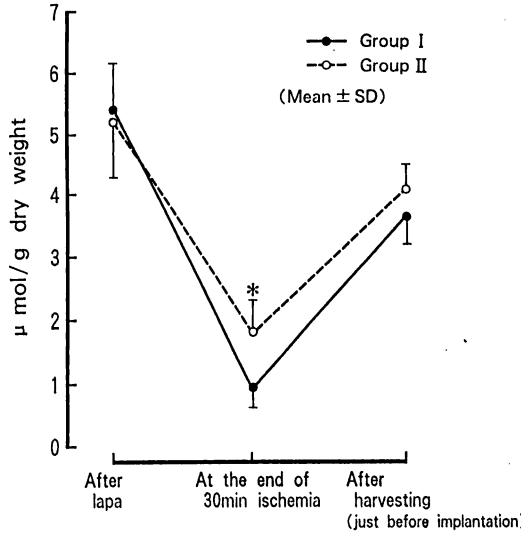


**Fig. 2.** Changes in hepatic cellular contents of adenosine triphosphate (ATP) in donor livers. Small specimens were taken at the times indicated during donor operation. Values are means for five experiments. (●) Group I; (○) Group II. *$P < 0.05$ vs Group I

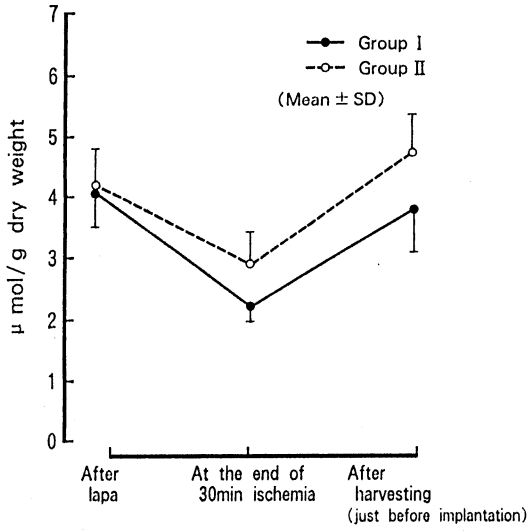


**Fig. 3.** Changes in cellular contents of adenosine diphosphate (ADP) in donor livers. The data were obtained from the same extracts as for Fig. 1. Values are means for five experiments. (●) Group I; (○) Group II

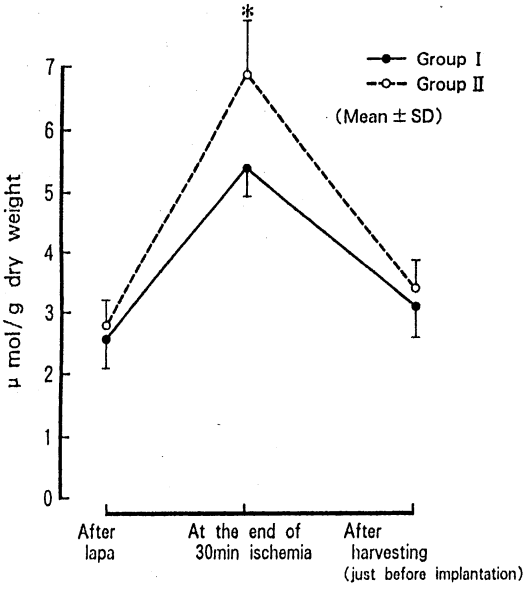


**Fig. 4.** Changes in cellular contents of adenosine monophosphate (AMP) in donor livers. The data were obtained from the same extracts as for Fig. 1. Values are means for five experiments. (●) Group I; (○) group II; $*P < 0.05$ vs group I

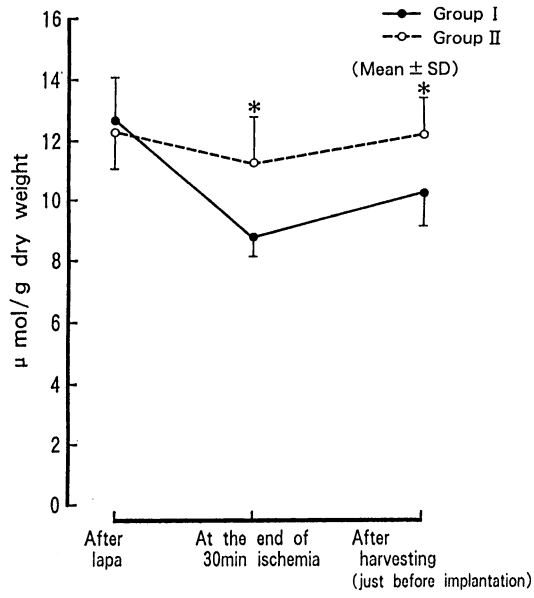


**Fig. 5.** Cellular total adenine nucleotides (TAN; ATP + ADP + AMP) levels soon after laparotomy, at the end of 30 min ischemia and after harvesting (just before implantation). Values are means for five experiments. (●) Group I; (○) group II; $*P < 0.05$ v.s. group I

## Discussion

In living-related transplantation, both the liver graft and the liver remaining in the living donor have to remain viable. Therefore, the grafts from living donors are easily exposed to warm IRD, in addition to cold ischemic injury. $PGI_2$ and its analogs protect the liver against warm ischemic insult [4] and are efficacious in cold preservation [5–7]. However, its major drawback is that it is highly unstable [12], with ureliable results. The synthetic compound, OP-41483 is stable for more than 20 days even at 60 °C and is independent of the pH of the solution and has similar pharmacological effects to those of native $PGI_2$ [13]. In a previous study, we succeeded in transplanting livers in the rat after cold preservation for 24 h with the combined use of OP-41483 and lactobionate solution [7]. In the present study, we tested the effects of OP-41483 on graft viability after warm IRD in a rat liver transplant model.

Sumimoto et al. [14] have indicated that 30 min is the critical time for warm ischemia during the donor operation in the rat liver transplant model. In our present study, the effects of OP-41483 on a 30-min ischemic insult and 10-min reperfusion injury during the donor operation were assessed by the survival of the transplanted rats, and by measuring cellular adenine nucleotides in the donor liver. Donor treatment with $PGI_2$ improved the 1-week survival (86% vs 25% in the control group), and this correlated with well maintained levels of TAN.

A relationship between energy metabolism and the extent of ischemic tissue damage has been demonstrated in experimental models [15, 16]. However, our results demonstrated that there were no significant differences in levels of ATP, ADP and AMP between $PGI_2$-treated liver

**Table 1.** One-week survival rates of rats grafted with fresh liver grafts, or liver grafts exposed to warm IRD

| | One-week survival (%) | Causes of death (xn) |
|---|---|---|
| Fresh liver grafts | 8/8 (100) | |
| Liver grafts exposed to IRD | | |
| Group I (vehicle treatment of donor) | 2/8 (25) | bleeding (1)<br>liver failure (3)<br>obstructive jaundice (2) |
| Group II ($PGI_2$ treatment of donor) | 6/7 (86)* | bleeding (1) |

* $P < 0.01$ vs group I
IRD, Ischemia reperfusion damage

grafts and untreated grafts after harvesting (just before implantation). Kamiike et al. [10] have demonstrated that TAN levels of human donor grafts correlated and predicted the graft viability after transplantation, in comparison with ATP levels. This was confirmed by our result that TAN levels of IRD-induced grafts were well maintained by $PGI_2$ treatment, and this reflected the graft survival after transplantation.

The exact cytoprotective mechanism of $PGI_2$ at the cellular level has been investigated by others [3, 17]. Sikujara et al. [4] have indicated that $PGI_2$ is capable of protecting the liver from 75-min ischemic insult. Although the livers were not subsequently transplanted, they concluded that elevated ATP and cyclic nucleotide levels of $PGI_2$-treated livers played an important role in successful cytoprotection during ischemia. In our present study, elevated levels not only of ATP but also AMP at the end of 30-min ischemia were observed in $PGI_2$-treated livers. During warm ischemia of the liver, AMP is degraded rapidly to xanthine via adenosine, inosine, hypoxanthine [18]. Although the levels of degradation products such as purine catabolites were not measured in our study, our results suggested that degradation of ATP to urate was more retarded by $PGI_2$ treatment at the AMP to adenosine step at the end of 30 min ischemia, which may contribute to well maintained TAN levels of the graft after harvesting (just before implantation).

In summary, our data suggested that $PGI_2$-treatment of donors maintained graft viability before and after transplantation. Future clinical application of $PGI_2$ during donor harvesting from not only cadavers but also living donors may contribute to maximal utilization of available donor grafts.

*Acknowledgements.* This work was supported by a grant for paediatric research (63-A-05) from the Ministry of Health and Welfare and Grant-in-Aid (A-01440054) for general scientific research from the Ministry of Education of Japan.

## References

1. Dusting GJ, Moncada S, Vane JR (1977) Prostacyclin (PGX) is the endogeneous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13: 3–15
2. Higgs EA, Higgs GA, Moncada S, Vane JR (1978) Prostacyclin ($PGI_2$) inhibits the formation of platelet thrombi in arteroles and venules of the hamster cheek pouch. Br J Pharmacol 63: 535–539
3. Araki H, Lefer AM (1980) Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. Am J Physiol 238: H176–H181
4. Sikujara O, Monden M, Toyoshima, Okamura J, Kosaki G (1983) Cytoprotective effects of prostaglandin $I_2$ on ischemia-induced hepatic cell injury. Transplantation 36: 238–243
5. Monden M, Fortner JG (1982) Twenty-four-and 48-hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 196: 38–42
6. Toledo-Pereyra JH (1984) Role of prostaglandins ($PGI_2$) in improving the survival of ischemically damaged liver allografts. Trans Am Soc Artif Intern Organs 30: 390–394
7. Goto S, Kim YI, Kodama Y et al. (1991) The beneficial effect of a stable prostacyclin analog (OP-41483) on rat liver preserved for 24 hours with lactobionate solution. Transplantation in press
8. Kawano K, Kim YI, Kaketani K, Kobayashi M (1989) The beneficial effect of cyclosporine on liver ischemia in rats. Transplantation 48: 759–764
9. Goto S, Kim YI, Shimada T, Kawano K, Kobayashi M (1991) The effects of pretransplant cyclosporine therapy on rats grafted with twelve-hour cold-stored liver. With special reference to reperfusion injury. Transplantation (in press)
10. Kamiike W, Budelski M, Steinhoff G, Ringe B, Lauchart W, Pichlmayer R (1988) Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. Transplantation 48: 138–143
11. Atkinson DE, Walton GM (1967) Adenine triphosphate conservation in metabolic regulation. J Biol Chem 242: 3239–3243
12. Dusting GJ, Moncada S, Vane JR (1977) Disappearance of prostacyclin in the circulation of the dog. Br J Pharmacol 62: 414–417
13. Yui Y, Takatsu Y, Hattori R, Kawai C, Osaki Y, Yoshida T (1985) A new stable prostacyclin analogue OP41483 (15-cyclopentyl-w-pentanor-5(e)-carbacyclin). Jpn Circ J 49: 571–575
14. Sumimoto K, Inagaki K, Yamada K, Kawasaki T, Dohi K (1988) Reliable indices for the determination of viability of grafted liver immediately after orthotopic transplantation. Transplantation 46: 506–509
15. Kamiike W, Watanabe F, Hashimoto T et al. (1982) Changes in cellular levels of ATP and its catabolites in ischemic rat liver. J Biochem 91: 1349–1356
16. Marubayashi S, Takenaka M, Dohi K, Ezaki H, Kawasaki T (1980) Adenine nucleotide metabolism during hepatic ischemia and subsequent blood reflow periods and its relation to organ viability. Transplantation 30: 294–298
17. Ozaki N, Tokunaga Y, Wakashiro S, et al. (1988) Evaluation of cytprotective drugs for liver preservation by pyridine nucleotide fluorometry. Surgery 104: 98–103
18. Harvey PRC, Iu S, McKeown CMB, Petrunka CN, Ilson RG, Strabrg SM (1988) Adenine nucleotide tissue concentrations and liver allograft viability after cold preservation and warm ischemia. Transplantation 45: 1016–1020