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# Efficacy of prostacyclin analogue (OP-2507) in viable hepatic grafts from pigs with non-beating hearts

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**Abstract** We investigated whether the stable prostacyclin analogue (OP-2507; OP) would ameliorate warm ischemia-related injury of the liver graft under conditions of a nonbeating heart. Thirty-six mongrel pigs were arranged into 3 groups of 6 pairs. Group 1 pigs underwent orthotopic liver transplantation from heart-beating donors (HBD). In group 2, animals received liver grafts from nonheart-beating donors (NHBD), defined as 30 min of cardiac arrest. Group 3 pigs received grafts from NHBD, but the donor had been pretreated with OP by intraportal infusion (2 μg/kg · min for 30 min immediately before the induction of cardiac arrest). The grafts were preserved at 4 °C in Euro-Collins solution in which OP was dissolved at 200 μg/l. Five-day survival rates after transplantation improved significantly in OP-treated animals (3/6, for group 3), compared with 0/6 for group 2 ($P < 0.05$, generalized Wilcoxon test). Five of 6 animals survived more than 5 days in the HBD group (group 1). Although the serum transaminase activities and bile production did not differ in the early phase of recirculation among the groups, there was a significant improvement in the hepatic microcirculatory environment in the surviving groups (groups 1 and 3). Analysis of arterial prostanoid levels showed a substantial suppression of $PGE_2$ release by OP treatment following reperfusion. Our data indicate that a stable prostacyclin analogue can be clinically useful for expanding the donor pool by improving the quality of the liver graft.

**Key words** Liver ischemia
Nonheart-beating donor · Prostacyclin analogue (OP-2507)
Cardiac arrest · Pigs · Euro-Collins
Hepatic microcirculation
Prostanoids (prostaglandins and thromboxane: $PGE_2$, $TXB_2$ and 6-keto-$PGF_1\alpha$) · Neutrophil infiltration

## Introduction

The shortage of acceptable donors greatly impedes liver transplantation [2]. The donor pool can be expanded by using organs from aged or unstable subjects [19]; however, this must be balanced against the possible primary dysfunction of the compromised graft [15, 22].

Prostacyclin ($PGI_2$) and its analogue are effective against ischemic liver injury [10, 23, 24] as a result of their properties of vasodilation [8], antiplatelet aggregation

[11], antileukocyte adherence [14], and cytoprotection [1, 25]. However, $PGI_2$ is highly unstable, with inconsistent biologic actions [7]. The recently developed $PGI_2$ analogue OP-2507 (OP; [15 *cis*-14-propylcyclohexyl)-16,17, 18,19,20-pentanor-9-deoxy-9α, 6-nitrilo-prostaglandin, methylester; Ono Pharmaceutical, Osaka, Japan) is chemically stable over 24 h in aqueous solution [17, 18].

We evaluated OP-2507 with regard to improving liver graft viability from unstable donors such as nonheart-beating pigs (NHBD) with special attention directed to the hepatic microcirculation and endogenous production of prostanoids.

## Materials and methods

### Animals

Eighteen pairs of female mongrel pigs weighing between 18 and 36 kg were fasted overnight before the experiment.

### Surgical procedure

Liver transplantation was performed according to a modification of Calne's model [5]. A mixture of oxygen (2 l/min), nitrous oxide (2 l/min), and halothane (0.2%) was used after intratracheal intubation for induction and maintenance of general anesthesia. After systemic heparinization (2000 U), one unit of blood was collected from the donor for transfusion into the recipient. The donor livers were cooled via the portal vein and celiac axis with 1.5 l of chilled Euro-Collins solution and stored for 4 h in the same solution at 4 °C. The hepatic grafts were implanted by means of a sutured upper caval anastomosis and a cuffed anastomosis for the portal vein and the vena cava below the liver. During the anhepatic phase, blood from the portal and the external iliac veins was shunted to the jugular vein using a Bio-Pump (Bio Medicus, Minneapolis, Minn.), under conditions of systemic heparinization (2000 U). Calcium gluconate (170 mg), sodium bicarbonate (40 mEq) and protamine sulfate (10 mg) were given intravenously at the time of graft recirculation. The animals were given free access to water for drinking immediately after awakening and were first fed 24 h after transplantation.

### Experimental protocol

The animals were arranged into three groups ($n = 6$ pairs in each group). In group 1, liver grafts were retrieved from heart-beating donors (HBD). Group 2 pigs were transplanted with livers taken from NHBD subjected to warm ischemia (WI) introduced for 30 min by cross-clamping the thoracic aorta with simultaneous cardiac standstill subsequent to ventricular injection of KCl (10 mEq). For the group 3 animals, OP (2 μg/kg · min) was given through a mesenteric vein branch for 30 min immediately prior to the induction of warm ischemia. OP was dissolved in the preservation solution at 200 μg/l.

### Parameters

The survival rate, serum biochemistry using an autoanalyzer (Clinalyzer MS-24), liver tissue flow (LFT) by laser Doppler flowmetry (BPM 403, TSI, St Paul, Minn) and histology were serially followed. Liver specimens were stained with hematoxylin and eosin. Endogenous plasma levels of prostanoids such as prostaglandin $E_2$ ($PGE_2$), 6-ketoprostaglandin $F_1$-alpha (6-keto-$PGF_1\alpha$), and thromboxane $B_2$ ($TXB_2$) were measured from arterial blood by randioimmunoassay [26].

### Statistical analysis

Data are expressed as mean ± SD. Comparisons for statistical significance were performed according to the generalized Wilcoxon test for survival and Student's *t*-test and Chochran Cox test for others. A $P$ value of less than 0.05 was considered significant.

## Results

The survival rates for each group are presented in Table 1. All animals in group 2 expired of liver failure. Three pigs

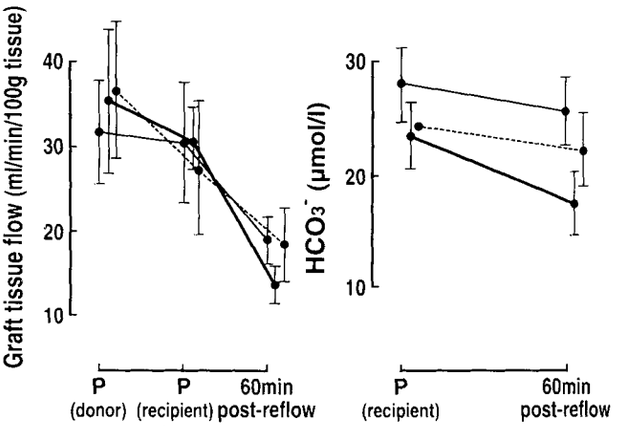


**Fig. 1** Changes in graft tissue flow and arterial blood bicarbonate levels after liver transplantation in group 1 (—●—, HBD, $n = 4$), group 2 (—●—), NHBD, $n = 6$), and group 3 (– – ●– – –, NHBD + OP, $n = 6$) ($P$ prior to operation)

**Table 1** Results of transplantation in three experimental groups (*WIT* warm ischemic time, *TIT* total ischemic time, *MST* mean survival time, (*N*) *HBD* (non) heart-beating donor, *OP* OP-2507)

| Groups ($n = 6$ pairs each) | WIT (min) | TIT (h) | Survival (day) | MST (day) | Five-day survival rate (%) |
|---|---|---|---|---|---|
| 1 (HBD) | – | 4.2±0.4 | 0, 5, 7, 7, 8, 30 | 9.5±10.4 | 83.3* |
| 2 (NHBD) | 30 | 4.4±0.6 | 0, 0, 0, 0, 0, 0 | 0 | 0 |
| 3 (NHBD+OP) | 30 | 4.3±0.6 | 0, 1, 1, 5, 7, 11 | 4.1±4.3 | 50.0** |

* $P < 0.01$, ** $P < 0.05$ vs. group 2

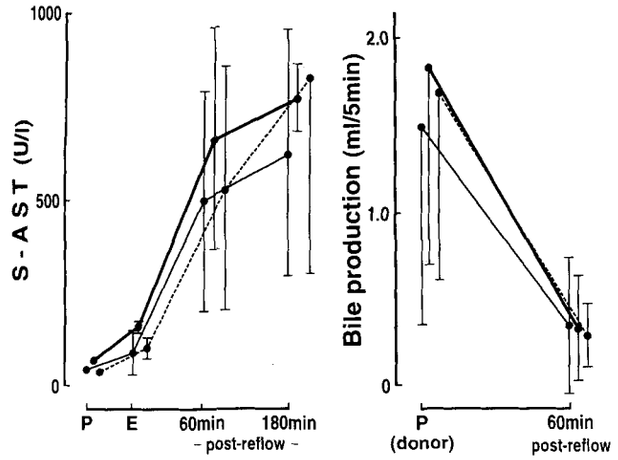


**Fig. 2** Bile production and serial changes in serum transaminase activities (S-AST) after liver transplantation in group 1 (—●—, HBD, $n=4$), group 2 (—●—, NHBD, $n=6$) and group 3 (– –●– –, NHBD + OP, $n=6$) (*P* prior to operation, *E* end of anhepatic phase)

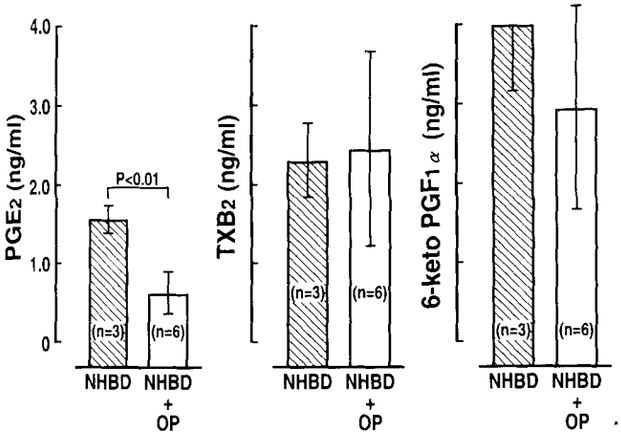


**Fig. 3** Arterial prostanoid levels at 15 min after liver transplantation from NHBD. The pretransplant values of $PGE_2$, $TXB_2$, and 6-keto $PGF_1\alpha$ were $0.22 \pm 0.17$ ng/ml (mean ± SD), $0.27 \pm 0.10$, and $0.31 \pm 0.23$, respectively ($n=6$). The *number in parenthesis* is the number of samples studied

in group 3 survived for more than 5 days, while the remaining three died between 12 and 36 h after transplantation. As for group 1, 83% (5/6) lived longer than 5 days.

Changes in LTF and arterial blood bicarbonate levels after liver graft recirculation are presented in Fig. 1. There were no differences in LFT between donor and recipient livers. At 60 min after the restoration of graft circulation, LFT in the untreated NHBD group substantially decreased to $13.3 \pm 2.6$ (ml/min per 100 g tissue, $n=6$) compared with $18.5 \pm 4.6$ ($n=6$) in the NHBD + OP

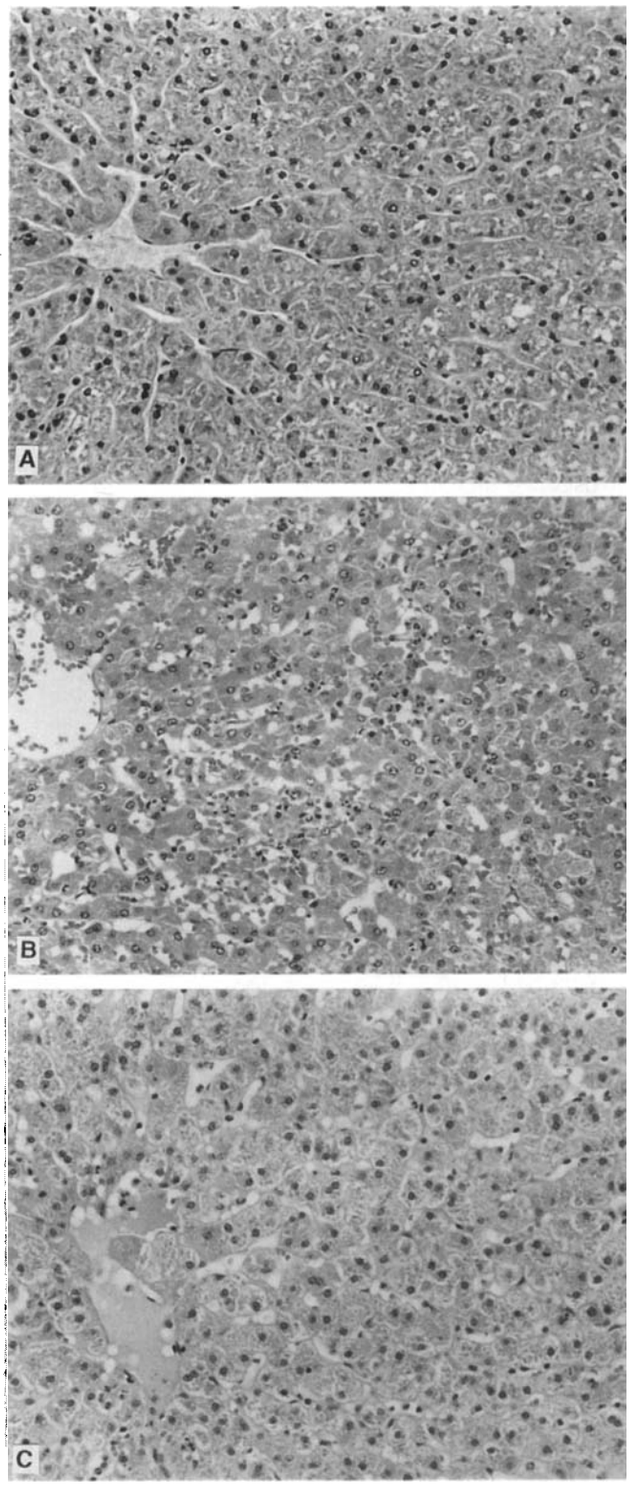


**Fig. 4A–C** Representative histologic alterations of liver grafts at 60 min after recirculation (H&E, ×180 times): **A** group (HBD), **B** group 2 (HNHBD), **C** group 3 (NHBD + OP)

group ($P < 0.05$). In parallel with LFT changes, the bicarbonate levels in group 3 recipients were significantly ameliorated ($22.0 \pm 3.4$ μmol/l for group 3 vs. $17.7 \pm 2.9$ for group 2, $P < 0.05$).

Figure 2 shows alterations in the serum activities of asparate aminotransferase (S-AST) and bile flow. The enzymatic activities of S-AST rose sharply in all groups from 60 min after graft recirculation, but the differences among the groups were not statistically significant. The immediate bile production 60 min post-reflow did not predict the outcome of transplantation.

Changes in prostanoid levels upon the restoration of graft circulation are presented in Fig. 3. In both NHBD groups, $TXB_2$ and 6-keto-$PGF_1\alpha$ activities significantly increased at 15 min after reperfusion, but there were no statistically significant differences among the groups. In contrast, the $PGE_2$ levels in the OP-treated animals were suppressed to approximately 40% of the corresponding value of nontreated pigs by 15 min of graft recirculation.

Histologic alterations in representative livers taken 1 h after liver graft reflow are illustrated in Fig. 4. In the NHBD group (group 2), eosinophilic changes with cytoplasmic vacuolization were manifest in the hepatocytes, and the hepatic sinusoids were remarkably destroyed, with parenchymal hemorrhage and infiltration of inflammatory cells such as polymorphonuclear neutrophils (Fig. 4b). In contrast, degenerative alterations in the parenchymal and nonparenchymal cells were ameliorated in group 3 pigs who survived for more than 5 days, as shown in Fig. 4c.

## Discussion

We found that the administration of the $PGI_2$ analogue OP lenghtened the survival time of pigs with a transplanted liver. In the immediate reperfusion period, the hepatic microcirculation was attenuated with respect to laser Doppler tissue flow-metry and was associated with an improvement of the arterial blood acidosis. Analysis of the arterial blood prostanoid levels showed that OP pretreatment substantially suppressed the production of $PGE_2$ but did not influence either the $TXB_2$ or 6-keto-$PGF_1\alpha$ concentrations. In the early period of reperfusion, S-AST activities and bile production were not affected by this agent.

Although the exact mechanisms of the cytoprotective effects of $PGI_2$ are unknown [1, 25], our data suggest that OP treatment alleviates the WS-related injury of the graft, as induced by cardiac arrest in the donor. As there were no differences in S-AST levels between the NHBD groups (with or without OP), other mechanisms unrelated to parenchymal hepatocyte injury are probably involved. Since it is evident that the integrity of the hepatic sinusoids plays a key role in ischemia/reperfusion injury of the liver graft [3, 20], the beneficial effect of OP might relate to a cytoprotection of nonparenchymal components such as endothelial cells. Other properties of OP, such as vasodilation and antiplatelet aggregation, may favour amelioration of the tissue microcirculation.

Investigations of ischemia/reperfusion injury in tissue have shown that oxygen radicals cause oxidative injury to the recirculated liver graft [6]. Reactive oxygen intermediates generated up on the restoration of blood weaken the integrity of the vascular endothelium [21]. Circulating neutrophils and platelets accumulate, and the hepatic microcirculatory state is seriously hampered. We noted histologic evidence of a remarkable infiltration of polymorphonuclear leukocytes in recirculated grafts. In effect, neutrophils contribute to the recirculation-related damage of an organ [13], and interactions of leukocytes and the sinosoidal system can account for the pathogenesis of hypoxia/recirculation insult to the graft [16]. It may be that OP alleviates the ischemia/reperfusion damage to the graft as this agent suppresses neutrophil infiltration, as based on evidence that $PGI_2$ attenuates the production of reactive oxygen intermediates by neutrophils and prevents leukocyte adherence to the vascular endothelium [9, 14].

In the present study, $PGE_2$ release in the systemic circulation was significantly suppressed in the OP-treated animals following graft recirculation. In this regard, we have evidence that in the same species, $PGE_2$ but not other prostanoids was progressively released from resident macrophages (Kupffer cells) in the liver after ischemia and reperfusion [unpublished data]. Since a Kupffer cell is activated to produce toxic mediators related to liver injury [4], we assume that Kupffer cells are the main source of $PGE_2$ production in our model. The cytoprotective properties of OP mean that the agent can effectively stabilize the activation of hepatic macrophages, an important component of the reperfusion damage after ischemia [12].

In conclusion, we were able to confirm that OP attenuates the WI-related injury of the liver grafted from NHBD. Preoperative treatment of a donor with a stable prostacyclin analogue can be clinically beneficial not only in improving the quality of liver grafts from unstable donors, but in expanding the suitable donor pool as a possible solution to the current organ shortage for liver transplantation.

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