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Effect of prostaglandin E_1 on preservation injury of canine liver grafts preserved in UW solution

K. Takahashi[1], N. Yamamoto[1], H. Egawa[1], T. Takeuchi[1], T. Yamaguchi[1], Y. Takada[1], F. Nishizawa[1], T. Takayasu[1], M. Narita[2], K. Mori[1], Y. Yamaoka[1], K. Ozawa[1]

[1] Second Department of Surgery, Faculty of Medicine, Kyoto University, 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto, 606 Japan
[2] Ono Pharmaceutical Company, Fukui, Japan

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Abstract. This study investigated whether prostaglandin E_1 (PGE_1) could reduce hepatic injury to the liver graft caused by harvesting and 24-h preservation in University of Wisconsin (UW) solution in a canine model. The PGE_1-treated group was intravenously administered 0.5 μg/kg per minute of PGE_1 for 30 min before harvesting, as well as a concentration of 1 mg/l PGE_1 in the washout and UW solutions. In both the PGE_1-treated and the control group, all recipients survived for 1 week or more after transplantation. Arterial ketone body ratio (AKBR) remained over 1.0 in the early postoperative period. The PGE_1 group showed significant reductions in guanase, GOT, and LDH during the early postoperative period compared to the untreated control group. Histological examination disclosed partial mitochondrial swelling, hepatocyte vacuolation, and necrosis in the control group, while such abnormalities were rarely seen in the PGE_1 group. These results suggest that PGE_1 can effectively reduce hepatic injury to liver grafts preserved in UW solution prior to transplantation.

Key words: Preservation, liver, canine – Liver transplantation, preservation, canine – Prostaglandin E_1, liver preservation – UW solution, liver preservation, canine

Introduction

A vital problem that needs to be overcome in order to improve the outcome of liver transplantation is that of securing the viability of the liver graft by appropriate harvesting and preservation. With the recent development of the University of Wisconsin (UW) solution [11, 28], the duration of safe, hypothermic preservation of liver grafts has been significantly prolonged, and 48-h preservation for canine livers has been reported [10]. Clinically, UW solution has also enabled successful simple cold storage of liver grafts for as long as 20 h with prompt, satisfactory function [12], and it is now widely used.

Correspondence to: Y. Yamaoka

However, it has been reported that the incidence of primary nonfunctioning grafts increases if clinical preservation in UW solution exceeds 20 h [5]. Therefore, it is necessary to devise a method to reduce injury to the liver during long-term preservation in UW solution.

Prostaglandin E_1 (PGE_1) reportedly exerts a cytoprotective effect [4, 13, 21, 29] and also suppresses thrombus formation by inhibiting platelet aggregation. It contains potent vasodilators that act directly on the vascular smooth muscle. Such actions would seem to be beneficial in the harvesting and preservation of liver grafts. In addition PGE_1, which is chemically more stable than PGI_2, is widely adopted clinically because it is easy to use. Before the advent of UW solution, the beneficial effects of prostaglandins were such that they were used to prevent preservation injury of liver grafts. However, aside from one study by Sanchez-Urdazpal et al. [22] on the effect of a PGI_2 analogue, no one has yet reported that prostaglandins can be used to reduce preservation injury of liver grafts preserved in UW solution.

In this study we assessed the levels of injury that might occur during long-term cold storage of canine liver grafts preserved for 24 h in UW solution, and we compared the results obtained between a group that was administered PGE_1 during harvesting and preservation and an untreated control group.

Materials and methods

Twelve pairs of adult beagle dogs, weighing 10–12 kg, were fasted for 12 h preoperatively but had free access to water.

Anesthesia

After induction of anesthesia by intravenous administration of ketamine hydrochloride (Ketalar) at 5 mg/kg of body weight, intubation was performed for subsequent mechanically assisted ventilation with an oxygen-air mixture by a respirator. Ketamine hydrochloride was later supplemented by additional 1 mg/kg dosages, as needed. Pancuronium bromide (Mioblock) was intravenously given as a muscle relaxant in 0.1 mg/kg dosages, as needed. During the oper-

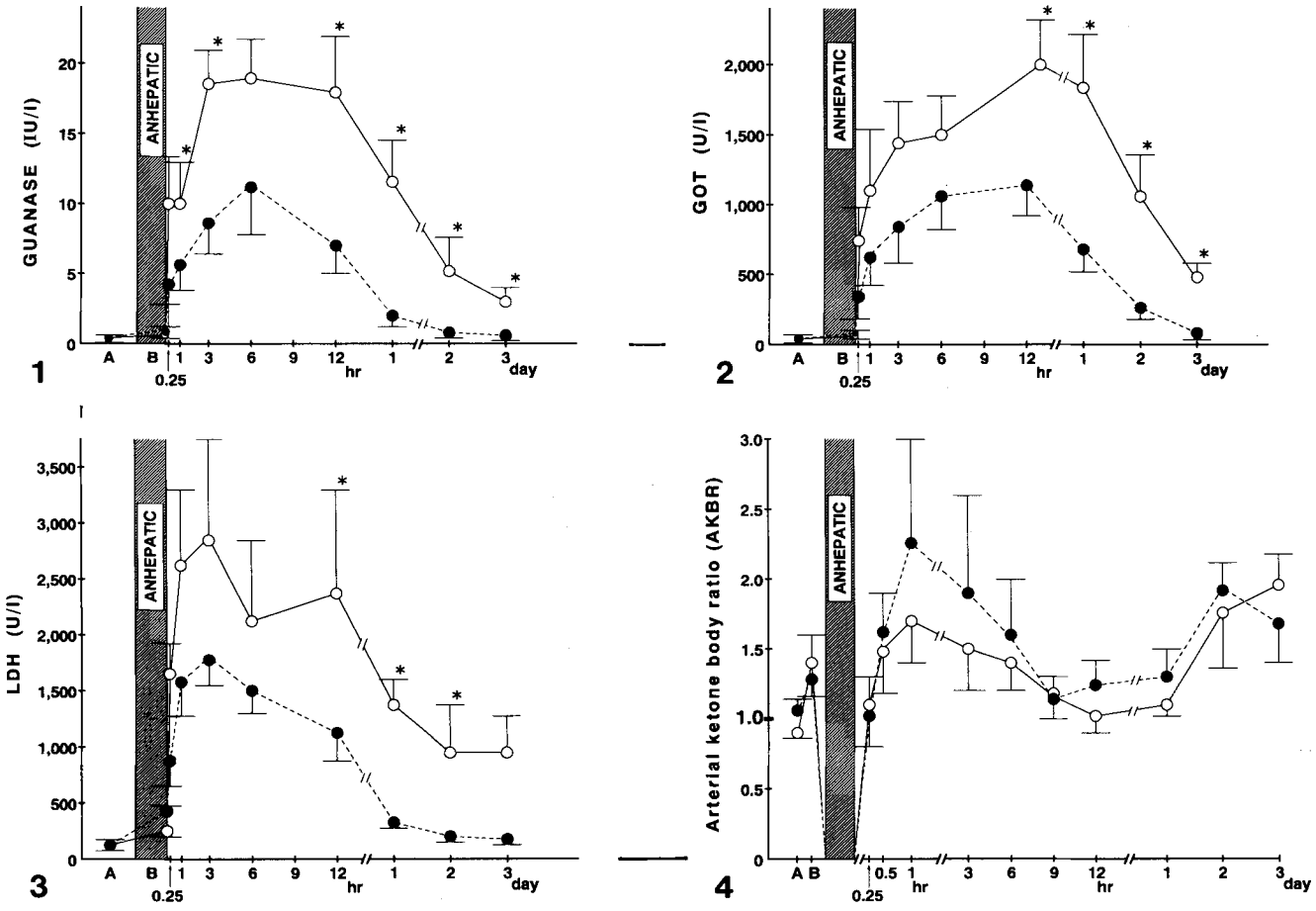


Fig. 1. Changes in the serum guanase level before and after transplantation. The serum guanase level increased in the early postoperative period and there was a subsequent reduction over time in both groups. The serum guanase level at 1 h after reperfusion differed significantly ($P < 0.05$) between the control (–○–) and PGE_1-treated (–●–) groups. *A*, Pretransplantation; *B*, pre-reflow

Fig. 2. Changes in the serum GOT level before and after transplantation. A significant ($P < 0.05$) intergroup difference in serum GOT was observed after reperfusion. Symbols as in Fig. 1

Fig. 3. Changes in the serum LDH level before and after transplantation. A significant ($P < 0.05$) intergroup difference in serum LDH was still observed after reperfusion. Symbols in Fig. 1

Fig. 4. Changes in arterial ketone body ratio. AKBR showed a marked elevation to over 1.0 at 15 min after reperfusion and remained over 1.0 thereafter in both groups. Symbols as in Fig. 1

ation, arterial pressure and central venous pressure were monitored via catheters inserted into the right carotid artery and the external carotid vein, respectively. Electrolyte and glucose solutions were administered intravenously.

Liver grafts were divided into a PGE_1 – treated group ($n = 6$) and an untreated control group ($n = 6$).

Harvesting and preservation

In the control group, 300 ml of lactated Ringer's solution (4 °C) containing 300 units of heparin was infused via the aorta and portal vein to wash out the donor liver. Thereafter, the washout solution was replaced with UW solution (4 °C) containing an equal amount of heparin, infused via the portal vein. The donor liver was then harvested and preserved for 24 h in UW solution (4 °C).

In the PGE_1-treated group, donor canines were intravenously treated with 0.5 μg/kg per minute of PGE_1, starting 30 min before washout. PGE_1 was also added to the same washout and UW solution used in the controls at a concentration of 1 mg/l.

Recipient operation

The procedure for the recipient operation was the same for both groups. After preparing a venovenous bypass with Biopump, the liver was removed. An end-to-end anastomosis was performed first for the suprahepatic vena cava and then for the portal vein, followed by the start of reperfusion. During the anastomosis of the suprahepatic vena cava, the UW solution was washed out of the liver with lactated Ringer's solution (4 °C). Then, an end-to-end anastomosis was performed for the intrahepatic vena cava and, subsequently, for the hepatic artery. Finally, the bile duct was anastomosed, and a T-tube was inserted.

Laboratory tests

Biochemical tests. Arterial blood samples were taken for biochemical tests. GOT, GPT, gamma-GTP, LDH, total bile acid, blood sugar, fibrinogen, free fatty acid, guanase, pyruvate, and lactate were all examined before ischemia, before reperfusion, after reperfusion (at 15 min, 1 h, 3 h, 6 h, and 12 h), and after surgery (on postoperative days 1–3). Prothrombin time, activated partial thromboplastin time (APTT), antithrombin III, and coagulation factors (II, V, VII, VIII, IX, X, and XI) were examined before ischemia, before reperfusion,

after reperfusion (at 1 h, 6 h, and 12 h), and after surgery (on postoperative days 1–3).

Arterial ketone body ratio (AKBR). The AKBR was measured following the enzymatic method using a Ketorex kit (Sanwa Chemical, Nagoya, Japan) and a KETO-340 semiautomatic spectrophotometer (Ihara-denshi, Kasugai, Japan) [15, 30]. AKBR was examined before ischemia, before reperfusion, after reperfusion (at 15 min, 30 min, 1 h, 3 h, 6 h, 9 h, and 12 h), and after surgery (on postoperative days 1–3).

Serum PGE_1 level. The serum PGE_1 level was measured using the methods of thin-layer chromatography and radioimmunoassay [9]. Crossreactivity of antiserum of PGE_1 against PGE_2 was not greater than 8%.

The serum PGE_1 level was determined in the donor canines before and at 15 and 30 min after intravenous administration of PGE_1 at 0.5 μg/kg per minute and in the recipient canines before and at 30 min, 3 h, 6 h, and 12 h after reperfusion and on postoperative days 1–3.

Histological examination

Both light and electron microscopy were performed. Tissue specimens were collected by wedge resection immediately before transplantation (after 24-h preservation) and 2 h after transplantation. For light microscopy, specimens were fixed in 10% formalin and stained with hematoxylin-eosin. For electron microscopy (JEM-100S, Nippon Denshi), specimens were double-stained with uranyl acetate and lead nitrate.

Immunosuppressive therapy

Immunosuppressive therapy was given with subcutaneous injections of cyclosporin A in both groups. The dosage was initially 5 mg/kg per day and was then reduced to 3 mg/kg per day.

Statistical analysis

The values of all parameters were expressed as the mean ± SEM. Significance of differences was determined by the Wilcoxon test. *P* values less than 0.05 were regarded as statistically significant.

Results

One recipient in each group died of excessive bleeding caused by technical failure. These canines were excluded from further evaluation. Results were therefore based on the five long-term survivors remaining in each group. The survival rate did not differ between the two groups. All canines survived for 1 week or more in both groups.

Biochemical tests

Analysis of changes in the serum guanase level before and after transplantation revealed an increase in the early postoperative period and a subsequent reduction over time in both groups (Fig. 1). Serum guanase at 1 h after reperfusion differed significantly ($P < 0.05$) between the untreated control group (10.66 ± 2.558 IU/l) and the PGE_1-treated group (5.48 ± 1.614 IU/l).

A significant intergroup difference in serum GOT was observed after reperfusion: 1988 ± 332 U/l in the untreated control group and 1136 ± 217 U/l in the PGE_1-treated group (Fig. 2). A significant intergroup difference in serum LDH was still observed after reperfusion (Fig. 3).

No significant differences were observed in any of the other parameters examined, including blood coagulation factors.

Table 1. Changes in serum PGE_1 level (ng/ml) in donors before and at 15 and 30 min after intravenous administration of PGE_1 and in recipients before and at 30 min, 3 h, 6 h, 12 h, and on days 1–3 after reperfusion. Values are expressed as mean ± SEM (ng/ml)

Time	Donor	Time	Recipient
Pre	0.053 ± 0.015	Pre	0.043 ± 0.01
15 min	0.163 ± 0.039	30 min	0.048 ± 0.012
30 min	0.18 ± 0.061	3 h	0.058 ± 0.012
		6 h	0.058 ± 0.012
		12 h	0.035 ± 0.007
		1 day	0.025 ± 0.007
		2 day	0.055 ± 0.007
		3 day	0.04 ± 0.014

Arterial ketone body ratio (AKBR)

AKBR showed a marked elevation to over 1.0 at 15 min after reperfusion and remained over 1.0 thereafter in both groups (Fig. 4).

Serum PGE_1 level

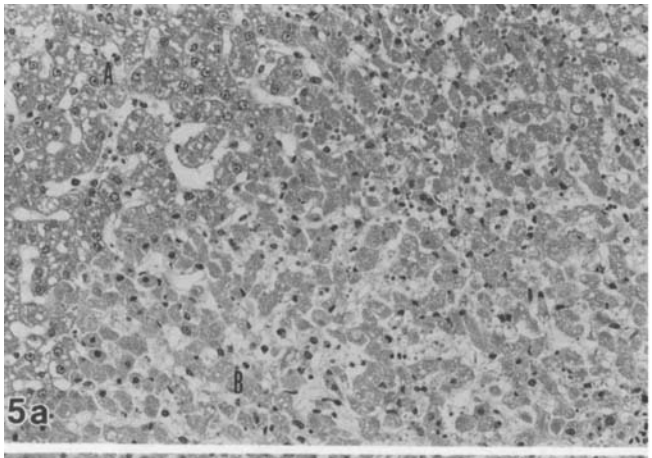
Serum PGE_1 levels are shown in Table 1. Thirty minutes after intravenous PGE_1 administration (0.5 μg/kg per minute) prior to donor graft harvesting, serum PGE_1 levels reached a peak (about 3.4 times the pretreatment level). After reperfusion, the serum PGE_1 level returned to the previous level in both groups, without any significant intergroup difference.

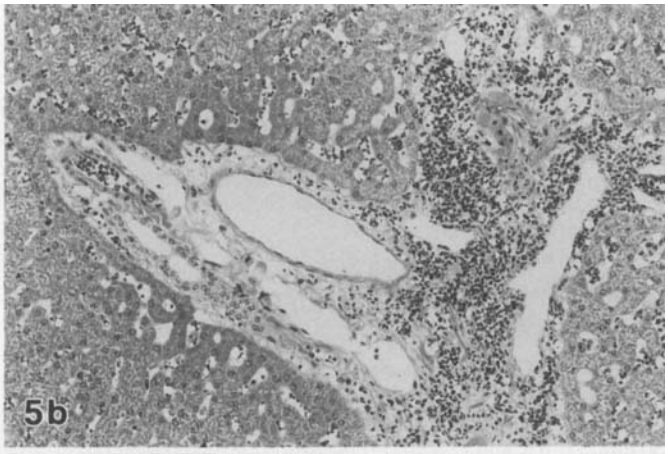
Histological examination

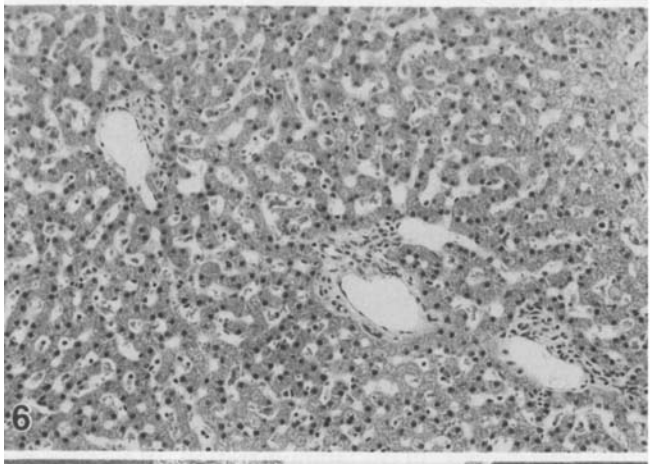
Histological examination by light microscopy revealed partial hepatocyte vacuolation, necrosis, and marked edema around the portal tract in the untreated control group 2 h after reperfusion (Fig. 5), while light microscopic findings from the PGE_1 group were near-normal (Fig. 6). Electron microscopy disclosed partial swelling of hepatic mitochondria as well as partial hepatocyte degeneration, necrosis (Fig. 7), and marked edema around the portal tract in the untreated control group 2 h after reperfusion, while hardly any change was observed in the PGE_1-treated group (Fig. 8). Sinusoidal endothelial cells appeared to be undamaged in both groups 2 h after reperfusion.

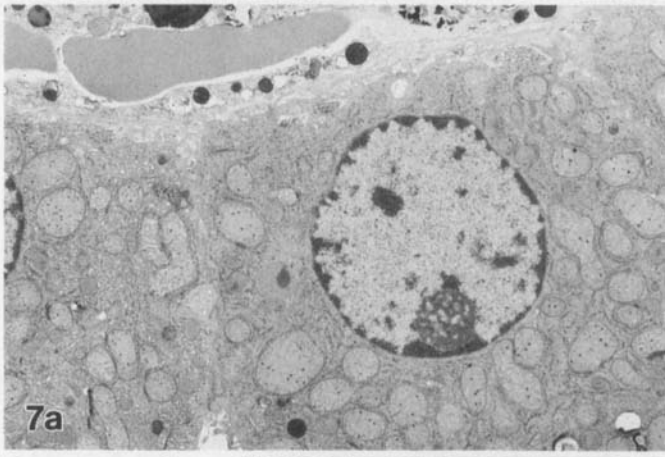
Discussion

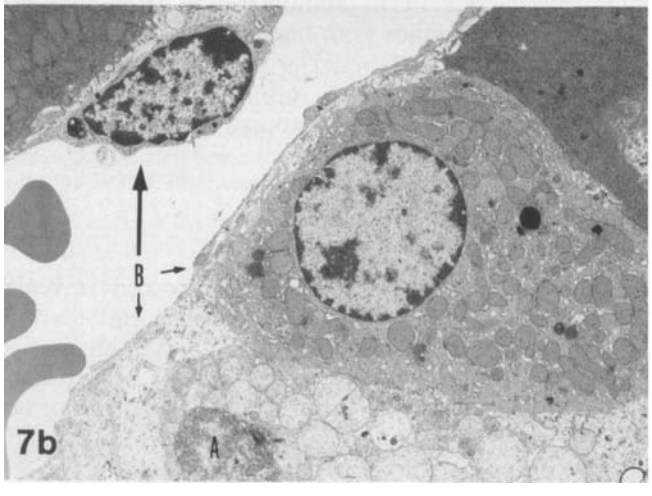
In both groups all canines survived for more than 1 week after transplantation. The post-transplant course of AKBR did not differ significantly between the two groups; it remained over 1.0 throughout the observation period. AKBR is the ratio of acetoacetic acid to beta-hy-











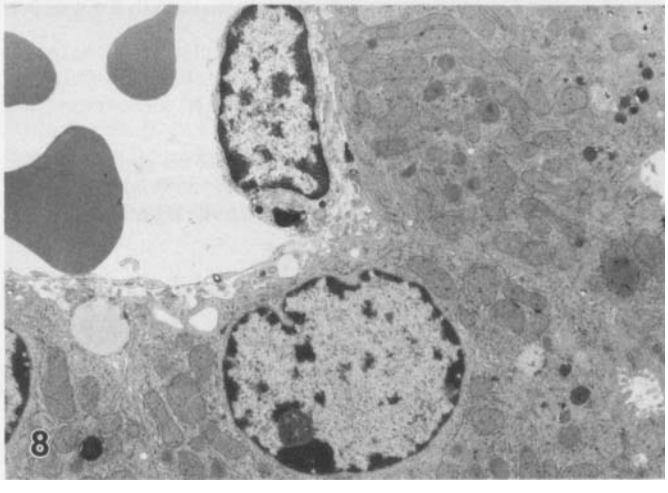


Fig. 5 a, b. Light micrograph of control group liver at 2 h after reperfusion: **a** hepatocyte vacuolation *(A)* and hepatocyte necrosis *(B)* are observed. × 170; **b** Marked edema around portal tract is observed. × 80

Fig. 6. Light micrograph of PGE_1 group liver at 2 h after reperfusion. Near-normal morphology is shown. × 130

Fig. 7 a, b. Electron micrograph of control group liver at 2 h after reperfusion: **a** Swelling of hepatic mitochondria is observed. × 4200; **b** Hepatocyte degeneration and necrosis are observed *(A)* Sinosoidal endothelial cell appears undamaged *(B, arrows)*. × 2800

Fig. 8. Electron micrograph of PGE_1 group liver at 2 h after reperfusion. Near-normal morphology is shown. × 4200

droxybutyric acid in arterial blood. This parameter is in equilibrium with the hepatic mitochondrial redox state (NAD^+/NADH). We have already demonstrated experimentally and clinically that AKBR is a useful index in evaluating graft liver viability after transplantation [1, 2, 19, 25, 26]. The favorable post-transplant courses of AKBR in the present study were reflected in the absence of fatal damage to livers during 24-h simple cold storage in UW solution and survival of all recipients after transplantation. Although partial swelling of hepatic mitochondria was observed electromicroscopically after 24-h preservation in the untreated control group, this change was considered to be sufficiently compensated for since AKBR

values did not decrease, resulting in no adverse effect on the survival of recipients.

In the present study, the elevation in liver enzymes (GOT), which occurred in the untreated control group in the early period, was significantly suppressed in the PGE_1-treated group. In the control group, GOT rose to about 2000 after transplantation. In this connection, Howard et al. reported that the incidence of rejection increased in cases in which GOT exceeded 2000 soon after transplantation [8]. In our histological examination, although the untreated control group showed partial mitochondrial swelling as well as partial degeneration and necrosis of hepatocytes, no rejection episodes were encountered.

The serum PGE_1 level rose to about 3.4 times the pretreatment level after intravenous administration of PGE_1 for 30 min during harvesting. At reperfusion, liver grafts were washed out with PGE_1-free, lactated Ringer's solution. Therefore, after reperfusion of the liver grafts, the serum PGE_1 level returned to its previous level in the PGE_1-treated group. This led us to conjecture that this particular action of exogenous PGE_1 might be present during harvesting and preservation.

Problems related to harvesting and hypothermic preservation may include vasoconstriction by the infusion of cold washout solution, microcirculatory plugging by platelet aggregation, and cell injury due to nonphysiological influx of Ca^{2+} into the cytoplasm caused by hypoxia [3], as well as the generation of oxygen-free radicals at reperfusion [14, 17, 20, 27] and thrombogenesis after reperfusion.

Exogenous PGE_1 is reported to promote the elevation of intracellular cyclic AMP [24, 31] and to be involved in the intracellular Ca^{2+} regulating system that prevents cell damage. That is, PGE_1 is thought to stabilize the cell membrane by reducing the intracellular Ca^{2+} by some as yet unknown mechanism [4, 13, 21]. PGE_1 is also reported to restore hepatic ATP to levels significantly higher than those in untreated controls [29]. These actions of PGE_1 may explain the cytoprotective effects that it exerts during harvesting and cold preservation. Actually, PGE_1 has been used experimentally to reduce hepatic injury during reperfusion [18] and has been used clinically during preflushing at harvesting [6]. Greig et al. reported on the clinical application of PGE_1 to treat primary nonfunctioning liver grafts following liver transplantation. They found that graft survival was 80% in the PGE_1-treated group and 17% in the untreated group, and that patient survival was 90% and 33%, respectively [7]. On the other hand, PGI_2 has generally been used for experiments because of its chemical instability: PGI_2 and its analogue have been used effectively during simple cold storage [16, 22] and warm ischemia [23], respectively.

These results suggest that PGE_1 is effective in reducing the preservation injury to liver grafts during long-term storage, even in UW solution.

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