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Prostaglandin E_1 Increases Survival with Extended Anhepatic Phase During Liver Transplantation

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Objective
The authors investigated the intraoperative treatment effects of Prostaglandin E_1 (PGE_1) for extension of the anhepatic phase and improvement of survival in a rat liver transplant model.

Background
Cross-clamping the inferior vena cava and the portal vein during liver transplantation causes severe pathophysiologic changes during surgery. The time of the anhepatic phase is strictly limited and results in a very tenuous period during the liver transplant operation.

Methods
Prostaglandin E_1 was infused at 0.5 μg/kg/min into five subgroups of rats with 20, 30, 40, 60, and 80 minutes of anhepatic phase during transplantation. Bile secretion, serum aspartate transaminase (AST), lactic dehydrogenase (LDH), and blood gas analysis were studied in the 30-minute subgroup. The results were compared with the sham-operated and control groups.

Results
Intraoperative treatment with PGE_1 extended the maximal anhepatic phase from 30 minutes in the sham-operated group up to 80 minutes, and increased survival. Significant changes in the PGE_1 treated rats in the 30-minute subgroup included an increase of bile flow and bile salt output and decrease of AST and LDH activities after surgery. Blood gas analysis showed a decrease in acidosis and hypercarbia at the end of the anhepatic phase.

Conclusions
The PGE_1 treatment increased survival with extended anhepatic phase during rat liver transplantation. The beneficial effects can be attributed to its biologic activities.

The anhepatic phase is the period between clamping and unclamping the portal vein and the abdominal portion of the inferior vena cava for removing the native liver and the donor liver implantation during transplantation. In spite of great improvement in the surgical techniques,[1] immunosuppression,[2,3] and preservation,[4] the anhepatic phase remains the most difficult period of the transplant operation and results in an early mortality and morbidity.[5] The time of the anhepatic phase is strictly limited because major vascular occlusion causes severe metabolic and hemodynamic disturbances, intestinal congestion, impairment of renal function, and

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other complications. Although venovenous bypass stabilizes hemodynamics during the anhepatic phase, complications caused by the technique can occur,[6] and the effects of the bypass for reduction of intraoperative risk and the post-reperfusion syndrome are controversial.[7,8] Therefore, the time limit of the anhepatic phase remains a challenge for successful liver transplantation.

This study investigated the effects of intraoperative treatment with prostaglandin E_1 (PGE_1) to extend the anhepatic phase and improve survival rate of rats undergoing liver transplantation.

MATERIALS AND METHODS

Animals

The experiment was conducted on male Sprague-Dawley rats weighing 302 ± 8 g obtained from Hilltop Lab Animals, Inc.(Scottdale, PA). Rats were housed in the standard animal room without fast before the surgery and were allowed free activity and chow after surgery or sample collection.

Experimental Protocol

The study was performed in three series of experimental groups. In group 1 (sham-operated), liver transplantations were performed in five subgroups of rats with 20, 30, 40, 60, and 80 minutes of anhepatic phase without any intraoperative treatment. In group 2 (control), five subgroups of liver transplanted rats were established under the same experimental conditions as group 1, except normal saline was administered intravenously with an infusion rate of 100 μL/min. In group 3 (PGE_1-treated), liver transplantations were done in five subgroups of rats under the same experimental conditions as group 2, except PGE_1 was infused instead of normal saline. The intravenous dose of PGE_1 was 0.5 μg/kg/min, with the same infusion rate (100 μL/min) as normal saline in controls (Table 1). Survival rates were compared among the three subgroups in each anhepatic phase time subgroup. Rats that survived 7 or more days after transplantation were considered survivors. Bile and blood samples in the subgroups with 30-minute anhepatic phase were collected for study of bile secretion, serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH) activities, and blood gas analysis.

Surgical Procedures

The jugular vein in the control and PGE_1-treated rats was cannulated using 10 cm of PE-50 polyethylene tubing (Clay Adams, Becton Dickinson & Co., Parsippany, NJ), filled with heparinized saline, and closed at one end.

Table 1. SURVIVAL RATES IN THE RAT WITH PGE_1-TREATMENT

Anhepatic Phase (min)	Survival Rate (> 1 week)		
	Sham-Operated	Control	PGE_1-Treated
20	87.5% (7/8)	100.0% (5/5)	100.0% (5/5)
30	37.5% (3/8)	66.7% (6/9)	75.0% (6/8)
40	0 (0/8)	12.5% (1/8)	87.5% (7/8)*
60	0 (0/3)	0 (0/6)	66.7% (4/6)*
80	0 (0/3)	0 (0/6)	16.7% (1/6)

Number of animals in parentheses. Sham-operated group: no intravenous infusion during liver transplantation. Control group: normal saline infused intravenously during transplantation. PGE_1-treated group: PGE_1 intravenous infusion instead of normal saline in the control group during surgery.
* $p < 0.05$ compared with the sham-operated and control groups.

The tip of the cannula was positioned at the superior vena cava. The closed end of the tube was exited on the back of the neck, opened, and connected to the Harvard Pump 22 (Harvard Apparatus, Inc., South Natick, MA) for the intravenous infusion.

Orthotopic liver transplantation was done using the cuff technique described by Kamada and Calne[9] under ether anesthesia in a 97 rats. Donor and recipient rats were matched for size. The hepatic artery was ligated without reconstruction. Heparinized normal saline at 1 C to 3 C was used to perfuse and store all donor livers. After clamping the suprahepatic vena cava, infrahepatic vena cava, and portal vein, we did not start the procedure of the donor liver implantation until the last 20 minutes of the designated anhepatic phase to give the liver equivalent periods of warm exposure during the operation. A small amount of the accumulated blood in the distal vena cava and portal vein was cleared out by opening and reclamping the clips quickly before reconnection of the vessels to prevent thrombosis. After revascularization, the vessels were unclamped simultaneously at the designated time. The anhepatic period was calculated from the time that the three vessels were clamped to the time of the unclamping. The common bile ducts were reconnected according to the method of Kamada and Calne,[9] except for the rats in the subgroups with 30-minute anhepatic phase, which were cannulated with a mini T tube for postoperative bile sample collection as described previously.[10] Five milliliters of 10% dextrose was given via the rats' penile veins after liver revascularization.

Sample Collection and Measurement

A 0.3-mL blood sample was collected from the cannulated jugular veins in the subgroups of rats after 30 minutes of the anhepatic phase, just before unclamping the

Table 2. BILE FLOW AND BILE SALT OUTPUT IN RATS WITH 30-MINUTE ANHEPATIC PHASE

	Bile flow (μL/100 g/h)	Bile Salt Output (μmol/100 g/h)
Sham-operated (n = 3)	169.7 ± 4.6	5.73 ± 0.63
Control (n = 6)	171.7 ± 6.7	5.87 ± 0.85
PGE$_1$-treated (n = 6)	192.2 ± 3.4*	7.93 ± 0.53*

Values are expressed as mean ± SEM.
* $p < 0.05$ compared with the sham-operated and control groups.

inferior vena cava and portal vein. The blood pH value, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), bicarbonate (HCO_3^-), oxygen saturation (O_2 SAT), and base excess (BE) in the sample were measured immediately using Corning 158 pH/blood gas analyzer (Ciba Corning Diagnostics Corp., Medfield, MA).

Blood samples (0.2 mL) from the rats' tail veins were collected daily for 6 days in the 30-minute subgroups. Serum AST and LDH activities were determined from each sample using research kits from Sigma Chemical Co. (St. Louis, MO).

Bile samples were collected from the mini T tube in the rat during the first postoperative hour. The concentration of the total bile salts was measured using a modification of the 3α-hydroxysteroid dehydrogenase technique[11] with sodium taurocholate as the standard. The enzymes, hydroxysteroid dehydrogenase, β-nicotinamide adenine dinucleotide, and sodium taurocholate were obtained from Sigma Chemical Co.

Statistical Analysis

Significant differences of the survival rate among three experimental groups were tested using the chi square test. Data, including rat body weight, bile volume, bile salt outputs, serum AST, LDH, and blood gas analysis are expressed as mean ± standard error of the mean (SEM). Significant differences between the two subgroups with 30-minute anhepatic phase in the control and PGE$_1$-treated groups were tested by the unpaired Student t test using the computer program Sigmaplot, version 4.0 (Jandel Scientific, Corte Madera, CA).

RESULTS

The time of the maximal anhepatic phase for survival of the liver transplant rat in the sham-operated group was 30 minutes, which resulted in a survival rate of 37.5%. No rats in the untreated groups survived for more than 7 days when the anhepatic phase was increased to 40 minutes or more. In control rats that had intraoperative saline infusion, survival rate in the subgroup of 30-minute anhepatic phase was increased to 66.7%. One of eight rats in the subgroup of 40-minute anhepatic phase survived (12.5%). With PGE$_1$ intraoperative treatment, survival rates in the rats that underwent liver transplantation increased significantly with extended anhepatic phase. The survival rate in the PGE$_1$-treated subgroup increased from 0% in the sham-operated and 12.5% in the control to 87.5% ($p < 0.05$) with 40-minute anhepatic phase, from 0% in the sham-operated and control subgroups to 66.7% ($p < 0.05$) with extended anhepatic phase to 60 minutes, from 0% in the sham-operated and control subgroups to 16.7% with extended anhepatic phase to 80 minutes (Table 1).

During the first hour after surgery, bile flow rate in the rats with 30-minute anhepatic phase increased from 169.7 ± 4.6 μL/100g body weight/hour in the sham subgroup and from 171.7 ± 6.7 in the control subgroup to 192.2 ± 3.4 in the PGE$_1$-treated subgroup, which was statistically significant ($p < 0.05$). Bile salt output increased significantly from 5.73 ± 0.63 μmol/100 g/hour in the sham subgroup and from 5.87 ± 0.85 in the control subgroup to 7.93 ± 0.53 in the PGE$_1$-treated subgroup (Table 2).

The PGE$_1$ treatment also significantly reduced serum AST activities in the rats with 30-minute anhepatic phase on day 2 and decreased serum LDH levels on days 1 and 2 (Table 2 and Fig. 1).

Table 3. SERUM AST AND LDH ACTIVITIES IN RATS WITH 30-MINUTE ANHEPATIC PHASE

Day	Sham-Operated (n = 3)	Control (n = 6)	PGE$_1$-Treated (n = 6)
Serum AST activity (U/L)			
1	565.5 ± 97.7	465.4 ± 70.1	367.3 ± 27.6
2	805.0 ± 73.2	672.7 ± 60.2	485.6 ± 51.4*
3	419.0 ± 45.7	363.7 ± 25.3	252.5 ± 48.1
4	258.9 ± 28.1	256.2 ± 55.5	170.1 ± 36.0
5	218.0 ± 25.2	205.5 ± 33.1	152.2 ± 25.5
Serum LDH activity (U/L)			
1	2133.5 ± 186.4	1890.5 ± 211.5	1256.9 ± 153.6*
2	961.1 ± 39.9	794.8 ± 67.9	465.6 ± 116.1*
3	450.1 ± 75.1	341.9 ± 41.6	308.7 ± 65.9
4	387.2 ± 30.1	458.5 ± 68.9	296.5 ± 49.1
5	324.7 ± 23.1	420.2 ± 45.1	292.0 ± 37.5

Values expressed as mean ± SEM.
* $p < 0.05$ compared with the sham-operated and control groups.

The results from blood gas analysis showed decreases of acidosis and hypercarbia at the end of the 30-minute anhepatic phase in the PGE_1-treated subgroup compared with the sham-operated and control subgroups. No significant differences of the blood PO_2, HCO_3^-, O_2 SAT, and BE were found among the three subgroups (Table 4 and Fig. 2).

Table 4. BLOOD GAS ANALYSIS IN RATS WITH 30-MINUTE ANHEPATIC PHASE

	Sham-Operated (n = 3)	Control (n = 6)	PGE_1-Treated (n = 6)
PH Value	6.90 ± 0.30	6.93 ± 0.02	7.04 ± 0.02*
PCO_2 (mmHg)	96.40 ± 5.63	95.33 ± 3.03	74.88 ± 2.73*
PO_2 (mmHg)	19.15 ± 1.53	21.03 ± 1.59	20.80 ± 1.79
HCO_3 (mmol/L)	17.68 ± 1.82	19.92 ± 0.99	19.67 ± 0.98
O_2SAT (%)	14.43 ± 3.2	15.83 ± 2.37	16.35 ± 3.55
BE (mmol/L)	−16.24 ± 2.12	−15.88 ± 1.25	−13.42 ± 1.23

Values are expressed as mean ± SEM.
* $p < 0.05$ compared with the sham-operated and control groups.

DISCUSSION

The cross-clamping of the inferior vena cava, hepatic artery, and portal vein during liver transplantation causes severe pathophysiologic changes in the recipient, including reduced cardiac output, increased systemic vascular resistance, visceral congestion and bleeding, tissue ischemia, intestinal edema, and renal function damage.[5,12–14] After liver revascularization, a large volume of cold, acidic, and hyperkalemic blood from the graft and obstructed venous beds suddenly returns into the systemic circulation and induces hypotension, bradycardia, systemic vasodilation, increased cardiac filling pressure, and decreased cardiac output,[8,13,15,16] so-called post-reperfusion syndrome.[8,14,15]

Because of the pathophysiologic disturbances, the time of the anhepatic phase is strictly limited, even if the transplantation is performed with the venous bypass.[8,17,18] Maximal time of the portal vein clamping was reported no more than 26 minutes in the rat liver transplantation model by Kamada and Calne.[19] No rats in the sham-operated subgroups survived when the anhepatic phase was extended to 40 minutes or more in our experiment. Maximal time of the anhepatic phase for human liver transplantation is unclear. The time required for removing the native liver and the donor liver revascularization is approximately 90 minutes in patients.[20]

Intraoperative treatment of the recipient rat with PGE_1 has extended dramatically the anhepatic phase from 30 minutes in the sham-operated rats to 80 minutes with increase of the rat survival. Also, the control animals exhibited 12.5% survival after 40-minute anhepatic phase, but the PGE_1-treated rats had 87.5% survival ($p < 0.05$) for anhepatic phase of the same duration. The mechanism of the effects resulting in prolongation of the anhepatic phase remains unclear, although several possibilities can be considered.

There is increased evidence of protective effects of prostaglandin in the donor liver cell from cold or warm ischemia.[21–23] Prostaglandin E_1 reduces bowel necrosis in a platelet-activating factor-induced intestinal necrosis model.[24] The cytoprotective effects are linked to stabilization of lysosomal membranes.[25] However, the exact mechanism of the stabilization is still unknown. Intraoperative treatment of PGE_1 decreases the degree of acidosis and reduces blood PCO_2, which accumulates with the blood stasis when inferior vena cava and portal vein are clamped. The phenomenon suggests that PGE_1 maintains the cellular integrity and metabolism under hypoxemia by a mechanism of membrane stabilization.

Prostaglandin E_1 also is a known vasodilator.[21,24] Several studies[21,25,26] have confirmed that PGE_1 improved hepatic microcirculation after liver cold storage. Bile flow is a function of blood flow through the liver,[27] and it may reflect the liver microcirculation after reperfusion in the liver transplant rat.[28] Increases in bile flow and bile salt secretion in our studies suggest that PGE_1 increases blood circulation in the liver. Prostaglandin E_1 treatment may improve the visceral microcirculation during the anhepatic phase.

Changes of renal hemodynamics after liver transplantation are characterized by rising arterial pressure, renal vasoconstriction, and a decrease in glomerular filtration rate.[14] Complete anuria or marked oliguria are frequent during the anhepatic phase.[26] Prostaglandin protects against excessive vasoconstriction.[14] Infusion of prostaglandin into the renal artery will decrease renal vascular resistance, increase renal blood flow, and augment sodium and water excretion.[22] Therefore, the effects of PGE_1 treatment in the present study may relate to its property of kidney function protection.

Under normal physiologic circumstances, blood cells and endothelial cells interact in complex ways to maintain an intact vascular system, in which platelets play a crucial role. Post-ischemic livers are sensitive to platelet, leukocyte, and lymphocyte adherence, which can lead to the destruction of the graft.[29–31] Prostaglandin E_1 significantly reduced serum AST and LDH activities in the PGE_1-treated rat in our experiment. Its biologic action of inhibition of platelet aggregation and the inhibition of thromboxane generation may be partly responsible for the improvement of the donor liver quality and graft survival.

Prostaglandins dilate vascular smooth muscle and act as potent vasodilators.[22] Prostaglandin E_1 infusion during the anhepatic phase reduced blood stasis by increased collateral circulation and stabilized hemodynamics expressed by increased blood oxygen saturation. Blood PO_2 and O_2 SAT levels measured in our experimental rats did not show any significant increase. Blankensteijn et al.[32] studied PGE_1 on intraoperative hemodynamic changes in the liver transplant pig and also found no protective effects of PGE_1 against the reperfusion syndrome. Therefore, the efficiency of PGE_1 for stabilization of hemodynamics during the liver transplantation remains obscure.

Prostaglandin E_1 significantly increases survival with extended anhepatic phase during rat liver transplantation. The beneficial effects can result from its biological

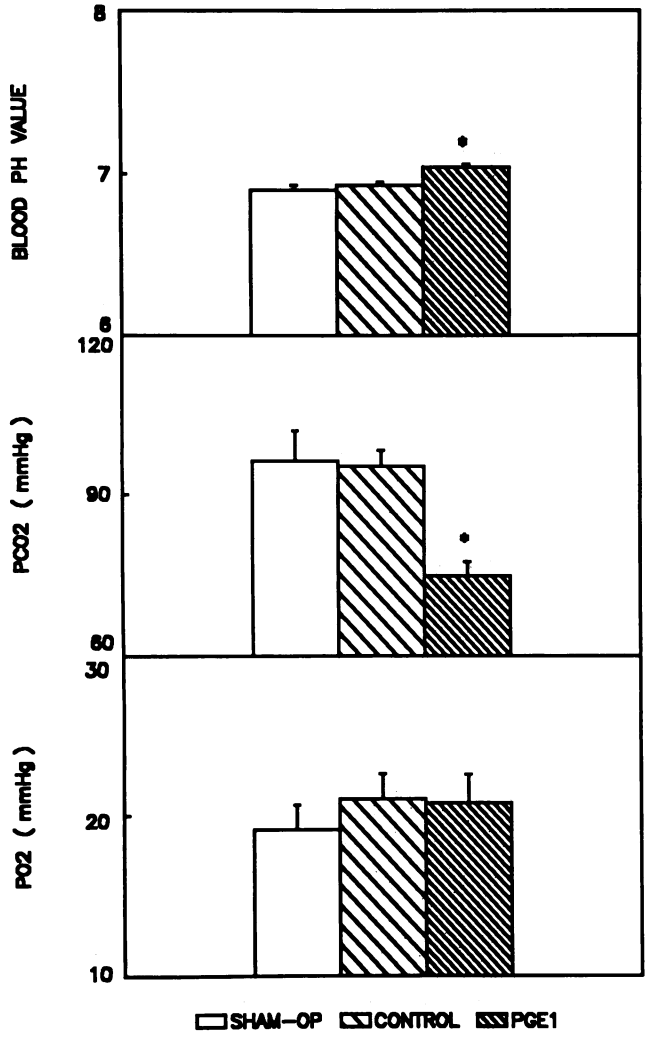

Figure 1. Changes of serum AST and LDH activities in the rats with 30 minutes of the anhepatic phase. SHAM: sham-operated subgroup; CONT: subgroup of control; PGE1: PGE_1-treated subgroup; Day 1: the day of the surgery; * = $p < 0.05$ compared with the sham-operated and control subgroups.

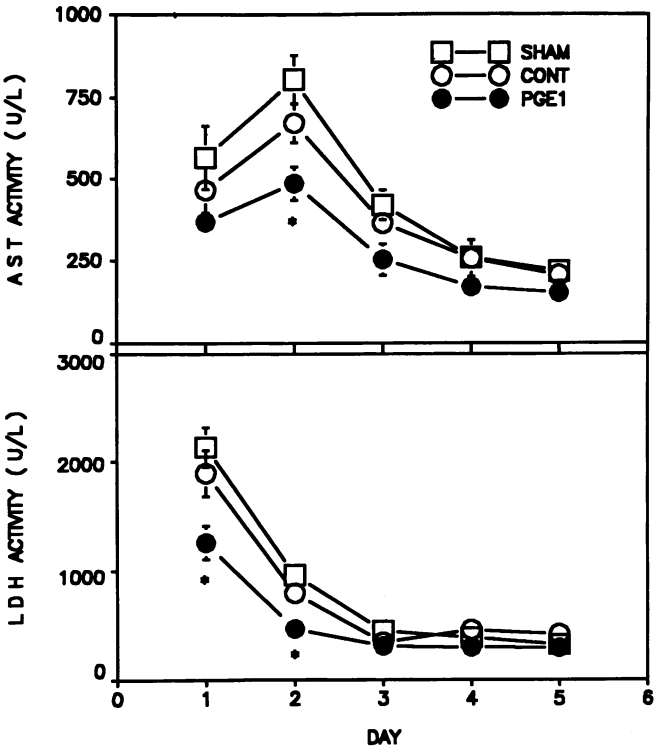

Figure 2. Comparison of blood pH PCO_2 and PO_2 in rats with 30 minutes of the anhepatic phase among the sham-operated, control, and PGE_1-treated subgroups. * = $p < 0.05$ compared with sham-operated and control rats.

activities, such as vasodilation, stabilization of lysosomal membrane, prevention of platelet aggregation, inhibition of thromboxane generation, and increase of blood flow to the splanchnic region. The results from our study also indicated that extension of the anhepatic phase using pharmacologic agents is a new strategy to improve survival rate after liver transplantation.

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