Blood pH and gas analysis

Blood for pH and gas analysis was obtained by aortic puncture, and was analyzed with an acid-base analyzer (ABL-30, Radiometer, Copenhagen).

Blood chemical analysis

Blood for chemical analysis was collected from the aortic puncture, was cooled immediately with ice, and centrifuged at 2,200 rpm for 20 min. Then the separated plasma was stored at −20 ℃ until measurement of the following parameters of liver and kidney functions with an autoanalyzer (Hitachi −736, Hitachi, Tokyo)[13]: glucose (Glu, glucose oxidase method), total protein (TP, Biuret method), albumin (Alb, Bromcresol green method), total bilirubin (TB, azobilirubin method), glutamic oxaloacetic transaminase (GOT, Ultraviolet method), glutamic pyruvic transaminase (GPT, Ultraviolet method), alkaline phosphatase (Alp, Bessey-Lowry method), blood urea nitrogen (BUN, urease ultraviolet method) and electrolytes (Na-K, electrode method).

Monitoring of systemic arterial blood pressure

The catheter for pressure recording was introduced into the caudal cut end of the right carotid artery, and systemic arterial pressure (SAP) was recorded with a pen recorder (SAN-EI, Type 1237, Tokyo; SR 6221, Graphtec, Tokyo).

Liver ischemia

After a midline laparotomy, the portal vein and the hepatic artery were exposed and clamped for 10 min with microsurgical clamps (Heifetz clip, Edward Weck & Company, NC).[14]

Administration and estimation of indocyanine green

Indocyanine green (ICG, Daiichi Pharmaceutical Co., Ltd., Tokyo) was dissolved in the aqueous solvent provided, and injected into the jugular vein as a bolus. ICG-K was obtained by the method described previously.[15] Briefly, an ICG monitor (RK-1000, Sumitomo Electric Industries Ltd., Osaka) was utilized, and a sensor was attached to the crus of the posterior foot. The ICG-K values were recorded 0, 30, 60, 120 and 180 min after liver ischemia in the same rats. It was first determined that the ICG 0.25 mg/kg used is suited to repetitive measurements.

Administration of Prostaglandin E_1 and related agents

PGE 1 (Ono Pharmaceutical Co., Ltd., Osaka) dissolved in the saline was injected into the cranial side of the clamped portal vein or into the right jugular vein. PGE 1 20 or 40 μg/kg was injected, and saline was used as the control. ONO-AE-829, the selective PGE 1 receptor antagonist (Ono Pharmaceutical Co., Ltd., Osaka) and 17-phenyl-trinor prostaglandin E_2, the selective PGE 1 receptor agonist (Biomol Research Laboratories, Inc., PA) dissolved in 5% glucose solution were also utilized to identify PGE 1 receptor specificity.[16] The amount of each injection was 100 μl, and all were completed as a bolus.

Statistical analysis

The statistical significance of the differences among values was evaluated by Duncan's multiple range test. $p<0.05$ was defined as significant.

RESULTS

Effects of PGE1 on blood pH and gas tension

No noticeable change in the blood pH or gas tension was seen when PGE 1 was injected into the portal vein or into the jugular vein under liver ischemia (Table 1).

ICG disappearance curves

The ICG disappearance curves under liver ischemia are shown in Fig. 1. The curve for the liver ischemia with PGE 1 40 μg/kg was downward compared to the curve in the liver ischemia with saline.

Effects of PGE1 on ICG-K

Comparison between PGE 1 rats and the control showed that ICG-K values at 60, 120 and 180 min after liver ischemia were higher in the portal PGE 1 rats than in the control rats. It was noted that ICG-K values 60 min after liver ischemia ranged in the order of portal PGE 1 rats, jugular PGE 1 rats, and control rats. ICG-K response 60 min after the ischemia in the portal PGE 1 rats was dose dependent (Fig. 2). ICG-K response due to PGE 1 was blocked by administration of ONO-AE-829, and this effect was mimicked by 17-phenyl-trinor prostaglandin E_2 administration (Fig. 2).

Table 1. Blood gas parameters 30 min after 10-min liver ischemia and PGE 1 (40 μg/kg) administration

	Portal injection		Jugular injection
	Saline	PGE 1	PGE 1
Temp (℃)	37.0±0.0	37.0±0.0	37.0±0.0
Hb (g/dl)	15.0±0.1	15.1±0.1	15.4±0.4
pH	7.459±0.018	7.461±0.022	7.491±0.010
Pco_2 (mmHg)	23.7±1.8	28.3±1.6	24.4±1.2
Po_2 (mmHg)	90.6±3.8	87.8±3.7	93.0±1.3
HCO_3 (mmol/l)	16.6±1.0	19.9±0.7	18.5±0.9
TCO_2 (mmol/l)	17.3±1.0	20.7±0.7	19.3±0.9
BE (mmol/l)	5.1±1.0	2.4±0.9	2.7±0.8

Values are the mean±SEM (n=6).

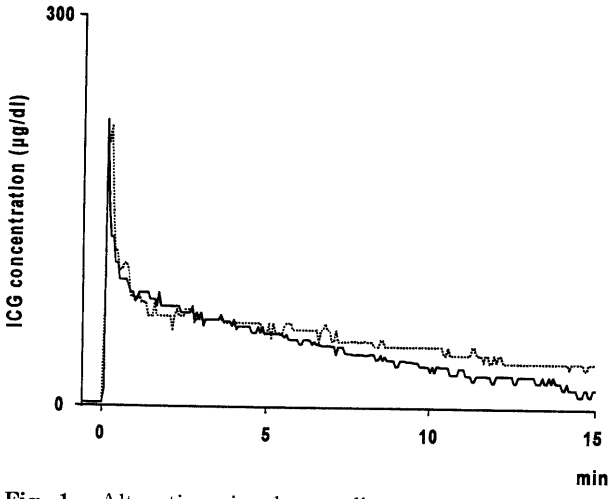

Fig. 1. Alterations in plasma disappearance curves of ICG after PGE 1 injection under liver ischemia. PGE 1 at 40 μg/kg (—) or saline (--) was injected into the portal vein immediately before liver vascular clamping.

Effects of PGE1 on serum liver and kidney functional scores

The GPT value increased by liver ischemia was suppressed by portal PGE 1 administration, but jugular injection of PGE failed to change the GPT value (Fig. 3). Liver functional parameters -- except for GPT -- were unchanged after PGE 1 administration. Kidney functional parameters were unchanged (Table 2).

Effects of PGE 1 on SAP

No significant differences between PGE 1 rats and the control were seen in the percent change in SAP when PGE 1 was given into the portal vein or into the jugular vein (Fig. 4).

DISCUSSION

We found that a prior administration of PGE 1 into the portal circulation supports the liver function associated with liver ischemia: PGE 1 injected into the portal vein immediately after liver vascular clamping enhanced ICG-K caused by liver ischemia. Because ICG-K has been shown to reflect the hepatocyte function,[17,18] this could mean that PGE 1 protects the hepatocytes from ischemia.

Because the selective PGE 1 receptor antagonist blocked the ICG-K response due to PGE 1 and the response was mimicked by selective PGE 1 receptor agonist, the observed phenomenon may be mediated through a specific PGE 1 receptor. Although the further mechanism is not easy to explain, there are at least three possibilities: 1) Oxygen free radicals generated by ischemia/reperfusion cause necrosis of the liver.[19,20] On the other hand, PGE 1 has been shown to stabilize the membrane of the hepatocytes,[11] so that PGE 1 may protect the membrane of the hepatocytes against damage caused by the oxygen free radicals; 2) Although the microcirculation has a primary role in controlling the viability of the parenchymal cells in the liver, intercellular adherence between the leukocyte and endothelium contributes to the maintenance of circulation.[21,22] Considering

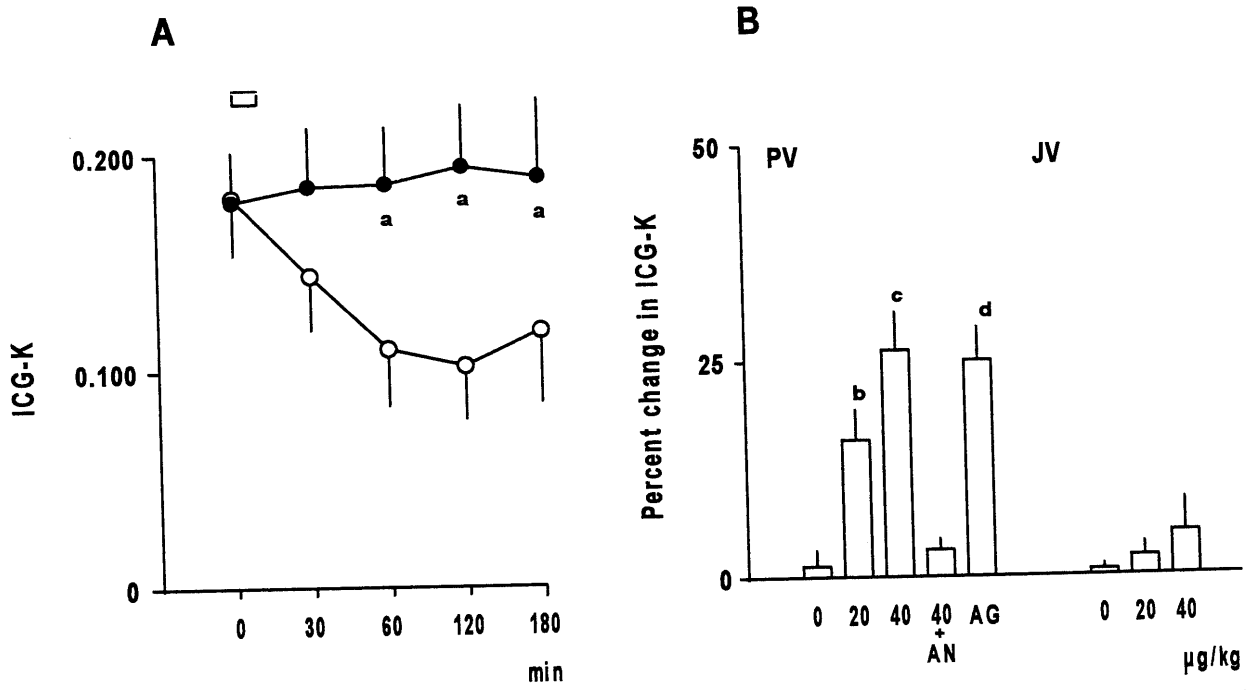

Fig. 2. **A.** Time courses for ICG-K after PGE 1 injection under liver ischemia. PGE 1 at 40 μg/kg (●) or saline (○) was portally administered. A bar indicates the time of liver ischemia. Values are the means±SEM (n=6). $^{a}p<0.05$ vs ○. **B.** ICG-K responses 60 min after PGE 1 injection. PGE 1 at 20 μg/kg (20) or 40 μg/kg (40) or saline (0) was injected into the portal vein (PV) or the jugular vein (JV). PGE 1 at 40 μg/kg (40) with ONO-AE-829 at 50 μg/kg (AN) or 17-phenyl-trinor Prostaglandin E_2 at 60 μg/kg (AG) was also given into the portal vein. Values are the means±SEM (n=8). $^{b}p<0.01$ vs 0 and 40+AN. $^{c}p<0.05$ vs 20. $^{d}p<0.01$ vs 0.

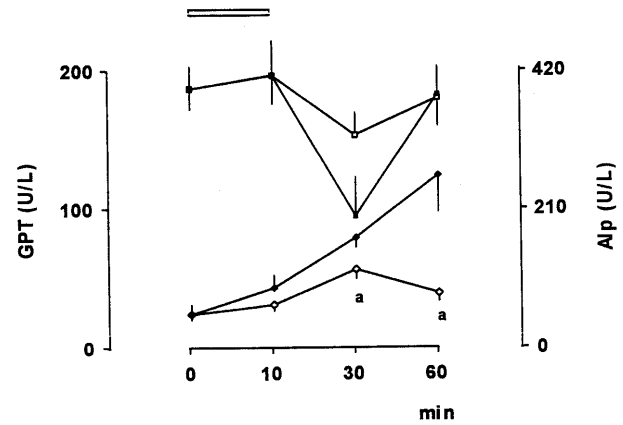

Fig. 3. Time courses for liver functional parameters after PGE 1 administration. PGE 1 (■-◆, 40 μg/kg) or saline (□-◇) was portally given, and GPT (◆-◇) and Alp (■-□) were estimated. A bar indicates the time of liver ischemia. Values are the means±SEM (n=6). $^{a}p<0.01$ vs saline.

these reports together with the finding that the production of cytokine moderating the adherence[23] can be regulated by PGE 1,[24,25] it is possible that PGE 1 determines the viability of the hepatocytes through a microcirculatory change; 3) Because mitochondrial function has been shown to be maintained by PGE 1 treatment,[12] PGE 1 may maintain hepatocyte function through the mitochondrial action of PGE 1. ICG-K is a parameter reflecting hepatocyte function.[17,18] PGE 1 has been shown to increase hepatic blood flow in rats, dogs and pigs,[7-9] but a direct circulatory effect of PGE 1 on the hepatocytes can be excluded in this case because, in the portal vein, there was no effective blood flow when PGE 1 was injected.

PGE 1 injected into the jugular vein failed to change ICG-K. This could mean either that PGE 1 could not reach the liver or that PGE 1 was diluted to an ineffective concentration in the systemic circulation.

GPT elevation signifies the destruction of the hepatocytes, and was increased after liver ischemia in this study. This is in keeping with reports indicat-

Table 2. Serum chemical parameters 30 min after 10-min liver ischemia and PGE 1 (40 μg/kg) administration

	Portal injection		Jugular injection
	Saline	PGE 1	PGE 1
Glu (mg/dl)	180±2	170±13	183±14
TP (g/dl)	4.9±0.1	4.8±0.1	4.7±0.1
Alb (g/dl)	1.9±0.0	2.0±0.0	1.9±0.0
TB (mg/dl)	0.1±0.0	0.1±0.0	0.1±0.0
GOT (U/L)	199±21	134±14	245±31
GPT (U/L)	79±6	56±6[a]	109±26
Alp (U/L)	199±65[b]	322±32	332±30
BUN (mg/dl)	18.9±0.9	17.4±0.8	14.1±1.1
Na^+ (mEq/l)	134.5±1.2	131.3±2.4	133.6±1.7
K^+ (mEq/l)	5.2±0.1	4.8±0.2	4.8±0.2

Values are the mean±SEM (n=6). [a]$p<0.01$ vs portal saline and jugular PGE 1. [b]$p<0.01$ vs portal and jugular PGE 1.

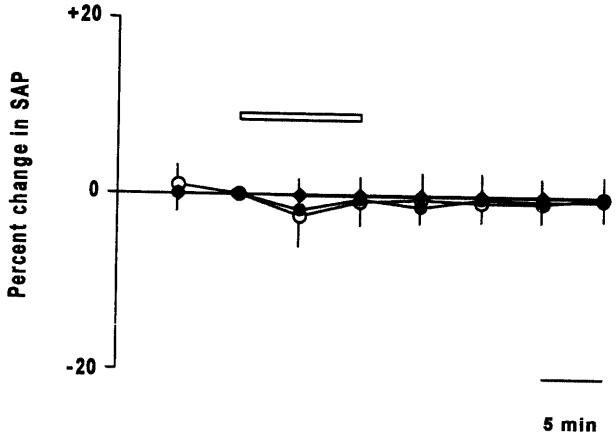

Fig. 4. Changes in SAP after PGE 1 injection under liver ischemia. PGE 1 at 40 μg/kg (●-◆) or saline (○-◇) was portally (●-○) or jugularly (◆-◇) administered. A bar indicates the time of liver ischemia. Values are the means±SEM (n=6).

ing that liver ischemia produces an increase in GPT.[26-28] ICG-K values also changed with liver ischemia; as shown in Figs. 1 and 3, there were similar time courses for GPT and ICG-K in response to the ischemia. ICG-K, however, can be estimated more easily than GPT. It appears that ICG-K estimation is suited for evaluating the recovery of liver function from ischemia.

The SAP changes when the liver has been reperfused.[4-6] Moreover, it has been shown that PGE 1 decreases the SAP by dilating the systemic vasculature,[5-8] but the biological half-life of exogenously administered PGE 1 has been estimated to be 2-5 min.[29,30] In this study, the concentration of PGE 1 effective in enhancing ICG-K evoked no further change in SAP when the liver was reperfused. An effect of PGE 1 on ICG-K can be expected without a great change in systemic arterial circulation.

This experiment simulated a clinical situation in which the protection of the hepatocytes is required when the vascular inflow to the liver is blocked. Consequently, it was noted that the prior administration of PGE 1 was useful in protecting the hepatocyte function and that this PGE 1 effect can be expected to be efficient when PGE 1 is injected into the portal vein.

From these observations, it was concluded that PGE 1 injection before liver circulatory occlusion is specifically effective in diminishing ischemic damage to the hepatocytes, and a portal injection of PGE 1 is more efficient than a systemic venous injection.

Acknowledgments. The authors are greatly indebted to Prof. Dr. Kazuhiro Tsukada (Second Department of Surgery, Toyama Medical and Pharmaceutical University) for his advice on this project. The PGE 1 and ONO-AE-829 used in this study were supplied by Ono Pharmaceutical Co., Ltd., Osaka.

REFERENCES

1) Pringle JH: Notes on the arrest of hepatic hemorrhage due to trauma. *Ann Surg* **48**: 541-549, 1908.
2) Goodall RGW, Hyndman WWB, Gurd FN: Studies on hypothermia in abdominal surgery. II. Occlusion of the vascular inflow to the liver. *AMA Arch Surg* **75**: 1011-1019, 1957.
3) Toledo-Pereyra LH: Liver transplantation reperfusion injury. Factors in its development and avenues for treatment. *Klin Wochenschr* **69**: 1099-1104, 1991.
4) Raffucci FL: The effects of temporary occlusion of the afferent hepatic circulation in dogs. *Surgery* **33**: 342-351, 1953.
5) Hemming AW, Scudamore CH, Shackleton CR, Pudek M, Erb SR: Indocyanine green clearance as a predictor of successful hepatic resection in cirrhotic patients. *Am J Surg* **163**: 515-518, 1992.
6) Jalan R, Plevris JN, Jalan AR, Finlayson NDC, Hayes PC: A pilot study of indocyanine green clearance as an early predictor of graft function. *Transplantation* **58**: 196-200, 1994.
7) Tsukada K, Katoh H, Iga Y, Tomiyama T, Okamura N, Sugimoto F, Ohtani T, Iiai T, Sakaguchi T, Yoshida K, Muto T: Prostaglandin E_1 enhances hepatic portal venous flow by dilating the portal vascular bed in 70% hepatectomized dog. *Gastroenterol Jpn* **27**: 341-347, 1992.
8) Nakadaira K, Tsukada K, Sakaguchi T, Shirai Y, Kurosaki I, Ohtake M, Yoshida K, Muto T: A pharmacological analysis of Prostaglandin E_1 on portal blood flow after partial hepatectomy in rats. *Surg Today* **23**: 277-279, 1993.
9) Ohtake M, Cui X, Sakaguchi T, Ishizuka D, Maruta T, Makino S, Shirai Y, Hatakeyama K: Suppression of liver blood flow by carbon dioxide pneumoperitoneum can be improved by prostaglandin E_1 in pigs. *Acta Med Biol* **47**: 147-153, 1999.
10) Sinclair SB, Greig PD, Blendis LM, Abecassis M, Roberts EA, Phillips MJ, Cameron R, Levy GA: Biochemical and clinical response of fulminant viral hepatitis to administration of prostaglandin E. *J Clin Invest* **84**: 1063-1069, 1989.
11) Beck PL, McKnight GW, Kelly JK, Wallace JL, Lee SS: Hepatic and gastric cytoprotective effects of long-term prostaglandin E_1 administration in cirrhotic rats. *Gastroenterology* **105**: 1483-1489, 1993.
12) Suzuki H, Suematsu M, Ishii H, Kato S, Miki H, Mori M, Ishimura Y, Nishino T, Tsuchiya M: Prostaglandin E_1 abrogates early reductive stress and zone-specific paradoxical oxidative injury in hypoperfused rat liver. *J Clin Invest* **93**: 155-164, 1994.
13) Aono T, Sakaguchi T, Nakadaira K, Ohtake M, Muto T: Orally administered prostaglandin E_1 derivative can enhance liver regeneration in partially hepatectomized rats. *Biochem Pharmacol* **46**: 767-769, 1993.
14) Foschi D, Castoldi L, Lesma A, Musazzi M, Benevento A, Trabucchi E: Effects of ischaemia and reperfusion on liver regeneration in rats. *Eur J Surg* **159**: 393-398, 1993.
15) Sakaguchi T, Nishimura A, Cui X, Aono T: Plasma indocyanine green disappearance induced by electrical stimulation of the hepatic vagal branch in rats. *Acta Med Biol* **46**: 89-92, 1998.
16) Takeuchi K, Ukawa H, Furukawa O, Kawauchi S, Araki H, Sugimoto Y, Ishikawa A, Ushikubi F, Narumiya S: Prostaglandin E receptor subtypes involved in stimulation of gastroduodenal bicarbonate secretion in rats and mice. *J Physiol Pharmacol* **50**: 155-167, 1999.
17) Aono T, Sakaguchi T, Tsukada K, Fujita N, Ishiduka D, Hatakeyama K: Prostaglandin E_1 increases indocyanine green disappearance rate in patients with chronic liver disease. *J Int Med Res* **23**: 299-307, 1995.
18) Tsukada K, Sakaguchi T, Aono T, Ishizuka D, Fujita N, Hatakeyama K: Indocyanine green disappearance enhanced by prostaglandin E_1 in patients with hepatic resection. *J Surg Res* **66**: 64-68, 1996.
19) Adkison D, Hollwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN: Role of free radicals in ischemia-reperfusion injury to the liver. *Acta Physiol Scand Suppl* **548**: 101-107, 1986.
20) Kawamoto S, Inoue M, Tashiro S, Morino Y, Miyauchi Y: Role of free radicals in entero-hepatic dysfunction caused by portal circulatory disturbance: effect of SOD derivative. *Transplant Proc* **21**: 1287-1289, 1989.
21) Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM: A monoclonal antibody to the adherence-promoting leukocyte glycoprotein, CD 18, reduces organ injury and improves survival from hemorrhagic shock and resuscitation in rabbits. *J Clin Invest* **81**: 939-944, 1988.
22) Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM: Inhibition of leukocyte adherence by anti-CD 18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. *Proc Natl Acad Sci USA* **87**: 2643-2646, 1990.
23) Winn RK, Vedder NB, Mihelcic D, Flaherty LC, Langdale L, Harlan JM: The role of adhesion molecules in reperfusion injury. *Agents Actions Suppl* **41**: 113-126, 1993.
24) Kunkel SL, Chensue SW, Phan SH: Prostaglandins as endogenous mediators of interleukin 1 production. *J Immunol* **136**: 186-192, 1986.
25) Harlan JM, Vedder NB, Winn RK, Rice CL: Mechanisms and consequences of leukocyte-endothelial interaction. *West J Med* **155**: 365-369, 1991.
26) Sumimoto K, Inagaki K, Ito H, Marubayashi S, Yamada K, Kawasaki T, Dohi K: Ischemic damage prevention by coenzyme Q_{10} treatment of the donor before orthotopic liver transplantation: biochemical and histologic findings. *Surgery* **102**: 821-827, 1987.

27) Ontell SJ, Makowka L, Trager J, Mazzaferro V, Ove P, Starzl TE: Pharmacologic modulation of experimental postischemic hepatic function. *Ann Surg* **209**: 200-210, 1989.
28) Stein HJ, Oosthuizen MM, Hinder RA, Lamprechts H: Oxygen free radicals and glutathione in hepatic ischemia/reperfusion injury. *J Surg Res* **50**: 398-402, 1991.
29) Ferreira SH, Vane JR: Prostaglandins: their disappearance from and release into the circulation. *Nature* **216**: 868-873, 1967.
30) Dawson W, Jessup SJ, McDonald-Gibson W, Ramwell PW, Shaw JE: Prostaglandin uptake and metabolism by the perfused rat liver. *Br J Pharmacol* **39**: 585-598, 1970.