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Amelioration of Normothermic Canine Liver Ischemia With Prostacyclin

S. Todo, H. Yokoi, L. Podesta, P. ChapChap, C. Pan, K. Okuda, Y. Kamiyama, J. Demetris, L. Makowka, S. Iwatsuki, and T.E. Starzl

RECENTLY, many agents that might protect the liver from ischemic insult have been developed. Validation of a protective effect with large animal liver transplantation, using either dogs or pigs, is too complex and expensive to be used routinely. In the present study, a new nontransplant model of normothermic liver ischemia was developed in dogs. The tolerance of canine liver to ischemia and the protective effect of prostacyclin (PGI2) was investigated using this system.

MATERIALS AND METHODS

Animal

Beagle dogs, weighing 15-18 kg were used. They were intubated and placed on a ventilator after intravenous (IV) injection of thiopental sodium 25 mg/kg. Anesthesia was maintained by IV ketamine 2 mg/kg, and pancuronium 0.1 mg/kg. Arterial blood pressure and central venous pressure were monitored throughout the operation.

Operative Procedures

The abdomen was entered through a midline incision. The liver was skeletonized, including division of all of the suspensory ligaments. Liver ischemia was induced by total occlusion of hepatic inflow with crossclamping the portal triad at the hilum. The splanchnic venous bed was decompressed by a pump-driven spleno-jugular bypass. The splenic vein and the external jugular vein were cannulated with standard No. 12-16 chest tubes (Argyle Division of Sherwood Medical, St Louis), which were connected by Tygon tubing (Norton Industrial Plastics, Akron, OH). A centrifugal pump (Bio Medicus, Minnetonka, MN) was placed in the circuit to drive the veno-venous flow. When liver ischemia was terminated by releasing the clamp, the spleen and the bypass system were removed. Lactated Ringer's solution, 2 to 4 L, and 1 unit of blood recovered from the bypass system were administered to animals. No heparin was used. Cephalosporin, 1 g, was given prior to the ischemia and continued for three days. The animals were fed, starting on the morning after operation.

Experimental Groups

Experimental groups and number of animals used were as follows:

1. Tolerance of liver ischemia
   Group 1: (N = 6) 1-hour ischemia
   Group 2: (N = 6) 2-hour ischemia
   Group 3: (N = 14) 3-hour ischemia
   Group 4: (N = 7) 3.5-hour ischemia

The experiment of three-hour ischemia, which had revealed an LD 50, was performed in two parts. The first seven experiments were by random insertion of the three-hour experiments with other groups. Then, confirmatory experiments were performed later with seven more animals.

2. Protective effect of PGI2
   Group 5: (N = 7) vehicle (glycine)
   Group 6: (N = 6) PGI2 (1 μg/kg/min)
   Group 7: (N = 6) PGI2 (2 μg/kg/min)

PGI2 was supplied as a crystalline powder from the Upjohn Company, Ltd (Kalamazoo, MI). It was dissolved into a glycine buffer (pH 10.5) and administered to the animals through a small mesenteric vein branch for one hour prior to ischemia. Severe, but not lethal, hypotension always occurred in animals receiving PGI2 infusion. Ordinarily, the mean blood pressure was in the 130 mmHg range before infusion. It decreased to the 70 mmHg range with 1 μg/kg/min of PGI2 and 50 mmHg with 2 μg/kg/min of PGI2. Blood pressure returned to the pretreatment level immediately after PGI2 was discontinued. No vasopressor was administered.

Peripheral venous blood samples were drawn serially for the determination of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), total biliru-

From the Departments of Surgery and Pathology, University Health Center of Pittsburgh, University of Pittsburgh, and the Veterans Administration Medical Center, Pittsburgh.

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Address reprint requests to Thomas E. Starzl, MD, PhD, Department of Surgery, 3601 Fifth Ave, Falk Clinic 4 West, Pittsburgh, PA 15213.

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bin, and blood sugar until the seventh postoperative day. Then the animals were killed. Postmortem examination of these dogs and those that died before then was carried out immediately after death.

Fisher's exact test and Student's *t* test were used for statistical analysis.

RESULTS

Tolerance of Liver Ischemia

None of the animals submitted to two hours of hepatic ischemia died within seven days, while one animal in group 1 submitted to one hour of hepatic ischemia died 18 hours postoperatively from pulmonary edema with minimal change in liver function and histology (Fig 1). With hepatic ischemia of three hours (group 3), half of the animals survived for seven days. All of the animals challenged with 3.5 hours of ischemia (group 4) died within 48 hours (Fig 1). Animals in group 3 and group 4 that died had significant oozing when the wound was closed or had a considerable amount of serosanguinous ascites at autopsy.

Postischemic derangements of liver function were well correlated with the duration of ischemia. Animals in group 3 and group 4 developed lactic acidosis immediately after the ischemia, which was highest at three hours, as well as hypoglycemia, with the lowest blood sugars at 12 hours. SGOT and SGPT were highest between 12 hours and 24 hours and gradually decreased thereafter, returning to the preoperative levels by seven days. The levels of SGOT and SGPT at 12 hours were 416 + 489 (SD) U/L and 550 + 844 (SD) U/L in group 1; 2,386 ± 1,829 and 2,196 ± 2,139 in group 2; 5,340 ± 4,774 and 7,662 ± 6,046 in group 3, and 11,749 ± 7,345 and 11,600 ± 10,168 in group 4. The differences were highly significant between groups. None of the animals had elevations of bilirubin.

Treatment by PGI2

Three-hour liver ischemia was chosen to test the effect of PGI2 pretreatment. PGI2 administration was associated with a dose-dependent improvement of animal survival (Fig 2). All of the animals that were given glycine buffer vehicle died within three hours (group 5). In contrast, none of the animals died within the first 48 hours when they were pretreated with PGI2 (groups 6 and 7). However, three dogs (50%) of the six who received 1 μg/kg/min of PGI2 and one animal (15%) of six who received 2 μm/kg/min of PGI2 died subsequently (Fig 2). The three animals that died in group 6 had serosanguinous ascites, and the one that died at six days in group 7 had increasing jaundice.

PGI2 treatment significantly inhibited the elevation of transaminases (Fig 3). Hypoglycemia commonly observed in untreated animals was prevented well by PGI2 administration. The mean blood sugar level at 12 hours

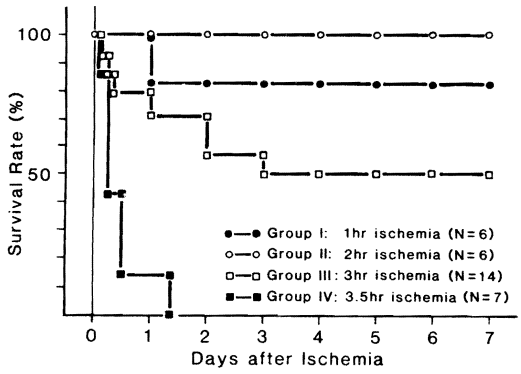

Fig 1. Survival of dogs after different periods of liver ischemia.

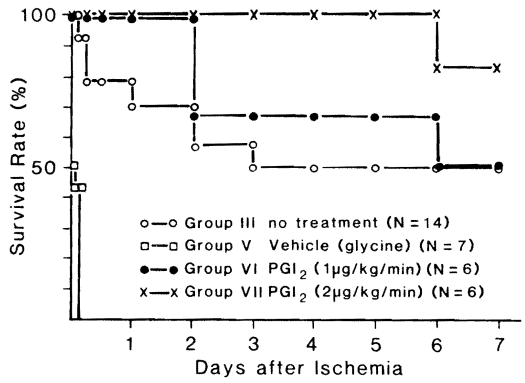

Fig 2. Survival of dogs after three hours of liver ischemia with PGI2 pretreatment.

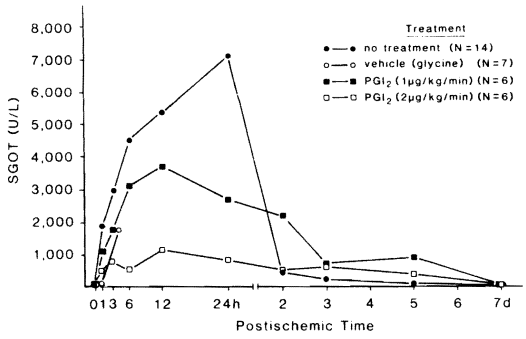

Fig 3. SGOT of dogs submitted to three hours of liver ischemia without (group 3) or with PGI2 pretreatment.

after ischemia was 43.9 ± 14.4 (SD) mg/dL in group 3, (the LD 50 controls), 78.6 ± 11.0 in group 6 and 98.6 ± 18.9 in group 7. No significant elevation of serum bilirubin was observed in treated animals except for the one animal in group 7 that died at day 6.

DISCUSSION

The liver generally has been considered to be highly sensitive to ischemia. Occlusion of hepatic inflow for longer than 20 minutes was lethal in dogs,[1] but the risk was principally due to acute portal hypertension. However, even when the splanchnic bed was decompressed with a spleno-femoral shunt[2] and side-to-side portacaval anastomosis,[3] the safe limit was reported to be less than 60 minutes.

In contrast, the present study using a pump-driven spleno-jugular bypass demonstrated that virtually all of the dogs tolerated two hours of hepatic ischemia and half of them survived after three hours of ischemia. Liver damage following one hour of ischemia in this study was mild and transient. Hepatic injury became significant only when the ischemia time was prolonged for longer than two hours. These findings were consistent with those previously reported in pigs.[4,5] It may be concluded that the liver is more tolerant to ischemia than previously realized. Huguet has come to the same conclusion about the human liver.[6]

The model developed in this study is simple and highly reliable. The results in the group 3 controls were compiled during two time periods. In the first time period, three dogs lived and four died. At the later time, four dogs lived and three dogs died. In the 14 dogs, an LD 50 was established. Other methods of inducing hepatic ischemia have been too extreme to permit survival of a significant fraction of animals,[2,3,7] and for that reason have not allowed the testing of potential therapeutic maneuvers or drugs.

A protective effect of PGI2 after liver ischemia and preservation has already been claimed by others.[8-10] Using PGI2 for graft flushing at the time of harvesting, Monden and Fortner[8] preserved canine livers for 24 hours and 48 hours with consistent survival. Attempts to confirm these observations in our laboratory were unsuccessful in that only one of eight animals survived operation, and that dog died after 68 hours. This disappointing experience led us to develop a nontransplant model for the evaluation of protective agents. Although PGI2 showed protection as reported herein, other prostaglandins tested to this time (PGE1 and PGE2) have not been effective (unpublished data). With PGI2, our results suggest that pretreatment of the donor with PGI2 may be a useful approach rather than simple flushing of the hepatic graft with the drug as was originally reported by Monden and Fortner.[8]

SUMMARY

A model of hepatic ischemia was developed in dogs using a pump-driven splanchnic-to-jugular vein bypass during crossclamping of the portal triad. An LD 50 was established with three hours of ischemia. PGI2 given for one hour before the ischemic insult ameliorated the ischemic injury and increased survival.

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