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 dis-
solved 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 infu-
sion; 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 dis-
continued. No vasopressor was administered.

Peripheral venous blood samples were drawn serially for the determination of serum glutamic oxaloacetic
transaminase (SGOT), serum glutamic pyruvic transami-
nase (SGPT), lactate dehydrogenase (LDH), total biliru-
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 PG12

Three-hour liver ischemia was chosen to test the effect of PG12 pretreatment. PG12 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 PG12 (groups 6 and 7). However, three dogs (50%) of the six who received 1 μg/kg/min of PG12 and one animal (15%) of six who received 2 μg/kg/min of PG12 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.

PG12 treatment significantly inhibited the elevation of transaminases (Fig 3). Hypoglycemia commonly observed in untreated animals was prevented well by PG12 administration. The mean blood sugar level at 12 hours...
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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, and for that reason have not allowed the testing of potential therapeutic maneuvers or drugs.

A protective effect of PG12 after liver ischemia and preservation has already been claimed by others. Using PG12 for graft flushing at the time of harvesting, Monden and Fortner 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 PG12 showed protection as reported herein, other prostaglandins tested to this time (PGE1 and PGE2) have not been effective (unpublished data). With PG12, our results suggest that pretreatment of the donor with PG12 may be a useful approach rather than simple flushing of the hepatic graft with the drug as was originally reported by Monden and Fortner.

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. PG12 given for one hour before the ischemic insult ameliorated the ischemic injury and increased survival.

REFERENCES