A New Technique of Hepatic Ischemia in the Rat*

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Abstract: A new ischemia model is described combining partial hepatectomy with clamping the remnant liver. Three clamps were placed across the pedicles of the median and left lateral lobe at the level of the hilum to achieve total ischemia after the resection of the caudate, right lateral, quadrate lobes, and papillary process. Mesenteric congestion was prevented with the spontaneous portal-caval shunts through the caudate branches and collateral vessels. Survival rate was studied following 60, 90 and 120 minutes ischemia. Liver function was tested. All rats survived over the resection of the liver tissues, and the procedure took 23 ± 3 minutes, and no bleeding of the resected tissue was observed. Spontaneous portal-caval shunts through the caudate branches and collateral vessels were demonstrated by the use of an intraportal dye. Sixty percent of animals survived 90 minutes of ischemia. This model is technically simple and applicable with an anhepatic period during ischemia and without the use of extra portal-systemic shunts.

Key words: liver ischemia; rat

Models of hepatic warm ischemia in rats have been instrumental in numerous experimental studies in liver transplantation, surgery, and regeneration, as well as in several forms of shock and endotoxemia [1-11]. Most techniques of hepatic ischemia in rats include segmental rather than total clamping of the hepatic blood supply to prevent mesenteric congestion [3, 4]. Partial hepatic ischemia, however, is not a method from which organ viability or animal survival can be assessed.

Hepatic ischemia procedures described in rats included either complete clamping of the hepatic pedicle associated with a portosystemic shunt (usually using an ex vivo bypass between a branch of the mesenteric vein and the jugular vein with the use of heparin) [2, 3, 12] or segmental hepatic ischemia, followed by resection of the nonischemic lobes at the time of reperfusion.

In the present study, we tried to establish a total liver ischemia model with clamping the pedicles of the median and left lateral lobe at the level of the hilum to achieve total ischemia after the resection of the caudate, right lateral, and quadrate lobes, and papillary process. Mesenteric congestion was prevented with the spontaneous portal-caval shunts through the caudate branches and collateral vessels.

MATERIALS AND METHODS

1. Animals

Male Wistar rats, weighing approximately 250 g, were used. The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). All surgical procedures were conducted with clean but not sterile instruments.

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2. Surgical procedures

The abdomen was shaved and a transverse incision was performed. The bowel loops were covered with saline-soaked gauze. The median and lateral left lobes were covered with saline-soaked gauze, and no-touch technique should be used during the isolation of these lobes. Liver lobes were isolated from their ligaments. The quadrate and the papillary process were freed from adjacent tissue, particularly the stomach, and were ligated at the level of their respective pedicle with 5-0 silk suture and resected, and the sutures were placed as close as possible to the vessels entering the lobes. Subsequently, isolation of the right lateral lobes was performed, and then the lobes were ligated and resected with the ties placed as close as possible to the vena cava. The caudate lobes were sutured and ligated as close as possible to the vena cava, and then resected. Total hepatic ischemia was achieved using three vascular clamps applied to the respective vascular supply of the remaining liver lobes. The incision was covered with saline-soaked gauze and plastic wrap in order to prevent dehydration.

3. Groups

Four groups, sham control (resection only), 60, 90, and 120-minute ischemia, were included in this study. Seventeen rats were included in every group, and ten rats were followed for 7 days for survival rate. Survival rates of the animals were studied. Autopsy was routinely performed in nonsurviving animals to evaluate the mechanisms of injury.

4. Liver weight measurement

Total liver weight, the weight of resected liver tissues, and the weight of the remaining liver tissue around the vena cava from resected liver lobes were measured.

5. Evidence of hepatic ischemia and spontaneous portal-caval shunts without mesenteric congestion

An intravenous dye was perfused into the portal vein during hepatic ischemia. Intraportal injection of trypan blue dye was used to evaluate all organ perfusion during the hepatic ischemia. Trypan blue dye dissolved in a 100:1 ratio with normal saline was injected through the portal vein until sufficient staining of perfused organs were observed.

6. Measurements of liver enzyme

Blood samples were taken from the inferior vena cava for liver enzyme measurements. Serum aspartate aminotransferase (AST), alanina aminotransferase (ALT), and lactic dehydrogenase (LDH) were measured using the serum separator biochemical analyzer.

7. Histological evaluation

Hepatic specimens were fixed in 10% formalin and embedded in paraffin. Hematoxylin and eosin (H&E)-stained sections were evaluated at × 400 magnification for severity of hepatic injury.

8. Statistical analysis

All values were expressed as mean ± SD. Statistical analysis was performed using student's t test and ANOVA. P values of less than 0.05 were considered to be statistically significant.

RESULTS

1. Survival rate

All animals survived after partial hepatic resection, 60 minute ischemia, and all rats died after 120 minute ischemia. Four rats died after 90 minutes ischemia.

2. Liver weight measurement

Only 0.2 g of liver tissue from the remaining liver tissue of the resected lobes was left around the vena cava.

3. Evidence of hepatic ischemia

Upon infusion of trypan blue dye, no stain was observed in the liver at any time during ischemia. The liver remained pale without any detectable change in color, while all other abdominal organs stained blue. The kidneys were the first abdominal organs to rapidly taking up trypan blue followed by the gut and other abdominal organs. The gut stained uniformly throughout its entire length, with no evidence of mesenteric stasis during the ischemic period.

4. Evidence of spontaneous portal-caval shunts without mesenteric congestion

Intraportal injection of trypan blue dye was used to evaluate all organ perfusion during the hepatic ischemia. Trypan blue dye has been used previously to perfuse and stain the liver in other types of models. The good portal flow in each animal that received the trypan blue dye indicated that portal vein thrombosis was not a factor of injury in this model.

The presence of portal-caval shunt in this model was demonstrated by the complete prevention of mesenteric congestion and the direction of the portal flow in the trypan blue experiment. An intravenous dye was perfused into the portal vein during hepatic ischemia. Portal pressure was also measured to evaluate possible change during the whole procedure. Before resection and clamping, the portal pressure was 5.6 ± 2.3 mmHg and increased to 7.3 ± 1.9 mmHg after resection of the right lateral, quadrate, and caudate lobes and papillary process. Portal pressure raised to 13.8 ± 5.1 mmHg for the first few minutes of clamping of the pedicles of the remaining liver lobes, and then dropped to 9.7 ± 4.3 mmHg within 30 minutes. Portal pressure remained at this level and returned to pre-resection level after removal of the clamps.

5. Measurements of liver enzyme

Serum enzymes were measured at 6 hours after reperfusion in each group and co-related the duration of ischemia. Blood samples were taken from the inferior vena cava for the measurements. Mean peak levels of AST were 2100 ± 310 U/L in the 60-minute ischemic group, 4300 ± 620 U/L in the 90-minute ischemic group and 7600 ± 870 U/L in the 120-minute ischemic group.

6. Histological evaluation

At 6 hours following reperfusion, the severity of sinusoidal congestion and cytoplasmic vacuolation was more evident in 120 minute ischemia group.

DISCUSSION

Hepatic ischemia-reperfusion injury occurs during surgical resections, liver transplantation, and hemorrhagic shock, and liver ischemia models in rats have been instrumental in lots of the experimental studies [1, 12-16]. Most procedures of liver ischemia in rats include a portosystemic shunt (usually using an ex vivo bypass between a branch of the mesenteric vein and the jugular vein) with the using of heparin, or segmental rather than total clamping of the hepatic blood supply to prevent mesenteric congestion. Partial hepatic ischemia is not a method from which organ viability or animal survival can be assessed. This studied was conducted to develop a simple and reproducible method of total hepatic ischemia in rat.

The model was validated by demonstration of complete occlusion of the hepatic blood supply to the whole organ without mesenteric congestion. The survival rates with this model are consistent with data available from other models of total hepatic ischemia and humans [14-16]. The technique described is attractive because there is no need for an extracorporeal bypass system as normally used in other studies. The techniques with ex vivo by pass are usually

precarious, requiring prolonged operation time and anticoagulation, which often results in substantial bleeding.

In this model, mesenteric congestion was prevented as shown by homogeneous anterograde staining of the gut mucosa with trypan blue, which established the presence of a portosystemic shunt through the caudate and quadrate branches. Although more proximal collaterals may exist, rapid staining of the cava and the kidneys after injection of the dye into the portal vein indicated that majority of the shunting occurred at the level of the remaining liver tissues of caudate and quadrate lobes. There was a mild increase in the portal venous pressure during ischemia, but, after removal of the clamps, the pressure dropped to the baseline of post resection levels. The resection of the caudate, right lateral and quadrate lobes and papillary process seemed not to be a critical factor in this model because the tissue removed represented less than 30% of the total hepatic mass. The resection of the lobes caused a slight increase in portal pressure and probably influenced survival only marginally after a prolonged period of ischemia.

We are currently using this model in our projects on the role of anti-neutrophil antibody in the ischemic liver of rats, and on the effect of FK506 on liver ischemia [2]. In these experiments, total liver ischemia was achieved similarly, and the results of the control group were consistent with the data available from other studies. Another advantage of the availability of such a simple model is the low cost compared with other models and the lack of a requirement for sophisticated expensive materials.

In conclusion, this study present a new and simple model of total hepatic ischemia in the rat, and the model may be instrumental in the research of the mechanisms of liver ischemia-reperfusion injury in different pathophysiological conditions.

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