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The Hepatoprotective Effect of Hypothermic Perfusion of Normal Saline in Period of Inflow Occlusion of Liver in Rats

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Background. **Liver damage in hepatic surgery from warm ischemia and reperfusion (I/R), especially in patients with underlying chronic liver disease, is still challenging. We propose a new method of perfusion of the liver by catheterizing the umbilical vein in the period of hepatic inflow occlusion, and evaluate the influence of transfusion of normal saline (NS) on liver injury in a modified I/R rat model.**

Methods. **Twenty-eight rats were randomized into four groups ($n = 7$): group I (sham-operated group): no I/R or transfusion; group II (I/R group): I/R + no transfusion; group III (37°C NS group): I/R + transfusion of 37°C NS ; group IV(24°C NS group): I/R + transfusion of 24°C NS. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as lactate dehydrogenase (LDH) were measured in rat serum. Light and electron microscopic examinations were performed on the liver tissues.**

Results. **Perfusion of 24°C NS in the period of inflow occlusion resulted in significant reductions of liver enzymes levels compared to the I/R alone group and 37°C NS group ($P < 0.001$ and $P < 0.001$, respectively). Histologic evaluation revealed the injury grade to be relatively lower in group IV compared to group II and III ($P < 0.001$ and $P < 0.001$, respectively).**

Conclusion. **This new hypothermic perfusion technique may be very useful in preserving the hepatocytes in hepatic surgery; it is an inexpensive and easy method, which makes it possible to increase its application.**

Key Words: **hypothermic perfusion; ischemia and reperfusion; liver; rats; normal saline.**

INTRODUCTION

Controlling of blood loss and protecting of the liver function are both critical for a successful hepatic operation. Since Pringle maneuver was first described a century ago, techniques of inflow and outflow occlusion are now commonly employed [1, 2]. With the control of intraoperative blood loss, major hepatic resections can be carried out without blood transfusion, but it has done so at the expense of liver damage from warm ischemia and reperfusion (I/R), especially in patients with underlying chronic liver disease [3]. Refinements of vascular exclusion methods [4–7], medications administration perioperatively [8–10], and preconditioning [11, 12] have been verified in reducing I/R injury. *In situ* hypothermic perfusion of the liver allows us to perform major hepatic resection in patients with diseased livers [13, 14]. However, it requires complicated procedures, including total vascular exclusion of the liver, veno-venous bypass, catheterizing of the portal vein, and cavotomy, which limit its application [15]. We propose a new method of perfusion of the liver by catheterizing the umbilical vein in the period of hepatic inflow occlusion, which can be easily manipulated. To verify the feasibility of the method, a modified I/R rat model was used, and an interesting manifestation was observed, that even transfusion of normal saline (NS) exhibits a significant effect on liver injury. A simple and effective approach of alleviating liver I/R injury may be accessed.

MATERIALS AND METHODS

Animals

Twenty-eight male Sprague-Dawley rats, weighing 220–250 g, were obtained from Animal Experimentation Centre of Second Military Medical University (Shanghai, China). The animals were kept

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in a standard housing facility and were supplied with ordinary laboratory chow and water. The experimental protocol was approved by the Local Animal Care Committee, and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Experimental Protocol

The animals were randomized into four groups (each group, $n = 7$). In group I, sham-operated animals received no transfusion or I/R but laparotomy, liver manipulation, and venepuncture. In group II, the animals underwent venepuncture and I/R (30 min ischemia and 120 min reperfusion) without any transfusion. In group III, ischemic animals were treated with NS of 37°C (1 mL/100 g body weight), and in group IV, ischemic animals were treated with NS of 24°C (1 ml/100 g body weight).NS was injected at the time of 0, 10, and 20 min after vascular control in group III and IV.

Surgical Procedure

As there is no umbilical vein available for cannulation in rat, we puncture the left portal vein instead. Under chlorohydrate anesthesia (300 mg/kg), a median laparotomy was performed. After dissection of the attachment of liver, the portal vein was punctured with a closed catheter system (22 G, BD Intima, Becton Dickinson, China), which was inserted into the vein of the left lateral and median lobes of the liver, and the blood supply to the left lateral and median lobes of the liver was interrupted for 30 min by a temporary suture ligation (only one knot) at the level of the hepatic artery and portal vein. The remaining right lateral and caudate lobes retained intact portal and arterial blood supply, preventing the development of intestinal venous hypertension [16]. Reflow was initiated by removal of the ligation. After 120 min of reperfusion, the right atrium was punctured, and blood was aspirated with a syringe. The blood sample was centrifuged (3000 rpm for 10 min at room temperature) to separate serum for storage at –40°C with subsequent analyses of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). Ischemic liver lobes were separated into two parts: one sector was washed in 0.9% saline solution and fixed in special solution for later electron microscopic examination (see details below); the other was fixed in formalin for histopathologic examination.

Temperature Control and Measuring

Rats were kept in supine position on a damp proofing pad and were covered by gauze after laparotomy and venepuncture. Environmental temperature was 24°C, maintained by air conditioners. The temperature of the surface of the liver was measured before, and 5, 15, 25, and 30 min after vascular control by an infrared ray thermometer (TD400; WGI Inc., Phoenix, AZ).

Measuring Serum Liver Enzymes

Serum concentrations of AST, ALT, and LDH were measured as indicators of hepatic I/R injury. These biochemical analyses were made by an autoanalyzer (Hitachi 7600; Tokyo, Japan) using commercial kits from Hitachi.

Light Microscopic Examination

Parts of isolated hepatic tissue were fixed in 10% formalin solution, then dehydrated in ascending grades of alcohol, and embedded in paraffin. Four micron-thickness sections were taken, stained with hematoxylin and eosin solution, and examined under light microscope by two pathologists, separately, who were both blinded to the treatment given. Analysis was performed on 10 randomly chosen fields in each slide under standard conditions at ×200 magnification. Grading of the severity of hepatic injury was as follows: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting of cytoplasm vacuolization and focal nuclear pyknosis; grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; and grade 3, severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration [17]. Averaging of the results from the two pathologists was regarded as the final value of scoring of liver injury.

Electron Microscopic Examination

Small pieces of liver tissue (about 1 mm^3) were fixed as soon as possible in 4% paraformaldehyde for 4 h. After three rinses in 0.1 mol/L phosphate buffer (each rinse for 15 min), the tissue was post-fixed in 1% buffered osmic acid for 2 h at 4°C. The specimens were then dehydrated in ascending grades of acetone and embedded in epon resin. Ultra-thin sections were cut, stained with uranium for 30 min, plumbum for 10 min, and examined with a transmission electron microscope (HITACHI H 800λ, Tokyo, Japan).

Statistical Analysis

All the values are expressed as the mean value ± SD. The results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey test for multiple comparisons using SPSS for Windows (version 13, Chicago, IL). Differences in P value < 0.05 were considered significant.

RESULTS

Results of biochemical analyses

The activities of AST, ALT, and LDH were significantly higher in rats exposed to ischemia/reperfusion-induced liver injury compared with sham-operated animals. Transfusion of 37°C NS in the period of ischemia had little influence on the results of liver enzyme,

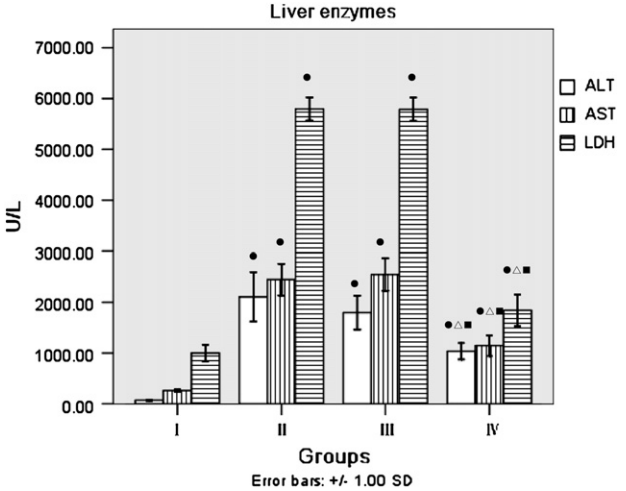

FIG. 1. Effect of ischemia/reperfusion (I/R) liver injury on serum alanine aminotransferase (ALT), aspartate aminotransferase(AST), and lactate dehydrogenase (LDH) levels in rats treated and untreated with normal saline. Data are expressed as mean ± SD. (Filled circle) $P < 0.01$ with respect to Sham-operated group (I), (open triangle) $P < 0.01$ with respect to I/R untreated group (II), (filled square) $P < 0.01$ with respect to I/R treated with 37°C normal saline group (III).