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Portal vein clamping alone confers protection against hepatic ischemia–reperfusion injury via preserving hepatocyte function in cirrhotic rats

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ABSTRACT

Background: Chronic liver diseases always increase the risk of liver failure after hepatectomy. We aimed to explore the protective effect of portal vein clamping without hepatic artery blood control (PVC) on a cirrhotic rat liver that underwent ischemia and reperfusion.
Methods: Carbon tetrachloride-induced cirrhotic rats were randomly assigned to four groups as follows: cirrhotic control, PVC, portal triad clamping (PTC), and intermittent portal triad clamping (IC). After 45 min of portal vascular clamping, hepatic injury and liver function were investigated by assessing the 7-d survival rate, liver blood loss, serum alanine aminotransferase, liver tissue malondialdehyde, liver tissue adenosine triphosphate, indocyanine green retention rate, and morphology changes of the rat liver.
Results: The 7-d survival rates in the PVC and IC groups were much higher than in the PTC group. The PVC group had more liver blood loss during the hepatectomy than the PTC group, but had much less than the cirrhotic control group ($P < 0.01$). In addition, there were no differences between the IC group and PVC group. The PVC rats had a significantly higher adenosine triphosphate level in the liver tissue and a markedly lower indocyanine green retention rate than the PTC and IC rats ($P < 0.05$). At 1, 6, and 24 h after reperfusion, the alanine aminotransferase and malondialdehyde levels in the PTC group were much higher than those in the PVC and IC groups ($P < 0.05$). Based on the histopathologic analysis, hepatic injury in the PVC and IC groups were similar but less prominent than in the PTC group.
Conclusions: Although both PVC and IC can confer protection against hepatic ischemic–reperfusion injury in cirrhotic rats, the PVC method is more efficient in preserving the energy and function of hepatocytes than the IC method, suggesting better prognosis after hepatectomy.

1. Introduction

Liver cancer in men is the fifth most frequently diagnosed cancer worldwide but the second most frequent cause of cancer death. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death [1]. Partial hepatectomy is the most important and efficient curative treatment for liver cancer. The amount of

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hemorrhage and degree of ischemia–reperfusion (I/R) injury have always been the key points of concern during hepatectomy and are clearly linked to morbidity and mortality post-operation [2]. An appropriate liver blood inflow occlusion method is vital for controlling intraoperative bleeding and I/R injury, especially for cirrhotic patients who unfortunately tolerate hemorrhage and I/R injury poorly [3,4].

The Pringle maneuver (clamping of the portal triad) has been acknowledged to be an effective means of reducing blood loss during liver resection [5]. However, severe I/R injury is inevitable if the operation is complicated and requires a prolonged occlusion time. The intermittent Pringle maneuver is an improvement to the Pringle method and is prevalent worldwide as it has proven to have better parenchymal tolerance [6]. At present, several portal occlusion strategies, such as ischemic preconditioning, selective liver inflow occlusion (involving hemihepatic vascular occlusion, and segmental vascular occlusion), and total hepatic vascular exclusion, have been designed for improvement but controversies still remain [7,8].

A previous animal study in our institution has demonstrated that portal vein clamping without hepatic artery blood control (PVC) could extend the tolerance time of liver ischemia without increasing the amount of bleeding significantly in hepatic transection [9,10]. Several studies have shown that a liver with underlying disease, such as cirrhosis or steatosis, tolerates I/R injury poorly and that injury mechanisms differ from those of the normal liver [11,12]. In addition, most of the hepatocellular carcinoma tends to occur in the background of liver cirrhosis [13]. Therefore, we designed the present study to explore whether PVC method is suitable for controlling liver blood inflow in cirrhotic rat livers. Furthermore, this PVC method was also compared with the classic Pringle and intermittent Pringle methods.

2. Materials and methods

2.1. Animal and experimental cirrhosis model

To avoid sexual interference, male Sprague–Dawley rats weighting 200 g obtained from the Experimental Animal Center of the Academy of Military Medical Science (Beijing, China) were used in this study. All rats were fed a standard rodent chow with water *ad libitum*. The rats were kept under constant environmental conditions with a 12-h light–dark cycle. Liver cirrhosis was induced by subcutaneous injection of 50% carbon tetrachloride (CCl_4) diluted with soybean oil at a dose of 0.3 mL per 100 g of body weight twice a week for 10 wk [14]. This study was conducted according to the guidelines set by the Guide for the Care and Use of Laboratory Animals and was approved by the Ethical and Research Committee of our hospital.

2.2. Experimental groups

All cirrhotic rats were randomly assigned into four groups as follows: cirrhotic control (CC) group ($n = 24$), CC rats; portal triad clamping (PTC) group ($n = 60$), rats with total PTC; PVC group ($n = 60$), rats with the PVC; and intermittent portal triad clamping (IC) group ($n = 60$), rats with IC with 15 min ischemia alternated with 5 min reperfusion for 3–5 cycles. To investigate the effect of different liver blood inflow control methods on animal survival, the clamping time was set for 45, 60, and 75 min in each group, and the 7-d survival rates were recorded.

2.3. Surgical procedures

Seven days after the last administration of CCl_4, each rat underwent surgery. A nonlethal model of 90% liver ischemia with portal blood bypass through the caudate lobe was used in all experiments [15]. After overnight fasting with water *ad libitum*, the cirrhotic rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (3 mL/kg). The abdomen was entered through a midline incision followed by dissection of the portal triads to the left and median lobes and to the right lobe. In the PTC group, two microvascular clamps were placed around the portal triads for 45 min. In the PVC group, the portal veins within the portal triads were isolated and clamped to maintain the arterial blood flow. In the IC group, three cycles of 15 min with the clamp on and 5 min with the clamp off were conducted with the same clamping method as in the PTC group (Fig. 1A). In the CC group, the hepatic pedicles were exposed but not clamped. Mesenteric congestion was avoided by allowing intestinal blood flow through the caudate lobe of the liver. During the ischemic period, the abdomen was covered with plastic film to prevent body fluid volatilization. After 45 min, the clamps were removed, and reperfusion was initiated. The caudate lobe was then resected, and the abdomen was closed with 3-0 sutures.

2.4. Sample collection

For sample collection, occlusion time was set for 45 min, and the collection time was 1, 6, and 24 h after reperfusion. At 15 min before each collection time point, indocyanine green (ICG) solution (2.5 mg/mL) dissolved in sterile water was injected into the right femoral vein (2.5 mg/kg). Blood samples were obtained from the inferior vena cava and centrifuged at 3000 rpm for 15 min, the supernatant was then stored at −20°C for serum testing. Each ischemic liver lobe was removed and cut into two pieces through the middle with a razor blade. One piece was immediately frozen in liquid nitrogen and stored at −80°C to measure the adenosine triphosphate (ATP) and malondialdehyde (MDA). The other piece was further sliced and fixed with 10% formaldehyde in 0.1 M phosphate buffer (pH7.4) for a histopathologic study.

2.5. Grading of the degree of liver cirrhosis

To identify the degree of liver cirrhosis among the groups, liver specimens of rats fixed in 10% formaldehyde solution were embedded in paraffin, cut into 5-μm sections, stained in 0.1% picrosirius red solution, and examined by a pathologist who was blinded to the experimental groups. The Ishak scoring system was used to grade the degree of liver cirrhosis [16].