Impact of ischemia-reperfusion injury on dimensional changes of hepatic microvessels

T. Kondo 1, T. Todoroki 2, T. Hirano 3, F. W. Schildberg 4, K. Messmer 1

1 Institute for Surgical Research, Klinikum Grosshadern, University of Munich, Marchioninistrasse 15, D-81377 Munich, Germany
2 Department of Surgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan
3 National Institute of Bioscience and Human Technology, Tsukuba, Japan
4 Department of Surgery, Klinikum Grosshadern, University of Munich, Munich, Germany

Received: 31 March 1998 / Accepted: 28 May 1998

Abstract. Dimensional alteration of hepatic microvessels was demonstrated during reperfusion after normothermic hepatic ischemia. Using a specially designed cover glass, it was possible to relocate selected sites of observation and microvessels repeatedly throughout the whole reperfusion time. Twenty minutes of hepatic ischemia resulted in a decrease of sinusoidal diameter (mean±SEM; 10.0±0.3 µm at baseline, 8.2±0.2 µm after ischemia) and diameter of postsinusoidal venules (26.4±1.2 at baseline, 23.0±1.0 after ischemia). In the control group (no ischemia induced) no changes of these parameters were observed. Thus, the reduction of hepatic microvascular cross section was present during the early phase of reperfusion. Hepatic dysfunction was characterized by increased serum activity of liver enzymes and reduction of bile flow in the ischemia-exposed animals. It has been suggested that postischemic dimensional microvascular changes are involved in postischemic liver dysfunction.

Key words: Ischemia-reperfusion injury – Microvascular dimension – Normothermic hepatic ischemia – Intravital fluorescent microscopy

Introduction

Using intravital fluorescence microscopy (IVM), it has been shown in vivo that ischemia reperfusion may lead to postischemic microvascular reperfusion failure. Furthermore, it was established that the ischemia reperfusion-induced hepatocellular damage and liver dysfunction correlate with the extent of microcirculatory reperfusion failure. The latter is characterized by a reduction in sinusoidal red blood cell velocity, impairment of sinusoidal perfusion, and stagnation of leukocytes in both postsinusoidal venules and in sinusoids [1–3]. However, the microvascular dimensions, such as the diameter of indi-
individual sinusoids or postsinusoidal venules, have not yet been systematically studied.

Since Rappaport et al. [4, 5] first described the microanatomy of the liver, only a few articles have focused on the changes in sinusoidal diameters by means of electron microscopy [6–9]. Using IVM to study hepatic microcirculation, in vivo observations have demonstrated sinusoidal constriction after induction of hemorrhagic shock [10], and exposure to endothelin-1 (ET-1) [11–13] in normal and ethanol fed rats [14]. Since serum levels of ET-1 were shown to increase after induction of hepatic ischemia, endothelin-1-induced constriction of sinusoids was suggested to be involved in postischemic liver dysfunction [12, 15]. Nevertheless, a direct comparison of the microvascular dimensions before and after induction of normothermic hepatic ischemia has not yet been performed. This may due to the difficulty involved in performing repetitive observations of identical liver areas and microvessels using IVM [16].

We recently described a new method which enables us to relocate selected sites of interest repeatedly using a specially designed cover glass with a grid system etched into it [17]. Using this grid method, we have demonstrated that during a 120-min reperfusion period there was a steady increase of leukocyte adhesion along with a partial recovery of the initially severely depressed sinusoidal perfusion. Moreover, no-reflow of sinusoids during the early phase of reperfusion, followed by subsequent reflow, was demonstrated. Accordingly, we hypothesized that the decrease in microvascular diameters occurs in the early phase of reperfusion and is associated with the microcirculatory perfusion failure; this means that dimensional changes of the hepatic microvessels appear to be involved in the postischemic liver dysfunction. The present study was performed to test this hypothesis through the quantification of postischemic microvessel dimensional changes in the liver using our grid method.

Materials and methods

Animal model and interventions

The study was performed after approval by the local ethics committee. After overnight fasting, with free access to tap water, 12 male Sprague-Dawley rats (Charles River Wiga, Sulzfeld, Germany; BW 286±31 g) were anesthetized with pentobarbital (50 mg/kg, ip.) and tracheotomized. The animals were ventilated mechanically (Harvard ventilator model 683, South Natick, Mass., USA). The absence of spontaneous breathing was confirmed every 5 min. When spontaneous breathing interfered with mechanical ventilation, additional pentobarbital (10 mg/kg) was given (approximately every 30–40 min). The animals were placed in a supine position on a heated pad in order to maintain the rectal temperature at 37.0°C. Polyethylene catheters (PE-50, 0.58 mm/0.96 mm inside/outside diameter; Portex, Kent, UK) were inserted into the left carotid artery and left jugular vein. These catheters were used for blood pressure monitoring, arterial blood sampling, continuous infusion of Ringer’s solution, and injection of fluorescent dye for microscopy. PaO2 and PaCO2 were maintained at 100–120 mmHg and 35–40 mmHg, respectively, by adapting the inspiratory O2 fraction (35–38%), tidal volume (0.8–1.2 ml 100 g body weight−1), and respiratory rate (50–60 min−1). After laparotomy had been performed by a transversal incision, the ligaments around the liver were dissected to mobilize the left lobe. At the same time, the hepato-duodenal liga-