Transpl Int (2000) 13 [Suppl 1]: S562–S564

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Reduction of hepatic reperfusion injury by antithrombin III and aprotinin

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Abstract Disturbance in hepatic microcirculation and leucocyte-endothelium interaction after warm ischaemia represents one of the leading mechanisms for postoperative organ dysfunction. Recent studies have shown that pretreatment with antithrombin III (AT III) and aprotinin reduces the leucocyte-endothelium interaction in ischaemic small intestine and during extracorporal circulation in cardiac surgery. Standardized warm hepatic ischaemia and intravital fluorescence videomicroscopy was performed in an experimental study with rats. Animals were pretreated with AT III or aprotinin. Analysis of intravital videomicroscopy showed that the hepatic microcirculation after warm hepatic ischaemia in rats was significantly enhanced by AT III and aprotinin, most likely by reducing the leucocyte-endothelium interaction. We concluded that drug application before the Pringle manoeuvre might reduce the reperfusion damage after liver resection.

Key words Ischaemia/reperfusion injury · Hepatic microcirculation · Leucocyte-endothelium interaction · Antithrombin III · Aprotinin

Introduction

Protecting the liver against ischaemic injury is a major concern during hepatic surgery because temporary occlusion of the hepatic blood supply is often necessary for the management of surgical procedures in the treatment of liver tumours, vascular lesions or even organ transplantation. Various agents have been investigated with regard to protecting the liver from ischaemia and reperfusion damage, but the effects of pretreatment with naturally occurring serine proteases have not been figured out. The present study was designed to examine the effects of antithrombin III and aprotinin on the hepatic microcirculation and the leucocyte-endothelium interaction in warm ischaemia and reperfusion damage in the rat liver.

Materials and methods

The study was approved by the committe on animal care, Regierungspräsidium Karlsruhe, Germany. Experiments were performed under general ketamine/phenobarbital anaesthesia in male Wistar rats ($n = 18$; weight 288.89 ± 26.03 g). The right jugular vein and carotid artery were cannulated for drug application and continuous monitoring of blood pressure and blood oxygenation. Standardized warm hepatic ischaemia was induced by the temporary (60 min) occlusion of blood vessels and bile duct of the left liver lobe. The animals were divided into three groups, and drug application was performed 30 min prior to the ischaemia: (A) AT III group ($n = 6$); 250 IU/100 g body weight (Kybernin®, Centeon Pharma, Germany), (B) aprotinin group ($n = 6$); 2700 KIU/100 g body weight for 20 min initially, followed by a continuous infusion of 670 KIU/100 g body weight per hour (Trasylol, Bayer Laboratory, Germany) and (C) control group ($n = 6$); an equivalent volume of Ringer solution was given.

The hepatic microcirculation was analysed 30 min after the onset of reperfusion by intravital fluorescence microscopy [5]. Sinusoidal and acinar perfusion were assessed after intravenous application of Na-fluorescin (2 μmol/kg; Sigma Inc, Germany). Leucocytes were visualized by Rhodamine G6 (0.1 μmol/kg; Sigma Inc.

Table 1 Results of intravital microscopy and assessment of liver enzymes. (Data are presented as mean ± SD)

	Controls	AT III group	Aprotinin group
Non-perfused sinusoids (%)	8.28 ± 0.45	4.46 ± 1.49*	6.39 ± 0.49*
Adherent leucocytes in sinusoids per mm^2 liver surface	47.44 ± 3.19	35.78 ± 1.25*	45.29 ± 1.51
Adherent leucocytes in postsinusoidal venules per mm^2 endothelial surface	217.19 ± 15.70	174.80 ± 9.16*	185.22 ± 8.83*
Serum GOT (U/l)	153.67 ± 51.56	92.50 ± 24.27*	175.50 ± 10.50
Serum GPT (U/l)	116.67 ± 72.25	47.33 ± 22.84*	175.0 ± 16.0

* Results significant vs. controls ($P < 0.05$)

Germany), and their accumulation and adherence were quantified by off-line analysis. Leucocyte stickers were defined as staining cells for 20 s (per square millimetre of liver surface or sqare millimetre of endothelial surface). Blood samples were taken at the end of the experiments for analysis of hepatocellular enzymes (GOT and GPT). Data are given as mean values ± SD. Statistical analysis was done by the Wilcoxon-Mann-Whitney *U*-test.

Results

The groups were comparable with regard to body weight, blood pressure and blood oxygenation. The disturbance in hepatic microcirculation was significantly reduced by pretreatment with AT III and aprotinin (Table 1). Sinusoidal perfusion was markedly improved in contrast to controls ($P < 0.05$). The leucocyte-endothelium interaction in hepatic sinusoids was significantly reduced in the AT III group. Leucocyte sticking in postsinusoidal venules was significantly inhibited in both groups. The serum levels of GOT and GPT were significantly reduced in the AT III group and were elevated in the aprotinin group.

Discussion

The results of our study suggested that pretreatment with AT III and aprotinin before hepatic warm ischaemia and reperfusion significantly enhanced hepatic microperfusion, most likely by reducing the leucocyte-endothelium interaction. There are still conflicting views on the exact mechanisms of ischaemia and reperfusion. Many investigators have analysed the pathogenesis and cascade of events that result in loss of hepatocellular integrity, tissue damage and organ dysfunction. One of the hypotheses is that hepatic ischaemia and reperfusion induces a state of hypercoagulability in response to activation of platelet, leucocytes and Kupffer-cells [1].

The effects of serine proteases such as AT III and aprotinin were examined in this study. The first experimental and clinical data concerning the use of AT III and aprotinin after ischaemia and reperfusion are available: pretreatment with AT III reduces leucocyte recruitment in microvessels following the reperfusion of post-ischaemic mesentery in rats [3]. Aprotinin administration reduces intraoperative bleeding in cardiac surgery when extracorporal circulation is required [8, 6]. A beneficial effect of aprotinin administration is also suggested in orthotopic liver transplantation and elective liver surgery [4, 7, 9, 10].

Aprotinin is a naturally occurring inhibitor of serine proteases. It inhibits human plasma and tissue kallikreins by forming reversible enzyme complexes. Kallikrein stimulates both fibrinolysis and coagulation, and leads to activation of the complement system [8]. AT III is a major anticoagulant that also inhibits the activity of serine proteases, such as thrombin, in the blood coagulation cascade. Experimental studies have shown that AT III can stimulate the production of prostacyclin by endothelial cells [2]. Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation. It might protect against the oxygen-free radicals released by activated neutrophils [11].

The exact mechanism of the hepatic protection described in this study due to pretreatment with AT III and aprotinin is not known. It could be that an increase in prostacyclin production following AT III adminstration improves tissue blood flow and provides the anti-inflammatory effect resulting in reduced leucocyte-endothelium interaction. Aprotinin, on the other hand, seems to reduce contact-mediated activation of fibrinolysis, resulting in haemodynamic stability, but it is not as effective as AT III in the leucocyte-endothelium interaction. Our results could have therapeutic implications in liver surgery and liver transplantation, but further studies need to be directed at the molecular mechanisms involved in these findings.

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