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In vivo microscopy reveals that complement inhibition by C1-esterase inhibitor reduces ischemia/reperfusion injury in the liver

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Abstract Complement plays a decisive role in postischemic tissue injury, a process responsible for severe damage after organ ischemia. Several pathophysiologic mechanisms initiated upon reperfusion are mediated by complement inducing microcirculatory disturbances. Here, we demonstrate the effects of complement inhibition using C1-esterase inhibitor (C1-INH) on microcirculation after liver ischemia by invivo microscopy (IVM). In rats, the left liver lobe was clamped for 70 min. C1-INH was given 1 min prior to reperfusion. Controls received Ringer's solution. IVM was performed 30–100 min after reperfusion. Nonperfused acini decreased and sinusoidal perfusion increased substantially after treatment. Leukocyte adherence to sinusoidal and venular endothelium was markedly reduced by C1-INH. Transaminases were significantly decreased by C1-INH. Our data obtained by IVM suggest that complement activation is an early key event of ischemia/reperfusion injury. These observations demonstrate for the first time that reperfusion related microcirculatory disorders can be minimized by C1-INH. This compound should be evaluated in clinical application.

Key words Microcirculation · Reperfusion injury · Liver · Complement · C1-esterase inhibitor

Introduction

Warm ischemia of the liver occurs during liver resection, hemorrhagic shock or operations for traumatic liver injury [5, 11]. The interruption of blood flow is followed by subsequent organ dysfunction caused by the irritability of the liver and additional cell injury initiated as well as further aggravated upon reperfusion [15]. Various pathophysiologic events have been identified to be involved in postischemic organ malfunction. Ischemia/reperfusion-induced microcirculatory disturbances play a pivotal role in the development of postischemic liver injury [15]. Moreover, leukocytes have been identified to contribute profoundly to hepatic I/R-injury [7]. Several mediators, whereof complement plays a central role, mediate these mechanisms.

The complement cascade is activated by the pathophysiologic event of ischemia/reperfusion (I/R) via both the classical and the alternative pathways [1]. During reperfusion complement-derived peptides (C3a and C5a), potent proinflammatory mediators, are released which contribute to neutrophil accumulation, contraction of smooth muscle cells, increased vascular permeability and activation of Kupffer cells within the liver [1, 4, 6, 8]. Moreover, endothelial cells are activated by complement, thus leading to fibrin deposition, platelet aggregation and adhesion of neutrophils to endothelium [13]. These mechanisms contribute to impaired hepatic microcirculation upon reperfusion [15].

The influence of complement inhibition on microcirculation after warm hepatic ischemia has not been investigated so far. Intravital microscopy is the technique of choice to study microvascular perfusion as well as temporary and firm leukocyte-endothelial interactions in sinusoids and venules [9]. The specific aim of this study was to evaluate the therapeutical potential of

complement inhibition by C1-INH on microcirculation in order to minimize liver damage and to prevent fatal organ failure after warm hepatic ischemia.

Materials and methods

Materials and animals

Male Wistar rats of 250–290 g were used. The fluorescent dyes sodium fluorescein and rhodamine 6G were dissolved in NaCl 0.9% to concentrations of 2 μmol/ml and of 0.2 μmol/ml, respectively. Experiments were performed in accordance with *Guide for the Care and Use of Laboratory Animals.*

Surgical procedure

Following anesthesia (ketamine 10 mg/kg IV, phenobarbital 18 mg/kg IP), polypropylene catheters were placed in the right carotid artery and internal jugular vein to monitor arterial blood pressure and for IV injections of C1-INH and fluorescent dyes. A laparotomy was performed by transverse abdominal incision beneath the costal arch. The left liver lobe was mobilized and its hilus including all vessels was clamped temporarily using a microvascular clip. After 70 min of warm ischemia the clip was removed. C1-INH (Berinert, Centeon, Marburg, Germany) was injected IV 1 min prior to reperfusion (100 IU/kg in NaCl 0.9%) ($n = 12$). Controls ($n = 12$) received an equivalent amount of NaCl 0.9%. At the end of the experiment, 100 min after reperfusion, blood samples were collected for further analysis and animals were killed by exsanguination. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by standard spectrophotometric procedures.

In vivo microscopy

Fifteen minutes after reperfusion, the lower surface of the left liver lobe was exteriorized on a mechanical stage and covered with Saran wrap. A modified Leitz orthoplan microscope for epi-illumination, a CCD video camera and a video tape recorder were used. Leukocytes were stained with rhodamine 6G (0.1 μmol/kg) 30 min after reperfusion. Assessment of sinusoidal as well as acinar perfusion was achieved by contrast enhancement of plasma upon the injection of sodium fluorescein (1 μmol/kg). Quantitations of acinar and sinusoidal perfusion and of leukocyte-endothelial interaction were performed off-line by frame-to-frame analysis of video recorded images [9].

Acinar perfusion

The left liver lobe was scanned at 30 and 90 min after reperfusion. One hundred acini were analyzed (magnification × 240) for assessment of acinar perfusion. Between 40 and 80 min after reperfusion, 15 acini and 12 postsinusoidal venules were randomly selected for video recording at higher magnification (× 600).

Sinusoidal perfusion and leukocyte-endothelial cell interaction

Perfusion of sinusoids within acini (% of all observed sinusoids) and "sticking" leukocytes (adherent leukocytes to endothelial cells), defined as stained cells located within blood vessels and not moving during an observation time of 20 s (standardized as number of cells per mm^2 liver surface) were analyzed [9]. In postsinusoidal venules, "sticking" leukocytes (calculated per endothelial surface area by $\pi \times$ diameter × length of observed vessel segment) and "rolling" leukocytes (defined as leukocytes moving with less than 30% of center-line velocity as percentage of all moving leukocytes) were counted.

Statistical analysis

All data are presented as mean ± SEM. Differences were considered significant for $P < 0.05$. All IVM data were calculated by nested design two-way analysis of variance (ANOVA) [9].

Results

General data

Complement inhibition by C1-INH had no influence on macrohemodynamics. Hematocrit, white and red blood cell counts were not different between groups. AST and ALT release showed significant differences ($P < 0.001$), reflecting reduced hepatocellular injury in the C1-INH group. AST values were decreased from 1069 ± 303 to 305 ± 102 IU by C1-INH, ALT values dropped from 719 ± 205 to 206 ± 65 IU.

Acinar and sinusoidal perfusion

Acinar perfusion was substantially improved by C1-INH-treatment, as reflected by decreased non-perfused acini, in comparison to controls (11.8 ± 0.7% versus 32.5 ± 2%; $P < 0.001$). Consequently, within perfused acini a significant increase in the number of well-perfused sinusoids for C1-INH-treated animals (90 ± 2.2% of all observed sinusoids) was observed in comparison to controls (71.9 ± 3.3%; $P < 0.001$).

Leukocyte-endothelial cell interaction

C1-INH treatment markedly reduced WBC adhesion in sinusoids (C1-INH: 96.5 ± 13.7 per mm^2 liver surface, controls: 198.8 ± 20; $P < 0.001$). In postsinusoidal venules, application of C1-INH resulted in a significant reduction of the number of leukocytes adherent to the venular endothelium (129.4 ± 12.2 in C1-INH versus 366.1 ± 31.9 in controls, $P < 0.001$). Rolling leukocytes in venules showed marked differences between both groups (7.3 ± 0.35% for the C1-INH treatment group versus 23 ± 0.8% of all leukocytes for the control group; $P < 0.001$).

Discussion

Complement inhibition by C1-INH improves microvascular perfusion

In this study, we demonstrated a marked increase in perfused sinusoids and acini following application of C1-INH. Due to complement inhibition, improved sinusoidal blood flow is explicable by decreased interstitial edema [8] and minimized endothelial cell swelling, hindering blood cell trafficking [16]. In addition, reduced fibrin deposition as well as inhibition of platelet and leukocyte aggregation may have prevented mechanical blockage of sinusoids with a concomitant breakdown of acinar perfusion resulting in parenchymal cell injury [3]. It may be concluded that inhibition of the classical complement pathway by C1-INH upon reperfusion of an ischemic liver is effective in maintaining sinusoidal integrity, thereby preventing microvascular perfusion failure. Consequently, improved microhemodynamics resulted in decreased liver damage.

Complement inhibition by C1-INH reduces leukocyte-endothelium interaction

Leukocyte adhesion in postischemic livers was effectively reduced by treatment with C1-INH. Leukocyte accumulation and adhesion to microvascular endothelium in postischemic tissues is important for ischemia/reperfusion injury by releasing toxic oxygen radicals and inflammatory cytokines and by proceeding transendothelial migration into damaged parenchyma [16]. Various adhesion proteins on leukocytes and on endothelial surfaces are upregulated during reperfusion in response to complement activation and mediate the sequence of cell interactions in postsinusoidal venules in the liver [2, 4, 6, 12, 14]. Here, the number of "rolling" and "sticking" leukocytes was significantly reduced upon complement inhibition. This could indicate decreased adhesion molecule expression as a result of complement inhibition in the current study. However, complete inhibition of leukocyte adherence upon complement inhibition could not be achieved, suggesting that leukocyte adhesion processes are also regulated by various other mediators [10]. Moreover, C1-INH selectively blocks the classical pathway and has no influence on the alternative pathway of the complement cascade. Thus, a complete blockade of the complement system using C1-INH cannot be expected. The effect of complement inhibition on leukocyte adhesion in sinusoids may not be related to decreased adhesion molecule expression due to lack of p-selectin on sinusoidal endothelial cells [14]. Therefore, reduced leukocyte "sticking" in the hepatic sinusoids after C1-INH treatment has to be explained by improved microvascular blood flow conditions.

In summary, our results provide further evidence that complement plays an essential role in mediating postischemic reperfusion tissue injury. This is the first report demonstrating a beneficial effect of C1-INH treatment to minimize postischemic liver injury. Inhibition of the classical complement pathway by C1-INH provides an effective means to reduce microcirculatory disturbances as well as proinflammatory events such as leukocyte-endothelial cell interactions in postischemic livers. Postischemic plasma levels of hepatocellular enzymes further support the effectiveness of C1-INH treatment. These findings are in accordance with the observed beneficial effect of complement inhibition following ischemia and reperfusion of liver and myocardium [2, 6]. Based on our observations, we conclude that treatment with C1-INH prior to reperfusion represents a suitable pharmacological intervention to protect livers against reperfusion injury.

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