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Impact of enalapril on microvascular perfusion and leukocyte adherence in a model of rat liver transplantation assessed by in vivo microscopy

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Abstract ACE inhibitors have been proven to be effective in the reduction of ischemia/reperfusion damage after myocardial ischemia. In an attempt to investigate this effect in a model of syngeneic liver transplantation in the rat, we compared a control group with an ACE inhibitor treatment group, in which enalapril was given i. v. before and during reperfusion. By means of in vivo microscopy, sinusoidal perfusion rate, permanent leukocyte sticking in sinusoids and postsinusoidal venules, and leukocyte rolling in postsinusoidal venules were assessed. Liver function was evaluated by measuring bile output. The sinusoidal perfusion rate was significantly improved by enalapril treatment. Leukocyte sticking in both sinusoids and postsinusoidal venules was found to be remarkably reduced in enalapril-treated animals; the fraction of rolling leukocytes remained unchanged. Bile output was increased in enalapril-treated animals. These results demonstrated, in a model of rat liver transplantation, that ACE inhibition by enalapril is effective in reducing hepatic ischemia/reperfusion damage as assessed by the leukocyte-endothelium interaction using in vivo microscopy and postreperfusion bile production.

Key words Liver transplantation · Ischemia/reperfusion damage · Leukocyte-endothelium interaction · ACE inhibition

Introduction

After more than 20 years of clinical experience, liver transplantation is an established treatment modality for end-stage liver disease, with overall 3-year survival rates of almost 70 % [1, 2]. Selection of suitable recipients and selection of donors with satisfactory liver function are crucial elements for a successful outcome. However, primary graft dysfunction develops in up to 22 %, initiating a cascade of severe postoperative complications with considerable impact on mortality rates [1–3]. The pathophysiological bases for graft dysfunction are damages from cold ischemia, warm ischemia, and reperfusion.

Angiotensin-converting enzyme (ACE) inhibitors have been proven to be effective in reducing ischemia/reperfusion injury in experimental models of warm myocardial ischemia, and this is attributed not only to their vasodilating effects, but also to the oxygen free radical scavenging properties of thiol-containing (SH-group) substances, for example captopril [4–6]. Vasodilation is mediated by inhibition of kininase II, leading to both a reduced production of vasoconstricting angiotensin II and an increase in bradykinin concentration [7]. Bradykinin by itself is the most potent endogenous stimulator of endothelial prostacyclin synthesis, which in turn enhances vasodilation [8, 9]. Furthermore prostacyclin has powerful antiaggregatory effects and, most importantly, possesses some, as yet not clearly understood, cytoprotective properties. In different models on ischemic, viral, and toxic liver damages, treatment with prostacyclin has resulted in amelioration of the insult to the liver [8–13]. On that basis and in an attempt to transfer the favorable effects of ACE inhibitors in myocardial ischemia to hepatic ischemia, we investigated in

Table 1 Body weight, liver wet weight, anhepatic period, and cold ischemic time in control (LTx) and enalapril-treated (LTx/Ena) groups. Results are given as mean (SEM)

Group	LTx	LTx/Ena
Body weight (g)	225.7 (6.9)	211.6 (3.0)
Liver wet weight (g)	7.2 (0.2)	7.6 (0.2)
Anhepatic period (min)	19.0 (0.3)	18.8 (0.7)
Cold ischemic time (h)	24.3 (0.0)	24.0 (0.2)

a model of syngeneic rat liver transplantation whether ACE inhibition by enalapril is effective in the amelioration of hepatic ischemia/reperfusion injury.

Material and methods

All experiments were performed with permission of the government authorities and in accordance with the German legislation on laboratory animal experiments.

Surgical technique

Syngeneic, male Lewis rats (donors: 150–200 g body weight; recipients: 190–250 g) underwent orthotopic liver transplantation according to the cuff technique described by Kamada and Calne [14]. In contrast to the original technique, the grafts were rearterialized as reported by Steffen with the modification described by Post, to allow for simultaneous arterial and portal-venous reperfusion [15, 16]. The total ischemic time was 24 h. Grafts were preserved by retrograde aortal flush with University of Wisconsin (UW) solution and stored in UW at a constant temperature of 4 °C. Prior to reperfusion the high-potassium UW solution was removed from the liver by flushing with Ringer's lactate solution at room temperature via the portal route.

A control group of untreated animals (LTx; $n = 10$), which received 0.9 % sodium chloride by an i.v. line in the internal jugular vein during reperfusion, was compared to a study group (LTx/Ena; $n = 8$) in which the animals were treated with enalapril (Xanef, Merck, Sharp & Dome, Munich, Germany) at a dosage of 0.1 mg/kg per hour i.v., starting 5 min before reperfusion and continuing until the end of the experiment. The mean arterial pressure was continuously monitored by an indwelling catheter in the left carotid artery.

In vivo microscopy (IVM)

For assessment of microvascular liver perfusion and the leukocyte-endothelium interaction, IVM was used according to the technique reported by Menger and coworkers [17]. In brief, 30 min after reperfusion and under the precondition of a stable mean arterial pressure of 50 mm Hg, the left liver lobe was exteriorized and almost immobilized on a specially designed stage. To avoid major fluid loss and drying, the abdominal cavity was covered with a saran wrap. Sodium fluorescein (2.0 μmol/kg) and rhodamin 6G (0.1 μg/kg) were injected i.v. for fluorescent staining of hepatocytes and leukocytes, respectively. The following IVM parameters were assessed in ten randomly selected acinar areas and postsinusoidal venules, each:

1. Sinusoidal perfusion rate: percentage of perfused sinusoids of all sinusoids of a defined acinar area
2. Permanent leukocyte adherence ("sticker") in sinusoids and postsinusoidal venules: number of stickers per liver lobule, number of stickers per square millimeter of venular endothelial surface sticking for more than 20 s
3. Temporary leukocyte adherence ("roller") in postsinusoidal venules: percentage of rollers of the total number of moving leukocytes observed during the observation period of 20 s

Bile flow

To assess the bile production of the reperfused graft, as an indicator of reestablished liver function, a drain was fixed in the bile duct. The amount of bile draining over a period of 60 min was weighed, and, to allow for comparison between individual animals and groups, was related to 100 g of liver wet weight.

Statistics

Statistical differences between groups were calculated using Student's t-test for parametric data and the Mann-Whitney U-test for nonparametric data. Differences were considered significant at $P \leq 0.05$.

Results

In regard to body weight, liver wet weight, anhepatic period, and total ischemic time, there were no significant differences between the study groups (Table 1). Microvascular perfusion was significantly improved in enalapril-treated animals. The sinusoidal perfusion rate almost reached levels of sham-operated animals, which is 98–100 %. In addition, the number of sticking leukocytes was remarkably reduced in both sinusoids and postsinusoidal venules, indicative of a lesser degree of ischemia/reperfusion injury (Figs. 1 and 2). The fraction of rolling leukocytes in the postsinusoidal venules, however, remained unchanged despite enalapril treatment [LTx vs. LTx/Ena: 10.0 (2.1) % vs. 7.2 (1.4) %, mean (SEM)]. Bile flow, as an indicator of restored, energy-dependent liver function, was increased; however, the observed difference failed to reach a significant level [1.13 (0.3) vs. 0.43 (0.1); $P = 0.06$].

Discussion

In the technical proceeding of cadaveric liver transplantation, the inevitable periods of hypothermic anoxia and partial normothermic hypoxia, lack of hepatotrophic substrates, and toxic insult of oxygen free radicals result in organ damage known as ischemia/reperfusion injury, which may, in up to 22 %, clinically present as graft dysor nonfunction. Introduction of the UW solution by Belzer marked a considerable progress in liver preserva-

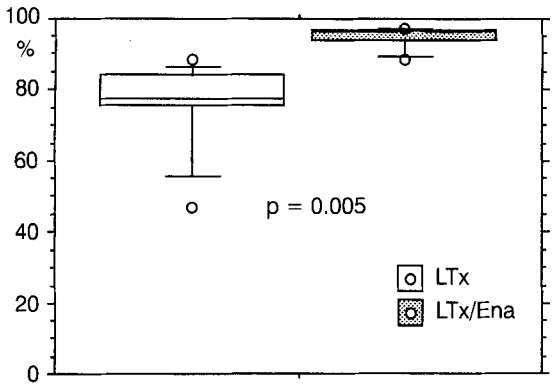

Fig. 1 Sinusoidal perfusion (box plot: horizontal bars depict 10th, 25th, 50th, 75th and 90th percentile, circles show outliers)

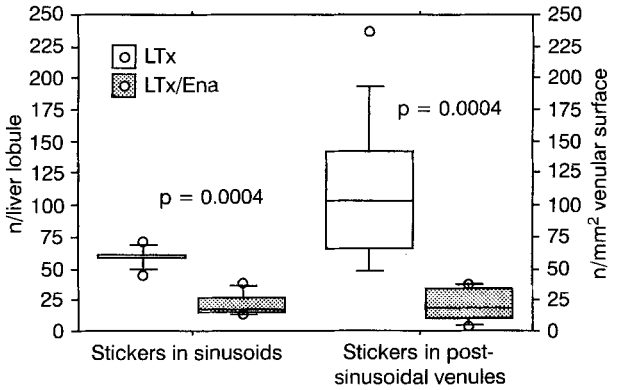

Fig. 2 Leukocyte adherence in sinusoids and postsinusoidal venules (box plot: horizontal bars depict 10th, 25th, 50th, 75th and 90th percentile, circles show outliers)

tion; however, there still seems to be a lack of an optimal preservation concept [18]. To the best of current understanding of optimal organ preservation, the goal is not only to preserve the primary graft viability by simply flushing the liver with an appropriate ice-cold solution, but also, and probably more importantly, to undertake therapeutic interventions during the more harmful periods of warm ischemia, removal of the high-potassium UW solution and reperfusion.

Former studies have clearly demonstrated, that, by not only changing the solution for flushout of the UW solution prior to graft reperfusion, but also by treatment of the recipient with scavengers or calcium channel blockers, improvements in graft function and structural integrity can be accomplished [19–22]. In different models of temporary coronary blood flow occlusion, ischemia/reperfusion damage has been effectively reduced by different types of ACE inhibitors. For thiol-containing drugs, such as captopril, the effects are attributed to their ability to inactivate reactive oxygen species [23, 24]. The common property of all ACE inhibitors is, however, their vasodilative action, which is based on both an inhibition of angiotensin II production and reduced bradykinin metabolism. Vasodilation and the bradykinin-induced endothelial synthesis of antiaggregatory and cytoprotective prostacyclin may explain the observed beneficial effects.

In terms of microvascular perfusion, we showed by means of IVM that enalapril treatment starting before reperfusion and continuing for 2 h improves nutritive liver perfusion remarkably, almost reaching values of nontransplanted livers. The leukocyte-endothelium interaction was significantly reduced in different areas of the microvascular liver network, indicating amelioration of ischemia/reperfusion damage. Because enalapril does not contain a thiol group in its molecule, scavenging actions cannot explain the effects. Presumably, an impact on the angiotensin, bradykinin, and prostacyclin metabolism is the underlying mechanism. As is known from earlier liver studies, the cytoprotective and platelet-inhibiting effect of prostacyclin may be of particular importance. Furthermore, prostaglandins of the E and I group are able to inhibit the activation of polymorphnuclear granulocytes, which in our model may explain the marked reduction in the leukocyte-endothelium interaction [25].

The findings of the IVM were in accordance with the result of the functional liver evaluation. Bile flow is known as a precise indicator of energy-dependent liver functions, and improvement may reflect better restoration of hepatic energy charge [26, 27]. Enalapril treatment was able to increase bile flow after reperfusion. The reason why the difference did not reach the level of significance may be related to the considerable variance in a group of only eight individuals and a collection period that was too short to demonstrate clearly the improvement.

Our study proved for the first time that the ACE inhibitor enalapril is able to reduce ischemia/reperfusion injury in a model of rat liver transplantation. The beneficial effects of enalapril observed in experiments on warm myocardial ischemia seem to be transferable to a model of rat liver transplantation, which includes a combination of cold and warm ischemia followed by reperfusion. However, these preliminary results need further confirmation. In particular, it has to be investigated in future studies whether the improvements can be attributed to changes in angiotensin, bradykinin, and prostacyclin metabolism, or whether enalapril, and possibly other substances of the ACE inhibitor group, possess some as yet unknown action of their own on ischemia/reperfusion injury.

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