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Reperfusion injury is dramatically increased by gentle liver manipulation during harvest

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Abstract Kupffer cell-dependent injury in livers gently manipulated during harvest develops upon reperfusion. The purpose of this study was to characterize this injury and to detect underlying mechanisms. Livers from female Sprague-Dawley rats were harvested for transplantation within 25 min. Minimal dissection was performed during the first 12 min, including freeing the liver from ligaments. After this, for further 13 min, livers were either left alone or manipulated gently. The livers were then cold-stored for 24 h in University of Wisconsin (UW) solution and perfused with oxygen-saturated Krebs-Henseleit buffer at 37 °C. Trypan blue in the buffer was used to index microcirculation. Cell damage was assessed with histology. Initial dissection during harvest and cold storage had minimal effects on sinusoidal lining cells; in contrast, the subsequent gentle organ manipulation dramatically increased cell death 6.5-fold, while the time for complete trypan blue distribution increased 2.3-fold ($P < 0.05$). Manipulation increased proteolysis 2-fold ($P < 0.05$). At harvest, manipulation increased portal venous pressure significantly by 68%. Treatment of donors with gadolinium chloride, a selective Kupffer cell toxicant, or with dietary glycine, an inhibitor of Kupffer cell activation, prevented effects of organ manipulation on all parameters studied. These findings demonstrate Kupffer cell-dependent reperfusion injury of sinusoidal lining cells caused by manipulation of the liver during its recovery. The mechanisms are those of proteolysis and impaired hepatic microcirculation.

Key words Organ retrieval · Reperfusion injury · Gadolinium chloride · Glycine

Introduction

Primary nonfunction and dysfunction frequently occur after liver transplantation, for reasons that remain unclear. They most probably involve harvest-related injury to the liver upon reperfusion. In clinical liver transplantation extensive dissection of the liver is associated with dysfunction of the graft after transplantation [11]. In experimental transplantation, in situ preparation of the portal vein area or gentle organ manipulation of the liver lobes disturbs microcirculation through mechanisms involving nerves to the liver [2, 6, 10]. Moreover, these microcirculatory disturbances cause hypoxia, which leads to activation of Kupffer cells and reperfusion injury after cold storage [7–10]. Once activated, Kupffer cells release numerous inflammatory mediators, including vasoactive substances and proteases, which cause graft injury after transplantation [7]. Microcirculatory disturbances and oxygen-dependent death of endothelial lining cells characterize Kupffer cell-dependent reperfusion injury after cold storage. This is a key event for primary nonfunction [7]. Our study was

designed to test the hypothesis that gentle in situ organ manipulation during harvest causes injury to sinusoidal lining cells via mechanisms involving proteolysis and hepatic microcirculation. To avoid difficulties with interpretation, which could occur in vivo, a blood-free liver perfusion model was used here.

Materials and methods

Experimental animals and treatment

Female Sprague-Dawley rats (200–230 g) were allowed free access to laboratory chow and tapwater. Some animals were given either $GdCl_3$ or dietary glycine prior to harvest for selective depletion or inactivation of Kupffer cells [3, 4]. Institutional guidelines on laboratory animal care were followed.

Harvest procedure

Donor livers were harvested within 25 min as described elsewhere [8]. Briefly, minimal dissection including freeing the organ from ligaments was performed in a standardized fashion during the first 12 min. During the following 13 min livers were either left alone or manipulated gently by touching, retracting and moving the liver lobes in situ. Prior to removal, livers were perfused with cold University of Wisconsin (UW) solution via the portal vein and subsequently stored for 24 h.

Enzyme assay

To detect proteolytic activity after cold storage, some livers were rinsed with 2 ml of cold UW solution prior to reperfusion. Arginine-specific proteolytic activity was measured in the effluent using H-D-Ile-Pro-Arg-pNA-2HCl (KabiVitrum, Sweden). Activity was determined from the rate of formation of *p*-nitroaniline (pNA) at 405 nm spectrophotometrically at 37 °C [8].

Liver perfusion

Livers were perfused via the portal vein at 3–4 ml/min per g of liver with Krebs-Henseleit bicarbonate buffer (118 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.7 mM KCl and 1.3 mM $CaCl_2$) at pH 7.6, saturated with 95 % O_2 and 5 % CO_2 at 37 °C using a peristaltic pump after 24 h of cold storage of the liver in UW solution [5].

Portal pressure

Livers were perfused in situ with oxygenated Krebs-Henseleit bicarbonate buffer (3–4 ml/min per g of liver at 37 °C), and the donor operation was performed as described above. Portal pressure was monitored continuously using a Digi-Med Low-Pressure-Analyzer™ Model 200 (Micro-Med, Louisville, Ky.).

Trypan blue infusion and histology

Following each experiment, trypan blue (500 μM; Aldrich) was infused into the liver. The time needed for trypan blue to stain the liver surface uniformly was measured to index microcirculation [5]. Livers were then flushed with additional perfusate to remove excess dye and fixed by perfusion with 4% paraformaldehyde in Krebs-Henseleit bicarbonate buffer at pH 7.6, embedded in paraffin, and processed for light microscopy using an eosin counterstain. The presence of trypan blue in the nuclei is indicative of irreversible loss of cell viability [5]. Five pericentral and five periportal fields (100 × magnification) were selected at random from at least four different sections per sample, and mean values of stained nuclei from nonparenchymal and parenchymal cells were calculated.

Statistics

Mean values ± SEM for various groups were compared using two-way analysis of variance (ANOVA) with the Student-Newman-Keuls post-hoc test as appropriate. $P < 0.05$ was selected prior to the study as the criterion of significance.

Results

Effect of gentle organ manipulation on proteolytic activity in the rinse effluent after cold storage

Gentle in situ organ manipulation during harvest significantly increased proteolytic activity in the graft rinse solution after cold storage 2-fold, from 0.14 ± 0.02 U/g in the non-manipulated group to 0.27 ± 0.05 U/g. In contrast, values for protease release in manipulated livers pretreated with $GdCl_3$ or glycine were similar to control values (0.11 ± 0.02 U/g).

Effects of gentle organ manipulation on hepatic microcirculation

Livers were perfused with trypan blue after 24 h cold storage, and the time needed for dye to distribute completely was recorded to index the hepatic microcirculation. In the unmanipulated group, livers turned uniformly blue 297 ± 28 s after the initiation of trypan blue infusion. This time was increased 2.3-fold by gentle organ manipulation ($P < 0.05$). However, if donors were pretreated with $GdCl_3$ or glycine before harvest, trypan blue distribution time of manipulated livers was not different from controls. Portal pressure was measured during perfusion of livers to index hepatic resistance. Gentle manipulation increased portal pressure to 68 % above baseline values; however, if donors were pretreated with $GdCl_3$ or glycine before manipulation, increases of portal vein pressure were significantly reduced, to 47 ± 7 % and 42 ± 8 % above baseline, respectively.

Effect of gentle organ manipulation on reperfusion injury after cold storage

After 24 h of cold storage in cold UW solution, livers were perfused with oxygenated buffer for 10 min. To de-

termine whether organ manipulation during harvest causes reperfusion injury to sinusoidal lining cells after cold storage, trypan blue was added to the perfusate. Gentle organ manipulation during harvest increased the number of trypan blue-positive sinusoidal lining cells per microscopic field 6.5-fold, from 3 ± 1 in the nonmanipulated group to 23 ± 3 ($P < 0.05$). Parenchymal cells were not affected by manipulation. Cell death upon reperfusion in livers manipulated during harvest was totally prevented if donors were pretreated with $GdCl_3$ or glycine before harvest.

Discussion

Gentle organ manipulation during harvest dramatically increases reperfusion injury

Recently, in situ organ manipulation during harvest was shown to dramatically reduce survival after transplantation [8]. In situ manipulation of the liver rapidly causes disturbances in hepatic microcirculation through stimulation of nerves to the liver [10]. This contributes to hypoxia and activates Kupffer cells [9] to release vasoactive mediators and proteases that in turn further impair circulation and cause additional injury. Indeed, in this study organ manipulation increased proteolytic activity in liver. The hepatic microcirculation was disturbed, as reflected by the longer time needed for trypan blue to distribute completely and also by an increased perfusion pressure. Both could potentially contribute to the development of Kupffer cell-dependent reperfusion injury after cold storage, a key event in primary nonfunction, which is characterized by oxygen-dependent death of endothelial lining cells [1, 7]. This view is supported in our study by a dramatically increased number of dead sinusoidal lining cells after 24 h of cold storage. Since endothelial cells comprise about 40% of sinusoidal lining cells and Kupffer cells and stellate cells initially retain viability [7], the exacerbated damage in manipulated livers shown here corresponds to virtually complete denudation of the endothelium.

Role of Kupffer cells in mechanisms of harvest-induced injury upon reperfusion

To test the hypothesis that Kupffer cells are involved in mechanisms of harvest-related reperfusion injury, donors were pretreated with $GdCl_3$, a rare earth metal and Kupffer cell toxicant [3], or dietary glycine, a nonessential amino acid that prevents activation of Kupffer cells [4]. These treatments prevented the effects of manipulation on reperfusion injury, suggesting a role for Kupffer cells. Taken together, the data from this study clearly demonstrate that gentle manipulation of livers during organ retrieval increases reperfusion injury to endothelial cells after cold storage. The effects of gentle organ manipulation can be prevented by modulation of Kupffer cell function with gadolinium chloride and glycine given to donors prior to organ harvest. If this finding is confirmed in humans, these pretreatments could improve the overall outcome of liver transplantation.

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