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Effects of warm Carolina rinse on microvascular reperfusion injury in rat liver transplantation

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Abstract Recently, it has been demonstrated that the use of both cold Carolina rinse (CR, 4 °C) as well as warm Ringer's lactate (RL, 37 °C) attenuates microvascular perfusion failure and leukocyte (WBC) accumulation in liver grafts. The aim of this study was to analyse in vivo whether warming of CR can also lead to a reduction in microvascular reperfusion injury in rat liver transplantation. Syngeneic orthotopic liver transplantation, including arterial reconstruction, was performed in male Lewis rats (180–300 g). Livers were stored in University of Wisconsin (UW) solution for 24 h and rinsed with 15 ml CR which was either cold 4 °C ($n = 7$) or warm 37 °C ($n = 8$) prior to reperfusion. Hepatic microcirculation and WBC accumulation were assessed by intravital fluorescence microscopy, and graft function was determined by analysis of bile flow during the 90-min reperfusion period. Warm CR yielded significantly ($P < 0.01$) improved sinusoidal perfusion when compared with cold CR; however, the extent of WBC adherence in both sinusoids and postsinusoidal venules did not vary between the groups. In addition, bile flow was slightly increased after warm CR. We conclude that after 24 h of cold storage in UW solution, warming of CR may offer additional benefit in the prevention of microcirculatory reperfusion injury without affecting WBC accumulation.

Key words Liver transplantation, rat · Preservation injury
Reperfusion injury · Carolina rinse
Intravital microscopy
Microcirculation

Introduction

The introduction of University of Wisconsin (UW) solution for hepatic cold preservation represented a major advance in clinical liver transplantation, extending the safe storage of human livers for transplantation to about 24 h [1]. However, liver grafts still fail as a consequence of storage injury. During extended cold storage, UW solution is well suited to preserving the integrity of parenchymal cells as well as Ito, Kupffer and bile duct cells, but not endothelial cells [2]. The endothelial cell damage, however, represents the primary manifestation of reperfusion injury, resulting in loss of organ viability and function [3].

The rinse of liver grafts with Ringer's lactate (RL) immediately before reperfusion is a necessary step in order to avoid cardiac complications due to the rich potassium content of UW solution [4]: However, by rinsing the graft, the components of UW solution which are meant to prevent the detrimental effects of toxic

oxygen products generated upon reperfusion/reoxygenation are flushed out just prior to the moment when they are most needed.

Special rinse solutions have been designed for direct pharmacological intervention during reperfusion, such as Carolina rinse (CR) whose formulation contains normal extracellular inorganic ions (NaCl, KCl, $CaCl_2$, KH_2PO_4, and $MgSO_4$), oncotic support against interstitial edema (hydroxyethyl starch), antioxidants (allopurinol, desferrioxamine, and glutathione), vasodilators to improve reflow (nicardipine and adenosine), substrates to regenerate ATP (fructose and glucose plus insulin), and buffer at a mildly acidic pH (MOPS, pH 6.5). The use of CR has been demonstrated to diminish endothelial cell damage and to improve survival after liver transplantation in the rat [5].

The rapid cooling during flush perfusion has been reported to provoke severe endothelial damage of sinusoidal lining cells, which is aggravated during reperfusion [6]. Although hypothermia at 4 °C (UW solution) is the best means to preserve liver viability and ultrastructure [7], pre-warming of the donor liver to 10 °C prior to reperfusion increased survival rates in rats [8]. Moreover, with the use of intravital microscopy (IVM), we have shown that warming of RL rinse to 37 °C attenuates microvascular perfusion failure after orthotopic rat liver transplantation (ORTL) [9]. Since morphological studies revealed evidence of the prevention of graft failure with the use of warm CR [10], we analyzed in vivo the effect of warm CR solution on microvascular reperfusion injury in rat liver transplantation.

Materials and methods

Surgical procedure

After premedication with atropine (0.1 mg/kg s.c.), the animals were anesthetized with ether. In recipient animals, the left carotid artery and jugular vein were cannulated with polyprophylene catheters for continuous recording of the arterial blood pressure, injections of fluorescent compounds, and volume replacement. The body temperature was monitored continuously and kept between 36.5 °C and 37.5 °C by means of a heating pad. Orthotopic liver transplantation was performed in 15 male Lewis rats of 180–300 g (Charles River Wiga, Sulzfeld, Germany). Donor livers were perfused in situ via the abdominal aorta with 10–15 ml UW solution at 4 °C under a pressure of 100 cm H_2O. Grafts were preserved for 24 h at 4 °C in UW solution. The technique of rat liver transplantation, including the reconstruction of the hepatic artery, has been described previously in detail [11]. The common bile duct of the graft was cannulated to measure bile secretion. Following IVM, the animals were killed 90 min after reperfusion by exsanguination for collection of blood and liver tissue.

Experimental groups

Two experimental groups were formed which differed in the temperature of the rinse solution before reperfusion: in group 1 ($n = 7$), grafts were flushed out with CR solution at 4 °C and in group 2 ($n = 8$), with CR at 37 °C. Liver grafts were rinsed with 15–20 ml CR via the portal vein under a pressure of 10 cm H_2O.

Intravital microscopy

The hepatic microcirculation was assessed by in vivo fluorescence microscopy 30–90 min after reperfusion. Contrast enhancement with sodium fluorescein (2 μmol/kg i.v.) allowed for the determination of sinusoidal perfusion. Leukocyte (WBC) adherence in sinusoids and postsinusoidal venules was assessed after in vivo staining with rhodamine 6G (0.1 μmol/kg i.v.).

Video-analysis and statistics

Quantitation of the microhemodynamics in the sinusoids and postsinusoidal venules was performed off-line by a frame-to-frame analysis of videotaped images [12]. Analysis of sinusoidal perfusion and WBC accumulation was performed within 8–12 perfused acini per animal. The following parameters were analyzed: sinusoidal perfusion as percentage of the total number of sinusoids observed; WBC adherence, defined as stained cells located within blood vessels and not moving during an observation period of 20 s (standardized in sinusoids/mm^2 liver surface, in postsinusoidal venules per endothelial surface). Data are presented as mean ± SEM. Comparisons between groups were performed by two-way nested design analysis of variance (ANOVA) after rank transformation of non-normally distributed data. Differences were considered significant for $P < 0.05$. Calculations were performed by the SAS procedures UNIVARIATE and GLM (SAS Institute, Cary, USA).

Results

Macrohemodynamics and bile flow

Mean arterial blood pressure during the observation period of 90 min after reperfusion was 76.8 ± 3.5 mmHg in group 1 and not significantly different from group 2 (76.1 ± 2.7 mmHg). Bile flow during the first 90 min after reperfusion was slightly (but not significantly) increased following CR at 37 °C when compared with CR at 4 °C (Fig. 1).

Perfusion of sinusoids

After cold CR (group 1), 11.4% ± 1.0% of sinusoids was not perfused after 60 min of reperfusion. Warming of CR to 37 °C (group 2) resulted in a significant ($P < 0.01$) decrease of the number of nonperfused sinusoids to 8.5%–1.1% (Fig. 1).

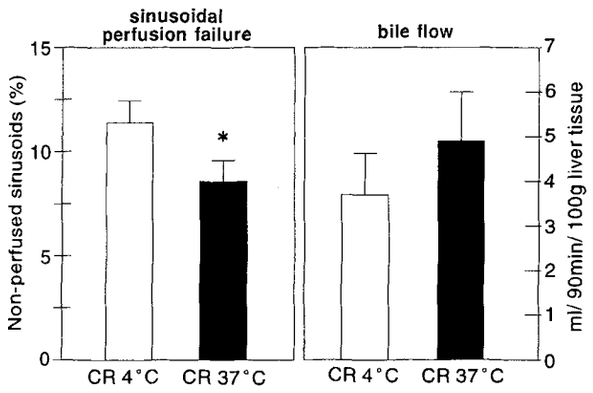

Fig. 1 *Left:* Percentage of nonperfused sinusoids within perfused acini as assessed by intravital microscopy (contrast enhancement with sodium fluorescein). A minimum of 75 acini was analysed within each group (□ CR/4°C, Carolina rinse at 4°C), (■ CR/37°C, Carolina rinse at 37°C, mean ± SEM, * $P < 0.01$ vs. CR/4°C). *Right:* Bile flow during the first 90 min of reperfusion

Leukocyte adherence

The use of CR at 37°C did not significantly influence WBC adherence in both sinusoids and postsinusoidal venules when compared with CR at 4°C. In group 1, WBC adherence amounted to $147 \pm 9\ mm^{-2}$ liver surface in sinusoids and $401 \pm 32\ mm^{-2}$ endothelial surface in postsinusoidal venules. In group 2, values were $161 \pm 11\ mm^{-2}$ and $482 \pm 34\ mm^{-2}$, respectively.

Discussion

The present study demonstrates that warming of CR (37°C) attenuates sinusoidal perfusion failure in rat liver transplantation compared with cold CR (4°C). These results are in agreement with an extend previous studies demonstrating that warming of RL has the potential to improve the hepatic microvascular perfusion after liver transplantation [9, 13]. The attentuation of microvascular perfusion failure by warming the rinse solution may be due to the prevention of ultrastructural damage to nonparenchymal/sinusoidal lining cells, in particular endothelial cells [10].

Previous studies from our laboratory have revealed that cold CR (4°C) when compared with cold RL (4°C) effectively reduces the number of WBC adhering to the endothelial lining of sinusoids and postsinusoidal venueles [14]. This study shows that warming of CR does not provide additional attenuation of WBC adherence in these microvessels. This may indicate that the specific components of CR which influence WBC adherence (adenosine, antioxidants) act similarly at 4°C and 37°C.

The tendency towars improved bile flow after warm CR may be explained by the improved sinusoidal perfusion rate, with consequently superior nutritional supply to parenchymal cells, and/or enhanced efficacy of the components of CR which influence the energy state of the hepatocytes (adenosine and fructose and glucose/insulin) [5].

In conclusion, we propose that cold storage in UW solution and flushing the graft with warm CR (37°C) represents a promising approach to attenuate microvascular reperfusion injury in liver transplantation.

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