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Resuscitation of cadaveric livers from non-heart-beating donors after warm ischemic insult: a novel technique tested in the rat

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Abstract. Clinical liver transplantation has become the therapy of choice in end-stage liver disease, but the limited availability of suitable donor organs still impedes its widespread application. In order to increase the availability of donor organs for liver transplantation, it would be advantageous if ischemically damaged livers could be resuscitated from cadavers in which the heart has stopped beating. A method for doing this has been developed in a rat model. Compared to livers excised from rats in which the heart is still beating, severe deteriorations of tissue integrity and functional performance were evident in predamaged livers after cold preservation without supplementary treatment. A treatment of those livers which included an antioxidant rinse with superoxide dismutase, and venous vascular insufflation of gaseous oxygen during preservation, completely prevented tissue alterations upon reperfusion, and promoted a functional recovery of the livers, making them comparable to organs harvested from heart-beating donors.
Key words. Non-heart-beating donor; liver; oxygen; persufflation; aerobic ischemia; transplantation; preservation; resuscitation; viability.

Liver transplantation has emerged from the experimental stage to become a clinical routine, and is the therapy of choice for patients with irreversible liver disease[1]. However, the shortage of donor organs from brain-dead donors whose hearts are still beating is still a limiting factor for the clinical use of organ transplantation.

In some countries, like Japan, where the problem of brain death remains unresolved, cadaveric liver transplantation has been seriously impeded compared with other countries[2]. In the present study a technique has been developed in rats to resuscitate livers damaged by warm ischemic insult from donors whose hearts were no longer beating. Viability of liver grafts could be achieved even if the organ was procured after cardiac arrest of the donor.

Materials and methods

Livers from fed male Wistar rats (250–300 g body weight) were isolated and rinsed blood-free via the portal vein with 20 ml of saline solution (50 U/ml heparin) and 10 ml of a standard organ preservation solution [University of Wisconsin (UW) solution, filtered through 4.5 μm pores before use]. Ischemic preservation was performed at 4 °C for 24 h in UW solution (group 1).

In groups 2 and 3 cardiac arrest was induced without application of heparin by phrenotomy of the anesthetized animal. Livers of groups 2 and 3 were excised 30 min after cardiac arrest of the donor. Livers of group 2 were then rinsed and preserved like the livers of group 1.

In group 3, the livers were connected to a gaseous oxygen supply according to techniques developed in our laboratory[3,4], to provide continuous aerobiosis of the tissue during ischemia. The humidified gas (pure oxygen) retrogradely entered the livers via the caval vein at a pressure limited to 18 mmHg in order to avoid barotrauma to the vasculature. Small pinpricks at the margin of the liver lobes served as outlets for the gas, but did not hamper post-ischemic reperfusion, and no leakage of perfusate was observed. Previously, 6000 U of superoxide dismutase (SOD) was added to the initial rinse solution in order to prevent oxidative tissue injury upon exposure to the high concentration of molecular oxygen.

In order to imitate the slow rewarming process every organ undergoes during the period of implantation into the recipient, all livers were immersed in physiologic saline at 25 °C for 30 min, during which no persufflation was performed in either group.

Reperfusion was performed after rinsing the liver with 10 ml of Ringer's solution with oxygenated Krebs–Henseleit bicarbonate buffer (95% O_2; 5% CO_2) at constant flow (3 ml g^{-1} min^{-1}) via the portal vein in a recirculating system at 37 °C for 45 min.

Livers were shock-frozen for determination of metabolic status at the end of the experiments. Energy-rich phosphates were determined from freeze-dried tis-

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sue samples by standard enzymatic tests. The energy charge potential (ECP) was calculated according to Atkinson[5] as

$$ECP = (ATP + 1/2\ ADP)/(ATP + ADP + AMP)$$

(Where ATP, ADP etc. are molar concentrations of adenosine triphosphate etc.) Liver injury mediated by oxygen-free radicals was assessed approximately from the tissue content of malondialdehyde (MDA), determined by high-performance liquid chromatography (HPLC) as detailed elsewhere[6]. Parenchymal liver injury was followed by the release of glutamate pyruvate transaminase (GPT) into the effluent, determined photometrically using a commercialized standard kit (Boehringer-Mannheim, Germany). Nonparenchymal cell injury was followed by the release of purine nucleoside phosphorylase (PNP) into the effluent. This enzyme has been shown to be indicative of vascular endothelial lesions after ischemic alteration of the isolated liver[7]. The determination of PNP in frozen perfusate samples was performed using HPLC techniques as described elsewhere[8].

Statistics. All values are given as the mean ± standard deviation (SD) of n = 5 experiments per parameter. Stochastical significance of differences between the groups was estimated using one-way analysis of variance and multiple comparison of the means by Dunnett's test. Differences were assumed to be significant if $p < 0.05$.

Results

Table 1 summarizes the results of the metabolic analyses. In contrast to freshly stored livers (group 1), livers harvested 30 min after cardiac arrest of the donor (group 2) exhibited a significantly impaired energetic status, with ATP tissue levels reduced to one half of those in group 1 and total adenine nucleotides (TAN) as well as energy charge potential (ECP) being concordantly diminished.

Table 1. Metabolic data of livers after 24 h of cold preservation, 30 min of rewarming and 45 min of postischemic reperfusion (mean ± SD).

	ATP (μmol/g)	TAN (μmol/g)	ECP	MDA (nmol/g)
Group 1 (n = 5)	4.94 ± 1.71	11.34 ± 1.27	0.59 ± 0.12	359 ± 201
Group 2 (n = 5)	1.38 ± 0.49**	7.37 ± 1.29**	0.34 ± 0.05**	326 ± 71
Group 3 (n = 5)	7.26 ± 1.37*	15.73 ± 1.35**	0.64 ± 0.07	304 ± 180

Tissue contents of metabolites refer to dry weight only. TAN, total adenine nucleotides (ATP + ADP + AMP); ECP, energy charge potential (ATP + 1/2ADP)/TAN; MDA, malondialdehyde. *$p < 0.05$, **$p < 0.01$ vs group 1 by analysis of variance and Dunnett's test.

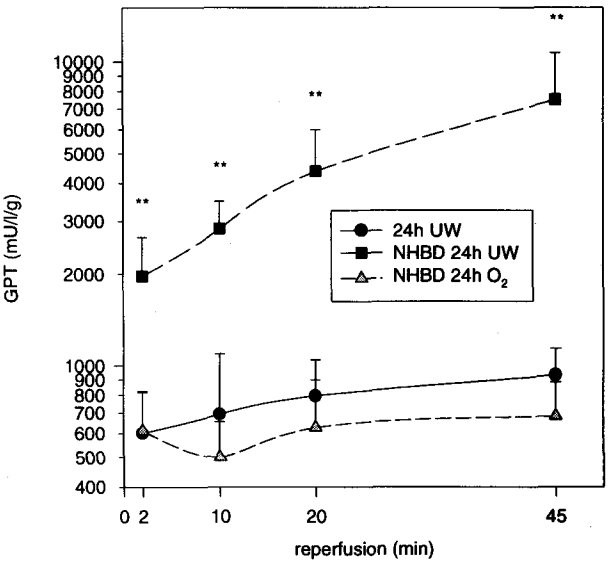

Figure 1. Release of GPT into the effluent after cold preservation of liver harvested from heart-beating donors (24 h UW), and livers harvested from non-heart-beating donors after 30 min of cardiac arrest with (NHBD 24 h O_2) or without (NHBD 24 h UW) oxygen insufflation for resuscitation. Values are given as mean ± SD. ** $p < 0.01$ vs 24 h O_2 by analysis of variance and Dunnett's test.

However, livers of group 3 showed significantly enhanced tissue levels of ATP, TAN and ECP, even compared with those in group 1, documenting the effectiveness of the resuscitation protocol in maintaining the level of hepatic energy metabolism, which is considered to be closely related to organ viability and subsequent graft function[9].

Lipid peroxidation, expressed as MDA tissue content of the livers, was comparable in all experimental groups. Thus it can be conjectured that no peroxidative tissue damage is associated with the insufflation of oxygen in warm ischemically damaged livers, previously rinsed with SOD, as performed in group 3.

Hepatic bile production during the 45 min of reperfusion was 17.7 ± 4.0 μl g^{-1} 45 min^{-1} in group 1, but decreased to only 4.0 ± 1.3 μl g^{-1} 45 min^{-1} in group 2. A significant and substantial improvement of bile flow to over five-fold the values of group 2 was seen in group 3, averaging 21.7 ± 4.7 μl g^{-1} 45 min^{-1} ($p < 0.05$ vs group 2; NS vs group 1).

Parenchymal enzyme loss during reperfusion of the livers is depicted in figure 1. While GPT release remained constant and of limited magnitude in groups 1 and 3 (peaking at 933 ± 208 and 684 ± 200 mU l^{-1} g^{-1}, respectively), a progressive increase up to 7492 ± 3208 mU l^{-1} g^{-1} was observed when predamaged livers were stored without oxygen insufflation (group 2).

A comparable pattern was observed with regard to the release of PNP. In contrast to livers harvested from

donors with beating hearts (group 1), where maximal PNP release was 2047 ± 854 mU l^{-1} g^{-1}, the loss of PNP in untreated livers from donors in which the heart was not beating (group 2) was significantly elevated to 7232 ± 2328 mU l^{-1} g^{-1} ($p < 0.05$). Resuscitation of predamaged livers by oxygen insufflation resulted in a massive reduction of PNP activity in the effluate to only 1192 ± 358 mU l^{-1} g^{-1}, which was even lower than that observed in group 1.

The portal perfusion pressure at the end of reperfusion averaged 6.6 ± 1.6 mmHg in freshly preserved livers (group 1) and 5.3 ± 0.8 mmHg in resuscitated livers (group 3), whereas untreated livers from non heart-beating donors (group 2) exhibited a significantly elevated perfusion pressure of 9.4 ± 1.0 mmHg ($p < 0.05$ vs group 1 and group 3).

Discussion

Retrograde oxygen insufflation through the vascular system, originally developed for the optimal storage of ischemic kidneys[10–12], has already also been used to improve the viability of freshly excised livers during cold ischemic preservation. In combination with the UW preservation solution this technique even provided a de novo synthesis of energy rich phosphates during cold storage, reaching supraphysiological tissue levels of ATP[13]. Thus it seems logical to use this effect in order to regenerate the energetic status of ischemically damaged livers by oxygen insufflation during cold storage.

However, introducing oxygen to previously anoxic liver tissue is known to bring risk of severe cellular damage due to the generation of oxygen free radicals, as has been documented in a large number of earlier studies (reviewed in ref. 14). Therefore we supplemented the initial rinsing solution with SOD in order to obviate the putative side effects of the high oxygen concentrations during persufflation. SOD is a potent scavenger of oxygen free radicals, which has successfully been used to reduce tissue injury during warm ischemia[15] or cold preservation[16] of the liver. The use of SOD in our study was based on previous experiences in our laboratory[17], and other antioxidants might also prove to be effective in this particular context of post hoc organ conditioning in conjunction with molecular oxygen.

Besides parenchymal damage to the hepatocytes, nonparenchymal cell alterations[18] and vascular dysfunction upon reperfusion may become a major determinant for the functional recovery of the liver from ischemic insult. In the present study, liver resuscitation by oxygen persufflation resulted in normalized perfusion characteristics and provided sufficient protection to the nonparenchymal cell population, as judged from the leakage of PNP, which was found to be even lower than in freshly preserved livers.

In conclusion, the combined medical treatment with SOD and subsequent aerobic preservation in UW solution was able to restore vascular and parenchymal integrity of the organ upon reperfusion. Thus the technique described has been shown to be a feasible means for resuscitation of predamaged livers from donors in which the heart has stopped beating, which requires only little additional equipment and would allow for transportation of the liver during storage. In view of these results, further studies in larger animals are strongly to be encouraged, and the use of predamaged donor livers for transplantation may be reconsidered.

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