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Endischemic oxygen persufflation to improve viability of marginally preserved donor livers

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Abstract The quality of cold-stored donor livers slowly declines beyond approximately 12 h, although these organs may still be used for clinical transplantation. The aim of the present study was to improve the energetic status and viability of long-term-preserved livers by short-term gaseous oxygen insufflation prior to implantation of the organ using a technique that has already been shown to promote aerobic energy metabolism during hypothermia. Livers from ten male Wistar rats were isolated, rinsed blood-free. Five livers (group 1) were stored for 48 h at 4 °C in UW preservation solution, and five livers (group 2) were isolated and stored in the same manner for 47 h, and were then, during the last 60 min of the preservation period, connected to a persufflation device and gaseous oxygen was introduced into the organ via the inferior caval vein, with the liver still immersed in cold UW solution. This technique of endischemic gaseous oxygenation resulted in a significant normalization of vascular resistance upon isolated reperfusion in vitro and a reduction in hepatic efflux of alanine aminotransferase as well as glutamate dehydrogenase, which led to improved recovery of the reperfused grafts of group 2 as evidenced by an elevated energy charge potential at the end of the reperfusion period. In conclusion, the technique described seemed effective in enhancing the preoperative viability of marginal donor grafts.

Key words Resuscitation · Viability · Oxygen · Persufflation · Preservation · Transplantation

Introduction

The introduction of University of Wisconsin (UW) solution for clinical liver preservation has extended the time of ischemic organ storage with respect to the formerly used Euro-Collins solution [3, 5, 6]. Although successful liver transplantation in humans is frequently reported after more than 24 h of cold preservation in UW solution [25, 28], the quality of cold-stored donor livers slowly declines beyond approximately 12 h of ischemia with a concomitant increase in the rate of primary nonfunction [1].

Progressive depletion of biochemical energy stores during ischemic storage entails the breakdown of hepatic energy metabolism and every energy-dependent metabolic process in the cell. Hepatic ATP is extensively degraded during ischemia [3, 15], and the catabolism of high-energy phosphates is followed by a time-dependent accumulation of membrane-permeable precursurs of adenine nucleotides such as inosine and adenosine [11]. The latter can permeate into the extracellular compartment where they are susceptible to being washed out upon vascular reperfusion and hence may become unavailable for later resynthesis of ATP. In particular endothelial cells are sensitive to a lack of oxygen, reacting with a fast breakdown of ATP and enhanced release of adenosine [23], and lethal injury to endothelial cells has been reported after 16 h of liver preservation in UW solution [4, 7].

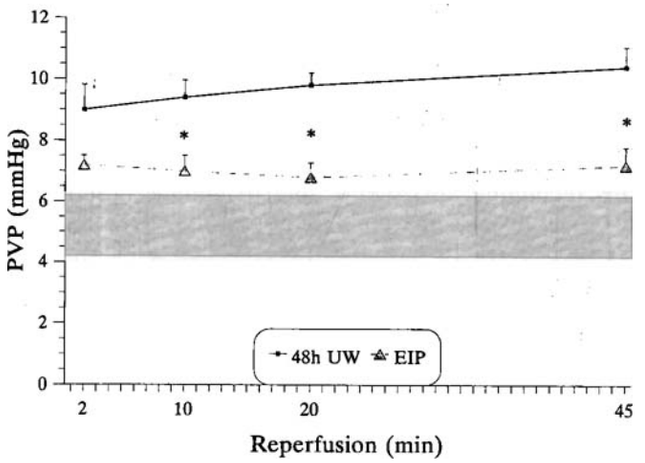

Fig. 1 Portal vascular perfusion pressure (PVP) upon postischemic reperfusion after long-term preservation (48 h UW solution, group 1) or with additional endischemic oxygen persufflation treatment (EIP, group 2). * $P < 0.05$

The lack of readily usable substrates for the rapid restoration of cellular energy stores may seriously hamper postischemic recovery of the preserved organ by retarding ion gradient re-equilibration and cell volume regulation upon reperfusion [24]. Extracellular/vascular edema is likely to further impair nutritional vascular reperfusion aggraveting the functional deterioration of the graft. Moreover, energetic support is also required for cellular repair mechanisms to counteract proteolytic tissue degradation and free radical-induced injuries [8, 20]. In previous studies we have established an effective method for gaseous oxygenation of ischemic liver, which avoids liquid perfusion and wash-out of the extracellular compartments [20, 21]. The objective of this study was to apply the technique of venous gaseous oxygen persufflation as a short-term regeneration protocol for long-term-preserved livers, which are likely to be of marginal viability, in order to improve the energetic status and tissue integrity of the graft prior to vascular reperfusion.

Materials and methods

Livers from ten male Wistar rats were isolated and rinsed blood-free via the portal vein with 10 ml UW solution. Five livers (group 1) were subjected to ischemic preservation at 4 °C in UW solution for 48 h, and five livers (group 2) were stored in the same manner for 47 h, and were then, during the last 60 min of the preservation period, persufflated with gaseous oxygen, which was introduced into the organ via the inferior caval vein at a pressure limited to 18 mm Hg with the liver still immersed in cold UW solution [13]. The dilated venules at the margin of the liver lobes were finely perforated using a fine accupuncture needle allowing the gas to leave the liver microvasculature. The technique has previously been shown not to bear the risk of sinusoidal air entrapment [29] and has been shown in an orthotopic liver transplant model to allow uncompromised nutritive perfusion in vivo [20].

To simulate the period of slow rewarming of the organ during surgical implantation in vivo [16], all livers were incubated at 25 °C in saline solution for 30 min prior to reperfusion while no persufflation was performed in either group. Immediately prior to vascular reperfusion, the organs were reflushed via the portal vein with exactly 10 ml Ringer's solution at ambient temperature. Reperfusion was carried out in vitro in a recirculating system at constant flow of approximately 3 ml/g liver per min with oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer at 37 °C. The liver was placed floating in 37 °C Krebs-Henseleit solution in order to assure homogeneous perfusion of the organ under extracorporeal conditions. The infrahepatic caval vein was ligated and a cannula was placed in the suprahepatic caval vein to allow collection and recirculation of the effluent perfusate.

Portal venous pressure (PVP) was measured during isolated perfusion by means of a Stadham pressure transducer connected to the portal inflow line and precalibrated to the calculated flow of 3 ml/g per min using PE catheters of length and size identical to the one used for the perfusion on the livers. As for the assay of enzyme activities in the effluent, glutamate dehydrogenase (GLDH) and alanine aminotransferase (ALT) were determined photometrically using commercial standard kits (Fa. Boehringer, Mannheim, Germany).

At the end of the experiment, the livers were freeze-clamped between precooled steel tongs according to the technique of Wollenberger et al. [30] for the determination of tissue levels of adenine nucleotides. Frozen tissue samples were preserved in a vacuum freezer (−45 °C; < 0.001 atm) to evaporate tissue water. The freeze-dried specimens were then homogenized and deproteinized with 0.33 *M* perchloric acid. The extract was centrifuged and the supernatant neutralized with 2 *N* KOH and frozen to remove $KClO_4$. Nucleotides were determined in the supernatant by standard HPLC techniques as described previously [18]. Total adenine nucleotides (TAN) were calculated as ATP + ADP + AMP. The energy charge potential (ECP) was calculated according to the method of Atkinson [2] as:

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

The results are expressed as means ± standard deviation, if not otherwise indicated. Stochastic significance of differences between the groups was evaluated using parametric comparison of the means with the alternate *t*-test. *P*-values < 0.05 were considered to be significant.

Results

The PVP during constant-flow reperfusion in vitro is shown in Fig. 1. It was found to be elevated to values above 9 mm Hg after extended ischemic preservation in group 1. This increased resistance to portal vascular perfusion was initially observed upon resumption of vascular reperfusion and remained essentially unchanged at this level until the end of the experiment. Short-term oxygen persufflation (group 2) resulted in a significant amelioration of hepatic vascular perfusion characteristics as evidenced by a reduced PVP.

The maximal enzyme leakage into the perfusate upon postischemic reperfusion was significantly different between the two groups (Fig. 2). Major losses of

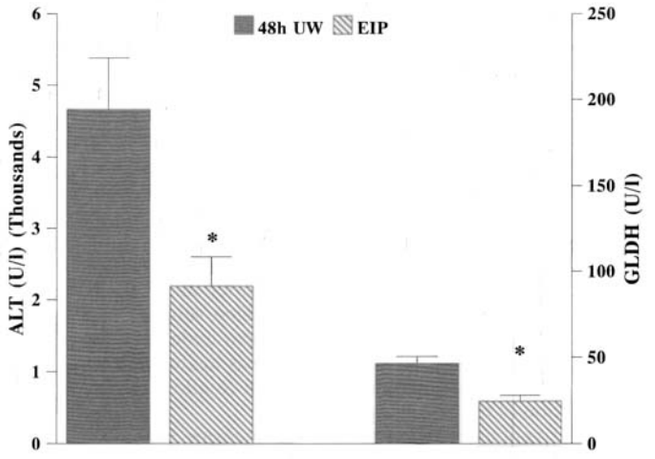

Fig. 2 Maximal release of alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH) during postischemic reperfusion of rat livers after long-term preservation (48 h UW solution, group 1) or with additional endischemic oxygen persufflation treatment (EIP, group 2). * $P < 0.05$

Table 1 Metabolic status and dry weight as a proportion of total wet weight of livers at the end of 45 min of reperfusion. Values are means ± SD. Group 1, 48 h of preservation in UW; group 2, 47 h of preservation in UW + 1 h of oxygen persufflation in UW at 4 °C (*TAN* total adenine nucleotides, *ECP* energy charge potential)

Group	ATP (μmol/g dw)	TAN (μmol/g dw)	ECP (1/1)	Dry weight/ total weight
1	1.85 ± 0.82	7.51 ± 1.48	0.380 ± 0.060	0.23 ± 0.01
2	3.28 ± 0.70	12.27 ± 2.09	0.482 ± 0.067	0.23 ± 0.02
P-value	< 0.05	< 0.05	< 0.1	NS

ALT were seen upon reperfusion of livers of group 1 and were taken as a general indicator of hepatocellular injury. However, ALT release was significantly reduced in livers of group 2 subjected to the endischemic oxygen persufflation protocol. A similar pattern was observed with respect to the intramitochondrial enzyme GLDH, the activity of which was markedly diminished in the perfusate of livers of group 2.

The results of the metabolic analysis are summarized in Table 1. Endischemic oxygen persufflation significantly improved hepatic restoration of tissue ATP concentrations after ischemic preservation and counteracted the decrease in TAN observed upon reperfusion of livers of group 1. The ECP at the end of the reperfusion period tended to the better in group 2 with respect to group 1, but this amelioration was only marginally significant. No differences were seen between the two groups concerning the development of tissue edema as judged from the dry weight to wet weight ratio of the liver specimens.

Discussion

Venous systemic oxygen persufflation has already been used to resuscitate cadaveric livers from non-heart-beating donors after warm ischemic insult [17, 22]. In the present study we demonstrated that this technique also seems able to enhance preoperative viability of marginal donor grafts after extended times of cold ischemic preservation in UW solution.

One major factor compromising the postischemic recovery of livers has been shown to be impaired vascular conductivity, leading to impaired vascular reflow and eventually deteriorating tissue integrity as a result of insufficient oxygenation [4, 10, 14]. Compromised vascular perfusion characteristics after long-term ischemic preservation were corroborated in the present study as evidenced by an abnormally increased PVP under constant flow conditions. Portal reflow, however, was significantly improved by endischemic gaseous oxygen persufflation. Gaseous oxygenation of the liver tissue using the persufflation technique described has been shown at low temperatures to result in a quite homogeneous oxygen distribution in the tissue, which is predominantly due to diffusion processes rather than the pressure-dependent insufflation of the entire vascular system [21]. Thus, endothelial cells within the liver microvasculature may be evenly oxygenated irrespective of vascular microcirculatory stasis, and hence enabled to restore cellular homeostasis with a consequent reduction in vascular edema upon reperfusion.

In analogy to this, the application of hyperbaric oxygen at the time of coronary reperfusion after experimental thrombosis has been reported to reduce the infarct size and to improve the functional recovery of the heart [26]. In postischemic skeletal muscle, hyperbaric oxygen treatment also decreases edema formation and improves metabolic recovery after tourniquet release [9].

Parenchymal effects after ischemic preservation were quantified by measurement of cytoplasmic and mitochondrial enzyme release upon reperfusion. Endischemic oxygen persufflation resulted in a marked improvement by both criteria. As it seems to be generally accepted that maintenance/recovery of cellular levels of energy-rich phosphates after revascularization [19, 24, 25, 27] is a primary predictor of organ viability subsequent to ischemic preservation, our findings on adenine nucleotide levels at the end of the experiments further confirm the results on parenchymal enzyme release. A single hour of endischemic gaseous oxygen persufflation nearly restored the levels of TAN to values which were not different from those found in nonischemic livers perfused in the same model [12].

In conclusion, the technique described seemed effective in enhancing preoperative viability of marginal donor grafts and might be particularly useful for providing energetic support to enable survival of the possibly relevant period of ischemic rewarming during implantation surgery.

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