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Reduction in nonparenchymal cell injury and vascular endothelial dysfunction after cold preservation of the liver by gaseous oxygen

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Abstract Reintroduction of oxygen to previously anoxic tissue may result in severe cell injury (oxygen paradox) and contribute to the so-called reperfusion damage of ischemic organs. Our study investigated the influence of simple gaseous oxygen supply during ischemia on nonparenchymal cell alterations upon reperfusion of the liver. Livers from male Wistar rats were isolated, rinsed blood-free and stored for 48 h at 4 °C in UW-preservation solution (group 1; $n = 6$). Gaseous oxygen was insufflated into a second group of livers (group 2; $n = 6$) during the storage period via the inferior caval vein at a pressure limited to 18 mmHg. To simulate the period of slow rewarming of the organ during surgical implantation in vivo, all livers were incubated at 25 °C in saline solution for 30 min prior to reperfusion. Reperfusion was carried out in vitro in a recirculating system with Krebs-Henseleit buffer. A control group was perfused immediately after harvest. The technique of aerobic storage (group 2) resulted in normal vascular perfusion characteristics without elevation of portal venous pressure (PVP) above control values, in contrast to group 1 livers which showed a significantly elevated PVP, averaging between 1.5 and 2 times the values of the control. Hepatic efflux of NO (nmol/ml) after 10 min of reperfusion was massively increased in group 1, while only low concentrations were found in group 2 and in control livers. Kupffer cell activation after ischemia was shown by a huge increase in acid phosphate release upon reperfusion compared with the control, with significantly lower values in group 2 after 10 min of reperfusion than in group 1. Thus, aerobic ischemia by gaseous oxygen persufflation seems an appropriate tool for long-term organ preservation, preventing vascular and parenchymal dysfunction upon reperfusion.

Key words Persufflation · Aerobic ischemia · Oxygen · Preservation · Liver

Introduction

Previous data from our laboratory have shown that gaseous oxygen insufflation into the venous system of cold-stored livers prevents anaerobic breakdown by hepatic parenchymal energy metabolism during preservation and improves the metabolic recovery of the organ upon postischemic reperfusion [16]. Moreover, circulatory disturbances are also significantly reduced after aerobic liver preservation by oxygen insufflation. Thus the purpose of this study was to determine the effects of the administration of gaseous oxygen during cold preservation on nonparenchymal tissue and on the functional and morphological integrity of the vascular system during postischemic reperfusion.

Materials and methods

Livers from male Wistar rats were isolated, rinsed blood-free and stored for 48 h at 4 °C in UW-preservation solution (group 1; $n = 6$). Gaseous oxygen was insufflated into a second group of livers (group 2; $n = 6$) during the storage period via the inferior caval vein at a pressure limited to 18 mmHg. The gas escaped through small pinpricks at the margin of the liver lobes [15]. To simulate the period of slow rewarming of the organ during surgical implantation in vivo [3, 18], all livers were incubated at 25 °C in saline solution for 30 min prior to reperfusion. Reperfusion was carried out in vitro in a recirculating system at a constant flow of approximately 3 ml/g liver per min with Krebs-Henseleit buffer, and no perfusate leakage was observed via the venous pricks. A control group ($n = 3$) was perfused immediately after harvest.

Effluent was collected during the in vitro perfusion by means of a short tube telescoped over a splint in the suprahepatic caval vein, allowing effluent perfusate samples to be taken for the determination of oxygen partial pressure with exclusion of room air contamination.

The following assays of enzyme activities in the perfusate were carried out. Alanine-aminotransferase (ALT) was determined photometrically using commercial standard kits (Fa. Boehringer, Mannheim, Germany). Purine-nucleoside-phosphorylase (PNP), which has been shown to be indicative of vascular endothelial lesions [21], was measured in frozen perfusate samples by high-performance liquid chromatography as described elsewhere [17].

The activity of acid phosphatase (ACP) in the effluent perfusate has been shown to provide an estimate of Kupffer cell activation during reperfusion of isolated livers [2] and was measured using a commercial kit (Fa. Boehringer).

The functional ability of the vascular endothelium to release the endogenous vasodilator NO was estimated by measurement of the stable oxidation products nitrate and nitrite ($NO_3 + NO_2$) in the effluent, based on the method described by Evans et al. [4].

Hepatic oxygen consumption was calculated from perfusate samples from the inflow and from the effluent which were immediately analysed using a pH blood gas meter (ABL 2 Acid-Base-Laboratory, Radiometer, Copenhagen). The results are expressed as means ± standard deviation (SD) if not otherwise indicated. Comparisons between multiple groups were performed with one way analysis of variance (ANOVA) and the Bonferroni t-test. Comparisons between two groups were done with the Mann-Whitney rank sum test or alternate t-test (GraphPad INSTAT); $P < 0.05$ was considered as significant.

Results

During postischemic reperfusion in vitro, untreated livers showed an increase in the portal venous perfusion pressure (PVP), which was about twice as high as in the control group and remained elevated throughout the observation period. In contrast, the PVP of livers persufflated with oxygen during the 48 h of ischemic storage did not differ from the values observed in the controls.

The endothelial release of NO was followed by the measurement of its stable oxidation products NO_x (Fig. 1). Interestingly, there was a large increase in NO_x production in untreated livers, corresponding to the elevated perfusion pressure in this group, while concentrations of NO_x in the perfusate of oxygen-persufflated livers were only slightly increased as compared with the controls.

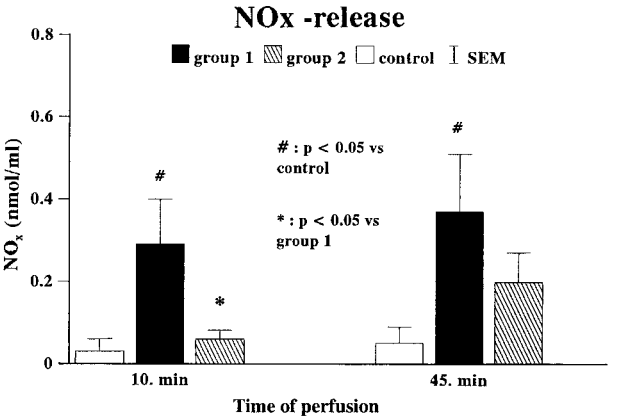

Fig. 1 Release of NO as approximated by its oxidation products NO_x after 10 min and after 45 min of isolated perfusion in vitro. Values are means ± SEM

The time course of enzyme leakage from the hepatic parenchyma into the perfusate is shown in Fig. 2. Control preparations exhibited low basal activities of ALT in the perfusate, which did not rise throughout the observation period. Upon reperfusion of ischemically preserved livers (group 1) sixfold higher activities of the enzyme were found during the first 10 min, after which time a steep and ongoing increase in ALT activities in the effluent was found until the end of the experiment. Gaseous aerobiosis during ischemia (group 2) resulted in a significant reduction in the initial ALT activities in the perfusate and completely suppressed the steep rise during the later reperfusion period.

Nonparenchymal enzyme release was followed by the activities of PNP from the vascular endothelium and acid phosphatase from activated Kupffer cells. While PNP was merely detectable in the perfusate of control livers, a progressive increase in PNP activity was observed in group 1 during postischemic reperfusion (Fig. 3). Livers of group 2 exhibited lower activities of this enzyme in the perfusate during the whole experimental course as compared to group 1, the differences being significant after 45 min.

Kupffer cell activation after ischemia was indicated by a huge increase in acid phosphatase release upon reperfusion in both experimental groups compared with the control, but significantly ($P < 0.05$) lower values in group 2 after 10 min of reperfusion compared with group 2 (0.22 ± 0.07 group 2; 1.07 ± 0.41 group 1; 0.02 ± 0.01 U/g per h control).

Oxygen consumption at the end of the perfusion period averaged 2.53 ± 0.19 ml/100 g per min in the control group. It recovered only to 1.47 ± 0.17 ml/100 g per min ($P < 0.01$) in group 1, whereas liver of group 2 reached

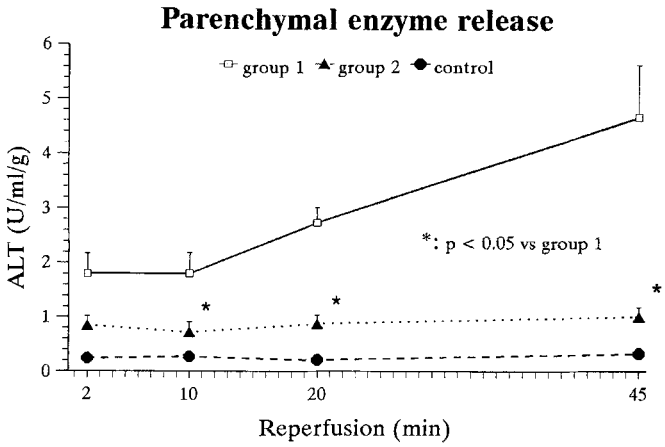

Fig. 2 Release of alanine aminotransferase (ALT) from livers during isolated perfusion in vitro. Values are means ± SEM

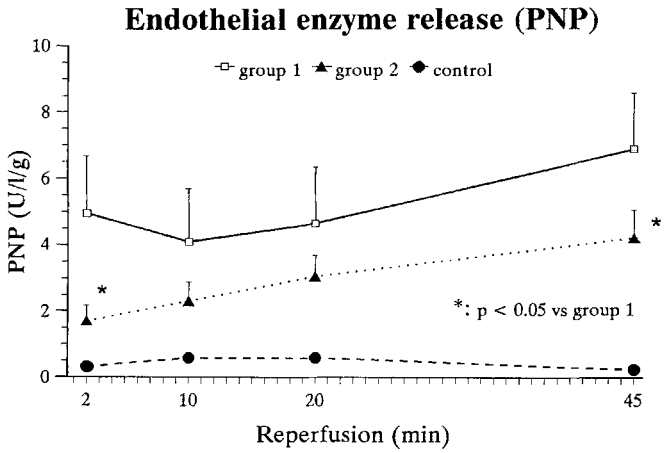

Fig. 3 Release of purine nucleoside phosphorylase (PNP) from livers during isolated perfusion in vitro. Values are means ± SEM

an average of 2.11 ± 0.24 ml/100 g per min, which was not significantly different from the controls.

Discussion

Artificial oxygenation of an ischemic organ has previously been tried in the early 1960s. Oxygen was applied at hyperbaric pressures to the nonperfused organ in order to achieve surface oxygenation of the tissue [12, 13]. However, the high oxygen partial pressures necessary to achieve sufficient supply to larger organs by diffusion require complicated devices and risk oxygen intoxication [24]. An alternative means to provide larger amounts of oxygen to a nonperfused organ has been developed using the arterial or venous vascular system, which avoids the inconvenience of hyperbaric pressure.

While orthograde oxygen persufflation, i.e. the application of oxygen via the arterial system, has been shown to achieve a highly significant improvement in the energy metabolism of ischemically preserved kidneys, this method does not result in functional recovery after reperfusion because of severe alterations in the arterial vascular system and the glomerula [10]. These side effects on the vascular system could be eliminated if the gas was introduced in a retrograde manner via the venous system. This method provides a sufficient aerobiosis of kidneys during ischemia as well as good postischemic function in vivo [5, 9, 22, 23]. Furthermore, no alteration in the portal vascular resistance have been observed upon reperfusion of livers which had previously been preserved in UW-solution using retrograde oxygen insufflation. On the contrary, livers in a persufflation group showed improved perfusion characteristics compared with untreated livers [16].

Endothelial cells play an important role in the regulation of vascular tone, producing and releasing several vasoactive substances including endothelium-derived vascular relaxing factor (EDRF), which has been identified as NO [19]. Alterations in the endothelial cells, which are most vulnerable in the sinusoidal and postsinusoidal veins [8, 25], may cause early microcirculatory disturbances and eventually result in early hepatocellular injury [8, 14].

In the present study we showed an increased production of NO in untreated postischemic livers, which was nevertheless not followed by an adequate reduction in vascular resistance. Endothelial NO is released in response to increased shear stress on the vascular wall [20] in order to normalize vascular flow characteristics. There was a strong stimulus for the release of NO in group 1, whereas only little or no need for vasodilative mediators was seen in control livers or livers kept oxygenated during ischemic storage. Since we did not measure the active form of NO, but only its oxidation products, it can be hypothesized that NO was rendered biologically inactive, e.g. by an excess of superoxide anion radicals [7], arising upon reoxygenation, thus preventing adequate vasorelaxation and an ensuing sustained stimulus to release NO.

Furthermore, it is known that the stress of ischemia/reperfusion can lead to an abnormal increase in endothelin production by the vascular endothelium [6, 11], leading to vasoconstriction and circulatory disturbances. We did not perform endothelin measurements and thus we cannot decided yet whether reduced stimulation of endothelin production or perhaps a simple reduction in endothelial cell swelling during preservation is the physiological mechanism responsible for the beneficial effect of aerobic ischemic storage on the hepatic vasculature. The significant improvement in the portal vascular conductance, however, underlines the overall protective ef-

fect of retrograde gaseous oxygen application on the vascular system.

Kupffer cell activation upon reperfusion occurs in response to factors released by injured endothelial cells [2], and eventually may contribute to microcirculatory disturbances in the liver [1]. Thus, the direct or indirect attenuation of hepatic activation of Kupffer cells obtained by oxygen persufflation during ischemic preservation is likely to be partially responsible for the improvement in hepatic perfusion characteristics in this group. In conclusion our findings show that aerobic ischemia by gaseous oxygen persufflation seems an appropriate and feasible tool for long-term liver preservation, preventing vascular and parenchymal dysfunction upon reperfusion.

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