Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Role of oxygen during hypothermic machine perfusion preservation of the liver

Bastian Lüer,* Martina Koetting,* Patrik Efferz and Thomas Minor

Surgical Research Division, University Clinic of Surgery, Bonn, Germany

Keywords
hypothermia, isolated rat liver, kidney, liver, machine perfusion, oxygen, preservation, transplantation.

Correspondence
T. Minor, University Clinic of Surgery, Div. Surg. Research, Sigmund Freud Str. 25, 53127 Bonn, Germany. Tel.: +49-228-287 1 5133; fax: +49-228-287 1 5199; e-mail: minor@uni-bonn.de

*Both authors contributed equally to this work.

Received: 24 November 2009
Revision requested: 21 December 2009
Accepted: 4 February 2010
Published online: 1 March 2010

doi:10.1111/j.1432-2277.2010.01067.x

Summary

Grafts from non-heart-beating donors are thought to be best preserved by hypothermic machine perfusion (HMP). Controversy exists concerning the role of oxygenation during HMP. In this study, we wanted to evaluate the relative role of oxygenation for graft integrity during and after HMP. Cardiac arrest was induced in male Wistar rats (250–300 g) by phrenotomy. Thirty minutes later, livers were flushed via the portal vein and subjected to 18 h of HMP at 5 ml/min at 4 °C. During HMP, the preservation solution was equilibrated with 100% oxygen (HMP100), with air (HMP20) or not oxygenated at all (HMP0). Graft integrity was assessed thereafter upon warm reperfusion *in vitro*. During preservation, oxygenation of the perfusate reduced alanine aminotransferase release by 50% compared with HMP0. HMP100 resulted in reduced oxygen free radical-mediated lipid peroxidation upon warm reperfusion compared with both HMP20 and HMP0. One hundred per cent oxygenation during HMP also significantly enhanced the activation of AMPK salvage pathway, and upstream activation of protein kinase A when compared with HMP0. Enzyme release during reperfusion was reduced by approximately 40% (HMP20) or approximately 70% (HMP100) after oxygenation compared with HMP0. Functional recovery (bile production) was only enhanced by HMP100 (approximately twofold increase vs. HMP20 and HMP0, $P < 0.05$). Efficiency of HMP might be markedly increased by additional aeration of the perfusate, most successfully by equilibration with 100% oxygen.

Introduction

In face of the increasing shortage of donor organs for clinical transplantation, the donor acceptance criteria for organ retrieval have been expanded towards inclusion of older donors and the use of 'extended donor criteria' grafts.

The latter comprise, for instance, livers with varying degrees of steatosis and livers that were retrieved after cardiocirculatory compromise of the donor, including grafts from non-heart-beating donors (NHBD), which are used in a growing number of centers as an alternative supply of donor organs [1–3], while retrieval of kidneys from NHBD has already become widely accepted as a clinical routine [4–6].

Despite all recent improvements in preservation solutions, donor treatment and harvesting protocols, preservation-associated ischemia and reperfusion injury prevail as pertinent factor responsible for primary dysfunction. Thus, any improvement in the preservation of grafts, which were procured from donors with compromised circulation or even from NHBD represents a valuable advance to enlarge the total number of viable donor organs available for transplantation.

Experimental as well as clinical reports have suggested hypothermic machine perfusion (HMP) to have advantages compared with conventional cold storage leading to improved functional recovery after transplantation [6–10].

© 2010 The Authors
Journal compilation © 2010 European Society for Organ Transplantation

Most recently, HMP has been shown to reduce significantly the risk of delayed graft function in a large prospective clinical multi-center study of patients undergoing kidney transplantation [10].

The advantage of HMP over cold storage, however, proved to be not as significant as expected and the exact mechanistic basis remains obscure. In that trial, HMP was performed without any oxygenation of the perfusate, neither with air nor with oxygen. After all, little remains known about the usefulness and optimal concentration of oxygen during hypothermic machine perfusion.

Positive effects after mere gaseous oxygenation of hypothermically stored livers [11] as well as recent studies on oxygenated machine preservation [12] suggest adequate restitution aerobic tissue homeostasis to be a useful adjunct to enhance graft integrity during HMP. The present study was therefore undertaken to evaluate the impact and adequate dosage of oxygenation during HMP for ulterior graft recovery.

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH Publication No. 85–23, revised 1985) were followed.

Male Wistar rats, weighing between 250 and 300 g, were anesthetized by intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg). The abdomen was opened by midline incision, and the liver was skeletonized and freed from all ligamentous attachments.

The portal vein was canulated, the livers were excised, rinsed via the portal vein with 20 ml of the histidine–tryptophan–ketoglutarate (HTK) solution and then put on a recirculating machine perfusion device with 125 ml of HTK. Hypothermic machine perfusion was performed for 18 h at 4 °C at a rate of 0.5 ml/g/min, delivered by a roller pump as detailed previously [13]. During HMP, the preservation solution was passed through an oxygenator, which was equilibrated with 100% oxygen (HMP100), with air (HMP20) or not oxygenated at all [i.e. no gas was introduced into the oxygenator (HMP0)]. This resulted in mean oxygen partial pressures of 600–700 mmHg in the HMP100 group, 200–250 mmHg in the HMP20 group and 50–100 mmHg in the HMP0 group.

An in-line filter (5 μm) was incorporated into the perfusion circuit to prevent recirculation of blood clots or cellular debris.

Postpreservation recovery of all livers ($n = 6$ per group) was evaluated thereafter upon warm reperfusion *in vitro* in a recirculating system for 120 min at 37 °C with oxygenated (95% O_2–5%CO_2; $pO_2 > 500$ mmHg) Williams E solution, supplemented with 3 mg/100 ml of bovine serum albumin (BSA; Fraction V; Sigma Chem, Munich, Germany), at a constant flow of 3 ml/g/min. This set-up has been previously validated and shown to allow for adequate approximation of tissue integrity and detection of structural changes in rat livers after hypothermic preservation [14,15]. To simulate the period of slow rewarming of the organ during surgical implantation *in vivo*, all livers were exposed to room temperature in a Petri dish for 20 min prior to reperfusion.

Perfusate samples were taken at the end of HMP and after 15, 45, 90 and 120 min of warm reperfusion, and enzyme activities of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were assessed photometrically using commercialized standard kits (Fa. Roche, Mannheim, FRG).

At the end of HMP, endothelin was determined in the preservation fluid using a commercialized standard ELISA kit according to the manufacturer's instructions (Biomedica, Vienna, Austria).

Portal venous pressure was measured during isolated perfusion by means of a water column connected to the portal inflow line and calibrated to the respective calculated flow using PE catheters of length and size identical to those used for the perfusion of the livers.

Total vascular resistance (TVR) was calculated from transhepatic flow and portal perfusion pressure and expressed in mmHg·min/ml.

Metabolic activity of the livers was approximated by the calculation of hepatic oxygen utilization. Perfusate samples were taken at the portal inflow and from the venous effluent, and the respective contents of O_2 were measured immediately using a pH-blood gas analyzer (ABL 500 acid-base laboratory; Radiometer, Copenhagen). Oxygen uptake by livers was calculated from the differences between portal and venous sites and expressed as μmol/g liver per min according to transhepatic flow and liver mass.

The common bile duct of the livers was canulated with a 27-gauge polyethylene tubing. Bile was collected during the whole reperfusion period, and hepatic bile production was calculated as μl/g/h.

Oxygen free radical-induced tissue injury was approximated by the degree of lipid peroxidation (LPO) in the tissue at the end of reperfusion. Liver samples were taken after completion of the experiment and LPO was evaluated by fluorimetry in the deproteinized tissue using the adduct formation with thiobarbituric acid as detailed elsewhere [16].

Western blot for phosphorylated AMPK and PKA

Whole tissue lysates were prepared from frozen tissue obtained after reperfusion, separated by gel electrophore-

© 2010 The Authors

sis and blotted onto nitrocellulose membrane as detailed previously [16].

Expression of specific proteins was analyzed by incubation overnight at 4 °C with the respective primary antibody and visualization on an X-ray film via chemiluminescence (Phototope®; New England Biolabs, Inc., Schwalbach/Taunus, Germany). Subsequently, blots were stripped and were probed with monoclonal anti-actin antibody to confirm equal amounts of protein loading.

Quantification of protein content was performed based on the ratios of individual signal and actin, determined densitometrically using UN-SCAN-IT gel v 6.1 (Silk Scientific Corporation, Orem, UT, USA).

Antibodies used were anti-phospo-AMPK and anti-phospho-PKA from Cell Signaling/New England Biolabs (Frankfurt, Germany) and anti-actin (AB-1; Calbiochem, Darmstadt, Germany).

Statistics

All values were expressed as mean ± SEM. After proving the assumption of normality and equal variance across groups, differences among groups were tested by analysis of variance (ANOVA) followed by the Student–Newman–Keuls (SNK) test, unless otherwise indicated. Statistical significance was set at $P < 0.05$.

Results

Liver function during machine perfusion preservation

Table 1 shows the results obtained upon oxygenated versus nonoxygenated machine preservation for 18 h.

Oxygen uptake by the livers during hypothermic machine perfusion was significantly influenced by the extent of oxygenation of the preservation solution.

In line with this, the degree of hypoxic metabolism actually differed between the three groups as lactic acid production significantly increased when oxygenation of the preservation solution was lowered.

However, parenchymal enzyme release during HMP did not show significant differences between HMP100 and HMP20 but was only increased after complete withdrawal of hypothermic aeration (HMP0).

Secretion of endothelin was investigated as indicator for vascular endothelial stress during prolonged HMP. Interestingly, the lowest levels were found upon HMP0. Complete oxygenation of the perfusate similarly prevented a rise in endothelin secretion, but equilibration with room air promoted a massive boost of perfusate endothelin levels, which was significant compared with both the other groups.

Maximal vascular resistance during HMP was also affected by the extent of hypothermic oxygenation. While it remained comparably low in the HMP100 as well as the HMP0 groups, a significant rise in vascular resistance was observed in the HMP20 group.

Table 1. Data obtained upon hypothermic machine perfusion with preservation solution equilibrated with 100% oxygen (HMP100), room air (HMP20) or without oxygenation (HMP0). Oxygen consumption (VO_2) was evaluated from pre- and posthepatic perfusate samples at the end of the HMP period. Accumulation of lactic acid, alanine aminotransferase (ALT) and endothelin was analyzed in the circulation perfusate as described under materials and methods. Maximal levels of total vascular resistance (TVR) of perfusion-preserved livers are calculated as the ratio of perfusion pressure and transhepatic flow.

	HMP100	HMP20	HMP0
VO_2 (µmol/g/min)	0.251 ± 0.015*#	0.082 ± 0.007*	0.023 ± 0.003
Lactic acid (mmol/l)	1.1 ± 0.1*#	2.5 ± 0.2*	4.6 ± 0.4
ALT (U/l)	8.2 ± 2.3*	8.8 ± 1.9*	25.7 ± 7.3
Endothelin (fmol/ml)	0.123 ± 0.041#	0.4104 ± 0.054*	0.032 ± 0.008
TVR (mmHg*min/ml)	0.72 ± 0.07#	2.18 ± 0.71*	0.80 ± 0.03

Data are given as mean and standard error.
*$P < 0.05$ vs. HMP0; #$P < 0.05$ vs. HMP20, anova + Student–Newman–Keuls test; $n = 6$ per group.
ALT, alanine aminotransferase; HMP, hypothermic machine perfusion; TVR, total vascular resistance.

Liver integrity upon postischemic reperfusion

Hepatic enzyme release upon reperfusion

Parenchymal integrity of the grafts after ischemic preservation was evaluated by the assessment of hepatic enzyme leakage upon reperfusion. A notable release of the cytosolic ALT into the perfusate was seen after anoxic HMP and was taken as a general parameter of hepatocellular injury (Fig. 1, upper panel).

Hypothermic machine perfusion with oxygenation significantly reduced these alterations to less than ¼ in the HMP100 group while lesser protection was conferred by HMP20.

Quite a similar pattern was observed with regard to the release of LDH (Fig. 1, lower panel). Again, machine-perfused livers showed lesser enzyme leakage in inverse correlation to the extent of oxygenation during preceding HMP.

Lipid peroxidation

The impact of oxygen free radicals on tissue integrity was approximated by fluorimetric detection of LPO in deproteinized liver homogenates.

Actually, it was observed that tissue LPO at the end of reperfusion was similar in the groups HMP0 and HMP20

© 2010 The Authors

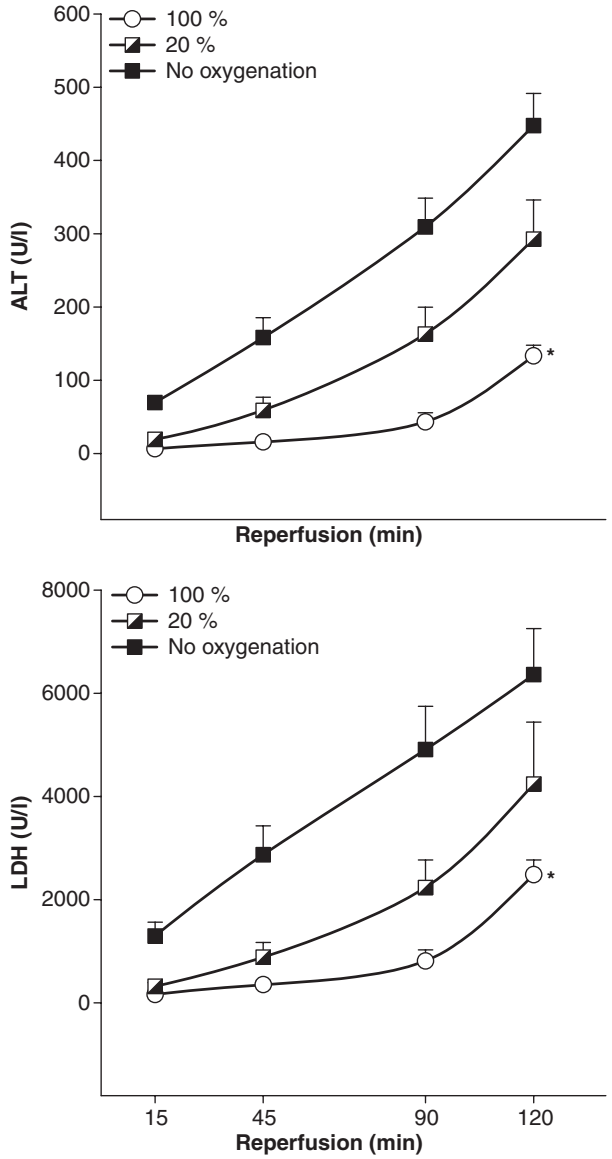

Figure 1 Parenchymal enzyme release of alanine aminotransferase (upper panel) and lactate dehydrogenase (lower panel) during normothermic reperfusion after 18 h of hypothermic machine preservation with different degrees of oxygenation. Values are given as mean ± SE (n = 6 per group); *P < 0.05 vs. no oxygenation.

(23.3 ± 6.3 and 24.1 ± 6.2 nmol/mg, respectively), while significantly lower values were measured in the HMP100 group (9.9 ± 1.8 nmol/mg; $P < 0.05$ vs. HMP20 and HMP0).

Hepatic functional recovery

In parallel to the lowering of parenchymal enzyme release, 100% oxygenation significantly improved functional recovery of the livers as evaluated by hepatocellular excretory function (Fig. 2). Hepatic bile production during

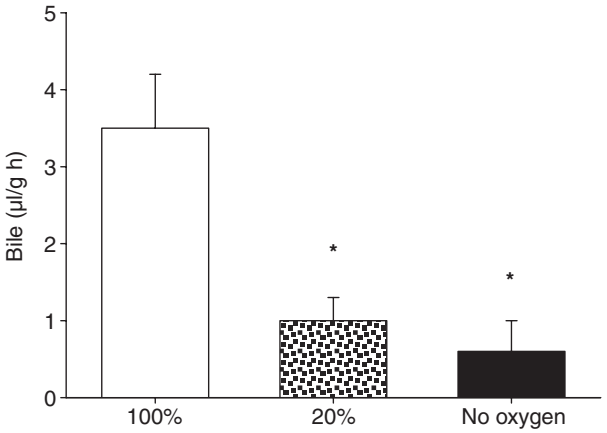

Figure 2 Functional recovery (bile production) during normothermic reperfusion after 18 h of hypothermic machine preservation with different degrees of oxygenation. Values are given as mean ± SE (n = 6 per group); *P < 0.05 vs. 100% oxygenation.

warm reperfusion was approximately threefold increased in the HMP100 group compared with any other preservation protocol.

No significant differences were observed between the groups concerning hepatic oxygen extraction, which amounted to an average of 1.8 ± 0.2, 1.6 ± 0.1 and 1.6 ± 0.1 µmol/g/min in the HMP100, HMP20 and HMP0 group respectively (NS).

Total vascular resistance during normothermic reperfusion was only slightly affected by the modalities of the preceding HMP protocol. While a tendency towards elevated values was seen in the HMP0 group, this difference was not statistically significant: 0.189 ± 0.019 vs. 0.184 ± 0.026 vs. 0.243 ± 0.020 mmHg·min/ml; HMP100 vs. HMP20 vs. HMP0, respectively).

AMPK-signalling pathway

Activation of the AMP-activated protein kinase (AMPK) following liver preservation and reperfusion was investigated by immunodetection of the phosphorylation state of AMPKα (Fig. 3). While phosphorylation of AMPK was found to be significantly depressed as compared with baseline values in the groups HMP0 and HMP20, complete normalization of AMPK phosphorylation occurred upon machine preservation with 100% oxygenation.

Concordant signal activation was disclosed with regard to the phosphorylation of protein kinase A (PKA), a putative upstream regulator of AMPK.

While over 50% reduction of PKA phosphorylation was observed after HMP0, oxygenation during HMP gradually normalized signal activation of PKA, reaching baseline values in the HMP100 group (Fig. 4).

© 2010 The Authors
Journal compilation © 2010 European Society for Organ Transplantation

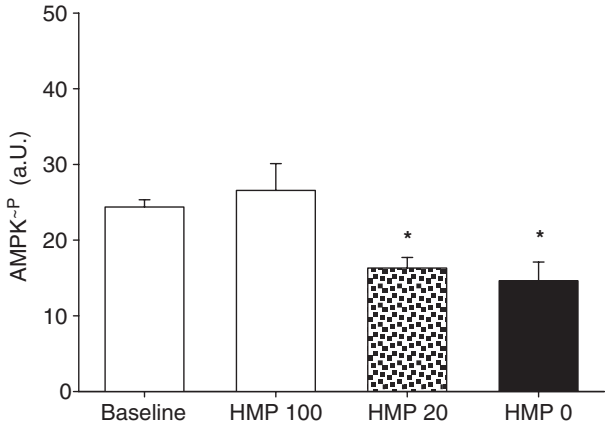

Figure 3 Phosphorylation rate of AMP-activated protein kinase (AMPK) during normothermic reperfusion after 18 h of hypothermic machine preservation with different degrees of oxygenation. Values are given as mean ± SE (n = 6 per group); *P < 0.05 vs. baseline and 100% oxygenation.

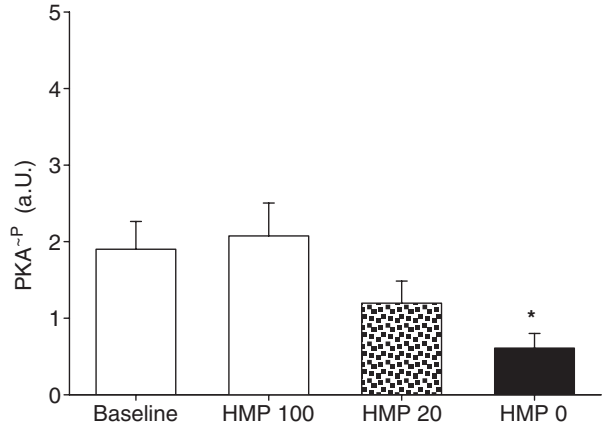

Figure 4 Phosphorylation rate of Protein Kinase A during normothermic reperfusion after 18 h of hypothermic machine preservation with different degrees of oxygenation. Values are given as mean ± SE (n = 6 per group); *P < 0.05 vs. baseline and 100% oxygenation.

Discussion

The present results indicate that the efficiency of hypothermic machine perfusion to preserve functional and structural liver integrity may be markedly increased by additional aeration of the perfusate during preservation. Therefore, it is of interest that the positive impact of oxygen was dose related and most successful preservation can be achieved by equilibrating the preservation solution with maximal oxygen concentration instead of room air.

As the advantages of HMP are most clearly seen in organs from marginal donors [6], we have used a model of non-heart-beating donation, representing a particularly important pool of organs attracting increasing attention [6,17].

Organ donation after cardiac arrest is particularly prone to formation of microthrombi and aggregating erythrocytes within the vascular system, impeding successful flush out upon harvest [18]. The benefit of machine perfusion preservation may hence relate to a progressive and better microcirculatory equilibration of the graft tissue with the preservation fluid. Ongoing perfusion after harvest gradually compensates for uneven perfusion of the tissue during initial flush out and eliminates regions of reduced protection because of incomplete equilibration with preservation solution.

Despite successful HMP of livers [8] and kidneys [10] without any additional oxygenation, HMP may, on the other hand, also fuel aerobic metabolism and energy homeostasis. Energy-consuming cellular processes have been shown to proceed even at low temperatures albeit at reduced rates [19]. It has been proposed that room air oxygenation should be sufficient for this purpose, supported by incomplete oxygen extraction from the perfusate [20], although no direct comparison with full oxygenation has been carried out.

Actually, 21% oxygenation has been found to be sufficient for maintaining functional viability of hypothermically preserved liver tissue slices with no additional benefit obtained through further increase in oxygen partial pressure [21]. As high oxygenation might favor the generation of oxygen free radicals, which in turn could impair tissue integrity, 't Hart and coworkers conclude 21% to be optimal for tissue slices [21].

In our experiments, however, we could not evidence any adverse effect of 100% oxygenation on oxygen free radical-induced LPO in the tissue samples. This is in line with the previous work from others similarly reporting high partial oxygen pressure during hypothermia to result even in reduced oxygen free radical formation upon reperfusion as judged from cytochrome c oxidation [22].

We further observed that 20% oxygenation did not prove sufficient for optimal outcome of whole liver grafts, preserved by HMP. In contrast to tissue slices or cell culture experiments, oxygenation of the intact graft is also dependent on vascular perfusion.

In this context, it is of note that microcirculatory disturbances occur during hypothermia, where heterogeneous flow patterns [23] may produce regions of no flow and thus undernutrition. Increased oxygenation of the perfusate with 100% oxygen would possibly help to aerate these nonperfused areas by gradient-driven gas diffusion via the tissue, similar to that has been shown during gaseous oxygen persufflation of the liver [24].

© 2010 The Authors

Vascular alteration resulting from continuous perfusion at hypothermia is a long discussed putative drawback of machine preservation [25]. Endothelin is secreted from sinusoidal endothelial cell as stress response and was found to be less elevated in the presence of 100% oxygen than that of 20% oxygen. This might reflect better endothelial protection through high oxygen delivery to the tissue, similar to that has been previously postulated for gaseous oxygen persufflation hypothermic organs [26]. Surprisingly, however, nonoxygenated perfusion also prevented elevated endothelin secretion. From the present results, it can only be speculated that anoxic endothelial cells might perhaps not be able to produce large amounts of endothelin due to energy deficiency.

In our model, only 100% oxygenation of the perfusate was operative in preventing the decline of AMPK activation during ischemia/reperfusion. AMPK is usually activated as an early response to an elevated AMP/ATP ratio caused by cellular and environmental stress. It has been shown to be operative in hepatic preconditioning [27] or attenuation of injury during cardiac ischemia [28]. Molecular mechanisms of the cell protective action of AMPK phosphorylation comprise activation of the p38 MAPK signalling pathway [28] and stimulation of autophagy [29]. Depressed cellular autophagy has been recently disclosed to be involved in postischemic liver damage in cell culture and whole organ models [15,30].

Autophagy allows the cell not only to recycle amino acids but also to remove damaged organelles thereby eliminating oxidative stress and allowing cellular remodeling for survival [31]. It occurs at low rates in cells to perform homeostatic functions (e.g. lysosomal degradation of defected proteins or injured organelles or depolarized mitochondria) and is involved in the recycling of denaturated proteins or metabolic catabolites [32].

The missing activation, or even depression of AMPK in livers, preserved without or only low oxygen support might indicate that the temporal extension of the ischemic insult is overwhelming the cellular response potential.

Upstream activation of AMPK also occurs independently of the AMP/ATP ratio [33], e.g. via the cAMP–PKA pathway. Previous experiments have shown that high oxygen partial pressures during hypothermic liver preservation result in maintenance of cellular signal levels of cAMP [34] and that the cAMP–PKA pathway significantly contributes to improved liver viability after cold preservation [35,36]. Using the phosphorylation state of PKA as a readout, we could here confirm the favorable effect of hypothermic oxygenation on the cAMP–PKA signal pathway and additionally show that lower oxygen partial pressures are less effective.

Because of the restrictions of this model, some aspects important for ulterior liver graft survival like the vanishing bile duct syndrome could not yet be addressed. Further studies *in vivo* will be required to delineate the differential impact of oxygenation during preservation on microcirculatory no reflow phenomena, cellular inflammation and repair mechanisms as to affect long-term survival of the blood reperfused graft.

Within the limits of our model, however, the presented data conclusively recommend additional aeration of the perfusate during HMP, best preserving cellular signal homeostasis by equilibration with 100% oxygen.

Authorship

MK: designed research and wrote the paper. BL: performed research and collected data. PE: performed research and collected data. TM: designed the study, performed research, analyzed data and wrote the paper.

References

1. D'Alessandro AM, Hoffmann RM, Knechtle SJ, *et al.* Liver transplantation from controlled non-heart-beating donors. *Surgery* 2000; **128**: 579.
2. Muiesan P, Girlanda R, Jassem W, *et al.* Single-center experience with liver transplantation from controlled non-heartbeating donors: a viable source of grafts. *Ann Surg* 2005; **242**: 732.
3. Reddy S, Zilvetti M, Brockmann J, McLaren A, Friend P. Liver transplantation from non-heart-beating donors: current status and future prospects. *Liver Transpl* 2004; **10**: 1223.
4. Cho YW, Terasaki PI, Cecka JM, Gjertson DW. Transplantation of kidneys from donors whose hearts have stopped beating. *N Engl J Med* 1998; **338**: 221.
5. Brook NR, Waller JR, Nicholson ML. Nonheart-beating kidney donation: current practice and future developments. *Kidney Int* 2003; **63**: 1516.
6. St Peter SD, Imber CJ, Friend PJ. Liver and kidney preservation by perfusion. *Lancet* 2002; **359**: 604.
7. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007; **83**: 1289.
8. Guarrera JV, Estevez J, Boykin J, *et al.* Hypothermic machine perfusion of liver grafts for transplantation: technical development in human discard and miniature swine models. *Transplant Proc* 2005; **37**: 323.
9. Dutkowski P, de Rougemont O, Clavien PA. Machine perfusion for 'marginal' liver grafts. *Am J Transplant* 2008; **8**: 917.
10. Moers C, Smits JM, Maathuis MH, *et al.* Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**: 7.
11. Minor T, Saad S, Nagelschmidt M, *et al.* Successful transplantation of porcine livers after warm ischemic insult

© 2010 The Authors

in situ and cold preservation including postconditioning with gaseous oxygen. *Transplantation* 1998; **65**: 1262.
12. Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved rat liver by hypothermic oxygenated machine perfusion. *Am J Transplant* 2006; **6** (5 Pt 1): 903.
13. Minor T, Manekeller S, Sioutis M, Dombrowski F. Endoplasmic and vascular surface activation during organ preservation: refining upon the benefits of machine perfusion. *Am J Transplant* 2006; **6**: 1355.
14. Minor T, Manekeller S. Assessment of hepatic integrity after ischemic preservation by isolated perfusion *in vitro*: the role of albumin. *Cryobiology* 2007; **54**: 188.
15. Minor T, Stegemann J, Hirner A, Kötting M. Impaired autophagic clearance after cold preservation of fatty livers correlates with tissue necrosis upon reperfusion and is reversed by hypothermic reconditioning. *Liver Transpl* 2009; **15**: 798.
16. Minor T, Kötting M. Gaseous oxygen for hypothermic preservation of predamaged liver grafts: fuel to cellular homeostasis or radical tissue alteration? *Cryobiology* 2000; **40**: 182.
17. Moers C, Leuvenink HG, Ploeg RJ. Non-heart beating organ donation: overview and future perspectives. *Transpl Int* 2007; **20**: 567.
18. Minor T, Tolba R, Neumann S, Schulz S, Sitzia M. Fibrinolysis in organ procurement for transplantation after cardiocirculatory compromise. *Thromb Haemost* 2003; **90**: 361.
19. Pegg DE, Foreman J, Hunt CJ, Diaper MP. The mechanism of action of retrograde oxygen persufflation in renal preservation. *Transplantation* 1989; **48**: 210.
20. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. *Lancet* 1967; **2**: 536.
21. 'T Hart NA, van der PA, Faber A, *et al.* Oxygenation during hypothermic rat liver preservation: an *in vitro* slice study to demonstrate beneficial or toxic oxygenation effects. *Liver Transpl* 2005; **11**: 1403.
22. Dutkowski P, Krug A, Krysiak M, Dunschede F, Seifert JK, Junginger T. Detection of mitochondrial electron chain carrier redox status by transhepatic light intensity during rat liver reperfusion. *Cryobiology* 2003; **47**: 125.
23. Lee CY, Zhang JX, deSilva H, Coger RN, Clemens MG. Heterogeneous flow patterns during hypothermic machine perfusion preservation of livers. *Transplantation* 2000; **70**: 1797.
24. Minor T, Klauke H, Vollmar B, Isselhard W, Menger MD. Biophysical aspects of liver aeration by vascular persufflation with gaseous oxygen. *Transplantation* 1997; **63**: 1843.
25. van der Plaats A, 'T Hart NA, Verkerke GJ, Leuvenink HG, Ploeg RJ, Rakhorst G. Hypothermic machine preservation in liver transplantation revisited: concepts and criteria in the new millennium. *Ann Biomed Eng* 2004; **32**: 623.
26. Minor T, Akbar S, Tolba R, Dombrowski F. Cold preservation of fatty liver grafts: prevention of functional and ultrastructural impairments by venous oxygen persufflation. *J Hepatol* 2000; **32**: 105.
27. Peralta C, Bartrons R, Serafin A, *et al.* Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. *Hepatology* 2001; **34**: 1164.
28. Li J, Miller EJ, Ninomiya-Tsuji J, Russell RR III, Young LH. AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB 1 in the ischemic heart. *Circ Res* 2005; **97**: 872.
29. Hoyer-Hansen M, Jaattela M. AMP-activated protein kinase: a universal regulator of autophagy? *Autophagy* 2007; **3**: 381.
30. Pagel H, Fandrey J, Schobersberger W, Fuchs D, Jelkmann W. Effects of neopterin and 7,8-dihydroneopterin on hypoxia-induced renal erythropoietin production. *Eur J Haematol* 1999; **62**: 341.
31. Yan L, Vatner DE, Kim SJ, *et al.* Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci USA* 2005; **102**: 13807.
32. Gustafsson AB, Gottlieb RA. Recycle or die: the role of autophagy in cardioprotection. *J Mol Cell Cardiol* 2008; **44**: 654.
33. Hutchinson DS, Summers RJ, Bengtsson T. Regulation of AMP-activated protein kinase activity by G-protein coupled receptors: potential utility in treatment of diabetes and heart disease. *Pharmacol Ther* 2008; **119**: 291.
34. Kötting M, Minor T. Biochemical effects and cyclic-amp second messenger signal upon venous oxygen persufflation of ischemically preserved livers. *Transplant Proc* 2000; **32**: 11.
35. Akbar S, Minor T. Significance and molecular targets of protein kinase A during cAMP-mediated protection of cold stored liver grafts. *Cell Mol Life Sci* 2001; **58**: 1708.
36. Arai M, Thurman RG, Lemasters JJ. Contribution of adenosine A(2) receptors and cyclic adenosine monophosphate to protective ischemic preconditioning of sinusoidal endothelial cells against storage/reperfusion injury in rat livers. *Hepatology* 2000; **32**: 297.

© 2010 The Authors
Journal compilation © 2010 European Society for Organ Transplantation