ORIGINAL ARTICLE

# Determination of an adequate perfusion pressure for continuous dual vessel hypothermic machine perfusion of the rat liver

Nils A. 't Hart,[1] Arjan der van Plaats,[2] Henri G. D. Leuvenink,[1] Harry van Goor,[3] Janneke Wiersema-Buist,[1] Gijsbertus J. Verkerke,[2] Gerhard Rakhorst[2] and Rutger J. Ploeg[1]

1 Surgery Research Laboratory, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
2 Department of BioMedical Engineering, University Medical Center Groningen, Groningen, The Netherlands
3 Pathology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

**Summary**

Hypothermic machine perfusion (HMP) provides better protection against ischemic damage of the kidney compared to cold-storage. The required perfusion pressures needed for optimal HMP of the liver are, however, unknown. Rat livers were preserved in University of Wisconsin organ preservation solution enriched with acridine orange (AO) to stain viable cells and propidium iodide (PI) to detect dead cells. Perfusion pressures of 12.5%, 25% or 50% of physiologic perfusion pressures were compared. Intravital fluorescence microscopy was used to assess liver perfusion by measuring the percentage of AO staining. After 1-h, the perfusion pressure of 12.5% revealed 72% ± 3% perfusion of mainly the acinary zones one and two. The perfusion pressure of 25% and 50% showed complete perfusion. Furthermore, 12.5% showed 14.7 ± 3.6, 25% showed 3.7 ± 0.9, and 50% showed 11.2 ± 1.4 PI positive cells. One hour was followed by another series of experiments comprising 24-h preservation. In comparison with 24-h cold-storage, HMP at 25% showed less PI positive cells and HMP at 50% showed more PI positive cells. In summary, perfusion at 25% showed complete perfusion, demonstrated by AO staining, with minimal cellular injury, shown with PI. This study indicates that fine-tuning of the perfusion pressure is crucial to balance (in)complete perfusion and endothelial injury.

## Introduction

Preservation has been a key factor for the success of transplantation. Since the development of the University of Wisconsin (UW) organ preservation solution, the limits in static cold-storage preservation are, however, reached [1–5]. As further improvements in procurement and preservation techniques are crucial to include 'extended' and non-heart-beating donors, it is important to explore other ways to diminish ischemia-reperfusion injury [1,3,6]. Brockmann et al. [7] showed in their meta analysis that the procurement method is important for effective organ retrieval. For procurement of abdominal organs, they recommended aortic perfusion above perfusion via both portal vein and aorta with a perfusion pressure which is higher than the normally used gravity controlled perfusion. Literature does, however, not provide an adequate level of evidence to draw conclusions about optimal perfusion pressures. Continuous hypothermic machine perfusion (HMP) preservation is another preservation method and could possibly improve preservation. HMP was first used by Belzer in the 1960s [2,8]. In his experiments, he applied continuous pulsatile perfusion for the canine kidney at 8–12 °C with perfusion pressures between 50/20 and 80/40 mmHg. A canine plasma-based solution was used as perfusate. In the past

decade, a number of transplant centers have reported the beneficial effects of clinical kidney HMP resulting in a 20% reduction in the incidence of delayed graft function when compared with cold-storage [9]. However, despite the fact that HMP is now being used, the required perfusion pressures to sustain an optimal viability without causing any injury to the vasculature have never been defined or justified.

To date, acceptance policies of abdominal organs have shifted from previously young cerebral trauma donors to older and more marginal donors. Inspired by the success of HMP in kidney transplantation, we gained interest in HMP of the liver. In the 1980s, a number of experimental studies already demonstrated that successful liver transplantation after HMP is feasible [10–12]. However, as normal liver circulation is largely dependent on portal perfusion at a low perfusion pressure of merely 12 mmHg, an exact definition of perfusion characteristics is probably even more important for the liver than that for the kidney. Moreover, the rheologic properties of the current standard UW-machine perfusion preservation solution (UW-MP) are significantly different from the original plasma-based perfusate [8,13]. As cold UW-MP is more viscous than blood, this could have its own effect on organ viability. In addition, our group recently reported that UW-MP containing hydroxyethyl starch induces aggregation of donor-blood erythrocytes [14,15] and thus currently used perfusion pressures for kidney HMP could be suboptimal for HMP [16].

The quality of HMP depends on adequate procurement, oxygenation of the preservation solution, and a complete and homogenous tissue perfusion [17–20]. In this study, we determined the appropriate perfusion pressure allowing complete perfusion of the liver and assessed the presence or absence of endothelial injury because of over-perfusion. We hypothesized that effective liver perfusion can be achieved at 60/40 mmHg, however, a more gentle approach may prevent shear–stress induced injury and thus improve donor organ viability.

## Materials and methods

Adult male WagRij rats (Harlan, Horst, The Netherlands) of 250–300 g, were used. All animals received care in compliance with the guidelines of the local Animal Care and Use Committee following National Institutes of Health Guidelines.

### Experimental design

Livers were procured and stored in either UW-MP or UW-cold storage solution (UW-CSS, Table 1). Preserva-

**Table 1.** Composition of UW-MP and UW-CSS.

| | UW-MP | UW-CSS |
|---|---|---|
| Adenine | 5 mM | |
| Adenosine | 5 mM | 5 mM |
| Allopurinol | 1 mM | 1 mM |
| CaCl$_2$·2H$_2$O | 0.5 mM | |
| Glucose | 10 mM | |
| Glutathione | 3 mM | 3 mM |
| Hepes | 10 mM | |
| HES | 5 g% | 5 g% |
| K-Gluconate | 10 mM | |
| KH2PO4 | 15 mM | 25 mM |
| K-Lactobionate | | 100 mM |
| KOH | | 100 mM |
| Mg-Gluconate | 5 mM | |
| MgSO4 | | 30 mM |
| Na-Gluconate | 80 mM | |
| NaOH | 15 mM | 20 mM |
| Raffinose | 30 mM | 30 mM |
| Ribose | 10 mM | |

tion was performed with the HMP technique, control livers were stored using static cold-storage. Different preservation solutions were chosen for HMP and cold-storage as they were considered to be a part of the preservation method. UW-MP or -CSS contained 13.5 μM of acridine orange (AO), to stain viable cells, and 14.9 μM of propidium iodide (PI), to detect dead cells [21]. The first part of the study describes 1-h pilot experiments. Three groups were defined and subdivided by perfusion pressures of the portal vein and hepatic artery. Group A which is 12.5% of normal perfusion pressure, consisted of continuous portal perfusion at 2 mmHg and a pulsatile arterial perfusion with a mean arterial pressure of 12.5 mmHg. Group B and C were defined to have portal perfusion pressures of 4 or 8 mmHg and mean arterial perfusion pressures of 25 or 50 mmHg, respectively. These pressures represent 25% (B) and 50% (C) of the physiologic pressures of the liver. In each group, a pulse rate of 360 bpm was used. In the second part of the study, we decided to compare 24-h HMP in group B and group C with 24-h cold-storage.

### Hepatectomy

For the hepatectomy procedure, inhalation anesthesia with isoflurane and a gas mixture of nitrous oxygen (66%) and oxygen (33%) was used. The celiac trunc was cannulated, the gastroduodenal artery and splenic artery were coagulated and the hepatic artery was dissected from the portal vein. One ml (0.9%) of NaCl with 500 iE/ml of heparin was administered. Ligation of the infra-hepatic

lower caval vein was followed by portal perfusion for an immediate *in situ* perfusion of the rat liver with ice-cold UW-MP or UW-CSS. Both preservation solutions were prepared according to the original recipe resulting in a viscosity of $11 \times 10^{-3} \pm 0.4 \times 10^{-3}$ Pa·s [15]. Fresh glutathione (3 mM) was added to both solutions just prior to initial blood wash-out. At the back-table, an additional 10 ml of UW was perfused via the portal vein and 5 ml via the hepatic artery.

### Hypothermic machine perfusion

Hypothermic machine perfusion was performed in a standard refrigerator using a thermostat with a hysteresis of $2 \pm 1$ °C (Omron Electronics BU; E5CN; Hoofddorp, The Netherlands). Both portal vein and hepatic artery were perfused in a recirculating fashion using a roller pump (Masterflex 7518-00; Cole-Parmer, Schiedam The Netherlands) for the portal vein and a pulsatile pump (Lab pump model OV; FMI, Syosset, NY, USA) for the hepatic artery. The arterial pulse amplitude increased in proportion with the increase in perfusion pressure with, because of the configuration of the pulsatile pump piston, a diastolic pressure of 0 mmHg. The portal and arterial flow (ultrasonic in-line flow probe, 1 N; Transonic Systems, Ithaca, NY, USA) and pressure (Truwave, Edwards Lifesciences, Irvine, CA, USA) were continuously monitored using a data acquisition program (Labview 5.0; National Instruments, Austin, TX, USA). Perfusion pressures ($P$) were corrected for cannulae resistances ($R$), that were considered to be a constant, using the following equation:

$$P_{total} = P_{liver} \cdot (1 + (R_{cannula}/(R_{total} - R_{cannula})))$$

In which $P_{total}$ is the total pump pressure, $P_{liver}$ is the perfusion pressure in the liver, $R_{cannula}$ is the cannula resistance and $R_{total}$ is the sum of liver and cannula resistance. Vascular resistance was calculated by dividing the perfusion pressure by flow and subtracting the cannula resistance. UW-MP was oxygenated with 100% of oxygen using an oxygenator consisting of 6-m silicon tubing with a 0.3-mm wall thickness (Rubber, Hilversum, The Netherlands).

### Intravital fluorescence microscopy

The intravital fluorescence microscopy technique was used to assess the amount of perfusion after 1-h preservation expressed in percentages. An inverted microscope (Leica DMil, Leica Microsystems AG, Rijswijk, The Netherlands) with a 100-W mercury lamp allowed epi-illumination of the intact hepatic surface. A 495/519-nm (FITC) filter was used to visualize AO staining of viable nuclei which is green fluorescent in concentrations

below 20 µM [22]. The liver surface was evaluated and images from 30 to 40 microscopic fields were recorded (Leica DC300F, Leica Microsystems AG, Rijswijk, The Netherlands) at 200 times magnification. Computer aided image analysis (Leica QWIN 2.8, Leica Microsystems AG, Cambridge, UK) was used to determine the percentage of perfusion.

### Microscopy

Cryosections (4 µm) were examined to disclose minute staining with AO and to identify the location and amount of dead cells. The exclusion-dye PI binds to DNA and RNA and is used to visualize nuclei of apoptotic or necrotic cells [21,22]. AO and PI both bind to nucleic acids but as the affinity of PI to nucleic acid is higher compared with AO, PI displaces AO from its binding site and results in a clear red staining, and not in a double staining pattern [23]. A fluorescent microscope with 495/519-nm (FITC) and 547/572-nm (TRITC) filters was used. PI positive cells, dead cells, were counted in 10 miscroscopic fields at a magnification of 200 times. Each field was recorded (Leica DC300F, Leica Microsystems AG, Rijswijk, The Netherlands) and analyzed using an image analysis technique (Leica Image Manager 500 1.2, Heerburgg, Switzerland). ED-1, marker for macrophages, staining was performed according to a previously described immuno-histologic technique using 3-amino-9-ethylcarbazole [24] as a chromogen. Rat endothelial cell antigen-1 (RECA-1) (dilution 1:20) staining was performed using rabbit-anti-mouse (1:50) and goat-anti-rabbit (1:50) as second and third peroxidase conjugated antibody with color-development using 3-amino-9-ethylcarbazole [25]. Light microscopy of hematoxyline and eosine stained sections was used to demonstrate the changes in morphology. Tissue was collected, fixed in 4% of formalin and subsequently embedded in paraffin. Sections were cut at 4-µm thickness. Liver morphology was scored after 1-h or 24-h preservation and tissue integrity was graded on a scale of one (excellent) to nine (poor) as previously described [26].

### Cellular viability

Adenosine triphosphate (ATP) was measured after 24 hours preservation using the ATP-bioluminescence assay kit CLS II (Roche Diagnostics GmbH, Mannheim, Germany). Samples were diluted in ethanol/EDTA to 200 µg protein/ml (Bio-Rad, München, Germany), subsequently 50 µl was further diluted in a 100 mM of phosphate buffer (pH 7.6–8.0) to a protein content of 20 µg/ml. Results were compared with normal liver tissue. Lactate dehydrogenase (LDH) activity in UW was measured using a kinetic photospectrometrical assay in which 100 µl of

pyruvaat is converted to lactate by LDH in the presence of 100 μl of NADH. Measurements were performed at 340 nm for 30 min at 37 °C with a lower detection limit of 0.25 U/ml.

### Statistical analysis

The 1-h ($n = 9$) and 24-h preservation experiments ($n = 18$) were performed with three and six livers in each group respectively. The results of the AO staining were analyzed using the nonparametric Kruskal–Wallis test. Marginal analyses were performed for PI positive staining

in 10 observations. One-way ANOVA was used with Bonferroni's correction for multiple comparisons. Data were considered to be statistically significant with a *P*-value of <0.05. Results are mean ± SEM.

### Results

The hepatectomy procedure took approximately 16 min. A complete initial wash-out in all livers was achieved. During preservation at $2 \pm 1$ °C, all livers showed an initial decrease in vascular resistance for both portal vein (Fig. 1a) and hepatic artery (Fig. 1b).
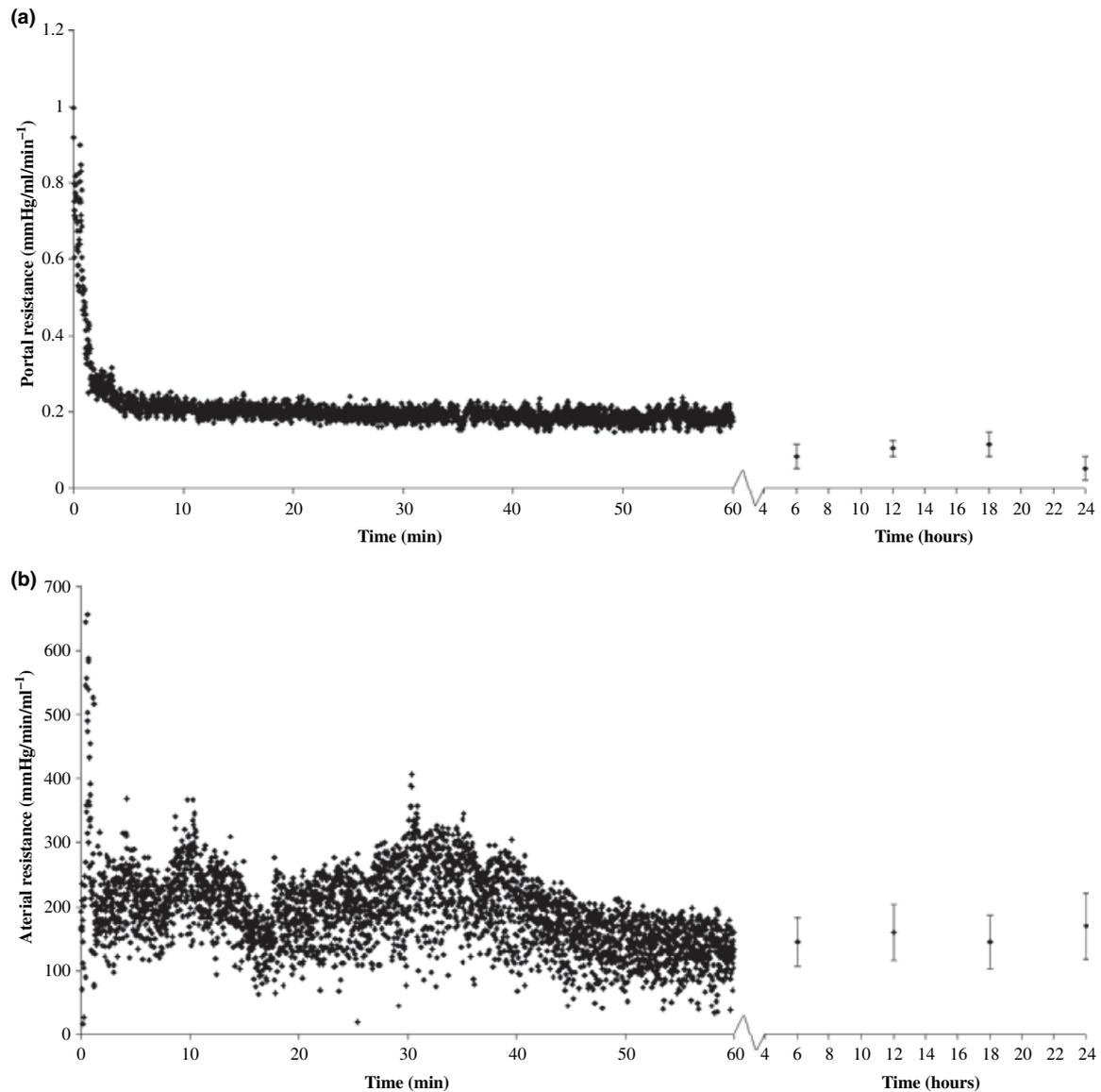


**Figure 1** (a) Example of portal resistance during hypothermic machine perfusion (1 sample/s). (b) Example of arterial resistance during hypothermic machine perfusion (1 sample/s), showing variation in resistance during the first 30 min, followed by a gradual decrease to a plateau of approximately 150 mmHg/min/ml$^{-1}$.

## Intravital fluorescence microscopy

Acridine orange staining of the liver was used as a marker for liver (micro)perfusion. A heterogeneous flow pattern was observed for 72% ± 3% of the liver surface, after 1-h preservation in group A, with a portal perfusion pressure of 2 mmHg and an arterial perfusion pressure of 12.5 mmHg (12.5%). The liver hilus showed a homogenous AO pattern (Fig. 2a), while a patchy AO distribution was found between the hilus and peripheral edges of the liver lobes (Fig. 2b) and staining was absent at the lobar edges (Fig. 2c). For group B (25%), 98% ± 1% of perfusion and for group C (50%), 100% ± 0% of perfusion was found, which was significantly more than the percentages found in group A ($P < 0.05$).

## Microscopy of 1-h preserved livers

Cryosections of livers were preserved with the lowest perfusion pressures confirmed intravital fluorescent microscopy findings and revealed complete AO staining around the hilum, however, AO staining was absent in the periphery of the liver lobes. Cryosections of livers from groups B (25%) and C (50%) showed evenly distributed AO stained nuclei of hepatocytes. PI staining was found for nonparenchymal cells, located in sinusoidal spaces and group C showed PI positive vascular structures as well. The use of low perfusion pressures resulted in a heterogeneous distribution of PI positive cells after 1-h HMP preservation, with a relatively large standard error of the mean. In contrast, the two more fiercely perfused groups showed a more homogeneous distribution and lower standard errors of the mean (Fig. 3). When high perfusion pressures were applied in group C, it resulted in PI staining in the arterial branches and to a lesser extent in portal veins, indicating cell death. Hematoxyline and eosine stained sections revealed marginal morphologic differences between the three groups, group A (12.5%) was classified with a mean integrity score of 6.5, group B (25%) with 4.0 and group C (50%) with 5.5. These differences were not statistically different. The morphologic features were: vacuolization mainly in zone one, picnotic nuclei of nonparenchymal cells and slight edema formation.

## Microscopy of 24-h preserved livers

In the second part of the study, 24-h preserved livers using HMP at the two highest settings (i.e. groups B and C) were compared with 24-h cold-storage preserved livers. Cold-storage preservation revealed 75.1 ± 6.2 PI positive cells (Fig. 3). Increasing the preservation time from 1 to 24 h resulted in an increase in PI positive cells from
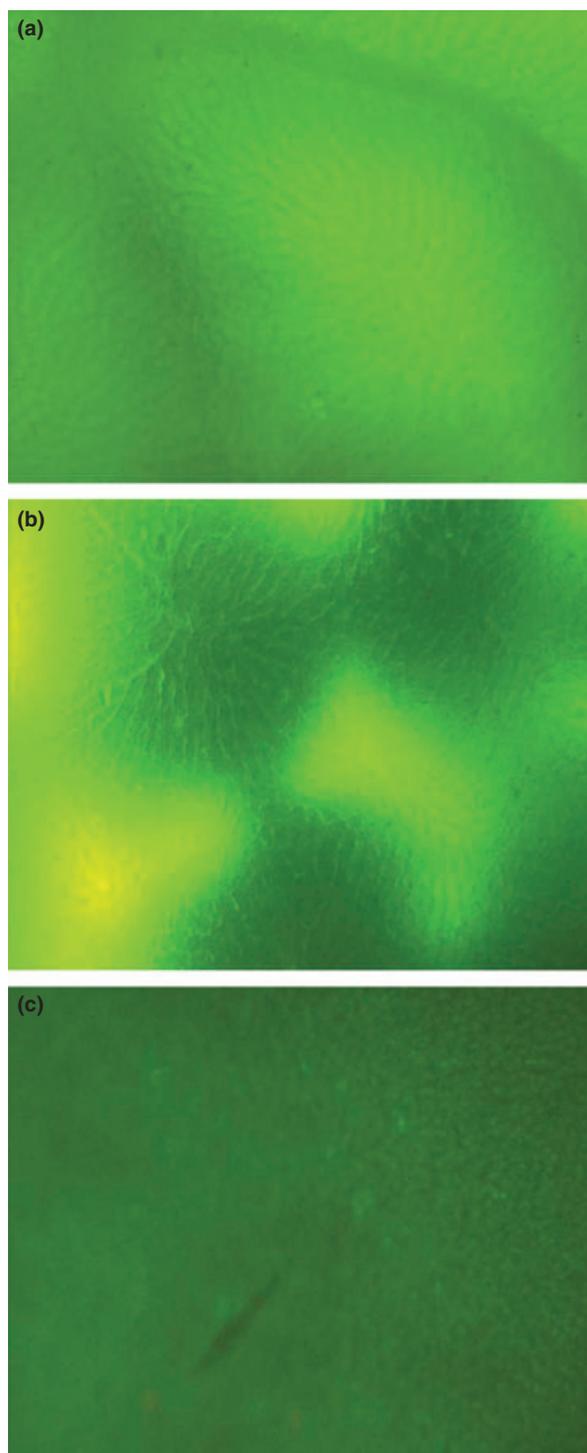


**Figure 2** (a) Acridine orange staining pattern in the hilar region of 1-h hypothermic machine perfusion preserved livers at a 2 mmHg portal pressure and a 12.5 mmHg arterial pressure (group A). (b) mid section of 1-h perfused livers in group A. (c) peripheral image of 1-h perfused livers in group A, note that this image is partly out of focus because of the concave liver edge.
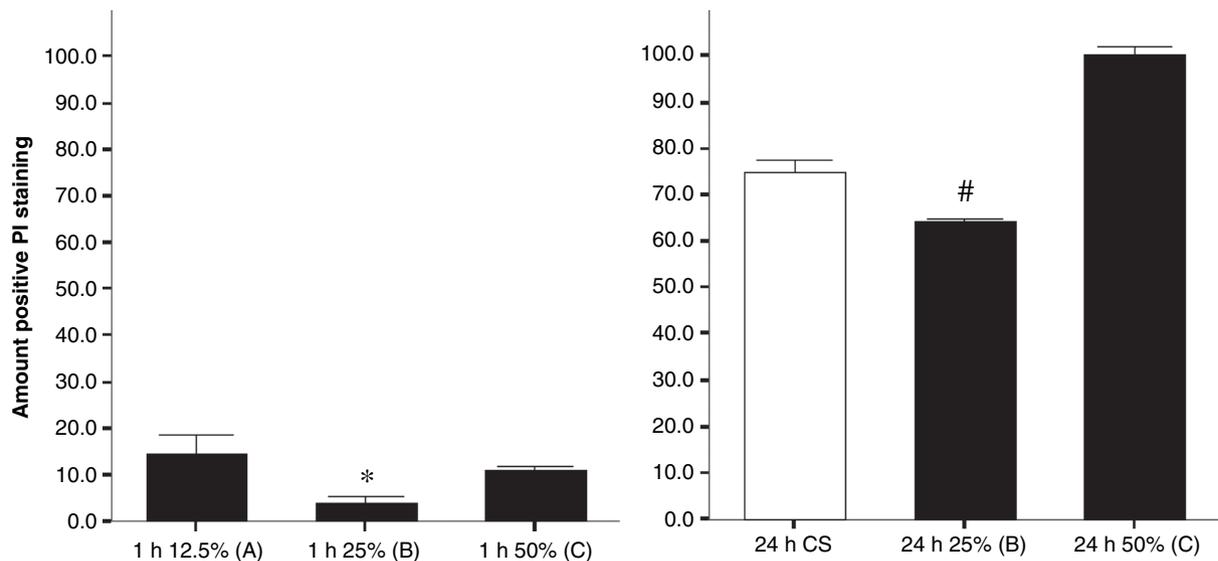
Figure 3 Marginal analyses of propidium iodide positive cell count in liver parenchyma (mean ± SEM) after 1-h and 24-h preservation. * = P < 0.05 versus groups A and C (25%), # = P < 0.05 versus 24-h cold-storage and 24-h preservation in group C (50%).

3.7 ± 0.9 at 1-h HMP to 64.4 ± 7.8 cells at 24-h HMP in group B (Fig. 3). Group B preserved livers showed less PI positive cells than was found in group C, 93.4 ± 5.9 cells after 24-h preservation (Fig. 3). After 24-h preservation, vascular PI staining was found in both groups B and C. All three groups showed the signs of compromised hepatocyte viability in mainly zone one. Hepatocytes showed vacuolization with occasional nuclear picnosis. The non-parenchymal cells had dense nuclei. The two groups in which the livers were preserved using the HMP technique showed wide sinusoids, edema formation around the portal triads and some hepatocyte detachment from their matrix.

The endothelial marker RECA-1 and the Kupffer cell marker ED-1 were used to characterize cells positively for PI. A completely perfused liver section with complete AO staining (Fig. 4a) showed PI staining patterns (Fig. 4b) resembling the same pattern as found with RECA-1 for the portal and arterial vascular endothelial cells and the peribiliary capillary plexus (Fig. 4c). PI positive cells were also found in bile ducts that were not RECA-1 positive. The PI positive staining pattern did not match with the ED-1 staining pattern (Fig. 4d).

### Cellular viability

Adenosine triphosphate is a marker for cellular energy and rapidly decreases during cold ischemia. After 24-h cold-storage, ATP levels were relatively low, 1.2 ± 0.5 pmol/μg protein. ATP preserved livers reached levels of 44.5 ± 5.9 for group B (25%) and 36.5 ± 2.8 pmol/μg protein for group C (50%). Two con-

trol samples of normal healthy livers showed ATP levels of 4.9 ± 0.4 pmol/μg protein. LDH release in UW was below detection limits in all groups. In addition, tissue samples showed LDH levels that were similar in all groups: group B, C and the CS group had LDH concentrations of 10.1 ± 0.6; 9.7 ± 0.4, and 9.7 ± 0.5 protein, respectively.

### Discussion

In the past, several groups have reported beneficial effects of HMP. HMP of the kidney improves kidney outcome after transplantation and facilitates the use of marginal and non-heart-beating donors. The mean perfusion pressure originally chosen by Dr Belzer in the early 1960s was 60 mmHg using a plasma-based solution [8]. Although the kidney perfusion pressure was based on an educated guess, transplantation results were good. In the years following its first introduction, perfusion pressures remained unchanged despite the later introduction of newer preservation solutions with higher viscosities than the original plasma-based solution. Surprisingly, transplant results remained similar, showing a 20% of reduction in delayed graft function compared with cold-storage. In this study, we postulated that similar settings could be applicable for HMP of the liver. It, nevertheless, should be considered that the low physiologic blood pressure in the portal vein might dictate a more gentle approach to prevent over-perfusion and tissue injury.

Perfusion pressure, route of perfusion, and perfusion characteristics (i.e. pulsatile- or continuous-flow) are important factors during HMP preservation. Several
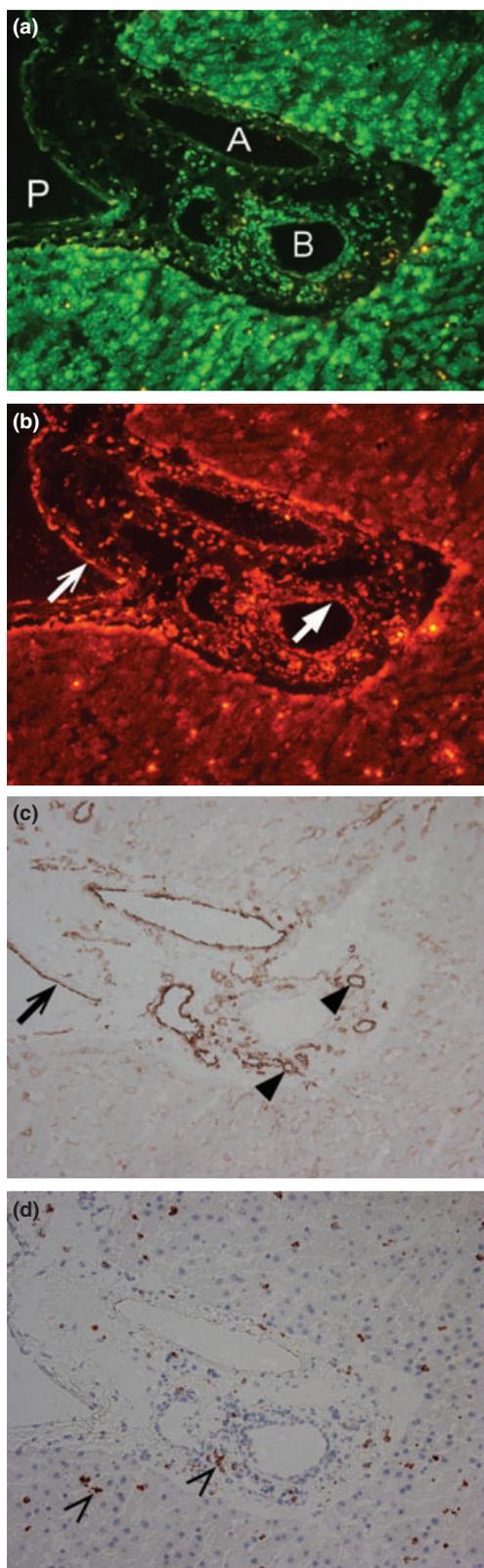
**Figure 4** (a) Acridine orange staining of a portal triad of 24-h preserved livers in group B (25%). P, portal vein; A, hepatic artery and B, bile duct. (b) Propidium iodide positive endothelial cells (open arrow) and bile duct cylindrical cells (closed arrow|) in a portal triad of 24-h preserved livers in group B (25%). (c) Rat endothelial cell antigen-1 positive endothelial cells (open arrow): portal vein, hepatic artery, and peribiliary capillary plexus (arrow head). (d) ED-1 positive Kupffer cells (open arrow head).

authors have described that arterial perfusion alone does not result in a completely perfused liver and is associated with more cellular injury compared with perfusion via the dual vessel approach [18,27]. Single vessel perfusion via the portal vein is shown to be sufficient during organ retrieval [7]. For continuous perfusion preservation for 24-h portal perfusion may result in an adequate preservation. Portal perfusion alone will not result in an efficient perfusion of the peribiliary capillary plexus and possibly enhances the chance of hepatic artery thrombosis or ischemic type biliary lesions. Our opinion is supported by the findings of Moench *et al.* They reported that an effective arterial wash-out allows better preservation and a reduction of the occurrence of ischemic type biliary lesions [28]. Retrograde perfusion or perfusion via both, portal vein and hepatic artery has been shown to be a good option for HMP of the liver [18,29]. In our study, we chose to perfuse the liver antegrade via both, hepatic artery and portal vein to mimic normal liver circulation and allow a better distribution of UW-MP in the peribiliary capillary plexus of bile canaliculi [30].

The design of this study involved a range of different combinations of perfusion pressures to assess results in a spectrum ranging from under perfusion to potentially detrimental edema formation and endothelial injury. Several groups attempted to preserve livers using a HMP setup. They mostly used a derivative of the UW organ preservation solution omitting hydroxyethyl starch to lower its viscosity [14,20]. However, the viscosity of UW-MP is $11 \times 10^{-3} \pm 0.4 \times 10^{-3}$ Pa·s and only a factor 1.3 higher than that of whole blood at 37 °C, which is $9 \times 10^{-3} \pm 1.7 \times 10^{-3}$ Pa·s [15]. The higher viscosity of UW-MP would result in a 1.3 times lower flow at a certain perfusion pressure when compared with physiologic conditions at 37 °C using whole blood. We consider these differences minor in respect to cold organ perfusion and chose to use the original UW-MP solution. The original UW-MP solution is effective in preventing edema formation and is widely used in kidney machine perfusion. In addition, HMP using the original UW-MP showed better preservation results than UW-MP without hydroxyethyl starch in experimental rat studies [31].

In our pilot experiment, 1-h preservation was used to compare three different perfusion pressures with a similar distribution ratio over the hepatic artery and portal vein. The period of 1 h was used to allow an adaptation of the liver vasculature to cold perfusion and dilatation of initially constricted vasculature. The lowest perfusion pressure of 12.5% resulted in a completely perfused liver hilum, however, no adequate perfusion and a scattered distribution of the preservation solution were obtained in the peripheral liver lobes. As vasodilation occurred within 10 min for the portal vein (Fig. 1), poor perfusion in group A (12.5%) cannot be explained by the cold or by UW-MP-induced vasoconstriction. Also, known irreversible vascular occlusions ought to be prevented by maintaining a continuous perfusion pressure throughout the preservation period. In the event of stasis, a resulting vascular occlusion will not be resolved during reperfusion and inevitably results in warm ischemia and consequently cell death [20]. AO staining demonstrated complete perfusion in groups B (25%) and C (50%). Thus, HMP using UW-MP allows complete and homogenous distribution of the solution at 25% and 50% of normal liver circulation. However, besides a complete liver perfusion, injury because of shear–stress on the endothelial cell layer should be prevented as well [32,33,16]. Shear–stress is a complicating phenomenon of cold perfusion as hypothermia renders endothelial cells more susceptible to injury because of a decrease in vascular compliance [34,35]. Obviously, shear–stress can easily negatively affect the integrity of vascular endothelial cells and induce cellular detachment [36,37]. PI results, as it stains dead cells, indicate that intermediate perfusion pressures at 25% are preferable, as less nonparenchymal cell injury was observed. High PI positive cell counts in the group perfused at high pressures (50%) were found and are associated with excessive shear–stress on the nonparenchymal endothelial cell layer.

In the second part of the study, we extended the preservation time to a more clinical relevant time of 24 h and compared HMP groups B (25%) and C (50%) with 24-h static cold-storage. The perfusion profiles of groups B and C were used, as the settings in group A (12.5%) were shown to be inferior and without complete tissue perfusion. The relevance of this part of the study was the assessment of the degree of cellular injury because of dynamic preservation in comparison with static cold-storage. Cold-storage preserved livers showed high levels of dead PI positive cells which were predominantly nonparenchymal. Livers preserved with HMP at 25% of physiologic perfusion showed a lower PI positive cell count than in the cold-storage group, and both groups of data were lower than HMP preserved livers using high perfusion pressures (50%). Thus, PI results showed that HMP

settings are critical for effective preservation. Changing perfusion pressures from 25% to 50% resulted in an increase in nonparenchymal cell injury. To reveal the nature of PI positive cells, further analysis was performed by comparing PI staining patterns with RECA-1 as an endothelial cell marker and ED-1 as a Kupffer cell marker. Identical staining patterns were found for PI positive nonparenchymal cells and RECA-1 indicating apoptotic or necrotic (sinusoidal) endothelial cells. Furthermore, PI staining in relation to RECA-1 also revealed PI positive cylindrical cells of bile ducts.

To assess the relevance of perfusion pressure for liver function and viability, we have now focused on an optimal liver perfusion during cold preservation. Perfusion parameters were determined together with a liver viability marker: ATP. This marker was selected as it can be a predictive marker for organ outcome after transplantation [6,38]. In addition, several authors have documented a better tissue ATP level during HMP [38,39]. In this study, we were able to confirm these data showing an accumulation of ATP after HMP with relatively high ATP levels compared with the samples from normal healthy livers. Oxidative phosphorylation showed significantly better results for HMP compared to standard cold-storage. Also, LDH release in UW was below our detection limit of 0.25 U/ml and tissue LDH content remained similar in all groups indicating that cellular cell membranes remained intact during CS and machine preservation.

In conclusion, optimal perfusion pressures for hypothermic machine preservation of the liver were determined and correspond with 25% of normal physiologic liver perfusion at 37 °C. Perfusion at 12.5% showed underperfusion and perfusion at 50% showed endothelial injury. HMP at 25% showed significantly lower PI counts than with static cold-storage, staining predominantly nonparenchymal cells. Hypothermic machine preservation of the liver can be performed at a mean pulsatile arterial perfusion pressure of 25 mmHg and a continuous portal perfusion pressure of 4 mmHg to achieve good cortical perfusion, minimize endothelial cell death and allow the generation of high ATP levels reflecting good donor liver viability.

## Acknowledgement

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