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ORIGINAL ARTICLE

In vivo quantification of oxygen-free radical release in experimental pancreas transplantation

Hannes P. Neeff,* Ernst von Dobschuetz,* Olaf Sommer, Ulrich T. Hopt and Oliver Drognitz

Department of General and Digestive Surgery, Transplantation Surgery, University Hospital Freiburg, Freiburg, Germany

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Correspondence
Hannes P. Neeff, Department of General and Digestive Surgery, Transplantation Surgery, University Hospital Freiburg, Hugstetterstr. 55, 79106 Freiburg, Germany. Tel.: +49 761 270 2806; fax: +49 761 270 2804; e-mail: hannes.neeff@uniklinik-freiburg.de

*Both authors contributed equally to this publication.

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Summary

Reactive oxygen species (ROS) were drawn to the attention in the setting of organ transplantation when the 'injury hypothesis' postulated a link between oxidative stress and the activation of the innate immunity of the recipient. While the occurrence of ROS during organ transplantation is undoubted, their onset and magnitude remain largely unknown. We therefore measured ROS using a novel cyclic hydroxylamine spin probe CMH (1-hydroxy-3- methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine) during syngeneic experimental pancreas transplantation in rats *in vivo*. Organs were subjected to two different cold preservation methods [University of Wisconsin preservation solution (UW) or normal saline] for 18 h. During the first 90 min of reperfusion, samples were collected and analysed using electron paramagnetic resonance signalling. Isolated blood-free perfused organs (IPO) were used for comparison. Analysis showed that it is feasible to detect ROS using CMH spin probes. While IPO organs displayed a very early ROS release, there was no ROS increase in the UW preserved group compared to NaCl. These findings were in line with conventional markers of organ damage such as serum lactate, glucose, potassium as well as tissue ATP levels. CMH spin probes might become a useful tool for the *in vivo* animal testing of antioxidative substances in models of solid organ transplantation.

Introduction

Two main problems remain in solid organ transplantation since it became the standard of care in various chronic diseases (e.g. chronic renal failure, type I diabetes) in the past decades: organ preservation and immunosuppression. In pancreas transplantation, today there is still considerable graft loss due to rejection [1,2]. This shortcoming has been addressed in two different ways. One is the improvement of organ procurement and preservation [3,4], the other is the introduction of new immunosuppression protocols [5,6]. Despite these efforts, organ preservation and immunosuppression were – in most cases – dealt with separately until the 'injury hypothesis' was formulated in 1994 by Land *et al.* [7]. Reactive oxygen species (ROS) were identified as key factors influencing outcome in solid organ transplantation by activating the innate immunity of the recipient. Possible sources of these reactive substances are the process of organ procurement, the time of cold organ storage, as well as the reperfusion period [8]. In general, oxidative reactions are facilitated during reperfusion (reoxygenation) whereas antioxidative pathways are inhibited mainly during hypoxia. Regardless of their site of origin, ROS generated during hypoxia/reoxygenation can lead to both direct and indirect cell damage. Lipid peroxidation for instance is caused by a chain reaction of a fatty acid with $^{\bullet}OH$ forming peroxyl radicals ($ROO^{\bullet}$). This in turn leads to a generation of lipid hydroperoxides (LOOH) and in the presence of trace amounts of iron to alkoxyl radicals ($RO^{\bullet}$) [9]. Peroxidation of the lipid bilayer of cellular membranes causes a rapid change in membrane permeability, the activity of membrane receptors and membrane bound enzymes, leading to severe dysfunction or cell death. Proteins in general and to a lesser extent nucleic acids are very susceptible to direct ROS-mediated damage. The reason for this is not only their sheer abundance in biological systems but also

their high rate constant for reaction with ROS [10]. While the single factors mentioned above have been thoroughly investigated, little is known about the overall effect of ROS in solid organ transplantation. So far, this has been looked at as a phenomenon occurring at a very early stage in transplantation – the reperfusion period. The question was raised if ROS-induced damage also extends into later stages of graft injury. Today, growing evidence supports the 'injury hypothesis' and the subsequent activation of the so-called innate immunity [11] leading to graft injury far beyond the reperfusion period itself. To prevent ROS-mediated ischaemia reperfusion damage and subsequent organ dysfunction, antioxidative strategies have been introduced into clinical practice. For the most part, these include antioxidative additives such as glutathione, allopurinol or histidine. Experimental trials have also proven the efficacy of using other antioxidants (e.g. *N*-acetylcysteine, L-arginine) administered to the recipient before reperfusion [12–14]. However, clinically used antioxidative strategies aimed at treating the recipient are only in the trial phase [7]. Because of the complexity of ROS-mediated injury, it becomes evident that not only proper targeting but also – because of the short-lived nature of ROS – proper timing of antioxidative therapy is mandatory. As data are lacking about the onset and magnitude of ROS release after reperfusion in the transplantation setting, we conducted this study on *in vivo* ROS release during experimental pancreas transplantation. A novel spin probe CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine) was used in conjunction with EPR [electron paramagnetic resonance – also called electron spin resonance (ESR)] to quantify dynamically total ROS release [15–18].

Materials and methods

All experiments were carried out in accordance with current German Animal Protection Law and were approved by the local governmental Animal Care and Use Committee. Male inbred Lewis rats (330 g) (Charles River WIGA, Sulzfeld, Germany) were used as donors and recipients. We used a modified technique [19] of syngeneic heterotopical pancreaticoduodenal transplantation firstly described by Lee *et al.* [20].

Anaesthesia

After induction with isoflurane (Forene; Abbott GmbH, Wiesbaden, Germany), spontaneously breathing rats were placed in a supine position on a heating pad (Effenberger, Med. Tech. Geraetebau, Pfaffing, Germany) to maintain a body core temperature of 36.5 °C throughout the experiment. Anaesthesia was maintained using 0.8–1.4 vol% of isoflurane in 100% oxygen, delivered using a Vapor 19.1 vaporizer (Draeger AG, Lübeck, Germany). Polyethylene catheters (ID 0.50 mm; Portex, Hythe, UK) were inserted into the left carotid artery and right internal jugular vein of both the donor and the recipient for continuous monitoring of arterial blood pressure (Sirecust 404-1; Siemens, Munich, Germany) and for continuous substitution of Ringers solution (4 ml/h) (Delta-Select GmbH, Pfullingen, Germany) via a syringe pump (Secura, B. Braun AG, Melsungen, Germany). In the recipient, continuous administration of the spin probe CMH (Noxygen Science Transfer & Diagnostics, Denzlingen, Germany) was also performed via the venous access. Arterial blood pressure was kept within normal limits of a mean arterial pressure (MAP) between 60 and 80 mmHg throughout procurement and transplantation. If necessary, volume depletion was substituted by 1-ml aliquots of Ringer's solution.

Procurement procedure

All surgical procedures were carried out under a microscope (OPMI1-FC, Carl Zeiss, Oberkochen, Germany) with 2.5–5× magnification. The pancreas was prepared through a midline incision skeletonizing it on an aortic segment together with the celiac trunk and the superior mesenteric artery. Venous outflow was provided by the portal vein. Care was taken not to subject the organ to longer periods of warm ischaemia (<1 min).

Organ preservation

After systemic administration of 300 U of heparin/kg body weight (Hoffmann-La Roche, Grenzach-Wyhlen, Germany), the procured organ was flushed at a hydrostatic pressure of 100 mmHg with either ice-cold normal saline (4 °C) (B. Braun AG) or University of Wisconsin preservation solution (UW) (4 °C) (Bristol-Myers Squibb GmbH, Munich, Germany). Special care was taken in using the UW solution. It was taken fresh daily from a bag containing aliquots of 100 ml of the solution, which was kept dark and cold until use to prevent any changes in glutathione concentration. The adjuncts required for using UW as Belzer's solution in a human setting, namely dexamethasone, insulin and penicillin, were not added in this set of experiments. After procurement, the organs were kept wrapped in gauze in a sterile tube in either normal saline or UW solution at 4 °C for 18 h.

Recipient operation

The recipient operation was carried out under the same perioperative circumstances in spontaneously breathing animals. In addition, a second arterial access was established using the femoral artery for blood pressure moni-

toring. The carotid artery was used for repeated blood sampling before and after reperfusion. After clamping of the aorta and V. cava, the pancreatic graft was anastomosed to the infrarenal aorta and the infrarenal vena cava respectively using 9/0 Nylon continuous running sutures (Serag Wiessner, Naila, Germany).

Isolated blood-free perfused organs

For reference, one set of organs ($n = 5$) was procured as described above. After procurement, the organs were not subjected to cold ischaemia, but were single-pass perfused *in vitro* in a heated perfusion chamber (37.5 °C) with cell-free-modified Krebs–Henseleit solution (pH 7.4) prepared according to Wang *et al.* [21] containing (in mM) glucose 5.5, NaCl 120, KCl 4, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, 6.5 g/l bovine serum albumin, and 36 g/l dextran. After a stabilization phase, perfusion was stopped for 30 min ('ischaemic' phase). ROS release was noted during the first 30 min at 1- and 5-min intervals.

Spin probe analysis

For spin probe analysis, a MiniScope MS 200 EPR device (Magnettech Ltd., Berlin, Germany) was used at the following settings: microwave frequency: 9.3–9.55 GHz, microwave: 20 mW, centre field: 335.9 mT, sweep: 4.8 mT, modulation amplitude: 0.1 mT, receiver gain: 500.

Data were collected and plotted using MiniScope Analysis Software (Magnettech Ltd). Data were recorded as arbitrary units [22]. ROS values are therefore given as a percentage change in total ROS with regard to prereperfusion baseline levels.

Fifteen minutes before completion of the anastomoses, spin probe CMH was added to the continuous venous infusion to achieve a stable state (5 mM CMH stock solution in phosphate-buffered saline; pH 7.4, 50 mmol; containing 50 μM desferoxamine). After application of a 1-mg bolus, CMH solution was given at a rate of 80 μg/kg/min. To minimize auto-oxidation, CMH solution was prepared fresh shortly before use and kept in a passively chilled syringe during the time of application. Likewise CMH was also added to the single-pass perfusion set-up for *in vitro* perfusion experiments.

The achievement of a stable state of continuous application of CMH was investigated in a separate set of experiments with nonoperated anaesthetized animals after establishing venous and arterial access as described above. Arterial blood samples as well as urine samples were collected.

Before and after restoring arterial blood supply to the transplanted organ, blood samples were taken in 50-μl glass capillaries. The time points are shown in Fig. 1. Samples were immediately put on ice. The time between collection and processing was kept to a maximum of 10 min. Additionally, arterial blood gas (ABG) samples were taken at the aforementioned time points. After 90 min, the animals were killed by exsanguination and pancreatic tissue was snap-frozen in liquid nitrogen. Native pancreata of each animal were also collected for reference.

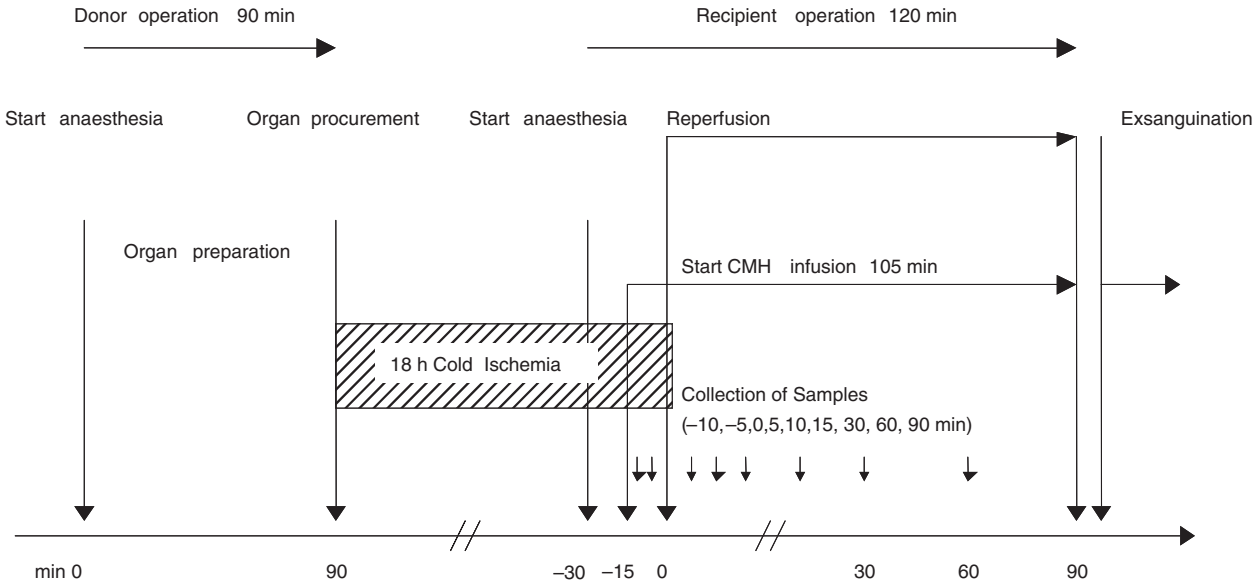

Figure 1 Timeline. Blood sampling at –10, –5, 0, 5, 10, 15, 30, 60 and 90 min (black filled arrows) during recipients' operation.

Measurement of myeloperoxidase

Sections of snap-frozen pancreatic tissue were taken from the transplanted organs of animals in both treatment groups (UW and NaCl) as well as from their native pancreas. After homogenization of the pancreatic tissue, myeloperoxidase (MPO) levels were analysed according to the method described by Fujimoto *et al.* [23]. Measurements were read with a spectrophotometer at 470 nm (Spectronic Genesys 5, Leeds, UK) for 1 min. The data were quantified using an MPO calibration curve [MPO from human polymorphonuclear (PMN) leucocytes: Calbiochem 475911, Merck KGaA, Darmstadt, Germany] and verified using an MPO-inhibitor (Calbiochem 475944, Merck KGaA). MPO levels are given in mU per mg protein.

Measurement of adenosine triphosphate

Analysis of adenosine triphosphate (ATP) levels was carried out using a bioluminescence assay for quantitative determination of ATP (ATP determination kit; Biaffin, Kassel, Germany) as follows: 100 mg of frozen pancreatic tissue was homogenized in chlorous acid ($HClO_2$; 0.6 M) and neutralized in potassium carbonate (K_2CO_3, pH 6.5–7.5). D-Luciferin was then oxidized in an ATP-dependent process, catalyzed by luciferase generating chemoluminescence at 560 nm (Microplate Luminometer MPL1; Berthold, Pforzheim, Germany). ATP levels are given as ng per mg protein.

Arterial blood gas analysis

Arterial blood gas analyses were carried out using a Radiometer Copenhagen ABL 715 unit (Radiometer GmbH, Willich, Germany). Blood samples (35 µl each) collected in glass capillaries were tested for haemoglobin/haematocrit, potassium, sodium, calcium, chloride, lactate, glucose and pH levels.

Groups

There were three groups of animals: NaCl ($n = 5$), UW ($n = 5$), isolated blood-free perfused organs (IPO) ($n = 5$). The detection of CMH and $CM^{\bullet}$ + CMH in plasma and urine was performed in a separate set of experiments.

Statistical analysis

All data were analysed using SIGMA STAT 3.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean values ± standard error of the mean (SEM). Values of $P \leq 0.05$ were considered significant. All data were first proven to fit the assumption of normality. Differences within a treatment group were tested by a repeated measurement analysis of variance (ANOVA), followed by the method of Holm–Sidak. Differences between treatment groups were tested using Student's *t*-test.

Results

CMH, stable state and excretion

Urine and plasma levels of CMH as well as $CM^{\bullet}$ + CMH were measured. A stable concentration of CMH and $CM^{\bullet}$ + CMH was noted after 15 min in the anaesthetized rat (Figs 2 and 3). The total concentration of $CM^{\bullet}$ + CMH in our samples was obtained by adding potassium superoxide (KO_2) to the reaction, thereby oxidizing any present CMH to $CM^{\bullet}$.

Organ transplantation

Five animals in each group (NaCl, UW) were included in this analysis. Any animals with gross signs of bleeding after reperfusion were excluded from further analysis. We analysed the changes of ROS after reperfusion with regard to the baseline values before reperfusion. Arbitrary units of ROS before reperfusion were averaged for each animal and set as 100%. This was referred to as 0 min. Any changes after reperfusion are therefore given as per cent of baseline values.

After reperfusion, there was a statistically significant increase in total ROS production in transplanted NaCl-preserved organs after 60 min of reperfusion (127.2 ± 5.6% of baseline; $P < 0.001$). In the early reper-

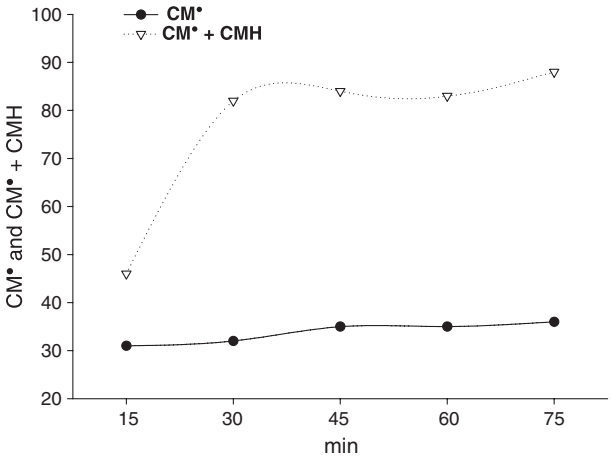

Figure 2 Renal clearance of $CM^{\bullet}$ and CMH causes a stable state of $CM^{\bullet}$ (filled black dots) and $CM^{\bullet}$ + CMH (open inverted triangles) in plasma of an anaesthesized rat.

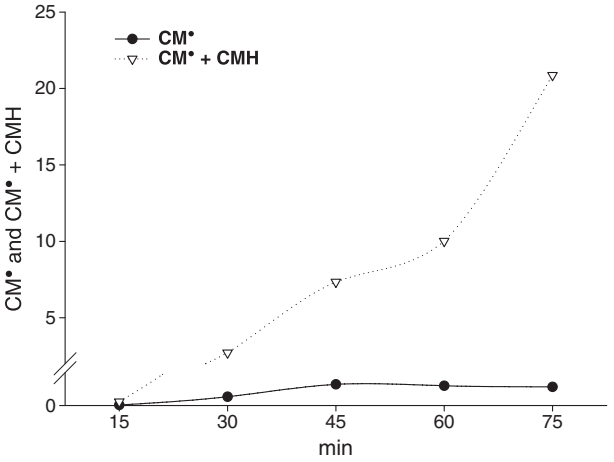

Figure 3 Renal clearance of CM• and CMH causes a stable state of CM• (filled black dots) and CM• + CMH (open inverted triangles) in urine of an anaesthesized rat during 60 min of infusion.

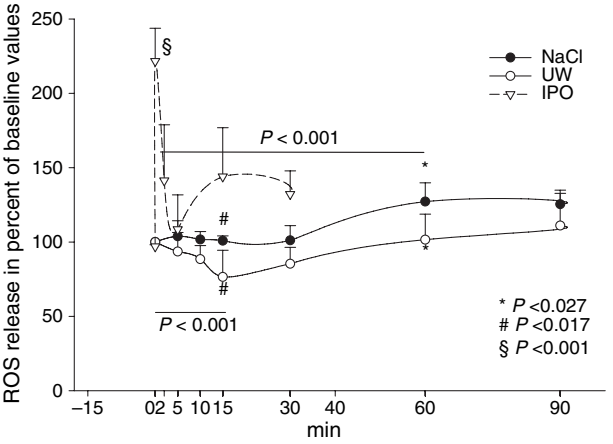

Figure 4 NaCl (filled black dots) versus UW (University of Wisconsin solution) (open white dots) preservation versus IPO (isolated blood-free perfused organs) (open inverted triangles) for reference (30 min of reperfusion). Percentage change of total ROS measured with CMH spin probe over a time period of 90 (30) min after reperfusion. There is a significant increase of total ROS in the NaCl group after 60 min ($P < 0.001$). Also, there is a significant decrease of total ROS in the UW group 15 min after reperfusion ($P < 0.001$). Total ROS levels are significantly different between groups at two time points (15 min (#$P < 0.017$) and 60 min (*$P < 0.027$)). In a blood-free environment, peak ROS release is after 1 min (§$P < 0.001$). Each group $n = 5$, ANOVA followed by Holm–Sidak method. Student's *t*-test between groups.

fusion period (5, 10 and 15 min), no significant change was observed in ROS production. The organs treated with UW solution showed a decreased total ROS production as early as 5 min after reperfusion (93.59 ± 5.7% of baseline; NS). This difference was statistically significant after 15 min (76.52 ± 8.0% of baseline; $P < 0.005$) as well as compared to the NaCl group (76.52 ± 8.0% vs. 100.92 ± 1.4%; $P < 0.005$). After 60 min of reperfusion, there was also a significant difference between the two groups (101.56 ± 2.4% vs. 127.2 ± 5.6%; $P < 0.001$). Notably, ROS levels in the UW groups had reached baseline values within 60 min, displaying a nonsignificant increase of total ROS only after 90 min of reperfusion. Results are displayed in Fig. 4.

Arterial blood gas analysis revealed a marked difference between the two transplanted groups (UW versus NaCl) with regard to glucose (290 ± 39 vs. 381 ± 14 mg/dl; $P < 0.05$) and lactate (3.4 ± 0.5 vs. 6.3 ± 0.9 mmol/l; $P < 0.028$) concentration. Serum potassium levels were significantly different in the NaCl-treated group before and after reperfusion (3.72 ± 0.4 vs. 4.5 ± 0.6 mmol/l; $P < 0.04$). This difference did not reach statistical significance compared to UW-treated organs. There was a statistically significant difference in haemoglobin levels between groups. Compared to baseline values, haemoglobin levels were higher in UW-preserved organs (9.4 ± 1.6 vs. 12.8 ± 0.9 mg/dl; $P < 0.01$). This difference is also reflected in two other markers of tissue damage: tissue levels of ATP and MPO. ATP concentration was statistically significantly lower in NaCl preserved organs compared with controls (475 ± 124 vs. 929 ± 168 ng/ml; $P < 0.05$). On the other hand, ATP levels were significantly elevated compared to controls in the UW group (1503 ± 163 vs. 929 ± 168 ng/mg; $P < 0.05$). Differences between treatment groups (NaCl versus UW) were also statistically significant (475 ± 124 vs. 1503 ± 163 ng/mg; $P < 0.002$). Compared to control values from the native organs, MPO levels were higher in NaCl-treated organs as well as UW-preserved organs (100 vs. 42.6 vs. 66.5 mU/mg). Because of a high variability of MPO concentration, results did not reach the level of statistical significance. Results are displayed in Fig. 5. Differences in all other markers tested (sodium, calcium, chloride and pH) were not statistically significant (data not shown).

Isolated blood-free perfused organs

For reference, we included data from five isolated blood-free perfused pancreata, which were procured in the same way as previously described. They were perfused with a modified Krebs–Henseleit buffer solution as described above in the absence of blood or plasma in a single-pass heated perfusion chamber after an 'ischaemic' time of no perfusion for 30 min. These isolated organs displayed marked ROS release during the first minute of reperfusion with a peak at 221 ± 22.2% after 1 min ($P < 0.001$) stabilizing at baseline levels after 5 min (Fig. 4).

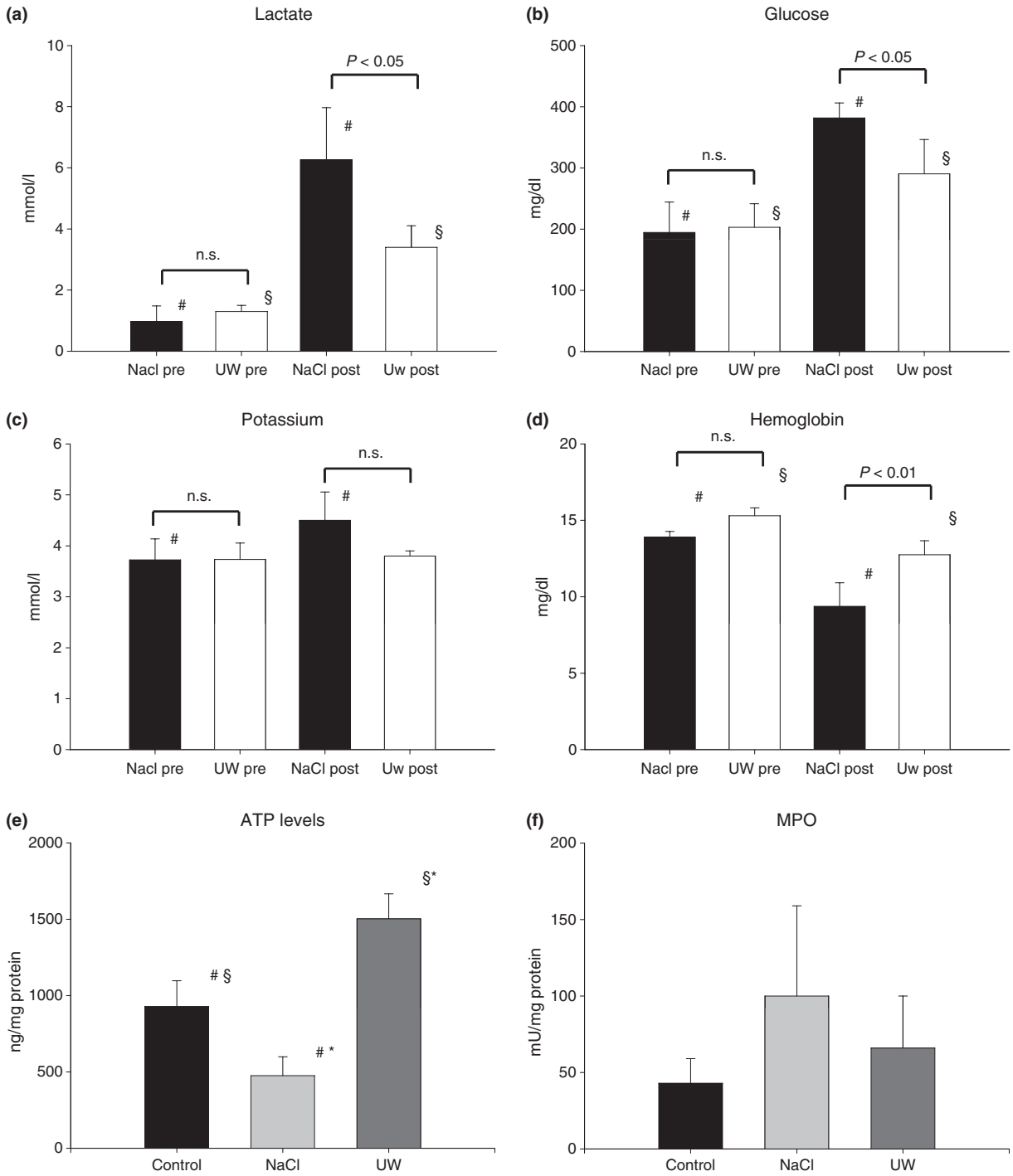

Figure 5 (a) Serum lactate levels in mmol/l (NaCl versus UW pre- and postreperfusion). Statistical difference postreperfusion between groups (see bar) and pre- and postreperfusion within groups (#, §$P < 0.05$). (b) Serum glucose levels in mg/dl (NaCl versus UW pre- and postreperfusion). Statistical difference postreperfusion between groups (see bar) and pre- and postreperfusion within groups (#, §$P < 0.05$). (c) Serum potassium levels in mmol/l (NaCl versus UW pre- and postreperfusion). Statistical difference in NaCl-preserved organs pre- and postreperfusion (#$P < 0.04$) No statistical difference between groups (NS). (d) Haemoglobin levels in mg/dl (NaCl versus UW pre- and postreperfusion). Statistical difference postreperfusion between groups (see bar) and pre- and postreperfusion within groups (#, §$P < 0.01$). (e) Tissue ATP levels at the end of experiment in ng/mg (control versus NaCl versus UW) (§$P < 0.05$, #$P < 0.05$, *$P < 0.002$). (f) Tissue MPO levels at the end of experiment in mU/mg (control versus NaCl versus UW) (NS).

Discussion

In vivo real time detection of ROS has proven to be a challenging task [24]. Until today, little is known about *in vivo* ROS release during the early ischaemia reperfusion period after transplantation. In this study, we showed that it is feasible to measure total ROS using a spin probe technique in combination with EPR in an experimental animal model of organ transplantation. Major care was taken to minimize the damage to the pancreas during procurement, as the generation of ROS is known to occur even at this early stage of a transplantation procedure [25]. We therefore closely followed the technique described by our group [19] which ensures that only minimal changes in microcirculation are noted after transplantation of a well-preserved organ with short ischaemic time. To achieve clinically relevant times of ischaemia, the cold storage period for organs in this study was lengthened to 18 h. This time span has been found to have a marked effect on organ viability after reperfusion [26]. We used normal saline (NaCl) for reference, because this clinically unused form of organ preservation has been shown to have detrimental effects on organ function [27].

The cyclic hydroxylamine CMH was chosen as spin probe because of its theoretically beneficial profile for dynamic *in vivo* measurement of ROS: the distinct advantage of cyclic hydroxylamines over nitrone spin traps such as DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) is their fast reaction with superoxides ($\sim 10^4$ M-1s-1) compared to that of nitrone spin traps ($\sim$55 M-1s-1) [28]. This allows for dynamic detection during the first seconds to minutes of reperfusion. In contrast to nitrone spin traps, CMH forms a stable nitroxide over the time of several hours even in the presence of antioxidants (e.g. ascorbic acid, GSH, cysteine). Only minimal biodegradation to EPR silent hydroxylamines or oxidation to secondary nitrones is noted with CMH [29,30]. CMH is i.v. applicable in rodents without any observable haemodynamic changes. After continuous i.v. administration of CMH, a stable state was reached and the substance was renally excreted (Figs 2 and 3).

Because of their high lipophilicity, intra- as well as extracellular ROS can be detected using cyclic hydroxylamines [31]. While the detection of ROS by cyclic hyroxylamines such as CMH is highly sensitive, its lack of specificity must be overcome by using superoxide dismutases or inhibitors of $O_2^{\bullet}$-production by NADPH oxidase, uncoupled eNOS (NOSIII), xanthine oxidase, apocynin, L-NAME, or oxypurinol [32,33]. This however was not the goal of this study.

After organ preservation in normal saline (NaCl), a vast increase of ROS was expected during the first minutes after reperfusion. However, this was only noted after 60 min. These results are in sharp contrast to our findings with isolated blood-free perfused pancreata which displayed a marked increase in total ROS release as early as 1 min after reperfusion (Fig. 4). This also holds true, for example, for the isolated perfused kidney [22]. We conclude that the absence of blood in the *in vitro* setting unmasks the extent of ROS release taking place during the reperfusion period. But even in the presence of blood, an increase in total ROS after reperfusion has been described by Pincemail *et al.* in human kidney transplantation [34]. These changes could be observed in the renal vein until 30 min after reperfusion. The early detection of ROS in the aforementioned study might be attributed to the fact that patients with end-stage renal disease suffer from a change in the redox state of blood [35,36] leading to decreased total antioxidative capacity.

Contrary to the late changes in ROS release after 60 min found with NaCl-preserved organs, we did not observe any increase in ROS during the first hour of reperfusion using UW-preserved grafts, suggesting a protective effect of this storage method also with regard to oxidative stress.

University of Wisconsin preservation solution is one of the most common organ preservation solutions which can be equally used for kidney, liver and pancreas preservation [3,37]. Originally, designed by F.O. Belzer *et al.* to address the issue of cold organ storage during the early years of organ transplantation, its current form, known as UW Belzer solution, was formulated in 1987 [38]. In addition to high levels of potassium (potassium-hydroxide 56%, 14,5 g/l; 100 mM and potassium-dihydrogenphosphate, 3,4 g/l, 25 mM) UW contains – apart from other ingredients – glutathione (0.922 g/l; 3 mM), allopurinol (0.136 g/l; 1 mM) and hydroxyl-ethyl-starch (50 g/l) as well as adenosine (1.34 g/l; 5 mM). Glutathione and allopurinol are well-known antioxidants. Especially, allopurinol is known to attenuate ROS production by inhibition of xanthine oxidase [39,40]. As the samples were taken from whole blood, UW washout after reperfusion might account for this additional antioxidative capacity of the recipient's whole blood, leading to a wholly attenuated ROS production when compared to a mere NaCl washout. On the other hand, serum potassium levels were not altered after UW treatment, excluding any substantial change of the recipient's homeostasis caused by UW washout.

Conventional markers of organ damage such as serum lactate, serum glucose and serum potassium levels showed a significant increase only after the reperfusion of NaCl-preserved organs, indicating a higher degree of cell damage.

Apart from its antioxidative potential, UW was designed to overcome the lack of substrates during the

cold ischaemic period. Adenosine, for instance, led to a better regeneration of ATP during the first 60–120 min after reperfusion in several studies [41–43]. This was also shown in the harvested organs after 90 min of reperfusion in our series.

Tissue MPO concentration was lower in the UW group. While PMN leucocytes are undoubtedly involved in organ damage after pancreas transplantation [43], differences in MPO concentration did not reach the level of statistical significance in this study ($P < 0.058$).

This study shows that it is feasible to detect systemically ROS in an experimental setting of whole organ transplantation in rats. ROS release was attenuated by a clinically used preservation solution (UW). These findings were in line with conventional markers of organ damage (lactate, glucose, potassium, ATP). While there is a renaissance of antioxidative therapy in organ transplantation, this method provides an important tool for the *in vivo* testing of antioxidative substances in a clinically relevant model.

It has to be pointed out that the use of CMH spin probes in the human setting is not an option at present, although it has been tried in large animals. The study is also limited by the fact that the total antioxidative capacity of the recipient's blood in the human transplantation setting (especially in renal failure but also in diabetes mellitus) is most likely different from that in healthy animals.

Authorship

HPN: performed research, collected data, analysed data and wrote the paper. EVD: designed research, performed research and analysed data. OS: performed research and collected data. UTH and OD: designed research.

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