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ORIGINAL ARTICLE

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Kidney transplantation from non-heart-beating donors after oxygenated low-flow machine perfusion preservation with histidine–tryptophan–ketoglutarate solution

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Abstract The aim of this study was to determine the potential benefit of aerobic machine preservation (MP) with non-colloidal histidine–tryptophan–ketoglutarate (HTK) solution compared with MP with Belzer machine perfusion solution (MPS) and standard cold storage, after marginal kidneys had been obtained from non-heart-beating donors. Cardiac arrest was electrically induced in anaesthetized German landrace pigs (20–25 kg bw). Their kidneys were harvested 40 min thereafter, flushed with HTK by gravity of 100 cm H_2O via the renal artery and then stored in HTK for 18 h at 4°C. Other organs were subjected to oxygenated ($pO_2 > 500$ mmHg) hypothermic pulsatile low-flow machine perfusion with HTK or MP with Belzer MPS at $P_{max} = 40$ mmHg, yielding transrenal flow values of 0.2–0.3 ml/min per g with HTK and approximately twice that amount with Belzer MPS. A well-preserved vascular endothelium and intact tubular epithelium were documented by electron microscopy at the end of perfusion preservation in both solutions as well as after cold storage. Concentrations of ATP (in micromoles per gramme) in tissue homogenates at the end of perfusion preservation with HTK were 1.18 ± 0.12 vs 0.16 ± 0.02 ($P < 0.05$) after simple cold storage and 2.43 ± 0.23 after perfusion with Belzer MPS, thus documenting a relevant effect of low-flow perfusion on tissue oxygenation. Viability of the grafts was followed for 1 week after heterotopic transplantation and bilateral nephrectomy in the recipient pigs. Machine perfusion with HTK significantly improved cortical microcirculation upon early reperfusion in vivo, as well as maximal serum levels of urea and creatinine, compared to recipients receiving cold-stored grafts. No differences could be found between MP with HTK or Belzer MPS. In conclusion, provision of oxygen during storage is possible by low-flow perfusion with HTK as with Belzer MPS and apparently improves graft viability after transplantation.

Keywords HTK solution · Machine perfusion · Oxygen · Preservation · Kidney · Transplantation · Non-heart-beating donor · Viability

Introduction

In view of the shortage of donor organs, retrieval of kidneys from non-heart-beating donors (NHBDs) has become widely accepted as a clinical routine to increase the number of grafts available for kidney transplantation [1, 2, 3]. Although the clinical outcome of these grafts, in terms of graft and patient

survival, has consistently been comparable to that of kidneys from heart-beating donors [1, 2, 4], an increased incidence of delayed graft function [5], as well as acute rejection episodes, [6] has been documented. Thus, any improvement in the preservation of grafts from donors with compromised circulation, or even from non-heart-beating donors, represents a valuable increase in the total number of viable donor organs available for transplantation. Pulsatile machine perfusion has been successfully introduced for the preservation of damaged donor kidneys [7, 8], allowing for viability assessment of damaged kidneys prior to transplantation and providing improved early and long-term renal function after transplantation [9, 10]. However, machine perfusion preservation is commonly performed with Belzer machine perfusion solution (MPS) [11], which is similar to the University of Wisconsin (UW) cold storage solution but is adapted to the requirements of oxygenated long-term perfusion of the graft. The Belzer MPS has a peculiar drawback in organ procurement from non-heart-beating donors because of its high viscosity at 4°C, which has been shown to preclude optimal microvascular tissue perfusion upon graft retrieval after the heart has stopped beating [12, 13]. Moreover, hydroxyethyl starch (HES) contained in the Belzer solution may provoke accelerated aggregation of erythrocytes, further deteriorating wash out of donor grafts [14].

Accordingly, kidneys from NHBDs have already been flushed initially with HTK, the viscosity of which is very near to that of water, before they are put on machine perfusion with Belzer's solution [2]. Experimental data, however, are suggesting that the use of crystalloids prior to storage with UW preservation solution may abrogate the protective effects of the latter [15]. Thus, the use of HTK for both initial flush out upon harvesting and subsequent long-term preservation by machine perfusion has been proposed, in order to minimize concentration shifts of electrolytes and substrates at the cellular level [16]. Previous data from our laboratory have shown that liver preservation [17] by continuous machine perfusion with HTK appeared equally effective as with Belzer MPS, both maintaining parenchymal viability and mitigating vascular expression of ICAM-1 and MHC-II complexes upon reperfusion. While the liver seems easily to support, or even benefit from, non-colloidal perfusion media, possibly due to rheological advantages of the low viscosity of saline solutions, the use of colloid has often been emphasized for hypothermic perfusion of the kidney [11, 18]. Our aim was, therefore, to assess the possible benefit of aerobic preservation of damaged kidneys by low-flow machine perfusion with HTK at high oxygen partial pressure.

Materials and methods

Kidney retrieval and storage

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.

German Landrace pigs, weighing between 20 kg and 25 kg, were starved for 24 h prior to the experiments. General anaesthesia was induced with ketamine and xylazine and maintained after incubation by mechanical ventilation with isoflurane in nitrous oxide/oxygen (2:1). Cardiac arrest was induced electrically. After 40 min of cardiac standstill the right kidney was removed from the donor and flushed via the renal artery with HTK solution.

Organ preservation

Kidneys from the control group ($n = 5$) were flushed by gravity (100 cm H_2O) and subjected to static cold storage at 4°C in 1 l of HTK solution for 18 h.

Other grafts were then subjected to oxygenated ($pO_2 > 500$ mmHg) pulsatile machine perfusion via the renal artery with modified HTK or Belzer MPS at 6°C–8°C.

The grafts were placed in a thermostatically cooled bath containing 1 l of the respective preservation solution, which was circulated by a roller pump, through an oxygenator, made of 8 m thin-walled silicon tubing in an atmosphere of medical-grade oxygen, and a bubble trap, finally to enter the kidney via the cannulated renal artery. Pulsatility was obtained by alternating compression of an air chamber incorporated into the arterial line. Hydrodynamic pressure at the renal artery was measured online with an electromechanical pressure transducer connected to the renal inflow tubing. For machine perfusion, 1 l of HTK was supplemented with glucose (1 g), ampicillin (0.5 g), heparin (5,000 U) and the osmolyte taurine (3 g). After 1 h to 2 h of stabilization, transrenal flow was adjusted to produce a pulsatile pressure pattern of approximately 40/20 mmHg at 50 cycles/min, and the kidneys were left overnight.

Recipient operation and kidney transplantation

Recipient animals were starved for 24 h prior to the operation but had free access to tap water. Surgery was started with the cannulation of the right jugular vein with a PE catheter for infusion and later collection of blood samples. Via a midline approach, both native kidneys were removed and the graft was transplanted

heterotopically into the groin. Vascular anastomoses were performed end to side (renal vein–vena cava) and end to end (renal artery–iliac artery), respectively, with 6-0 running sutures. Prior to the clamping of the iliac artery for completion of the arterial anastomosis, 3,000 IU of heparin were injected to prevent vascular thrombosis. The ureter was cannulated and drained with PE tubing. Reperfusion was established by the release of the venous clamp and then the arterial clamp, followed by infusion with 50 ml–100 ml of mannitol 20% to induce osmotic diuresis.

Renal tissue perfusion 10 min after reperfusion was assessed non-invasively as mean erythrocyte flux, determined by laser Doppler flowmetry (blood flow monitor DRT4, Moor Instruments, Axminster, England) detailed previously [19]. To account for temporal variations in blood flow, we calculated the mean flux value over 10 s of recording, and in order to eliminate the influence of regional heterogeneity, we performed measurements on four distinct places of the renal surface. All flux measurements were taken in each individual animal as percent variation from the baseline value obtained from the non-ischaemic native kidney.

Postoperatively, the animals were given 1 l of saline solution via the jugular catheter and had free access to water; standard food was provided on the next morning.

The recipients underwent immunosuppression with continuous doses of FK506 (5 mg/day) and a single dose of methylprednisolone (250 mg i.v.) on the day of the operation. Anti-thrombosis therapy was provided by 5,000 U of heparin, given on a daily basis. Antibiotic treatment consisted of the perioperative application of 500 mg ampicillin (i.v.) and daily doses of 2×200 mg during the postoperative period.

Renal function was assessed by serial blood urea and creatinine measurements. Total urine output was measured every day after transplantation.

Tissue preparation and biochemical analysis

For electron microscopy, tissue samples were fixed with 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide and embedded in Epon 812. Semi-thin sections were stained with methylene blue and Azure II as described by Richardson et al. [20]. Ultra-thin sections were stained with uranyl acetate and lead citrate and were examined with a Zeiss EM 900 electron microscope (Leo, Oberkochem, Germany).

Tissue specimens for assessment of metabolic status were taken with a cooled clamp from the perfused kidney and were immediately immersed in liquid nitrogen. They were then transferred in a vacuum freezer. After tissue water had been evaporated during at least 5 days of freeze drying, the samples were weighed to determine total dry weight. Tissue content of high-energy phosphates was determined by standard enzymatic tests, as described previously [21].

Statistics

Results are expressed as the means ± standard error of five measurements per observation point unless otherwise indicated. Statistical significance of differences was assessed by non-parametrical comparison, by the Mann–Whitney U test.

Results

Following 40 min of warm ischaemia due to cardiac standstill of the donor, preservation by hypothermic perfusion with HTK showed intra-renal resistance (IRR) values of 1.26 ± 0.22 mmHg min g per ml, ensuing in a mean renal flow of approximately 0.2 ml/g per min. In contrast, the use of Belzer MPS resulted in an IRR of only 0.63 ± 0.04 mmHg min g per ml and increased the transrenal flow by a factor of 2. Nonetheless, while avoiding systolic pressure values exceeding 40 mmHg, in our hands vascular resistance did not rise during the 18 h of oxygenated machine perfusion preservation in either group.

Renal tissue oxygenation provided by the low-flow perfusion preservation was assessed by the determination of parenchymal levels of ATP at the end of the preservation period, in supplementary experiments. While simple cold storage resulted in mean ATP tissue levels of 0.16 ± 0.02 μmol/g, energy status was significantly improved after oxygenated low-flow perfusion with HTK (1.18 ± 0.12 μmol ATP/g; $P < 0.001$) or after MP with Belzer's solution (2.43 ± 0.23 μmol ATP/g; $P < 0.001$).

The possible impact of non-colloidal machine perfusion on renal ultrastructure was investigated by electron microscopy from tissue slides, also taken at the end of the preservation period.

Tubular epithelial cells and glomeruli were as well preserved after cold storage as after machine perfusion with HTK as well as Belzer MPS. Mitochondria were also of normal appearance in both groups. Glomerular capillary endothelium was found to be rather undisturbed by the hypothermic low-flow perfusion with HTK, and there were no signs of endothelial detachment, but structural integrity of the endothelium was equally preserved at the end of simple cold storage (Fig. 1).

Microcirculatory tissue perfusion upon renal reperfusion in vivo showed, however, significant differences between the groups: cortical erythrocyte flux averaged $101 \pm 18\%$ (HTK) and $101 \pm 2\%$ (Belzer MPS) of controls in perfusion-preserved kidneys, respectively repre-

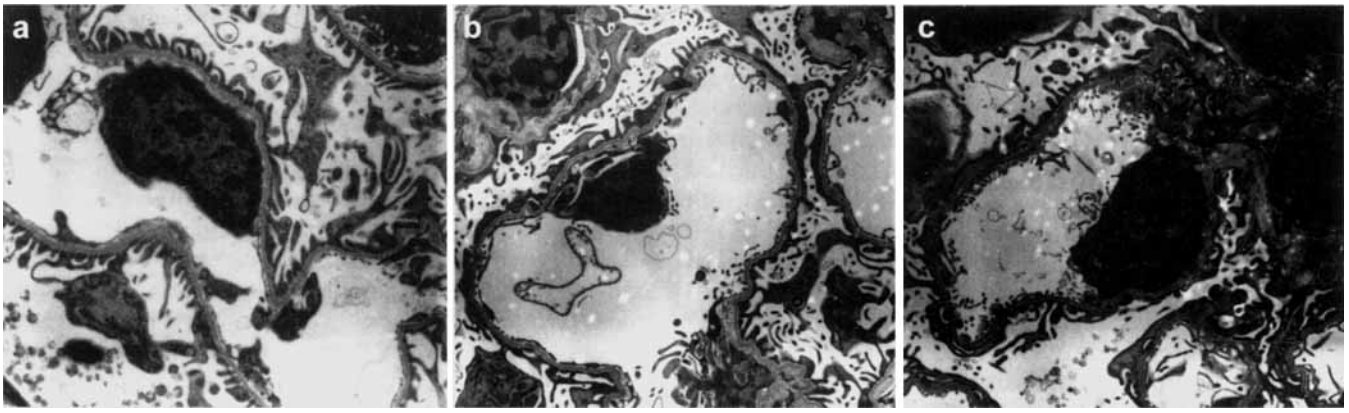

Fig. 1a-c Preservation of glomerular endothelium in the two experimental groups. Simple cold storage (**a**) resulted in good preservation of endothelial cell lining; however, vascular integrity was also maintained after 18 h of hypothermic low-flow perfusion with oxygenated HTK solution (**b**) as well as after perfusion preservation with Belzer MPS (**c**). Electron micrographs ×4,400

senting significant enhancement, compared with the 79 ± 14% observed in cold-stored grafts ($P < 0.05$).

All kidneys preserved by machine perfusion showed spontaneous urine production > 500 ml/day, while delayed graft function was evidenced in all cold-stored kidneys by oligo-anuria consistently observed during the first 2 days after transplantation.

In the cold-storage group, four of five animals survived the study period of 1 week. Of the pigs receiving perfusion-preserved kidneys, all survived.

Serum creatinine rose after kidney transplantation, but levels remained significantly lower in the machine perfusion groups as from day 2 (Fig. 2). No differences were seen between perfusion preservation with HTK or Belzer MPS.

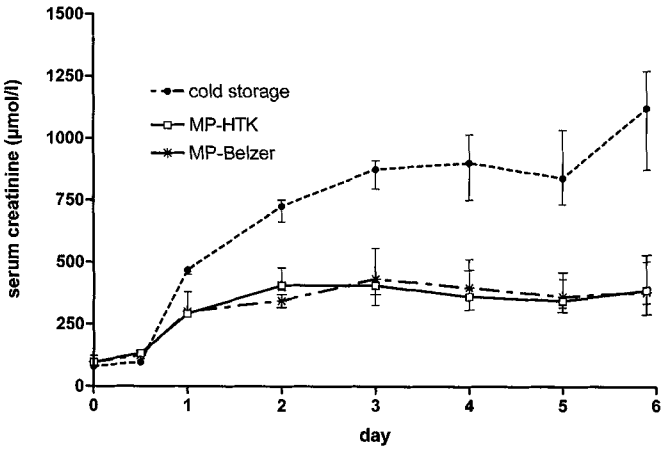

Fig. 2 Median serum creatinine levels (± interquartile range 25%–75%) after kidney transplantation from non-heart-beating donors. As from day 2 machine perfused grafts showed significantly lower values than the grafts of the cold storage group

Median values of maximal concentrations of urea were accordingly found to be significantly reduced by machine perfusion to 39.6 (34.2–46.4) mmol/l with HTK and to 36.7 (35.3–47.1) mmol/l with Belzer MPS, in contrast to 87.1 (74.9–101.4) mmol/l after cold storage (median ± interquartile range 25%–75%; $P < 0.05$).

Discussion

The shortage of donor organs is becoming a pivotal challenge in clinical transplantation. While the number of patients on the waiting list for kidney transplantation rises steadily, the rate of available donor organs remains rather static. The recent upsurge in retrieval from donors after the heart has stopped beating helps to enlarge the donor pool but might create difficulties concerning organ viability after warm ischaemic insult [8]. The quality of organ preservation during ischaemic storage may have a significant effect on renal allograft outcome after transplantation and may modulate the risk of long-term complications.

Whereas considerable controversy exists with regard to whether kidney preservation is best performed by simple cold storage or by hypothermic pulsatile machine perfusion [11], there has been renewed interest in pulsatile machine perfusion for the preservation of damaged kidneys. Our study demonstrates that kidneys preserved by oxygenated perfusion show initial graft function after transplantation and improved integrity during the postoperative follow up superior to such function and integrity in simply cold stored kidneys.

In contrast to previously reported data, that indicated that long-term liver perfusion at hypothermia was easily possible without the addition of colloid to the perfusion medium [16, 22], we found that vascular resistance of the damaged kidneys used in this study allowed for flow values of only approximately 0.2–0.3 ml/g/min if perfusion pressure was to be limited to 40 mmHg for longer periods of time. Hence, the IRR of about 1.26 mmHg g min/ml that we observed seems approximately twice as high as that seen under comparable conditions with the

colloid containing Belzer MPS, the latter being in line with data calculated from colloid-enriched perfusion of kidneys as reported previously [10, 23].

However, the low viscosity of the crystalloid HTK solution allowed for a rapid flush out and cooling of the kidneys after cardiac standstill, and the use of the same solution for subsequent machine preservation prevents any prejudice of mixing preservation fluids [15].

Interestingly, long-term perfusion of the kidneys was possible even with colloid-free HTK solution, as flow was reduced to lower values, still providing significant aerobiosis to the renal cortex during hypothermia, as could be evidenced by surface pO_2 measurements yielding 5–10 mmHg in cold-stored kidneys, in contrast to values between 100 mmHg and 150 mmHg pO_2 during low-flow perfusion with HTK (data not shown). The metabolic relevance of this difference is confirmed by the nearly eightfold increase in tissue levels of ATP compared with simple cold storage.

Moreover, we could demonstrate improved microvascular tissue perfusion upon normothermic reperfusion after MP–HTK, whereas depressed erythrocyte flux in the CS group was indicative of impaired vascular conductance of these kidneys. This is in line with previous observations that high oxygen availability during ischaemia maintains structural integrity of sinusoidal endothelial cells [24]. Endothelial cells are specifically sensitive to energy deficiency [21], and anoxic swelling of vascular endothelium may account for microcirculatory dysfunction upon reperfusion after ischaemic storage [25]. Therefore, we conjecture that aerobiosis during extra-corporal preservation might be more important to maintain tissue viability than mere hydrodynamic perfusion itself.

This is in line with previous experimental data, which stated the equivalent superiority of gaseous oxygenation during preservation [26] and machine perfusion over simple hypothermic storage of livers from non-heart-beating donors [16, 27].

In conclusion, provision of oxygen during storage of damaged kidneys is possible by low-flow perfusion with HTK and apparently contributes to improved graft viability after transplantation.

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