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ORIGINAL ARTICLE

Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys

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Summary

Delayed graft function still represents a major complication in clinical kidney transplantation. Here we tested the possibility to improve functional outcome of cold stored kidneys *a posteriori* by short-term hypothermic machine perfusion immediately prior to reperfusion. A total of 18 kidneys from female German Landrace pigs was flushed with Histidine-Tryptophan-Ketoglutarate solution and cold-stored for 18 h (control). Some grafts were subsequently subjected to 90 min of hypothermic reconditioning by hypothermic machine perfusion with ($HR+O_2$) or without ($HR-O_2$) oxygenation of the perfusate. Early graft function of all kidneys was assessed thereafter by warm reperfusion *in vitro* ($n = 6$, respectively). Renal function upon reperfusion was significantly enhanced by $HR+O_2$ with more than threefold increase in renal clearances of creatinine and urea. $HR+O_2$ also led to significantly higher urinary flow rates and abrogated the activation of caspase 3. By contrast, $HR-O_2$ was far less effective and only resulted in minor differences compared to control. It is derived from the present data that initial graft function can be significantly improved by 2 h of oxygenated machine perfusion after arrival of the preserved organ in the transplantation clinic.

Introduction

Renal transplantation remains the therapy of choice for patients with end stage renal disease. However, the number of patients, waiting for a kidney graft continues to increase and far exceeds the availability of donor grafts [1–3].

A large number cadaver organ transplants manifest a degree of early dysfunction leading to the clinical syndrome of delayed graft function (DGF) [4].

Delayed graft function (DGF) represents a significant problem in clinical kidney transplantation affecting upto 50% of all cadaveric graft recipients [5]. This has an impact on short-term management, including the requirement of haemodialysis treatment and an increased risk of acute rejection. Moreover DGF has been shown in multivariate analyses to independently favour the incidence of chronic nephropathy and later graft loss [4,6].

In face of this conflicting shortage of donor organs, the expansion of donor criteria has led to accept an increasing number of older donors, and more marginal donor grafts [7], which poses even higher challenges on organ preservation prior to transplantation.

An more and more accepted method to maintain donor organ viability is hypothermic machine perfusion (HMP), which has shown to improve kidney function in experimental models [8,9] and to reduce DGF in expanded criteria donor (ECD) kidneys [10,11].

Notwithstanding its protective properties on renal parenchyma extensive application of HMP might be prone to adverse vascular side effects and still remains subject to debate [12–14]. We have previously shown in the liver, that the outcome of potentially critical marginal grafts could be changed for the better by post-hoc revitalization applied only at the end of the cold ischemia period [15]. The revitalization comprises artificial

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oxygenation of the hypothermic organ by aerobic machine perfusion or gaseous oxygen insuffflation [16].

In this report, our objective was to evaluate the restorative capacity of an only end-ischemic in-house treatment of previously conventionally stored kidneys by 2 h of HMP, immediately prior to warm reperfusion. The putative influence of perfusate oxygenation during HMP should be highlighted in this context as well.

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals.

Female German Landrace pigs ($n = 18$), weighing between 25 and 30 kg were premedicated with ketamine (90 mg/kg), xylazine (10 mg/kg) and atropine (10 μg/kg) administered intramuscularly (i.m.) 10 min before induction of anaesthesia. General anaesthesia was induced by midazolam (0.5 mg/kg), pancuronium (0.2 mg/kg) and fentanyl (12.5 μg/kg) administered intravenously (IV) and maintained after intubation by mechanical ventilation with isoflurane in air/oxygen. Fentanyl was administered for improved analgesia.

The right kidney was removed and flushed by gravity (100 cm H_2O) on the back table with 500 ml of histidine-tryptophan-ketoglutarate (HTK) solution at 4 °C. After static cold storage at 4 °C in HTK for 18 h the kidneys were randomly assigned to one of the following groups:

1 Group 1: ($n = 6$); cold stored kidneys were used for the experiments without further treatment and served as controls.

2 Group 2: ($n = 6$); cold stored kidneys were subsequently subjected to 'hypothermic reconditioning' ($HR+O_2$) consisting of 2 h hypothermic machine perfusion on a pulsatile perfusion pump (RM3, Waters Medical Systems, Minneapolis, MN, USA). Perfusion was done at 4–6 °C with UW-MP solution while systolic pressure was maintained at 40–50 mmHg and the perfusate was oxygenated by superfusion with 100% oxygen, yielding a mean perfusate pO_2 of ≥300 mmHg.

3 Group 3: ($n = 6$); cold stored kidneys were treated like in group 3, but with the exception, that only room air equilibration of the perfusate was performed during machine perfusion ($HR-O_2$).

Isolated kidney perfusion

Immediately prior to reperfusion, all organs were exposed to no flow conditions at room temperature for 30 min in order to simulate the period of slow rewarming of the graft during surgical implantation *in vivo* [17].

Graft integrity was tested thereafter by isolated reperfusion *in vitro* via the renal artery in a recirculation system for 90 min. The perfusion medium consisted of 500 ml freshly prepared Krebs–Henseleit buffer containing a mixture of two oncotic agents [18], 0.6% bovine serum albumin and 3.6% polyethylene glycol (PEG 20,000). Creatinine (0.1 g/l) and urea (2 g/l) were added to the perfusate to allow for calculation of respective renal clearances. The ureter had been canulated with PE-tubing and urine was collected throughout the reperfusion period.

Kidneys were placed in a moist temperature chamber and perfused at 37 °C. Perfusate was oxygenated in a temperature controlled hollow fibre oxygenator (Minimax Plus, Medtronic, Minneapolis, MN, USA) with a mixture of 95% oxygen and 5% carbon dioxide. Temperature was regulated by a circulating thermostat, connected to perfusion chamber and oxygenator.

Kidney perfusion pressure was set at 100 mmHg and automatically maintained by servo-controlled roller pump, connected to a pressure sensor placed in the inflow line immediately prior to the renal artery.

Perfusate enzyme activities of lactate dehydrogenase (LDH) were assessed photometrically using commercialized standard kits (Fa. Roche, Mannheim, Germany).

Concentrations of creatinine and urea were determined in perfusate and corresponding urine samples in a routine fashion at the laboratory centre of the University Hospital Bonn.

Metabolic activity of the grafts was approximated by the calculation of renal oxygen utilization. Perfusate samples were taken at the arterial inflow and from the venous effluent and the respective contents of O_2 were measured immediately in a pH-blood gas analyser (ABL 500 acid–base laboratory, Radiometer, Copenhagen. Denmark). O_2-uptake was calculated from the differences between arterial and venous sites and expressed as μmol/g per min according to transrenal flow and kidney mass.

Activity of caspase 3

Functional activity of caspase 3 was analysed from homogenized tissue lysates in 96 well plates using a fluorimetric assay kit (Calbiochem), based on the detection of the cleavage product 7-amino-4-trifluoromethyl coumarin (AFC), which emits yellow–green fluorescence (max. at 505 nm) upon excitation at 400 nm.

Measurements were done on a fluorescence micro plate reader (Tecan, Grailsheim, Germany) and enzymatic activities of caspase 3 in the experimental groups are presented as the percentage increase with respect to the baseline values obtained from nonischemic control tissue.

Statistics

All values were expressed as means ± SEM of $n = 6$ animals per group. After proving the assumption of

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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

Kidney perfusion

Renal vascular flow upon pressure controlled isolated reperfusion did not show relevant variations over time nor were there any differences between the groups. On average, renal perfusion rates were 231 ± 23 ml/min vs. 210 ± 19 ml/min vs. 197 ± 24 ml/min (control versus $HR+O_2$ vs $HR-O_2$, respectively).

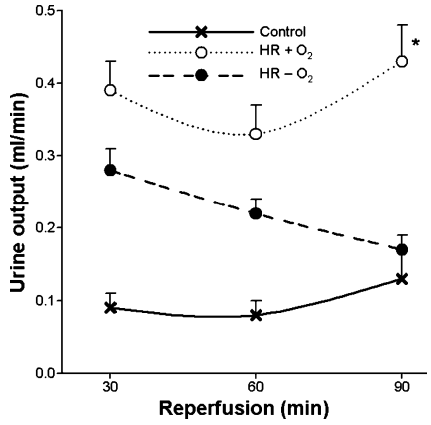

Figure 1 Renal urine production upon reperfusion after 18 h of cold storage (control) or after additional 2 h of hypothermic reconditioning with ($HR+O_2$) or without ($HR-O_2$) oxygenation of the preservation solution ($n = 6$ per group; $*P < 0.05$ versus control).

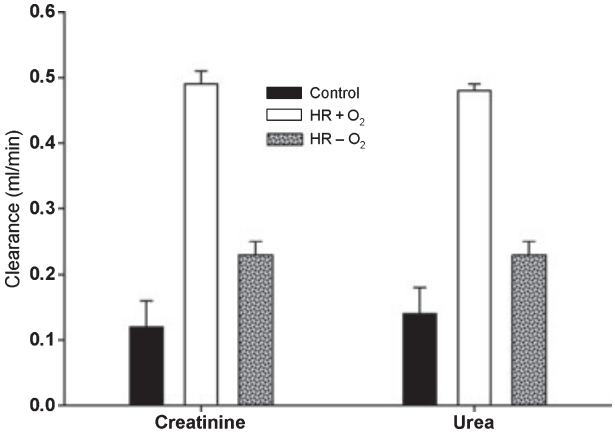

Figure 2 Early graft function upon reperfusion after 18 h of cold storage (control) or after additional 2 h of hypothermic reconditioning with ($HR+O_2$) or without ($HR-O_2$) oxygenation of the preservation solution ($n = 6$ per group; $*P < 0.05$ versus control).

Urine output

Significant changes in urine output were noted over time (Fig. 1). $HR+O_2$ resulted in a notable increase in renal urine production during the whole observation period as compared to the controls. However, $HR-O_2$ only lead to minor and temporary elevations of urinary flow, which fell to control values after 90 min.

Renal function

There was a major and significant effect of $HR+O_2$ treatment on primary graft function upon warm reperfusion (Fig. 2). An approximately three-fold increase in renal clearances of both, creatinine and urea was seen after hypothermic reconditioning ($HR+O_2$) during the observation period of the experiment. $HR-O_2$, again, was found to be far less effective.

By contrast, no significant differences could be detected in postischemic oxygen consumption of kidneys, which were only cold stored, and those, which were subjected to $HR+O_2$ or $HR-O_2$ (0.42 ± 0.02 vs. 0.42 ± 0.03 vs. 0.45 ± 0.05 mmol/min per 100 g; control versus $HR+O_2$, versus $HR-O_2$, respectively).

Enzyme release

Renal enzyme release of LDH was followed during the time course of reperfusion as parameter of structural cell injury. Perfusate activities of LDH increased over time in all experimental groups, but there were no significant differences between controls and the respective treatment groups (Fig. 3).

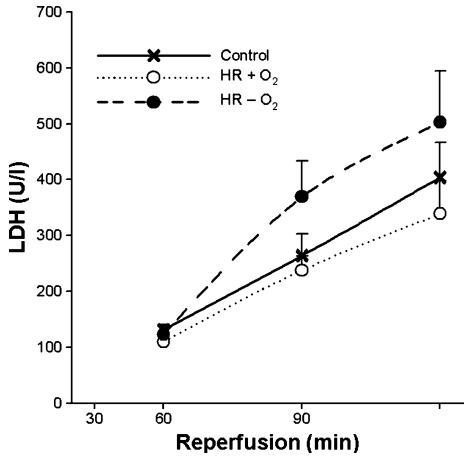

Figure 3 Enzyme release into the perfusate upon reperfusion after 18 h of cold storage (control) or after additional 2 h of hypothermic reconditioning with ($HR+O_2$) or without ($HR-O_2$) oxygenation of the preservation solution ($n = 6$ per group; $*P < 0.05$ versus control).

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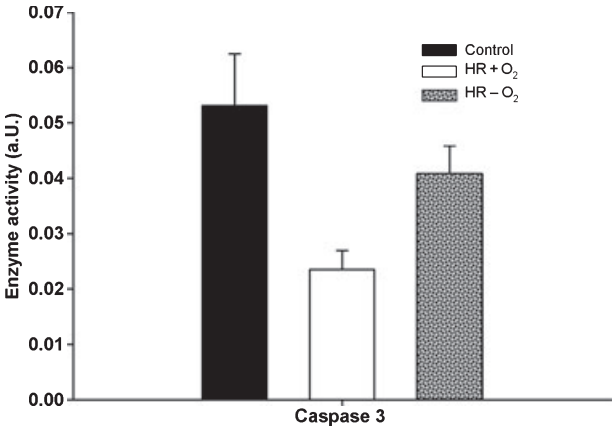

Figure 4 Apoptosis induction in renal cortical tissue upon reperfusion after 18 h of cold storage (control) or after additional 2 h of hypothermic reconditioning with (HR+O_2) or without (HR−O_2) oxygenation of the preservation solution (n = 6 per group; *P < 0.05 versus control).

Activation of caspase 3

After reperfusion of control kidneys we observed an approximately two-fold increase in caspase 3 activity with respect to baseline values, while postischemic enzymatic activation of caspase 3 was found to be significantly mitigated by HR+O_2 (Fig. 4).

By comparison, HR−O_2 did not significantly change induction of the executioner enzyme of apoptosis as compared to the controls.

Discussion

The optimization of marginal organs for transplantation continues to be an important problem for renal transplant programs [19]. Any improvement of the quality of organ preservation may have a significant effect on renal allograft outcome after transplantation and modulate the risk of long term complications [20].

However, irreversible structural damage of the ischemically preserved tissue basically occurs not during the storage period but rather unfolds in the course of reperfusion after transplantation [21–24].

An expedite recovery of oxidative phosphorylation after ischemic storage is of pivotal importance for the resumption of energetic homeostasis and a prerequisite for organ viability after transplantation [25]. Thus, mitochondrial dysfunction and consecutive failure to adequately comply with energetic demands upon reperfusion could be incriminated as a pivotal step in post-transplant organ dysfunction [26].

Continuous aerobiosis during graft storage effectively prevents energetic breakdown and deterioration of mitochondrial redox homeostasis and has often been acknowledged as an important beneficial feature of HMP [27]. Notwithstandingly recent data from a large multicenter study actually also established limited but significant superiority of nonoxygenated machine perfusion over simple cold storage in clinical kidney transplantation [11].

In our setting, perfusion *per se* did not bring up described protection and failed to provide functional improvements upon reperfusion. Oxygen is thus conjectured to play a central role of pivotal importance for hypothermic reconditioning after ischaemic storage. Similar results have also been reported in the liver, where endischaemic oxygenation in hypothermia was able to improve graft function upon reperfusion, while mere vascular perfusion did not [28].

It is therefore conjectured, that restitution of adequate mitochondrial redox status and cellular homeostasis by only short-term tissue oxygenation during hypothermic hibernation positively influences graft recovery upon beginning of metabolic work load to the parenchyma in the moment of warm reperfusion. Hypothermic reconditioning may hence represent an attractive and feasible adjunct to organ preservation, which enables a selective improvement of tissue integrity in for particularly endangered grafts, locally and immediately prior to transplantation.

A drawback for direct extrapolation of our study to the clinical situation could be seen in the fact that we did procure the grafts from nonbrain dead donor animals. Any effects related to brain death, like up-regulation of genes related to inflammatory responses and reparative mechanisms prior to organ harvest [29] were thus not accounted for in this study. Owing to the limited observation period of 90 min reperfusion, it is also unlikely that our model would have accounted for cellular repair pathways, aiming at long-term recovery of the renal graft and which might be differentially activated in the respective study groups. Further studies using *in vivo* models are strongly encouraged, which might establish additional parameters for viability testing during the time of HMP and provide early feedback of therapeutic success.

Authorship

MK: wrote the paper and analysed data. CF: performed research/study, collected and analysed data. TM: designed research/study, analysed data and wrote the paper.

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References

1. 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.
2. Lindell SL, Compagnon P, Mangino MJ, Southard JH. UW solution for hypothermic machine perfusion of warm ischemic kidneys. *Transplantation* 2005; **79**: 1358.
3. Brasile L, Stubenitsky B, Booster M, Kootstra G. The cadaveric kidney and the organ shortage – a perspective review. *Clin Transplant* 2001; **15**: 369.
4. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* 2004; **364**: 1814.
5. Szwarc I, Garrigue V, Delmas S, Deleuze S, Chong G, Mourad G. Delayed graft function: a frequent but still unsolved problem in renal transplantation. *Nephrol Ther* 2005; **1**: 325.
6. Hauet T, Gibelin H, Richer JP, Godart C, Eugene M, Carretier M. Influence of retrieval conditions on renal medulla injury: evaluation by proton NMR spectroscopy in an isolated perfused pig kidney model. *J Surg Res* 2000; **93**: 1.
7. Frei U, Noeldeke J, Machold-Fabrizii V, *et al.* Prospective age-matching in elderly kidney transplant recipients – a 5-year analysis of the Eurotransplant Senior Program. *Am J Transplant* 2008; **8**: 50.
8. Hernandez A, Light JA, Barhyte DY, Mabudian M, Gage F. Ablating the ischemia-reperfusion injury in non-heart-beating donor kidneys. *Transplantation* 1999; **67**: 200.
9. Maathuis MH, Manekeller S, van der PA, *et al.* Improved kidney graft function after preservation using a novel hypothermic machine perfusion device. *Ann Surg* 2007; **246**: 982.
10. Buchanan PM, Lentine KL, Burroughs TE, Schnitzler MA, Salvalaggio PR. Association of lower costs of pulsatile machine perfusion in renal transplantation from expanded criteria donors. *Am J Transplant* 2008; **8**: 2391.
11. 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.
12. Cerra FB, Raza S, Andres GA, Siegel JH. The endothelial damage of pulsatile renal preservation and its relationship to perfusion pressure and colloid osmotic pressure. *Surgery* 1977; **81**: 534.
13. Pegg DE. Organ preservation. *Surg Clin North Am* 1986; **66**: 617.
14. Nicholson ML, Hosgood SA, Metcalfe MS, Waller JR, Brook NR. A comparison of renal preservation by cold storage and machine perfusion using a porcine autotransplant model. *Transplantation* 2004; **78**: 333.
15. Stegemann J, Minor T. Energy charge restoration, mitochondrial protection and reversal of preservation induced liver injury by hypothermic oxygenation prior to reperfusion. *Cryobiology* 2009; **58**: 331.
16. 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.
17. Minor T, Yamaguchi T, Isselhard W. Effects of taurine on liver preservation in UW solution with consecutive rewarming in the isolated perfused rat liver. *Transplant Int* 1995; **8**: 174.
18. Wang J, Nation RL, Evans AM, Cox S. Isolated rat kidney perfused with dextran and bovine serum albumin: a stable model for investigating renal drug handling. *J Pharmacol Toxicol Methods* 2004; **49**: 105.
19. Hosgood S, Harper S, Kay M, Bagul A, Waller H, Nicholson ML. Effects of arterial pressure in an experimental isolated haemoperfused porcine kidney preservation system. *Br J Surg* 2006; **93**: 879.
20. Land W. Impact of the reperfusion injury on acute and chronic rejection events following clinical cadaveric renal transplantation. *Clin Investigator* 1994; **72**: 719.
21. Menger MD, Lehr HA, Messmer K. Role of oxygen radicals in the microcirculatory manifestations of postischemic injury. *Klin Wochenschr* 1991; **69**: 1050.
22. Lemasters JJ. V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. *Am J Physiol* 1999; **276**: G1.
23. Minor T, Sitzia M, Dombrowski F. Kidney transplantation from NHBD after oxygenated low flow machine perfusion preservation with HTK. *Transplant Int* 2005; **17**: 707.
24. Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved rat liver by hypothermic oxygenated machine perfusion. *Am J Transplant* 2006; **6**: 903.
25. Vajdova K, Graf R, Clavien PA. ATP-supplies in the cold-preserved liver: a long-neglected factor of organ viability. *Hepatology* 2002; **36**: 1543.
26. Legrand M, Mik EG, Johannes T, Payen D, Ince C. Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. *Mol Med* 2008; **14**: 502.
27. Fuller BJ, Lee CY. Hypothermic perfusion preservation: the future of organ preservation revisited? *Cryobiology* 2007; **54**: 129.
28. Manekeller S, Minor T. Possibility of conditioning predamaged grafts after cold storage: influences of oxygen and nutritive stimulation. *Transplant Int* 2006; **19**: 667.
29. Bouma HR, Ploeg RJ, Schuurs TA. Signal transduction pathways involved in brain death-induced renal injury. *Am J Transplant* 2009; **9**: 989.

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