TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Kidney transplantation after oxygenated machine perfusion preservation with Custodiol-N solution

Thomas Minor,[1] Andreas Paul,[2] Patrik Efferz,[1] Jeremias Wohlschlaeger,[3] Ursula Rauen[4] and Anja Gallinat[2]

1 Surgical Research Division, University Clinic of Surgery, Bonn, Germany
2 Clinic of General, Visceral and Transplantation Surgery, University Hospital of Essen, Essen, Germany
3 Department for Pathology, University Hospital of Essen, Essen, Germany
4 Institute of Physiological Chemistry, University Hospital Essen, Essen, Germany

Keywords
Custodiol-N, kidney, machine perfusion, preservation, solution.

Correspondence
Thomas Minor, Surgical Research Division, University of Bonn, 53127 Bonn, Germany.
Tel.: +49 228 287 15133;
fax: +49 228 287 15199;
e-mail: minor@uni-bonn.de

Conflicts of interest
U.R. is a consultant of Dr. Franz Köhler Chemie. All other authors have declared no conflicts of interest.

Received: 26 February 2015
Revision requested: 27 March 2015
Accepted: 15 April 2015
Published online: 30 April 2015

doi:10.1111/tri.12593

Summary

Custodiol-N, a new preservation solution, has been shown particularly suitable for hypothermic machine perfusion preservation (HMP) in isolated porcine kidneys. These preliminary results should be confirmed in an actual transplant model *in vivo*. Kidney function after 21 h of HMP was studied in an autotransplant model using Landrace pigs (25–30 kg; $n = 6$ per group). Perfusion was performed with oxygenated perfusate, using either Custodiol-N solution including 50 g/l dextran 40 (CND) or kidney perfusion solution 1 (KPS-1) as gold standard. Viability of the grafts was followed for 1 week after bilateral nephrectomy in the recipient pigs. HMP with CND resulted in less acute tubular injury, evaluated by levels of fatty acid-binding protein and better clearance function during the first 24 h after Tx than with KPS-1 ($P < 0.05$, resp.). Serum creatinine tended to be lower in the CND group during the whole observation period. Histological tissue scores one week after Tx were similar in both groups. Expression of endothelin-1 as well as of Toll-like receptor 4 15 min after reperfusion was lower in the CND group ($P < 0.05$), suggesting less endothelial stress response. The data provide first *in vivo* evidence for the suitability of Custodiol-N as an effective perfusate for renal machine perfusion.

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]. More transplantation centers accept nowadays extended criteria donor organs for transplantation; organs of a lesser quality that would have been discarded in the past. It is obvious that the extension of donor criteria might have an impact on graft function after transplantation.

Renal dysfunction can be caused by donor- and recipient factors, many of which are pre-existing, but preservation associated tissue injury prevail as pertinent and influenceable factor that can potentiate renal tissue alterations and increase the risk of primary organ dysfunction. Thus, any improvement in organ preservation technique will represent a valuable advance to enlarge the total number of viable donor organs available for transplantation.

Experimental as well as retrospective clinical studies have suggested that preservation of the kidney by hypothermic machine perfusion (HMP) may have advantages compared to conventional cold storage (CS) and lead to improved functional recovery after transplantation [4–8]. Most recently (HMP), instead of CS has been shown to significantly reduce the risk of delayed graft function along with a better 1- and 3-year survival in a randomized controlled prospective multicenter study [9,10].

Research on HMP has mainly focused on delineating the optimal physical perfusion characteristics, as pressure [11], pulsatility [12,13], or temperature [14] of perfusion, but

little effort has been spent into the development or optimization of the perfusion fluid used for HMP.

Custodiol-N is a new CS solution, recently developed by Rauen and de Groot as improved modification of the former HTK solution [15–17], that could actually been shown to promote superior organ protection when used as perfusate for hypothermic machine perfusion in the rat liver [18]. Based on primary *in vitro* findings, we previously proposed the use of Custodiol-N supplemented with dextran 40 as a colloid, as protective perfusate to be also used in hypothermic machine perfusion of the kidney [19].

These should now be corroborated *in vivo* using a preclinical autotransplant model. Thus, postoperative organ function could be followed without interference of any antigenic mechanisms.

This study was thus undertaken to investigate the putative benefit of Custodiol-N plus dextran for hypothermic machine perfusion of the kidney by comparison with the gold standard in kidney preservation that is Belzer machine perfusion solution, nowadays distributed under the name kidney perfusion solution 1 (KPS-1).

Materials and Methods

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

German landrace pigs weighing 25–30 kg were used for the study. All animals had free access to tap water and standard pellet food. Solid food was withdrawn 24 h before beginning of the experiment. A porcine autotransplantation model was used as previously established in our laboratory and detailed elsewhere [11, 20]. In brief; the right internal jugular vein was cannulated under general anesthesia, with polyethylene tubing for infusion and daily collection of blood samples. After left nephrectomy, the kidneys were flushed on the backtable with HTK solution from a height of 100 cm till the effluent was clear.

All grafts were preserved overnight by pulsatile machine perfusion in the Lifeport Kidney Transporter System. Machine perfusion was performed using either Custodiol-N solution supplemented with dextran 40 (CND, $n = 6$) or KPS-1 ($n = 6$) as gold standard. Custodiol-N (Dr. F. Köhler Chemie, Bensheim, Germany) was supplemented with 50 g/l dextran 40 (MW 40,000 Da; AppliChem, Darmstadt, Germany) and sterilized by filtration (0.022-μm filter, Steritop-GP Filtereinheit; Millipore, Schwalbach, Germany). KPS-1 was purchased from Organ Recovery Systems, Chicago, IL, USA. The composition of the respective solutions is listed in Table 1. A modification of the perfusion circuit allowed for interposition of a membrane oxygenator and oxygenation of the perfusate ($pO_2 > 500$ mmHg) during preservation [20].

Table 1. Composition of CND and KPS-1.

	CND (Custodiol-N with dextran)	KPS-1
Sodium	16	100
Potassium	10	25
Magnesium	8	5
Calcium	0.02	0.5
Chloride	30.04	
Histidine	124	
N-Acetylhistidine	57	
Mannitol	–	30
Sucrose	33	
α-Ketoglutarate	2	
Aspartate	5	
Glycine	10	
Alanine	5	
Tryptophan	2	
Arginine	3	
Deferoxamine (μmol/l)	15.3	
LK 614 (μmol/l)	6.2	
Phosphate		25
Gluconate		85
Glucose		10
Adenine		5
Glutathione		3
HEPES		10
Ribose		5
Hydroxyethyl starch (g/l)		50
Dextran 40 (g/l)	50	
pH	7.0	7.4
Osmolarity (mosm/l)	306	320

Composition of CND (Custodiol-N supplemented with 50 g/l dextran 40) and kidney perfusion solution 1 (KPS-1), as used in the study. Values are given in mmol/l unless stated otherwise.

After 21 h of preservation, the kidneys were auto-transplanted subsequent to removal of the native contralateral kidney. Vascular anastomoses were performed end to side (renal vein–vena cava) and end to end (left renal artery–right renal artery), respectively. At the time of reperfusion, 50 ml of glucose 50% was infused to induce osmotic diuresis. No other diuretics were given. The ureter was cannulated with polyethylene tubing, which was tunneled through the abdominal wall, allowing continuous visual inspection of urine production.

Renal tissue perfusion was assessed noninvasively 10 min after reperfusion as mean cortical erythrocyte flux, determined by LASER Doppler flowmetry as detailed previously [21, 22]. To account for temporal variations in blood flow, we calculated the mean flux value over 10 s of recording and to eliminate the influence of spatial heterogeneity, we performed measurements on four distinct places of the

renal surface. All flux measurements were taken as percent variation from the baseline values obtained from the nonischemic native kidneys. Cortical renal biopsies were taken 15 min after onset of reperfusion, snap-frozen in liquid nitrogen and stored at −80 °C until later analysis.

Analyses

Using a jugular catheter left in place after the operation, venous blood samples were taken daily from the beginning of the intervention until the animals were sacrificed on postoperative day 7. At the same time, urine samples were collected for biochemical analyses. These were used for measuring the levels of creatinine, urea, and sodium determined with methods of the clinical routine.

Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis of inflammation-related genes was performed on renal cortical wedge biopsies taken 15 min after the onset of blood reperfusion after transplantation. Total RNA was isolated from snap-frozen samples using TRIreagent (Applied Biosystems Darmstadt, Germany). Equal amounts of RNA were quantified by Nano Drop (Thermo Fisher, Waltham, MA, USA), complementary DNA by incubation with High Capacity cDNA RT Kit (Applied Biosystems). The PCR mix was prepared using TaqMan GenEx Master Mix (Applied Biosystems). The amount of specific mRNA in the tissue was expressed in arbitrary units after normalization for the respective individual quantities of transcripts of ribosomal protein L19 (RPL19), which was analyzed as housekeeping gene.

Primers for RPL19 (no. Ss03375624_g1), endothelin-1 (ET-1; no. Ss03392455_m1), endothelial nitric oxide synthase (no. Ss03383940_u1), von Willebrand factor (vWF; no. Ss03376198_u1), Toll-like receptor 4 (TLR-1; no. Ss03389780_m1), and TNF-alpha (no. Ss03391318_g1) were purchased from Applied Biosystems.

Oxygen free radical-induced tissue injury was approximated by the amount of thiobarbituric acid-reactive substances (TBARS), breakdown products of lipid peroxidation (LPO), released into the circulation upon reperfusion. TBARS were evaluated by fluorimetry from deproteinized serum samples using the adduct formation with thiobarbituric acid as detailed elsewhere [23].

ELISA kits to analyze *L*-type fatty acid-binding protein (LFABP) were purchased from USCN Life science, Wuhan, China) and used according to the instructions of the manufacturer. Measurements were taken on a fluorescence micro plate reader (Tecan, Grailsheim, Germany).

Light microscopy

In all animals, renal tissue was collected at the conclusion of the experiments, cut into small blocks (3 mm thickness), and fixed by immersion in 4% buffered formalin. The blocks were embedded in paraffin and cut into 2 μm sections using a microtome. Hematoxylin and eosin staining was used to judge morphological integrity of the parenchyma.

Renal injury was scored by an experienced pathologist blinded for the groups. Assessment was carried out following Torras *et al.* [24]: In each slide, 10 visual fields were investigated assessing six morphological parameters indicating renal parenchyma injury (tubular dilatation, epithelial vacuolization, epithelial shedding, epithelial necrosis, interstitial edema, and inflammation). A 5-point scale was applied for each parameter: 0 = no damage; 1 = lesions affecting < 10% of the field; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; and 5 > 75%.

Statistics

All values were expressed as means ± SEM. After proving the assumption of normality and equal variance, differences between groups were tested by unpaired *t*-tests, unless otherwise indicated. Statistical significance was set at $P < 0.05$.

Results

No significant differences in cold ischemic times (CIT) and anastomosis times were observed between groups. The mean (±SEM) CIT for the CND and KPS-1 groups were 20.4 ± 0.3 and 20.5 ± 0.2 h, respectively. The mean ± SEM anastomotic times were 31 ± 2 and 32 ± 2 min, respectively. The first warm ischemia time (time between arterial clamping and cold flush out) ranged from 2 to 4 min and did not differ significantly between groups. In both groups, all six animals survived the 7-day follow-up period.

Cortical tissue perfusion upon transplantation

Microcirculatory tissue perfusion upon renal reperfusion after transplantation showed small but significant differences between the two experimental groups. Cortical erythrocyte flux tended to be higher after HMP with CND as compared to KPS-1 (85.9 ± 2.2 vs. 73.1 ± 4.7% of baseline; CND vs. KPS-1, respectively, $P < 0.05$).

Reperfusion injury

Measurement of TBARS, markers of lipid peroxidation, in the systemic circulation on day one after transplantation allowed detection of oxygen free radical-mediated tissue injury. Serum levels of TBARS after transplantation were found significantly ($P < 0.05$) higher in the KPS-1 group

than after preservation in CND, 95.6 ± 14.4 vs. 29.3 ± 5.3 nmol/ml, respectively.

At the same time, tubular cell injury as assessed by serum levels of LFABP was significantly higher in the KPS-1 group with values of 28.0 ± 1.4 ng/ml as compared to 17.7 ± 2.9 ng/ml in the CND group ($P < 0.05$). A similar tendency was observed concerning the cellular loss of HMGB-1 (0.82 ± 0.38 vs. 1.76 ± 0.63 ng/ml; CND vs. KPS-1), but here differences between the two groups did not reach statistical significance ($P = 0.28$).

Functional graft recovery

Renal clearance of creatinine was found to be transiently depressed during 1 day after transplantation subsequent to preservation in KPS-1, but significantly better after preservation in CND (cf. Fig. 1).

Accordingly, serum creatinine levels at POD 1 were significantly lower in recipient animals of the CND group than in the KPS-1 group. Renal function subsequently normalized in both groups. However, serum creatinine tended to be lower in the CND group during the whole observation period.

Similar differences could be substantiated between the groups with respect to postoperative clearance of urea. A transient depression to 17 ± 3% of baseline values was evident in the KPS-1 group during postoperative day 1 that was significantly ($P < 0.05$) less pronounced (57 ± 10%) in the CND group.

However, differences of peak serum levels of urea after transplantation did not reach statistical significance (144.8 ± 41.4 mg/dl vs. 87.7 ± 16.2 mg/dl; KPS-1 vs. CND, respectively) and clearance of urea quickly normalized during the observation period in both groups.

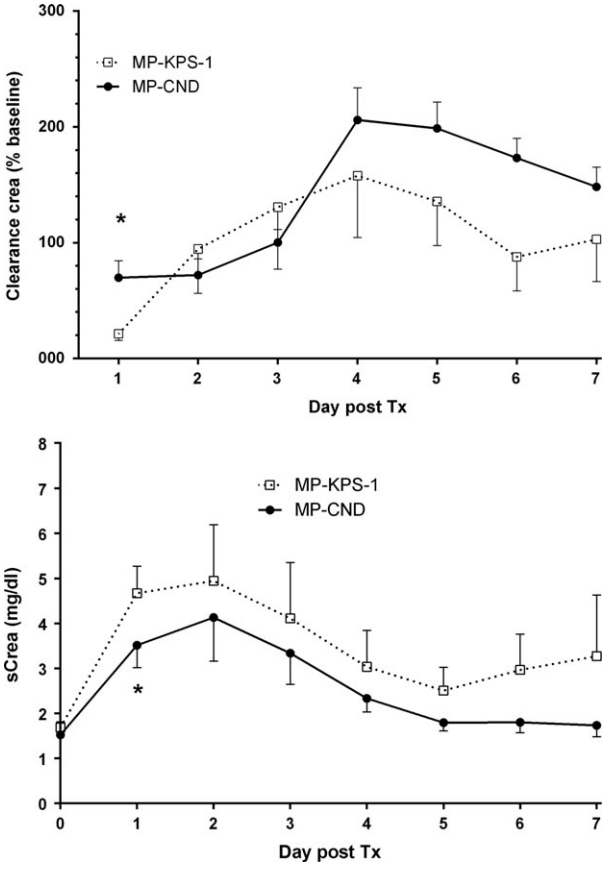

Figure 1 Autograft function after transplantation of kidneys preserved by machine perfusion (MP) with Custodiol-N solution including 50 g/l dextran 40 (CND) or kidney perfusion solution 1 (KPS-1). Upper panel: creatinine clearance; lower panel: serum creatinine. Values are given as mean ± SE; *$P < 0.05$ vs. MP KPS-1.

Endothelial activation and inflammation

Four endothelial activation markers and one proinflammatory cytokine were studied to examine the influence of the preservation modalities on endothelial activation and inflammation (cf. Fig. 2). Upregulation of ET-1 as well as of TLR-1 was significantly lower in the CND group than after preservation with KPS-1 ($P < 0.05$), suggesting less endothelial stress response. No differences were seen with regard to vWF or endothelial nitric oxide synthase.

The increase in the expression of TNF-alpha was more than twofold higher the KPS-1 group than in the CND group; however, due to the high variation in the former group, this difference did not quite reach statistical significance ($P = 0.11$).

Structural tissue integrity

Light microscopy performed on tissue samples obtained after conclusion of the experiments did not disclose signifi-

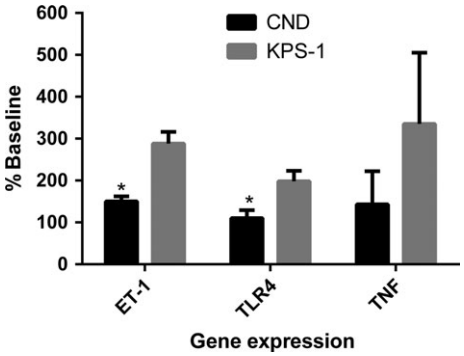

Figure 2 Gene expression of endothelin-1 (ET-1), Toll-like receptor 4 (TLR-4) and tumor necrosis factor (TNF)-alpha in the postreperfusion biopsy as described under methods. Data are given as mean and standard error of $n = 6$ experiments; *$P < 0.05$.

cant differences among the groups, although epithelial vacuolization tended to be higher in the KPS-1 group (score: 1.7 ± 0.3 vs. 1.2 ± 0.2, $P = 0.28$). Overall, only slight alterations of normal structural appearance were observed in any group comprising moderate epithelial vacuolization, and slight interstitial edema (Fig. 3). Changes indicative of irreversible cellular damage such as epithelial shedding or necrosis were not observed.

Discussion

Renal preservation by hypothermic machine perfusion has gathered increasing acceptance and its advantages compared with conventional CS have been suggested by several clinical studies [10, 25, 26], especially in organs originating from expanded criteria donors, which exhibit a reduced resilience to ischemia–reperfusion injury.

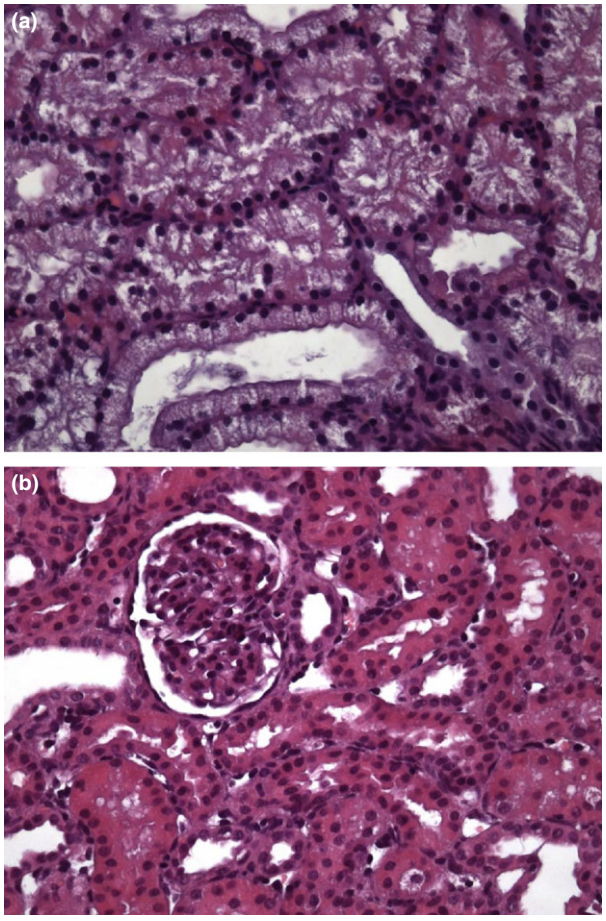

Figure 3 Renal histology by light microscopy 1 week after transplantation. H&E staining, 400× original magnification. (a) after hypothermic machine perfusion (HMP) with kidney perfusion solution 1 (KPS-1), (b) after HMP with Custodiol-N solution including 50 g/l dextran 40. Note slightly increased epithelial vacuolisation in the KPS-1 group but overall similarly well-preserved renal architecture in both groups with only limited edema and the absence of tissue necrosis.

Newer insights into the pathophysiology of preservation injury tend to favor the use of a pulsatile perfusion pattern [13, 27], as well as active oxygenation during hypothermic perfusion [21, 20–30]. One advantage of machine perfusion is seen in the ability to maintain energetic homeostasis during preservation, the importance of which seems to increase with the vulnerability of the grafts to be preserved [31]. Technical performance of machine perfusion in our study complied with this knowledge. Using a preclinical model of porcine autotransplantation, postoperative organ function could be followed *in vivo* without interference of any antigenic mechanisms.

Although Custodiol-N has originally been developed for static organ preservation, the solution appears to be suited also for oxygenated perfusion and has thus already been used with success in liver from rodents and pigs [18, 32].

The major features that render the solution particularly suitable for oxygenated preservation [33] are the inclusion of iron chelators, the partial substitution of histidine by *N*-acetylhistidine, and possibly the higher chloride concentration as compared to KPS-1. The iron chelators, inhibit cold-induced, free radical-mediated injury [34, 35], and have already been shown effective to protect isolated rat livers during HMP [18]. The partial substitution of histidine by the histidine derivative *N*-acetylhistidine reduces buffer toxicity [15] and is likely to account for the observed superiority of Custodiol-N base solution (without iron chelators) over HTK solution during HMP of rat livers [18]. Chloride-free conditions surprisingly proved to be injurious to the endothelium [16, 35], the cell type most endangered during machine perfusion.

However, as the kidney is held to be particularly prone to edema development upon noncolloidal perfusion [21], dextran 40 was added as a colloid for theoretical reasons to provide the necessary vascular oncotic support to counteract hydrostatic capillary pressure during continuous perfusion. The concentration of 50 g/l was chosen in line with previous experiences in porcine lung transplantation [33] and had proven to be effective in isolated porcine kidney perfusion [19].

In the present study, it could be shown for the first time in an *in vivo* setting that CND was as least equally effective as compared to KPS-1 as machine perfusate in kidney preservation. Moreover, HMP with CND resulted in less acute tubular injury and better clearance function during the first 24 h after transplantation than HMP with KPS-1. These findings were associated with discretely better renal cortical tissue perfusion shortly after transplantation and less endothelial dysfunction as judged from the expression profiles of ET-1.

Circulatory dysfunction upon early reperfusion represents a pivotal event in the pathophysiology of renal ischemia–reperfusion injury [36, 37]. Microcirculatory perfusion deficits are likely to decrease glomerular filtration rate [38] and may elicit secondary warm hypoxic tissue injury and mitochondrial failure [36] that increase the risk for acute tubular necrosis (ATN) [39].

Inhibition of the formation of highly reactive oxygen free radicals by effective iron chelation [34, 40] leads to better maintenance of vascular endothelial integrity and homeostasis after preservation with CND, and thereby likely also to a decreased inflammatory reaction upon reperfusion [34]. Superoxide anions as produced by activated/invading inflammatory cells at the vascular lumen have been described to counteract adequate vascular conductance by inactivation of the endothelium-derived nitric oxide through formation of peroxynitrite [41].

Vascular protection from oxygen free radicals by CND, as already suggested from earlier *in vitro* experiments [19], is hence conjectured to promote better glomerular perfusion and secondary improvements in filtration rate.

Notwithstanding that, equal resumption of renal function has been found after 1 week, irrespective of the perfusate used in this study and both solutions seem to provide adequate resumption of long-term recovery in this model. However, in this experiment, fresh, young kidneys were used that intrinsically tend to be less vulnerable to ischemia/reperfusion injury than much of the clinically prevailing grafts from extended criteria donors. Moreover, the present data might not cover conclusions extended on nonoxygenated machine perfusion. The potential of CND to minimize transient early dysfunction after transplantation might point at a more pertinent protection of tissue integrity that might become increasingly relevant upon more critical preservation times and/or oxygenated HMP of more marginal door organs.

In conclusion, CND has been shown to be biologically save *in vivo* and previous data from *in vitro* studies, indicating CND to provide an interesting organ protective potential for HMP of the kidney, could be validated in a clinically relevant large animal model.

Authorship

TM, AP, UR and AG: participated in research design. TM, UR and AG: participated in the writing of the paper. TM, PE, JW and AG: participated in the performance of the research. TM, PE, JW and UR: participated in data analysis. AP, PE and JW: critical revision of the manuscript. TM and AG: research supervision. UR: provided important reagents. TM, AP and AG: original idea.

Funding

This study was supported by Institutional Funds and a nonrestricted grant from Dr. Franz Köhler Chemie. The design, performance, data interpretation, and manuscript preparation was under the complete control of the authors and not influenced by the company.

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. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007; **83**: 1289.
5. 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.
6. Polyak MM, Arrington BO, Stubenbord WT, Boykin J, Brown T, Jean-Jacques MA, *et al.* The influence of pulsatile preservation on renal transplantation in the 1990s. *Transplantation* 2000; **69**: 249.
7. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. *Lancet* 1967; **2**: 536.
8. Balupuri S, Buckley P, Mohamad M, Chidambaram V, Gerstenkorn C, Sen B, *et al.* Early results of a non-heartbeating donor (NHBD) programme with machine perfusion. *Transpl Int* 2000; **13**(Suppl. 1): S255.
9. Moers C, Smits JM, Maathuis MH, Treckmann J, van Gelder F, Napieralski BP, *et al.* Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**: 7.
10. Moers C, Pirenne J, Paul A, Ploeg RJ. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2012; **366**: 770.
11. Maathuis MH, Manekeller S, van der Plaats A, Leuvenink HG, 'T Hart NA, Lier AB, *et al.* Improved kidney graft function after preservation using a novel hypothermic machine perfusion device. *Ann Surg* 2007; **246**: 982.
12. Timsit MO, Tullius SG. Hypothermic kidney preservation: a remembrance of the past in the future? *Curr Opin Organ Transplant* 2011; **16**: 162.
13. Gallinat A, Fox M, Luer B, Efferz P, Paul A, Minor T. Role of pulsatility in hypothermic reconditioning of porcine kidney grafts by machine perfusion after cold storage. *Transplantation* 2013; **96**: 538.

14. Hoyer DP, Gallinat A, Swoboda S, Wohlschlaeger J, Rauen U, Paul A, *et al.* Subnormothermic machine perfusion for preservation of porcine kidneys in a donation after circulatory death model. *Transpl Int* 2014; **27**: 1097.
15. Rauen U, Klempt S, de Groot H. Histidine-induced injury to cultured liver cells, effects of histidine derivatives and of iron chelators. *Cell Mol Life Sci* 2007; **64**: 192.
16. Fingas CD, Wu S, Gu Y, Wohlschlaeger J, Scherag A, Dahmen U, *et al.* Assessment of a chloride-poor versus a chloride-containing version of a modified histidine–tryptophan–ketoglutarate solution in a rat liver transplantation model. *Liver Transpl* 2011; **17**: 650.
17. Rauen U, Wu K, Witzke O, de Groot H. Custodiol-N—a new, mechanism-based organ preservation solution. *Cryobiology* 2008; **57**: 331.
18. Stegemann J, Hirner A, Rauen U, Minor T. Use of a new modified HTK solution for machine preservation of marginal liver grafts. *J Surg Res* 2010; **160**: 155.
19. Gallinat A, Lüer B, Swoboda S, Rauen U, Paul A, Minor T. Use of the new preservation solution Custodiol-N supplemented with dextran for hypothermic machine perfusion of the kidney. *Cryobiology* 2013; **66**: 131.
20. Gallinat A, Paul A, Efferz P, Luer B, Kaiser G, Wohlschlaeger J, *et al.* Hypothermic reconditioning of porcine kidney grafts by short-term preimplantation machine perfusion. *Transplantation* 2012; **93**: 787.
21. Minor T, Sitzia M, Dombrowski F. Kidney transplantation from NHBD after oxygenated low flow machine perfusion preservation with HTK. *Transpl Int* 2005; **17**: 707.
22. Minor T, Isselhard W, Yamaguchi T. Involvement of platelet activating factor in microcirculatory disturbances after global hepatic ischemia. *J Surg Res* 1995; **58**: 536.
23. Minor T, Koetting M. Gaseous oxygen for hypothermic preservation of predamaged liver grafts: Fuel to cellular homeostasis or radical tissue alteration? *Cryobiology* 2000; **40**: 182.
24. Torras J, Herrero-Fresneda I, Lloberas N, Riera M, Ma CJ, Ma GJ. Promising effects of ischemic preconditioning in renal transplantation. *Kidney Int* 2002; **61**: 2218.
25. Cannon RM, Brock GN, Garrison RN, Smith JW, Marvin MR, Franklin GA. To pump or not to pump: a comparison of machine perfusion vs cold storage for deceased donor kidney transplantation. *J Am Coll Surg* 2013; **216**: 625.
26. Yuan X, Theruvath AJ, Ge X, Floerchinger B, Jurisch A, Garcia-Cardena G, *et al.* Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. *Transpl Int* 2010; **23**: 561.
27. Gracia-Sancho J, Villarreal G Jr, Zhang Y, Yu JX, Liu Y, Tullius SG, *et al.* Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. *Transplantation* 2010; **90**: 142.
28. Mangino JE, Lindell S. Hypothermic machine perfusion of kidneys. In: Uygun K, Lee CY *Organ preservation and reengeneering*. Norwood, MA: Artech House; 2011. p 35–37.
29. Thuillier R, Allain G, Celhay O, Hebrard W, Barrou B, Badet L, *et al.* Benefits of active oxygenation during hypothermic machine perfusion of kidneys in a preclinical model of deceased after cardiac death donors. *J Surg Res* 2013; **184**: 1174.
30. Hoyer DP, Gallinat A, Swoboda S, Wohlschlaeger J, Rauen U, Paul A, *et al.* Influence of Oxygen Concentration During Hypothermic Machine Perfusion on Porcine Kidneys From Donation After Circulatory Death. *Transplantation* 2014; **98**: 944.
31. Hoffmann T, Minor T. New strategies and concepts in organ preservation. *Eur Surg Res* 2015; **54**: 114.
32. Minor T, Efferz P, Fox M, Wohlschlaeger J, Lüer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am J Transplant* 2013; **13**: 1450.
33. Pizanis N, Petrov A, Heckmann J, Wiswedel I, Wohlschlager J, de Groot H, *et al.* A new preservation solution for lung transplantation: evaluation in a porcine transplantation model. *J Heart Lung Transplant* 2012; **31**: 310.
34. Rauen U, de Groot H. New insights into the cellular and molecular mechanisms of cold storage injury. *J Investig Med* 2004; **52**: 299.
35. Wille T, de Groot h, Rauen U. Improvement of the cold storage of blood vessels with a vascular preservation solution. Study in porcine aortic segments. *J Vasc Surg* 2008; **47**: 422.
36. 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.
37. Hellberg POA, Kallskog O, Wolgast M. Red cell trapping and postischemic renal blood flow. Differences between the cortex, outer and inner medulla. *Kidney Int* 1991; **40**: 625.
38. Brezis M, Heyman SN, Epstein FH. Determinants of intrarenal oxygenation. II. Hemodynamic effects. *Am J Physiol* 1994; **267**(Pt 2): F1063.
39. Wilhelm SM, Simonson MS, Robinson AV, Stowe NT, Schulak JA. Endothelin up-regulation and localization following renal ischemia and reperfusion. *Kidney Int* 1999; **55**: 1011.
40. Schroder C, Heintz A, Pexa A, Rauen U, Deussen A. Preclinical evaluation of coronary vascular function after cardioplegia with HTK and different antioxidant additives. *Eur J Cardiothorac Surg* 2007; **31**: 821.
41. Minor T, Isselhard W. Role of the hepatovasculature in free radical mediated reperfusion damage of the liver. *Eur Surg Res* 1993; **25**: 287.