Preclinical evaluation of a new organ preservation solution

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Chapter 7

Evaluation of a novel system for hypothermic oxygenated pulsatile perfusion preservation
Evaluation of a novel system for hypothermic oxygenated pulsatile perfusion preservation

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Abstract

Background
Recently, a novel innovative machine perfusion (MP) system for hypothermic oxygenated pulsatile perfusion called the Airdrive (AD) has been developed. The aim of the study was to evaluate the biological safety of the AD system for perfusion preservation of kidney grafts in a porcine autotransplantation model using the low-viscosity perfusion solution Polysol (PS) in comparison with cold storage (CS) using PS or the University of Wisconsin solution (UW). In addition, we evaluated real-time microcirculation parameters. At sacrifice, grafts were retrieved for histological analysis and immunohistochemistry.

Methods
After assessment of the microcirculation, left kidneys were retrieved. Following the washout, kidneys were preserved for 20 hr using AD-PS, CS-PS or CS-UW. Thereafter, contralateral kidneys were removed followed by heterotopic autotransplantation of the preserved graft. Seven days after transplantation animals were sacrificed with retrieval of the grafts for histological analysis. Renal function, renal microcirculation and tissue injury including the proliferative response of tubular epithelial cells (TECs) were compared.

Results
Preservation using AD-PS or CS-PS resulted in higher microcirculatory flow compared with CS-UW. Improved recovery of renal function was seen in the AD-PS and CS-PS groups compared with CS-UW. Structural integrity was better preserved using AD-PS compared with both CS groups. Proliferative response of TECs was higher in CS-UW preserved grafts compared to grafts preserved using AD-PS.

Conclusion
This study demonstrates the biological safety of the AD system in a porcine autotransplantation model. Also, the microcirculation was better preserved and less morphological injury was observed after 20 hr MP compared with CS.
Introduction

Due to the continuous shortage of donor organs, optimal use of the organs donated for transplantation is of critical importance. In order to expand the donor pool, optimization of organ preservation methods is imperative. At the present time, the preservation methods employed are cold static storage (CS) and machine perfusion (MP). Since the introduction of CS in the late 1970s, the University of Wisconsin solution (UW, Viaspan®, Bristol Myers Squibb, New York, NY, USA) is considered the golden standard for the preservation of cadaveric kidney grafts1-5. However, the alternative modality of hypothermic MP has been shown to have beneficial effects on preservation quality6-9. Utilization of MP for the preservation of kidney grafts is associated with a reduction of delayed graft function in the recipients of these grafts10. Moreover, the use of MP is correlated with lower costs for hospitalization of transplant recipients as expressed by a reduced need for postoperative dialysis and the length of hospital stay11,12. In contrast to CS, hypothermic MP holds the potential of viability assessment and allows for pharmacological intervention13,14.

Currently, three MP systems are commercially available: the RM3 (Waters Medical Systems LLC, Plymouth, MN, USA), the Kidney Assist (Organ Assist BV, Groningen, the Netherlands) and the Lifeport (Organ Recovery Systems Inc., Des Plaines, IL, USA). In these systems, Belzer Machine Perfusion Solution (KPS-1, Organ Recovery Systems Inc., Des Plaines, IL, USA), an extracellular-type solution (high sodium, low potassium content) with a high viscosity is used. Although this solution was developed over thirty years ago and is currently the only MP preservation solution commercially available, MP solutions developed since have not been able to significantly improve preservation quality of donor grafts15,16.

Several pre-clinical studies have demonstrated beneficial effects from oxygenation of the perfusion medium17,18. However, the widely-used Lifeport MP system does not allow for active oxygenation of the perfusion medium.

Recently, an innovative perfusion system called the Airdrive (AD, Fig. 1) has been developed. As opposed to the Lifeport, the AD allows for oxygenated perfusion of kidney grafts19. Whereas the RM3, Kidney Assist and Lifeport employ conventional electrical fluid pumps for perfusion, the novel pulsatile perfusion pump used in the AD is propelled by oxygen pressure. The perfusion medium used in the AD is Polysol (PS), a colloid-based solution with a low viscosity. PS contains Polyethylene glycol (PEG), a low molecular-weight colloid which is known to have a beneficial effect in renal preservation and allows for continuous perfusion of organs for transplantation20. In addition, PS is a physiological extracellular type solution (high sodium, low potassium content) and has an enhanced buffering capacity through the addition of N-2-Hydroxyethylpiperazine-N’-2-Ethanesulfonic acid (HEPES). In experimental studies, PS demonstrated favorable results in both MP and CS preservation of the liver21-23 and CS preservation of the kidney24 and small bowel25.
The aim of this study was to assess the biological safety and efficacy of the AD as an MP system for the preservation of kidney grafts in a porcine autotransplantation model. Since the AD is not comparable to current MP systems due to the unconventional pump, the AD was compared with the golden standard in clinical transplantation of heart-beating donor kidneys, namely, CS using UW and with CS using PS. Furthermore, in order to assess the effects of perfusion on microcirculation, real-time parameters of the microcirculation were evaluated at various time points pre-retrieval and posttransplant using the oxygen-to-see (O2C) Laser Doppler flowmetry and remission spectroscopy system. In order to evaluate the structural integrity of the grafts, histological analysis of renal tissue was performed as well as immunohistochemical staining for proliferating tubular endothelial cells using anti-Ki67 antibody.

Materials and methods

Animals and experimental protocol
All experiments were performed in accordance with German law governing animal studies following the Principles of Laboratory Animal Care (NIH publication 85-23, revised 1985). Female German landrace pigs from a disease-free barrier breeding facility at the University of Bonn were housed in metabolic cages and allowed to acclimatize to their surroundings for a minimum of 1 week before surgery. The animals, weighing 24.3 ± 3.2 kg (mean ± SD), were fasted 24 hours prior to the experiments. All animals demonstrated normal renal function before start of the experiments. This study was performed using a porcine renal autotransplantation model and involved three groups: MP preservation using the AD in combination with PS (AD-PS, n=7) or CS preservation with either PS (CS-PS, n=7) or UW (CS-UW, n=7).
Surgical Procedures
Ten minutes before induction of anesthesia, the animals were premedicated with ketamine (90 mg/kg BW), xylazine (10 mg/kg BW) and atropine (0.01 mg/kg BW) administered intramuscularly (IM). General anesthesia was induced by midazolam and fentanyl (12.5 μg/kg BW), muscle relaxation was provided by pancuronium (0.2 mg/kg BW), all administered intravenously (IV) by means of a butterfly cannula inserted into an external marginal ear vein. After intubation, anesthesia was maintained by mechanical ventilation with isoflurane. Thereafter, the right internal jugular vein was cannulated with a polyethylene (PE) catheter for infusion and daily collection of blood samples. Left nephrectomy was performed after assessment of the microcirculation at 4 pre-defined locations on the renal surface using a combined laser Doppler and flowmetry system (oxygen-to-see, O2C system, LEA Medizintechnik, Giessen, Germany).

Kidney preservation
After retrieval, the kidneys were immediately flushed ex vivo with 500 mL of either PS solution or UW solution at 4°C at a hydrostatic pressure of 100 cmH₂O. Thereafter, CS kidneys were stored in a sterile bag with 500 mL of PS or UW and placed in a cold water bath (4°C, Lauda, Königshofen, Germany) for a period of 20 hours.

Airdrive perfusion system
Machine-perfused kidneys were washed out with PS followed by 20 hours of MP using the AD. The system contains a 2 L medical oxygen cylinder which is the only reusable part of the apparatus. The oxygen pressure is used to propel the novel membrane pump. Furthermore, the perfusion medium is actively oxygenated by a membrane oxygenator and an overpressure is created in the organ chamber in order to maintain sterility. To prevent injury to the microcirculation, the AD system is pressure-controlled at a mean arterial pressure of 25 mmHg and produces a pulsatile waveform over the renal artery at a temperature of 2°C to 6°C for a period up to 24 hours. The perfusion pressure parameters, flow and renal resistance (mmHg/ml/100 gr kidney weight) were continuously recorded.

Renal autotransplantation model
Twenty hours after the first operation and induction of general anesthesia, the abdomen was reopened and the contralateral kidney was removed. Immediately thereafter, the preserved kidney was heterotopically transplanted; the renal artery was anastomosed end-to-end to the right renal artery and the renal vein end-to-side to the inferior vena cava using 6-0 running Prolene® sutures. Cold ischemic times and times needed for performing both anastamoses were recorded. To prevent vascular thrombosis, a bolus of 3,000 IU of heparin was injected prior to reperfusion. Subsequently, 250 mL of glucose 20% was administered intravenously to
induce an osmotic diuresis. To allow free outflow of urine, the ureter was cannulated with a PE tube and an ureterocutaneostomy was applied. Renal microcirculation was re-assessed at 10 minutes after reperfusion. Postoperatively, the animals were supplemented with 1 L of 0.9% NaCl infusion (IV) and allowed free access to water. Food was provided the next day. Postoperative analgesia was provided with Tramadol (1 mg/kg BW) administered IM every 6 to 8 hours for up to 72 hours posttransplant. Ranitidine 50 mg (IV) was also given up to 72 hours postoperatively. Antibiotic treatment consisted of perioperative and subsequent administration of Ampicillin 2 x 500 mg on a daily basis. Anti-thrombotic therapy was provided daily by 500 mg of Aspirin IV. Animals were observed for seven days after transplantation. On a daily basis, venous blood samples were taken for the measurement of renal function by serum creatinine, urea and electrolytes. Also, 24-hour urine production was collected and creatinine clearance was calculated using the following formula: creatinine clearance = ((urine creatinine x 24 hr Volume) / (plasma creatinine x 24 x 60 min)).

Seven days after transplantation, under general anesthesia, the transplanted kidney was removed after reassessment of the renal microcirculation. Immediately thereafter, animals were euthanized by injection of T61 IV (Hoechst Roussel Vet, Wiesbaden, Germany).

Noninvasive evaluation of microcirculation
To evaluate the microcirculation noninvasively, a combined Laser Doppler and flowmetry device was used, the O2C. The O2C system allows for simultaneous recording at 2 mm and 8 mm tissue depths of capillary blood flow (flow, arbitrary units, AU) and capillary blood flow velocity (velocity, AU)\(^{26}\). The O2C has been validated previously in various surgical disciplines\(^{27,28}\). To prevent the influence of regional heterogeneity and temporal blood flow variations, measurements were performed at 4 pre-defined locations on the renal surface for 30 seconds each.

Renal morphologic studies
Sections of the cortex, medulla and the corticomedullary junction were fixed in neutral 10% buffered formalin and embedded in paraffin. Tissue injury was assessed on periodic acid-Schiff (PAS)-stained 4 µm thick sections by scoring glomerular ischemic damage (shrinkage), inflammatory cell infiltrates, tubular damage and interstitial edema. Injury of each specimen was graded to the extent of regional involvement in 10 randomly chosen, non-overlapping fields (original magnification, x400). Injury was scored by a pathologist blinded for the groups using a 5-point scale: 0 = no damage, 1 = lesions affecting ≤ 10% of the field, 2 = 10%-25%, 3 = 25%-50%, 4 = 50%-75% and 5 = > 75% \(^{29}\).
Immunohistochemistry
For the detection of proliferating cells, immunostaining was performed on 4 µm sections of paraffin-embedded tissue by applying a rabbit polyclonal antibody against nuclear antigen Ki67 (Abcam plc, Cambridge, United Kingdom) using the Dako REAL™ Detection System (Peroxidase/DAB+, Rabbit/Mouse, K5001, Dako, Hamburg, Germany). After deparaffinization and rehydration, slides were boiled in citrate buffer (pH 6) and peroxidase (H2O2) blocking solution was applied. Subsequently, slides were incubated with rabbit polyclonal anti-Ki67 (1:1000) for 30 minutes followed by Dako Wash Buffer (Dako, S3006) and biotinylated with secondary antibodies (Dako Real Link). Slides were finally developed using 1% DAB (Dako REAL™ DAB+ Chromogen) diluted in buffered solution containing hydrogen peroxide. The amount of Ki67+ cells was quantified in 10 non-overlapping fields in the corticomedullary junction\textsuperscript{30}.

Statistical Analysis
Mean values were calculated for each group with standard deviation (mean ± SD). Statistical analysis of microcirculation parameters and renal function was carried out using analysis of variance (ANOVA) for repeated measurements (RM) followed by the Bonferroni posttest. Area under the curve (AUC) for both serum creatinine and blood urea levels were calculated individually using the GraphPad Prism 5.0 statistics package (GraphPad Software, San Diego, CA, USA). When a non-parametric test was needed, the Kruskal-Wallis test was applied, followed by the Dunn's Multiple Comparison test. A \( P \)-value of < 0.05 was considered statistically significant.

Results
The weight of the animals did not differ between the three groups (kg; CS-UW, 25.6 ± 2.1; CS-PS, 24.4 ± 2.7; AD-PS, 22.9 ± 4.4; \( P = 0.335 \)). All animals demonstrated normal renal function before start of the experiments (serum creatinine, mg/dL; CS-UW, 1.25 ± 0.13; CS-PS, 1.12 ± 0.22; AD-PS, 1.10 ± 0.13; \( P = 0.187 \)). Cold ischemic times (CITs) of the CS-UW group were shorter when compared with the AD-PS group but comparable to the CS-PS group (CS-UW, 19:56 hr ± 0:24 min; CS-PS, 20:05 hr ± 0:25 min; AD-PS, 20:33 hr ± 0:05 min; \( P = 0.015 \), CS-UW vs. AD-PS, \( P < 0.05 \)). Times required for both anastomoses were comparable between all groups (minutes; CS-UW, 00:37 ± 0:06; CS-PS, 00:39 ± 0:05 and AD-PS, 00:42 ± 0:07, \( P = 0.192 \)). At the end of the 500 mL wash-out, all kidneys showed a macroscopically asanguinous effluent. Kidney weights after wash-out did not differ between the groups (gr; CS-UW, 58.6 ± 5.9; CS-PS, 65.0 ± 4.5; AD-PS, 66.8 ± 11.6; \( P = 0.246 \)). Kidney weights after preservation were higher in the AD-PS group compared to...
both cold stored groups (gr; CS-UW, 61.3 ± 7.1; CS-PS, 60.0 ± 2.4; AD-PS, 96.6 ± 11.4; \( P < 0.0001 \), RM ANOVA, CS-UW vs. AD-PS, \( P < 0.001 \) and CS-PS vs. AD-PS, \( P < 0.001 \)).

All animals in the study groups survived seven days. No differences were seen between the three groups and none of the experiments were terminated early. No adverse effects of the solutions used could be identified.

**AD MP system**

In this study, all grafts in the AD-PS group were perfused for 20 hours at a mean pressure of 25 mmHg. During perfusion, all grafts showed an increase in flow rate whereas perfusion resistance decreased over time. The temperature of the perfusion solution after 20 hours MP was 3.8°C ± 0.8°C.

**Microcirculation**

Compared with the CS-UW group, grafts preserved using AD-PS or CS-PS showed overall a higher cortical microcirculatory flow at 2 mm depth, ten minutes after reperfusion as well as 7 days posttransplant prior to sacrifice (\( P = 0.045 \), RM ANOVA, CS-UW vs. CS-PS, \( P < 0.05 \); CS-UW vs. AD-PS, \( P < 0.05 \)). Improvement of microcirculation was seen as expressed by a relatively higher cortical capillary blood flow post-reperfusion and prior to sacrifice in the CS-PS and AD-PS groups versus pre-retrieval, whereas in the CS-UW group, post-reperfusion, a decline in blood flow was recorded at both 2 mm and 8 mm tissue depths (Fig. 2A). Also, blood flow velocity post-reperfusion showed the same effect, i.e. an increase in the CS-PS and AD-PS groups versus a decline of blood flow velocity in the CS-UW group (Fig. 2B). Directly after revascularization, the AD-PS and CS-PS preserved grafts showed a homogenous perfusion, in contrast to the CS-UW preserved grafts which macroscopically showed perfusion defects.

**Renal function**

Overall, posttransplant serum creatinine values in the AD-PS and CS-PS groups were lower than posttransplant serum creatinine levels in the CS-UW group (\( P = 0.0001 \), RM ANOVA, CS-UW vs. CS-PS, \( P < 0.001 \), CS-UW vs. AD-PS, \( P < 0.001 \), Fig. 3A). In comparison with the CS-UW group, peak serum creatinine levels in the AD-PS and CS-PS groups were lower (mg/dL; CS-UW, 12.8 ± 6.4; CS-PS, 4.8 ± 1.3; AD-PS, 3.4 ± 1.9; CS-UW vs. CS-PS, \( P < 0.01 \); CS-UW vs. AD-PS, \( P < 0.001 \)). Also, times to peak creatinine (\( T_{\text{peak}} \)) were shorter in the AD-PS and CS-PS groups compared to CS-UW (days; CS-UW, 5.0 ± 1.8; CS-PS, 2.4 ± 0.8; AD-PS, 2.0 ± 1.9; CS-UW vs. CS-PS, \( P < 0.05 \); CS-UW vs. AD-PS, \( P < 0.01 \)). At sacrifice, serum creatinine levels differed significantly between both CS-PS and AD-PS preserved groups compared with the CS-UW group, with more favorable results in the AD-PS and CS-PS preserved grafts.
Fig. 2. Microcirculation (A) Capillary blood flow values registered at 2 mm and at 8 mm tissue depths, CS-UW, CS-PS and AD-PS group (flow at 2 mm, $P = 0.045$, RM ANOVA, CS-UW vs. CS-PS, *$P < 0.05$; CS-UW vs. AD-PS, *$P < 0.05$). (B) Blood flow velocity values registered at 2 mm and at 8 mm tissue depths, CS-UW, CS-PS and AD-PS group.
Fig. 3. Renal function. (A) Serum creatinine values posttransplant in the CS-UW, CS-PS and AD-PS group ($P = 0.0001$, RM ANOVA, CS-UW vs. CS-PS, ***$P < 0.001$; CS-UW vs. AD-PS, ***$P < 0.001$). (B) Blood urea levels posttransplant in the CS-UW, CS-PS and AD-PS group ($P = 0.001$, RM ANOVA, CS-UW vs. CS-PS, **$P < 0.01$; CS-UW vs. AD-PS, **$P < 0.01$). (C) Creatinine clearance rates posttransplant in the CS-UW, CS-PS and AD-PS group ($P = 0.039$, One-way ANOVA AUC creatinine clearance rates, CS-UW vs. AD-PS, **$P < 0.01$).
Posttransplant blood urea levels were significantly lower in the AD-PS and CS-PS groups \((P = 0.001, \text{RM ANOVA})\); CS-UW vs. CS-PS, \(P < 0.01\); CS-UW vs. AD-PS; \(P < 0.01\), Fig. 3B). In comparison with the CS-UW group, peak urea levels in the AD-PS and CS-PS groups were significantly lower \((P < 0.01); \text{CS-UW vs. AD-PS, } P < 0.01\). Time to peak urea was also significantly lower for the AD-PS and CS-PS preserved groups \((P < 0.01); \text{CS-UW vs. AD-PS, } P < 0.01\).

Blood urea at sacrifice was significantly lower in the AD-PS and CS-PS groups compared with the CS-UW group \((P < 0.05); \text{CS-UW vs. AD-PS, } P < 0.05\). Urine production did not differ significantly between the three groups. All animals produced urine every day. Overall, posttransplant creatinine clearance rates were higher in the AD-PS preserved kidneys compared with CS-UW preservation \((P < 0.01, \text{Fig. 3C})\). At sacrifice, creatinine clearance rates in both AD-PS and CS-PS preserved groups were significantly higher than creatinine clearance rates in the CS-UW group \((P < 0.01); \text{CS-UW vs. AD-PS, } P < 0.05\) and \(P < 0.05\).

**Histological examination**

Histological examination showed overall less tubular damage in the AD-PS and CS-PS preserved grafts, compared with grafts stored in UW solution. Glomeruli were well preserved using the AD-PS, whereas CS-UW and CS-PS preserved grafts showed significantly more glomerular shrinking. In addition, glomeruli of grafts preserved using CS-PS showed less shrinking when compared with grafts preserved using UW solution. Inflammatory infiltration was more pronounced in the CS-UW and CS-PS groups compared with the AD-PS group. Moreover, AD-PS preserved grafts demonstrated less tissue edema in comparison with both CS-UW and CS-PS preserved grafts (Table 1). Overall, structural integrity was best preserved in grafts preserved using AD-PS.
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<th>Table 1. Quantification of morphological data</th>
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<td><strong>Glomerular shrinking</strong></td>
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|                             | 1.3 ± 0.1 | 0.4 ± 0.2 | 0.1 ± 0.2 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS ** |
| **Tubular damage**           |       |       |       |          |
| Cortex                       | 2.0 ± 0.2 | 1.1 ± 0.2 | 1.7 ± 0.3 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| Medulla                      | 2.3 ± 0.3 | 1.7 ± 0.2 | 0.2 ± 0.2 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| Corticomedullary junction    | 2.0 ± 0.2 | 1.8 ± 0.2 | 1.9 ± 0.3 | NS |
| **Inflammatory Infiltration**|       |       |       |          |
| Cortex                       | 1.5 ± 0.2 | 1.0 ± 0.2 | 0.3 ± 0.2 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| Medulla                      | 2.2 ± 0.2 | 2.2 ± 0.2 | 1.1 ± 0.1 | CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| Corticomedullary junction    | 1.8 ± 0.3 | 1.5 ± 0.3 | 0.5 ± 0.2 | CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| **Edema**                    |       |       |       |          |
| Cortex                       | 0.9 ± 0.1 | 0.5 ± 0.1 | 0.2 ± 0.1 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |
| Medulla                      | 1.5 ± 0.2 | 2.0 ± 0.2 | 1.5 ± 0.2 | CS-UW vs. CS-PS ***  
CS-PS vs. AD-PS *** |
| Corticomedullary junction    | 1.3 ± 0.1 | 0.8 ± 0.3 | 0.3 ± 0.2 | CS-UW vs. CS-PS ***  
CS-UW vs. AD-PS ***  
CS-PS vs. AD-PS *** |

Semiquantitive scale: 0 = no injury, 1 = lesions affecting ≤ 10% of the field, 2 = 10%-25%, 3 = 25%-50%, 4 = 50%-75% and 5 = involvement of > than 75% of the field
Data expressed as mean ± SD, **P < 0.01, ***P < 0.001, NS, not significant
**Immunohistochemistry**

The amount of proliferating tubular epithelial cells as determined by Ki67-positive cells in the corticomedullary junction was reduced in the AD-PS preserved grafts when compared with grafts preserved using CS-UW (Fig. 4A-D).

![Graph](image)

**Fig. 4.** Proliferative response (A) The amount of Ki67+ cells as counted in 10 randomly selected fields in the corticomedullary junction of the CS-UW, CS-PS and AD-PS groups (P=0.020, Kruskal-Wallis, CS-UW vs. AD-PS, *P<0.05). Immunohistochemistry (B) CS-UW group (C) CS-PS group (D) AD-PS group (original magnification, x20).
Discussion

Several pre-clinical and clinical studies comparing MP versus CS have shown benefits in favor of MP. Recently, the first randomized controlled clinical trial as reported by Moers et al. has confirmed these findings and could lead to universal acceptance and employment of hypothermic MP preservation of kidney grafts.

The Lifeport MP system used in this study, however, lacks the capability to oxygenate the perfusate. Whereas hypothermic preservation is known to aggravate ischemic injury, the effects of hypothermia and ischemia can potentially be reversed by MP through addition of oxygen to the perfusate. However, to our knowledge, clinical studies comparing machine perfusion with oxygenated machine perfusion have not been published.

In our study, the novel AD MP system was used for hypothermic oxygenated pulsatile perfusion of kidney grafts. Manekeller and colleagues showed that oxygenated hypothermic perfusion preservation can result in excellent preservation quality by preventing oxygen deprivation and restoration of energy status. With respect to functional integrity, hypothermia and shortage of energy substrates are known to be detrimental to the graft, as the metabolism, although reduced, is still active at hypothermia. By enabling kidney grafts to restore tissue homeostasis, glomerular and tubular function can be maintained during ischemia.

In the AD-PS preserved grafts, functional integrity was better preserved as expressed by a significantly higher creatinine clearance rate and lower posttransplant blood urea AUC in the AD-PS preserved grafts. Moreover, evidence for improved preservation of the renal tubules in the AD-PS group can be derived from a significantly lower expression of the Ki67 antigen.

It has been reported that the expression of the Ki67 is low in normal tubular epithelium but upregulated in injured renal tubular epithelial cells. In a different porcine study performed by our group, the amount of Ki67+ cells in normal, non-transplanted kidneys was 55.3 ± 24.0 (mean ± SD, data not shown).

A major drawback associated with hypothermic MP is the aggravation of endothelial injury during hypothermic preservation caused by sheer stress, leading to attenuation of perfusion flow. In this study, however, a decrease in perfusate flow during perfusion preservation was not observed. The low preset mean perfusion pressure (25 mmHg) applied in the AD MP system could be partly accountable for the maintenance of circulatory parameters in our study. Using a similar experimental model, application of a low perfusion pressure of 30/20 mmHg, which resembles the mean arterial pressure used in the AD MP system, resulted in less perturbation of endothelial cells compared to a perfusion pressure of 60/40 mmHg. Maathuis and co-workers also postulated that preset perfusion pressures are critically important for successful outcome after MP of kidney grafts.
Directly after revascularization, the AD-PS and CS-PS preserved grafts showed a homogenous reperfusion with blood, in contrast to the CS-UW preserved grafts which macroscopically showed reperfusion deficits. The obstruction of blood flow leading to perfusion deficits is possibly due to incomplete washout and tissue edema. This phenomenon was previously encountered in experimental studies when describing the potential adverse effects of UW in CS preservation of porcine kidneys\(^7,24,38\).

Immediately after the preservation period, graft weights were higher in the AD-PS group. This phenomenon was investigated by Wilson et al. in a retrospective clinical study \((n = 97)\) on kidneys preserved by hypothermic MP\(^39\). All perfused kidneys gained weight during MP. A correlation between weight gain and immediate function, primary non-function or duration of delayed graft function could not be identified. Wilson et al. concluded that kidney grafts which gained over 30% of weight on hypothermic MP preservation can be transplanted successfully.

The weight gain is more likely to result from a reversible intravascular pooling than from extravasation of the perfusion medium. The histological examination at 7 days posttransplant supports this hypothesis since machine perfused grafts showed significantly less tissue edema compared to CS preserved grafts. The favorable results of MP in our study with regards to posttransplant function and in particular structural integrity corroborate with others that weight increase does not seem to correlate with graft viability\(^39,40\). In addition, microcirculation in both the AD-PS and CS-PS groups was better preserved compared to the CS-UW group as assessed noninvasively by Laser Doppler flowmetry. In several preclinical studies, graft blood flow could be reliably monitored by Laser Doppler flowmetry during transplantation, whereby a strong correlation was also observed between microcirculation of the graft during preservation and graft function after transplantation\(^24,41\). Our findings corroborate a previous study by Minor et al. which demonstrated improved microcirculatory parameters during preservation due to high oxygen availability\(^6\).

The preservation solution employed for perfusion as well as for cold storage in our study is the recently developed PS solution. PS, a low-viscosity colloid-based solution, appears to meet the requirements for efficient perfusion preservation, as the low viscosity allows for an effective washout whereby tissue edema is prevented by the colloid and impermeants. Potential explanations for the higher efficacy of PS over UW, albeit in the setting of CS, have been described previously\(^24\).

The AD MP system has been designed for portability and accessibility in order to facilitate application of MP. Also, the disposable aspect of the system obviates the high costs and complex logistics associated with current MP systems since return transport, maintenance and dedicated personnel involved in operation and handling are no longer required. The AD MP system uses a novel low-pressure, low-flow oxygen pressure-driven perfusion pump. High viscosity of the perfusion medium was found to negatively influence the performance and reliability of both the
perfusion pump and electronic control system. Since the viscosity of both UW and KPS-1 solution is higher than the viscosity of PS (5.7 vs. 2.5 vs. 1.8 centipoise, respectively), we decided not to use UW or KPS-1 solution in combination with the AD MP system.

Using the autotransplant model, in this study we opted to evaluate the biological safety and posttransplant function of kidney grafts during MP or CS without interference of alloantigen-dependent mechanisms. In order to assess the performance of the preserved kidney, the contralateral kidney was explanted prior to heterotopic re-implantation of the graft. In our study, using a 7-day follow-up, we focused on the events directly after transplantation. Cold ischemic time was limited in this study to 20 hours since porcine kidneys are known to be particularly susceptible to ischemia. Our findings are in accordance with previous experimental studies using porcine autotransplant models which all showed a distinct benefit from hypothermic MP over CS using UW as well as large variations of results within the CS-UW groups.

In addition to the encouraging results obtained in previous CS preservation studies using PS, this porcine kidney transplant study demonstrates that the microcirculation of kidney grafts was better preserved after 20 hours when using the AD MP system for hypothermic oxygenated pulsatile perfusion preservation compared with CS preservation. Moreover, in the perfused grafts overall less morphological injury at 7 days posttransplant was observed compared with kidney grafts preserved using CS. Renal function was improved in both AD-PS and CS-PS preservation as compared to CS-UW. Whether MP preservation of marginal donor grafts as well as clinical application will lead to favorable results will be the subject of future studies.

In conclusion, this study demonstrates the biological safety of the AD as a MP preservation system for renal grafts in a large animal model. Clinical data are needed to confirm the safety of the device and the perfusion solution used.

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