Improved preservation and microcirculation with POLYSOL after transplantation in a porcine kidney autotransplantation model

Marie-Claire J. M. Schreinemachers¹, Benedict M. Doorschodt¹, Sandrine Florquin², Marius A. van den Bergh Weerman², Johannes B. Reitsma³, Wei Lai⁴, Mario Sitzia⁵, Thomas M. Minor⁴, Rene H. Tolba⁵,⁶∗ and Thomas M. van Gulik¹

¹Surgical Laboratory, ²Department of Pathology, ³Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands, ⁴Surgical Research Division, Department of Surgery, ⁵House of Experimental Therapy, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn and ⁶Institute for Laboratory Animal Science and Experimental Surgery, RTWH-Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany

Abstract

Background. The most widely used preservation method for kidney grafts is cold static storage (CS) using the University of Wisconsin (UW) solution. To date, new preservation solutions have not been able to significantly improve preservation quality of grafts. The aim of this study was to compare POLYSOL, a recently developed low viscosity preservation solution, and the UW solution for CS of porcine kidney grafts.

Methods. In a porcine autotransplantation model, real-time parameters of the renal microcirculation were evaluated using the novel oxygen-to-see (O2C) combined laser Doppler and flowmetry system. Thereafter, kidneys were retrieved and washed out with POLYSOL or UW followed by 20-h CS. After the preservation period, the contralateral kidneys were removed and the preserved kidneys autotransplanted. The microcirculation was re-assessed at 10 min after reperfusion and at 7 days posttransplant, prior to removal of the grafts for histological evaluation.

Results. POLYSOL was able to better preserve the microcirculation compared to UW as expressed by higher values of capillary blood flow, blood flow velocity and tissue oxygen saturation values. In addition, CS using POLYSOL resulted in improved functional recovery demonstrated by lower posttransplant serum creatinine and blood urea values in comparison to the UW group. Also, structural integrity was better preserved in the POLYSOL group, compared to UW.

Conclusions. This study in a clinically relevant large animal model showed that a new preservation solution, POLYSOL, resulted in improved preservation quality of kidney grafts compared to the UW solution.

Keywords: kidney; microcirculation; pig; preservation solution; transplantation

Introduction

Currently, cold static storage (CS) using the University of Wisconsin (UW) solution is considered the gold standard for the preservation of abdominal organs [1–6]. Although UW was developed over 20 years ago, clinically applied preservation solutions devised since have not been able to significantly improve the preservation quality of donor organs. A drawback of the UW solution, however, is its high viscosity, mainly due to the colloid hydroxyethyl starch (HES). This high-molecular-weight molecule is known to cause obstructions of the microvasculature by accelerated aggregation of erythrocytes, resulting in incomplete washout of the donor graft [7,8].

Recently, POLYSOL has been developed for hypothermic perfusion preservation. POLYSOL is a preservation solution with low viscosity and a high buffering capacity, which contains 60 components, consisting of impermeants, antioxidants, vitamins, energy substrates and amino acids [9–16]. One of the main components is polyethylene glycol (PEG, 35 kDa), a low-molecular-weight colloid that does not increase viscosity as seen with HES-containing solutions (Table 1).

In experimental studies in rats, POLYSOL demonstrated favourable results in both Machine Preservation (MP) and CS preservation of the liver [9–15] and CS preservation of the small bowel [16]. The aim of this study was to compare, in a prospective randomized fashion, POLYSOL and UW for CS preservation of kidney grafts in a porcine autotransplantation model. Furthermore, we evaluated real-time parameters of the microcirculation, pre-retrieval, at 10 min post-reperfusion and 7 days posttransplant at sacrifice using the novel...
Improved preservation and microcirculation with POLYSOL after transplantation

### Subjects and methods

#### Experimental protocol

All experiments were performed in accordance with the German legislation governing animal studies. The Principles of Laboratory Animal Care (NIH publication 85-23, revised 1985) was followed.

Female German landrace pigs, from a disease-free barrier breeding facility of 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 pigs, weighing 25.0 ± 2.6 kg (mean ± SD), were fasted 24 h prior to the experiments. All animals demonstrated normal renal function before the start of the experiments.

#### Experimental design

This study was performed using a porcine renal autotransplantation model as previously established in our institution and described by Maathuis et al. [18]. After left nephrectomy, kidneys were flushed ex vivo and preserved for a period of 20 h by CS at 4°C in the respective solution. After preservation, the contralateral kidney was removed, followed by immediate heterotopic transplantation of the preserved kidney. This study involved two experimental groups, preservation with POLYSOL (n = 6) and UW (n = 6), as was conducted in a prospective randomized fashion.

Animals were premedicated with ketamine (90 mg/kg), xylazine (10 mg/kg) and atropine (10 µg/kg) administered intramuscularly (IM) 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. Intraoperatively animals were monitored by means of pulse oximetry using a tail probe.

Prior to nephrectomy, the right internal jugular vein was cannulated with a polyethylene (PE) catheter for infusion and daily collection of blood samples. Through a midline laparotomy, the left kidney was approached and microcirculation was assessed at four pre-determined locations on the renal surface using a combined laser Doppler and flowmetry system (O2C system, LEA Medizintechnik, Gießen, Germany). After recovery, kidneys were immediately washed out with 500 ml of either POLYSOL (POLYSOL, Doorzand Polysol B.V., Amsterdam, The Netherlands) or UW solution (Viaspan®, Bristol-Myers Squibb B.V., Woerden, The Netherlands) at 4°C using a cold water bath (Lauda, Königshofen, Germany) during a 20-h period.

### Renal autotransplantation

After the 20-h preservation period, the contralateral kidney was removed, followed directly by heterotopic autotransplantation of the left kidney. 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. Both anastomoses were performed with 6–0 running Prolene® sutures. Times needed for performing both anastomoses were recorded. Prior to completion of the arterial anastomosis, a bolus of 3000 IU of heparin was injected (IV) to prevent vascular thrombosis. Following reperfusion, 250 ml of glucose 20% was administered IV to induce osmotic diuresis. The ureter was cannulated with a PE tube to allow free outflow of urine through an ureterocutaneostomy. Ten minutes after reperfusion, renal microcirculation was re-assessed.

#### Postoperative care

Postoperatively, the animals were given 1 litre of saline infusion via the jugular catheter. The animals had free access to water, and food was provided the next day. All animals were subjected to a standard medication regimen. Postoperative analgesia was provided with tramadol (1 mg/kg)-administered IM every 6–8 h for up to 72 h posttransplant. Ranitidine 50 mg (IV) was also given up to 72 h postoperatively. Antibiotic treatment (ampicillin 2 × 500 mg daily) was started prior to kidney retrieval and continued during the observation period. Anti-thrombotic therapy was provided daily by 500 mg of aspirin IV.

### Table 1. Most important components of preservation solutions used

<table>
<thead>
<tr>
<th>Component</th>
<th>POLYSOL</th>
<th>UW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloid</td>
<td>HES (g/l)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>PEG-35 (g/l)</td>
<td>20</td>
</tr>
<tr>
<td>Impermeants</td>
<td>Lactobionic acid (mM)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Raffinose (mM)</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Trehalose (mM)</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Sodium gluconate (mM)</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Potassium gluconate (mM)</td>
<td>30</td>
</tr>
<tr>
<td>Anti-oxidants</td>
<td>Allopurinol (mM)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Glutathione (mM)</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Alpha-tocopherol (mM)</td>
<td>5.4 x 10^-6</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid (mM)</td>
<td>0.11</td>
</tr>
<tr>
<td>ATP precursor</td>
<td>Adenosine (mM)</td>
<td>5.0</td>
</tr>
<tr>
<td>Buffer</td>
<td>Potassium phosphate (mM)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Sodium phosphate (mM)</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>Histidine (mM)</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>HEPES (mM)</td>
<td>24</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>Na^+ (mM)</td>
<td>120/15</td>
</tr>
<tr>
<td></td>
<td>K^+ (mM)</td>
<td>27/125</td>
</tr>
<tr>
<td>Amino acids</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Vitamins</td>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td>Viscosity at 5°C (cP)</td>
<td>1.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

cP: centipoise; HES, hydroxyethyl starch; PEG, polyethylene glycol; UW, University of Wisconsin.

aComposition developed and patented by B.M. Doorschodt.

bThe following amino acids are present in POLYSOL (mM): alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine.

*cThe following vitamins are present in POLYSOL (mM): ascorbic acid, biotin, Ca-pantothenate, choline chloride, inositol, ergocalciferol, folic acid, menadione, nicotinamide, nicotinic acid, pyridoxal, riboflavin, thiamine, vitamin A, vitamin B12 and vitamin E.

oxygen-to-see (O2C) laser Doppler flowmetry and the remissiion spectroscopy system [17].
Following transplantation, animals were observed for 7 days. Venous blood samples were taken for measurement of renal function by serum creatinine, urea and electrolytes daily. The internal jugular vein catheter was flushed with saline and blocked with heparin 1000 IU/ml after each sampling procedure. Total 24-h urine production was collected, and creatinine clearance was calculated.

At Day 7 posttransplant, animals were sacrificed, and the transplanted kidney was removed for histological evaluation. Prior to removal, renal microcirculatory measurements were performed. Immediately after removal of the graft, animals were euthanized (T61 injection IV, Hoechst Roussel Vet, Wiesbaden, Germany).

Noninvasive evaluation of microcirculation

In this study, a combined laser Doppler and flowmetry device was used to noninvasively evaluate the microcirculation at 2 and 8 mm tissue depths. The O2C system allows simultaneous recording of the following microcirculatory parameters: tissue oxygen saturation (SO2 percentage, absolute value), capillary blood flow [flow, (AU)] and capillary blood flow velocity [velocity, (AU)] [17]. The O2C has been validated clinically in various surgical disciplines [19,20]. To prevent the influence of regional heterogeneity and temporal blood flow variations, measurements were performed at four pre-determined locations on the renal surface for 30 sec each.

Histological examination

Tissue samples of cortex, medulla and the corticomedullary boundary were collected. For light microscopy, sections were fixed with neutral 10% buffered formalin and embedded in paraffin. Conventional staining (haematoxylin and eosin, periodic acid-Schiff) was applied. Tissue sections were examined by a pathologist blinded for the experimental conditions using a Philips CM10 transmission electron microscope (FEI, Philips, Eindhoven, The Netherlands). Images were acquired using a digital transmission EM camera (Morada 10-12, Soft Imaging System, RvC, Soest, The Netherlands) using the software Research Assistant.

Statistical analysis

Mean values were calculated for each group with standard deviation (mean ± SD). Statistical analysis of the microcirculation parameters, serum creatinine and blood urea values of the two groups was made using a two-way analysis of variance (ANOVA) for repeated measurements and the Bonferroni posttest correction. Where applicable, the unpaired two-tailed t-test was performed. When a non-parametric test was needed, the Mann–Whitney U-test was applied. A P-value of <0.05 was considered statistically significant.

Results

The weight of the animals did not differ between the two groups (POLYSOL, 24.4 ± 2.9 kg; UW, 25.6 ± 2.3 kg, P = 0.472). Cold ischaemic times (CITs) and times required for both anastomoses were comparable (POLYSOL, 20:05 h ± 0.27 min; UW 19:56 h ± 0.27 min, P = 0.596 and POLYSOL, 00:41 ± 0.06; and UW, 00:38 ± 0.06 min, P = 0.420, respectively). At the end of the 500 ml washout, all kidneys showed a macroscopically asanguinous effluent. All animals in both groups survived 7 days, and no differences were seen between both groups. No adverse effects of the solutions used could be identified.

Microcirculation

Compared to the UW group, POLYSOL-preserved grafts showed better preservation of microcirculation, 10 min after reperfusion as well as 7 days posttransplant prior to sacrifice. Improvement of microcirculation was seen as expressed by a relatively higher capillary blood flow post-reperfusion in the POLYSOL group versus pre-retrieval, whereas in the UW group, post-reperfusion, a decline in blood flow was recorded at both 2 and 8 mm tissue depths. Also, blood flow velocity post-reperfusion showed the same effect, i.e. an increase in the POLYSOL group versus a decline of blood flow velocity in the UW group. In addition, tissue oxygen saturation values were higher in the POLYSOL-preserved grafts as well at 10 min after reperfusion as at 7 days posttransplant compared to grafts preserved using UW (Figure 1A–C).

Renal function

Overall, posttransplant serum creatinine values in the POLYSOL group were lower than posttransplant serum creatinine levels in the UW group (P = 0.041, two-way ANOVA, Figure 2). Creatinine area under the curve (AUC) values were significantly different in favour of the POLYSOL group. In comparison with the UW group, peak serum creatinine levels and time to peak creatinine (Tpeak) in the POLYSOL group were lower. At sacrifice, serum creatinine levels differed significantly between both groups with more favourable results in the POLYSOL-preserved grafts (Table 2).

Posttransplant blood urea levels were significantly lower in the POLYSOL group (P = 0.048, two-way ANOVA,
Figure 3). AUC for blood urea was significantly less for POLYSOL-preserved grafts. In comparison with the UW group, peak serum urea levels in the POLYSOL group were significantly lower. Time to peak urea was also significantly lower for the POLYSOL group (Table 2).

Urine production did not differ significantly between both groups. All animals produced urine every day. Mean posttransplant creatinine clearance rates were numerically higher in the POLYSOL-preserved kidneys at all timepoints compared to UW (creatinine clearance rate AUC: POLYSOL, 135.2 ± 58.0; UW, 76.8 ± 67.5, P = 0.139). At sacrifice, however, creatinine clearance in the POLYSOL-preserved grafts were significantly higher than creatinine clearance rates in the UW group (POLYSOL, 33.09 ± 6.7 ml/min; UW, 12.30 ± 21.3 ml/min; P = 0.046).

**Histological examination**

Histological examination showed overall less tubular damage in the POLYSOL-preserved grafts, compared to grafts

---

Table 2. Summary of posttransplant results

<table>
<thead>
<tr>
<th></th>
<th>POLYSOL</th>
<th>UW</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC creatinine during 7-day follow-up</td>
<td>25.0 ± 8.1</td>
<td>60.1 ± 35.8</td>
<td>0.041</td>
</tr>
<tr>
<td>Peak creatinine during 7-day follow-up (mg/dl)</td>
<td>4.79 ± 1.45</td>
<td>13.01 ± 7.00</td>
<td>0.018</td>
</tr>
<tr>
<td>$T_{peak}$ creatinine (days)</td>
<td>2.3 ± 0.8</td>
<td>5.5 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum creatinine at sacrifice (mg/dl)</td>
<td>2.02 ± 0.69</td>
<td>10.21 ± 8.34</td>
<td>0.037</td>
</tr>
<tr>
<td>AUC urea during 7-day follow-up</td>
<td>426 ± 134</td>
<td>846 ± 440</td>
<td>0.049</td>
</tr>
<tr>
<td>Peak urea during 7-day follow-up (mg/dl)</td>
<td>81.5 ± 35.1</td>
<td>225.8 ± 147.5</td>
<td>0.042</td>
</tr>
<tr>
<td>$T_{peak}$ urea (days)</td>
<td>2.7 ± 1.6</td>
<td>5.7 ± 1.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Blood urea at sacrifice (mg/dl)</td>
<td>40.5 ± 22.5</td>
<td>195.0 ± 173.2</td>
<td>0.056</td>
</tr>
</tbody>
</table>

$T_{peak}$, time to peak level; UW, University of Wisconsin; AUC, area under the curve; sacrifice, day 7 posttransplant.
stored in the UW solution. Also, glomeruli were well preserved using POLYSOL, whereas UW-preserved grafts showed significantly more glomerular shrinking. More inflammatory cell infiltrates were present in the cortical region of the UW-preserved grafts compared to the POLYSOL group. Overall, integrity of the cortical region was better preserved in the POLYSOL group (Table 3, Figure 4A–F; additional colour images available online as Supplementary material).

Electron microscopical evaluation showed well-preserved structural integrity of renal grafts stored using POLYSOL. Tubular epithelial cells and glomerular capillary endothelium were well preserved whereas UW-preserved grafts showed vacuolization of tubular cells and debris in the tubular lumen. Also, mitochondrial structures were better preserved in the POLYSOL group compared to the UW group (Figure 5A, B).

Discussion

In this pre-clinical study, the autotransplant model was chosen to assess posttransplant function of kidney grafts in the absence of alloantigen-dependent mechanisms. A contralateral nephrectomy was performed prior to heterotopic re-implantation of the kidney in order to evaluate solely the performance of the preserved kidney. Noninvasive evaluation of the microcirculation was used as a tool to assess the viability of the graft as described by Anaise et al., who showed a direct correlation between the integrity of the cortical microcirculation during preservation and life-sustaining function of the kidney after experimental transplantation [22]. A recent clinical study by Lisik et al. demonstrated the predictive value of Laser-Doppler measurements of parenchymal blood flow after reperfusion on postoperative function [23]. In several clinical studies, noninvasive assessment of the microcirculatory status using Laser-Doppler flowmetry proved to be a reliable method of monitoring graft blood flow perfusion during transplantation [24].

Faure et al. showed that renal function of autotransplanted porcine kidneys can vary widely according to the preservation solution used [25]. The exposure of juvenile animals to two major operations under general anaesthesia within a period of 24 h resulted in a considerable catabolic response, reflected in the postoperative biochemistry. In the POLYSOL group, the results of the various parameters were homogenous showing early and rapid posttransplant recovery of all animals. The results in the UW-preserved group, however, showed a wider variation. The cause of non-recovery of renal function was most likely due to the difference in the preservation solution used. In a porcine kidney autotransplant study by Badet et al. comparing the UW solution with IGL-1, in which Na+ and K+ concentrations are inverted and HES is substituted by PEG-35, non-recovery of UW-preserved grafts was also observed. One animal died 2 days after transplantation and the remaining five showed a wide variation of renal function within the group [26].

A possible explanation for the less favourable results and wider variation within the UW group could be an incomplete washout of the graft due to the high viscosity of the UW solution as compared to the low viscosity of POLYSOL. Previous experimental studies suggested that an initial flush with cold UW may result in poor perfusion of the graft because of its high viscosity and may also induce microcirculatory disturbances thereby influencing graft function after transplantation. Conversely, an initial flush with a low-viscosity solution is suggested to effectively wash vasoactive substances and inflammatory mediators out of the graft [27,28].

In addition, the HES molecule in the UW solution is known to extend and accelerate the aggregation of erythrocytes, resulting in stasis of blood and thus in an incomplete washout of the donor graft [7,9,10]. Directly after revascularization, all POLYSOL-preserved grafts showed a macroscopically homogenous perfusion, in contrast to the UW-preserved grafts that macroscopically showed perfusion defects and tissue oedema. The latter was confirmed by histological analysis as significantly more tissue oedema in the cortical regions of UW grafts as opposed to POLYSOL grafts. The hypothesis of an incomplete washout causing impaired (re)perfusion of UW grafts was also confirmed by the O2C measurements performed at 10 min after reperfusion, demonstrating a significant decrease in blood flow, blood flow velocity and oxygen saturation in the UW group in contrast to the POLYSOL group. This finding of deprived microvascular tissue perfusion upon reperfusion using UW in a porcine autotransplant model is in line with a recent study by Maathuis et al. [18].

Another potential reason for the superiority of POLYSOL over UW in our study is the low potassium versus high sodium ratio in POLYSOL. Extracellular-type solutions that contain a low potassium and high sodium content are more effective in cold storage preservation of kidneys than

| Table 3. Quantification of morphological data at sacrifice |
|----------------|----------------|----------------|
|               | POLYSOL        | UW             | P-value |
| Glomerular damage (shrinkage) |               |                |         |
| Cortex        | 0.4 ± 0.2      | 1.3 ± 0.1      | ***     |
| Inflammatory infiltration |               |                |         |
| Cortex        | 1.0 ± 0.2      | 1.5 ± 0.2      | ***     |
| Medulla       | 2.2 ± 0.2      | 2.2 ± 0.2      | n.s.    |
| Cortico-medullary boundary | 1.5 ± 0.3      | 1.8 ± 0.3      | n.s.    |
| Tubular damage |               |                |         |
| Cortex        | 1.1 ± 0.2      | 2.0 ± 0.2      | ***     |
| Medulla       | 1.7 ± 0.2      | 2.3 ± 0.3      | ***     |
| Cortico-medullary boundary | 1.8 ± 0.2      | 2.0 ± 0.2      | 0.044   |
| Oedema        |               |                |         |
| Cortex        | 0.5 ± 0.1      | 0.9 ± 0.1      | ***     |
| Medulla       | 2.0 ± 0.2      | 1.5 ± 0.2      | 0.0001  |
| Cortico-medullary boundary | 0.8 ± 0.3      | 1.3 ± 0.1      | 0.0001  |

UW, University of Wisconsin; sacrifice, Day 7 posttransplant.

Semi-quantitative scale: 0 = no abnormality; 1 = mild, lesions affecting 0–10% of the field; 2 = moderate, lesions affecting 10–25% of the field; 3 = severe, lesions affecting 25–50% of the field; 4 = very severe, lesions affecting 50–75% of the field and 5 = extensive damage, involvement of >75% of the field.

***P < 0.001, n.s.: not significant.
Fig. 4. Light microscopy (additional colour images available online as Supplementary material). (A) POLYSOL group cortex: well-preserved glomerulus, no inflammation (original magnification ×20, PAS stain). (B) UW group cortex: shrinking of glomerulus, tubular dilatation and interstitial inflammation (original magnification ×20, PAS stain). (C) POLYSOL group medulla: well-preserved tubuli without clear pathology (original magnification ×20, PAS stain). (D) UW group medulla: tubular dilatation, denudation of tubular basal membrane and cellular casts (original magnification ×20, PAS stain). (E) POLYSOL group cortico-medullary border: minor shrinking of glomerulus, tubuli intact, minor oedema (original magnification ×20, PAS stain). (F) UW group cortico-medullary border: shrinking of glomerulus, simplification of tubular epithelium (original magnification ×20, PAS stain).
intracellular-type solutions, such as UW. The use of a low-
K$^+$ solution in a transplantation study of rat kidneys re-
sulted in reduced peritubular endothelial cell death due
to ischaemia-reperfusion injury (IRI) and improved graft
survival [29]. In a porcine autotransplant study, analysing
the effects of preservation solutions on the function of
autotransplanted kidneys, the Hopital Edouard Herriot so-
lution (UW solution modified to high Na$^+$, low K$^+$ content)
proved to be more effective than the regular UW solution
[30]. Since both solutions consisted of similar components,
the protective effect could be directly related to the Na$^+$,
K$^+$ ratio. As both the low level of K$^+$ and the high Na$^+$
concentration limit the entry of Ca$^{2+}$ into the cell, Ca$^{2+}$
overload is prevented, thereby avoiding a depolarization of
smooth muscular cell membrane leading to vasoconstric-
tion. This limitation of vasoconstriction favours a more
homogeneous diffusion of the solution within the organ.
Moreover, extracellular-type solutions maintain an equilib-
rium between the extracellular and intracellular compart-
ment to prevent osmotic shifts and cell swelling.

Also, the colloid PEG with a molecular weight of 35 kDa
is a vital component of POLYSOL. PEG is known to have
anti-oxidative properties and has led to the improvement
of immediate function of kidney grafts as it protects renal
tubular cells against the effects of cold ischaemic injury.
The addition of PEG to the preservation solution reduced
inflammatory infiltration in autotransplanted kidneys after
reperfusion [31]. In our study, using a 7-day follow-up, we
focused on the events shortly after transplantation. The use
of POLYSOL resulted in significantly less morphological
injury 7 days posttransplant compared to kidney grafts pre-
served using UW. Whether PEG was responsible for the
protective effect as suggested by others [31,32] is unclear.

Although the chain length of the PEG molecule (20 kDa)
and the concentration used differed from our study, Faure
et al. demonstrated that PEG exerts its protective effect by
improving morphological features throughout a 12-week
period of follow-up. This group also investigated the protec-
tive effect on renal cells of PEG in different concentrations
and molecular weight added to a variety of preservation
solutions. Only PEG 35 kDa exhibited abolishment of re-
active oxygen species, preservation of ATP synthesis and a
clear protective effect on tubule cell morphology [33]. From
these studies one could conclude that the optimal concen-
tration and molecular weight of PEG as a component of
preservation solutions are still a matter of debate.

Furthermore, other components of POLYSOL could play
an important role. POLYSOL consists of 60 components
in total. POLYSOL contains histidine, sodium phosphate
and HEPES in order to provide enhanced buffer capac-
ity. HEPES demonstrated superior results in a non-animal
in vitro study comparing the buffer capacity and efficacy
of various preservation solutions by standard electrometric
titrations methods [34]. Furthermore, POLYSOL contains
impermeants, free radical scavengers, energy substrates,
amino acids and vitamins; the substantial value of many of
these components has been previously demonstrated [1,3–
5,35–37].

Moreover, in addition to the favourable results obtained
in previous MP and CS preservation studies of the rat
liver using POLYSOL, this porcine kidney transplant study
demonstrates that POLYSOL can be used effectively as
a CS preservation solution for renal grafts. Whether MP
preservation of porcine kidneys using POLYSOL in the
same model will result in favourable results compared to
POLYSOL CS will be the subject of future studies.
Other studies have also shown the potential adverse effects of UW in CS preservation of porcine kidneys resulting in a rise of serum creatinine and blood urea levels which, if encountered in clinical practice, would inevitably lead to dialysis treatment [26,38]. Nicholson et al. suggested to limit the CIT in porcine transplantation models to a maximum of 18–20 h, because porcine kidneys are more susceptible to ischaemic-reperfusion injury than canine kidneys, which have been preserved successfully for 72 h [39]. On the other hand, Hauet et al. successfully demonstrated application of longer preservation times in a porcine kidney autotransplantation model [31]. Therefore, future transplantation studies using POLYSOL should focus on prolonged CITs and transplantation of kidneys subjected to warm ischaemic injury. This is of importance in order to translate the results from our experimental study to the clinical setting in which CITs of $>$20 h are by no means exceptional.

Whether the superiority of POLYSOL above UW as demonstrated in this study is based on the lower viscosity as achieved by substituting HES with PEG, or on the extracellular composition of the solution or on other components of POLYSOL, remains unclear and will be the subject of further investigations.

In summary, this study demonstrates that using POLYSOL, microcirculation was better preserved and renal function was improved after 20-h CS preservation compared to the UW solution in a clinically relevant large animal model. We could also demonstrate biological safety in vivo in this model. Therefore, this study shows that the use of POLYSOL as a preservation solution for hypothermic preservation of kidney grafts is feasible. Its superiority to UW should be assessed in a prospective clinical trial.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

Acknowledgements. The authors thank Ute Lohmer and Koichiro Hata for their continuous support. This study was supported in part by Doorzand Medical Innovations B.V.

Conflict of interest statement. M.C.J.M. Schreinemachers and B.M. Doorschodt are research fellows at the Surgical Laboratory of the Academic Medical Center sponsored by Doorzand Medical Innovations. B. M. Doorschodt is also a shareholder of Doorzand Medical Innovations. S. Florquin, M.A. van den Bergh Weerman, J.B. Reitsema, W. Lai, M. Sitzia, T.M. Minor, R.H. Tolba and T.M. van Gulik have nothing to declare.

References


Received for publication: 10.6.08
Accepted in revised form: 12.9.08