Preclinical evaluation of a new organ preservation solution
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Chapter 4

Improved renal function of warm ischemically damaged kidneys using Polysol
Improved renal function of warm ischemically damaged kidneys using Polysol

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Abstract

Objective
We sought to assess the efficacy of POLYSOL, a low-viscosity, colloid-based organ preservation solution, for the preservation of warm ischemically damaged kidney grafts compared with histidine-tryptophane-ketoglutarate (HTK) solution.

Methods
Pigs (25–30 kg) underwent a left nephrectomy after clamping the renal vessels for 30 minutes. Kidney grafts washed out with Polysol ($n = 6$) or HTK ($n = 6$) were cold stored (CS) for 20 hours at 4°C. After the preservation period, the contralateral kidney was removed and the preserved kidney implanted heterotopically. Renal function was assessed daily for 7 days. Thereafter, animals were killed and the kidney grafts removed for histologic analysis.

Results
All animals survived for 7 days. All Polysol CS–preserved grafts showed immediate function, as demonstrated by urine production within 24 hours after reperfusion as compared with 3/6 grafts in the HTK CS group. Overall, the Polysol CS group showed improved renal function compared with HTK CS. Also, peak serum creatinine and blood urea values were lower in the Polysol CS group compared with HTK-preserved grafts. Histologic evaluation of warm ischemically damaged grafts showed less glomerular shrinking, less tubular damage, less edema, less inflammatory infiltration, and less necrosis in Polysol compared with HTK-preserved grafts.

Conclusion
Application of Polysol solution for washout and CS preservation of warm ischemically damaged kidney grafts resulted in improved renal function and structural integrity when compared with HTK.
Introduction

Preservation of warm ischemically damaged kidneys commonly employs cold storage (CS) in histidine-tryptophane-ketoglutarate (HTK) solution. Because the low-viscosity of HTK solution facilitates easy wash-out of the graft, it is widely accepted as the preservation solution of choice for non-heart-beating donor kidneys. Recently, a new low-viscosity perfusion preservation solution Polysol has been shown to be efficacious in small and large animal organ preservation models. In a previous study using a porcine renal autotransplantation model, preservation of heart-beating donor kidney grafts using Polysol resulted in improved renal function compared with University of Wisconsin solution. Therefore, the aim of this study was to assess the efficacy of Polysol, a colloid-based organ preservation solution, versus HTK solution to preserve warm ischemically damaged kidney grafts using a porcine autotransplant model.

Materials and methods

All experiments were performed in accordance with the Dutch legislation governing animal studies. The Principles of Laboratory Animal Care (NIH publication. 85-23, revised 1985) were followed. This study was approved by the Animal Ethical Committee of the University of Amsterdam. Female Landrace pigs (25-30 kg) were allowed to acclimatize to the laboratory environment for 7 days under standardized conditions. Before the experiments, pigs were fasted overnight with free access to water. This study on warm ischemically damaged kidney grafts was performed using an autotransplant model and involved 2 experimental groups: CS preservation with Polysol (Polysol CS, n = 6) versus CS preservation using HTK (HTK CS, n = 6).

General anaesthesia was induced by inhalation of a mixture O₂/air and isoflurane. After intubation, anaesthesia was maintained by mechanical ventilation and IV administration of ketamine (5-10 mg/kg body weight [BW] per hour), sufenta forte (5-10 µg/kg BW per hour), pavulon (50-100 µg/kg BW per hour), and, if necessary, isoflurane (0%-2%). For infusion and daily collection of blood samples, the right internal jugular vein was cannulated with a polyethylene catheter. Through a midline laparotomy, the left kidney was mobilized and the renal pedicle clamped for 30 minutes (warm ischemic time [WIT] 30). After 30 minutes, the kidney was retrieved followed by an immediate ex vivo wash-out with 500 mL of either Polysol (Doorzand Polysol B.V., Amsterdam, The Netherlands) or HTK solution (Dr. F. Köhler Chemie GmbH, Alsbach-Hänlein, Germany) at 4°C at a hydrostatic pressure of 100 cm H₂O. Kidney grafts were placed in a sterile bag with 500 mL of either Polysol or HTK solution and stored on melting ice. After the 20-hour preservation period, the contralateral kidney was
removed and the preserved kidney was heterotopically transplanted. The renal artery was anastomosed end-to-end to the right renal artery and the renal vein anastomosed end-to-side to the inferior vena cava. Before completion of the arterial anastomosis, a bolus of 3000 IU of heparin was injected IV to mitigate vascular thrombosis. Following reperfusion, 500 mL of 5% glucose was administered IV to induce an osmotic diuresis. The ureter was cannulated with a PE tube (CH 10) to allow free outflow through the ureterocutaneostomy. After transplantation, animals were observed for 7 days. Venous blood samples were taken for the measurement of renal function by serum creatinine, urea and electrolytes every morning. Total urine production was collected and creatinine clearance was calculated. At posttransplant day 7, the transplanted kidney was removed for histological evaluation and the animals were humanely killed. Histology was assessed using light microscopy. Sections fixed in neutral 10% buffered formalin were embedded in paraffin for staining with hematoxylin/eosin and periodic acid-Schiff. Tissue sections were examined by a pathologist blinded for the experimental conditions and scored for glomerular damage (shrinking), inflammatory cell infiltrates, tubular damage, edema, and necrosis. Injury in each specimen examined in 10 randomly chosen, nonoverlapping fields (original magnification, X400) was graded on a scale from 0 to 5: 0 = no abnormality, 5 = extensive damage, involvement >75% of the field depending on the extent of region involvement in 10 randomly chosen, nonoverlapping fields (original magnification, X400). Data are expressed as mean values ± standard errors of the mean (SEM). For statistical evaluation we performed ANOVA for repeated measurements (RM) and the unpaired 2-tailed Student $t$-test. For nonparametric comparisons we used the Mann-Whitney test. Area under the curve (AUC) was calculated individually using the GraphPad Prism 5.0 statistics package (GraphPad Software, San Diego, CA, USA). $P < .05$ was considered significant.

Results

In terms of animals weight, cold ischemic time (CIT), and anastomosis time (second warm ischemic time [WIT]), the groups were comparable (Polysol CS 28.8 ± 0.6 kg; HTK CS, 27.5 ± 0.6 kg, $P = .132$; for CITs, Polysol CS, 20:08 ± 10 minutes; HTK CS, 20:09 ± 5 minutes; and 2nd WIT, Polysol CS, 46.7 ± 9.9 minutes; HTK CS, 42.0 ± 4.0 minutes). All animals survived for 7 days.
Renal function

Serum creatinine and blood urea values after transplantation were significantly lower in warm ischemically damaged kidney grafts preserved using Polysol compared with CS preservation using HTK (Fig. 1).

In addition, peak serum creatinine values and time to peak ($T_{\text{peak}}$, days) were lower in the Polysol CS group. Peak blood urea values and $T_{\text{peak}}$ urea were also lower in Polysol-preserved grafts when compared with HTK (Table 1). After reperfusion, immediate urine production was seen in 5 animals in the Polysol CS group (1.6 ± 0.4 minutes after reperfusion), whereas only 1 animal in the HTK group produced urine shortly after reperfusion (5 minutes postreperfusion). Moreover, all animals in the Polysol CS group produced urine within 24 hours after transplantation, whereas only 3 out of 6 animals produced urine in the HTK CS group.
Table 1. Summary of posttransplant results

<table>
<thead>
<tr>
<th></th>
<th>HTK CS WIT 30 (n = 6)</th>
<th>Polysol CS WIT 30 (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived (n)</td>
<td>6</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Urine production within first 24 hours posttransplant (n)</td>
<td>3</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Peak creatinine (µmol/L)</td>
<td>1016 ± 39.6</td>
<td>629.8 ± 64.8</td>
<td>***</td>
</tr>
<tr>
<td>$T_{peak}$ creatinine (days)</td>
<td>3.2 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>**</td>
</tr>
<tr>
<td>Serum creatinine AUC</td>
<td>4262 ± 402</td>
<td>2630 ± 320</td>
<td>**</td>
</tr>
<tr>
<td>Peak urea (mmol/L)</td>
<td>23.8 ± 2.1</td>
<td>16.5 ± 2.2</td>
<td>.037</td>
</tr>
<tr>
<td>$T_{peak}$ urea (days)</td>
<td>3.0 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>**</td>
</tr>
<tr>
<td>Blood urea AUC</td>
<td>116.6 ± 18.9</td>
<td>66.5 ± 10.4</td>
<td>.042</td>
</tr>
</tbody>
</table>

$T_{peak}$, time to peak level; AUC, area under the curve; NA, not applicable; NS, not significant.

**P < .01, ***P < .001.

Overall, urine production during follow-up did not differ between the groups (AUC urine production): Polysol CS, 10,650 ± 753 versus HTK CS, 9216 ± 1153 ($P = .324$). However, on the first 3 days after transplantation, urine production in the Polysol CS group was significantly greater than the HTK CS group. For posttransplant days 4 through 7, urine production in the HTK CS group was numerically higher compared with the Polysol CS group at all times, albeit not significantly (data not shown). Creatinine clearances in Polysol-preserved grafts were higher on days 2 and 3 posttransplantation compared with HTK-preserved grafts (Fig. 2).

**Histological evaluation**

Overall, Polysol CS-preserved grafts showed less glomerular shrinking, less tubular damage, less inflammatory infiltration, and less interstitial edema compared with HTK CS-preserved grafts. In addition, warm ischemically damaged kidney grafts preserved using HTK showed significantly more necrosis than Polysol-preserved grafts (Table 2).
Table 2. Morphologic data

<table>
<thead>
<tr>
<th></th>
<th>HTK CS WIT 30</th>
<th>POLYSOL CS WIT 30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular shrinking</td>
<td>1.4 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>*</td>
</tr>
<tr>
<td>Tubular damage</td>
<td>3.1 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>***</td>
</tr>
<tr>
<td>Inflammatory infiltration</td>
<td>1.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>***</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>2.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>***</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.7 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>***</td>
</tr>
</tbody>
</table>

Semiquantitive 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; 5 = extensive damage, involvement of > 75% of the field.
CS, cold storage; HTK, histidine-tryptophane-ketoglutarate solution; WIT, warm ischemic time.

*P < .05, ***P < .0001.
Discussion

This study of the preservation of porcine kidney grafts subjected to 30 minutes of warm ischemic damage followed by 20 hours of CS preservation demonstrated that the renal function of warm ischemically damaged grafts was better using the new Polysol solution compared with HTK solution. Also, the structural integrity of Polysol-preserved grafts proved to be better than that of HTK-preserved grafts.

Our experimental design assessed the efficacy of CS preservation using Polysol versus HTK. In clinical practice in most institutions, non-heart-beating donor kidneys are washed out and preserved using the low-viscosity HTK solution as opposed to the less favorable wash-out obtained with the high-viscosity UW solution. In a porcine autotransplantation study, Nicholson et al. observed less favorable results using UW to preserve kidneys subjected to 30 minutes of warm ischemic injury: only 1 of 5 animals survived for > 7 days. Polysol includes 60 components; the value of most of which has been previously demonstrated. Moreover, Polysol is an extracellular-type solution with low potassium and high sodium contents, which are known to benefit cold storage preservation of kidney grafts. Because both the low concentration of K⁺ and the high concentration of Na⁺ limit the entry of Ca²⁺ into the cell, Ca²⁺ overload is prevented, thereby avoiding depolarization of the cell membrane of smooth muscle cells that otherwise would produce vasoconstriction. The blockade of vasoconstriction favors more homogeneous diffusion of the solution within the organ, which could in part account for the results of our study. In addition to the favorable results obtained with previous CS and machine perfusion preservation studies using Polysol, the porcine kidney transplant study described herein demonstrated that Polysol is a useful CS preservation solution for warm ischemically damaged renal grafts. Also, because the combination of warm and cold ischemic damages is known to lead to marked injury to porcine kidney grafts, the results of this study suggested a potential beneficial effect of Polysol in clinical practice.

In conclusion, this study of a clinically relevant, large animal kidney transplant model, clearly demonstrated that Polysol wash-out and CS preservation improved kidneys subjected to 30 minutes of warm ischemic damage compared with HTK. This improvement was reflected by better recovery of renal function and preservation of structural integrity among the Polysol versus HTK preserved grafts.
References