Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Static cold storage preservation of ischemically damaged kidneys. A comparison between IGL-1 and UW solution

Mark-Hugo J. Maathuis,[1] Petra J. Ottens,[1] Harry van Goor,[2] Jacco J. Zwaagstra,[1] Janneke Wiersema-Buist,[1] Theo A. Schuurs,[1] Rutger J. Ploeg[1,3] and Henri G. D. Leuvenink[1]

1 Surgical Research Laboratory, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
2 Pathology and Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
3 Department of Surgery, Renal Transplant Unit, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Keywords
IGL-1, organ preservation, rat model, static cold storage, University of Wisconsin cold storage solution.

Correspondence
Mark-Hugo Maathuis MD, Surgical Research Laboratory, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel.: +31 50 3619028; fax: +31 50 3632796; e-mail: m.h.j.maathuis@chir.umcg.nl

Received: 14 November 2007
Revision requested: 10 December 2007
Accepted: 21 December 2007

doi:10.1111/j.1432-2277.2007.00634.x

Summary

Especially in damaged organs, adequate organ preservation is critically important to maintain viability. Institut Georges Lopez-1 (IGL-1) is a new preservation solution, with an extracellular sodium/potassium ratio and polyethylene glycol as a colloid. The influence of warm and cold ischemia was evaluated in a rat Lewis–Lewis transplant model with a follow up of 14 days. Eight groups of donation after cardiac death donor kidneys were studied with warm ischemia of 0 and 15 min followed by 0- or 24-h cold storage (CS) preservation in IGL-1 or UW-CSS. Blood was collected daily during the first week and at day 14. Recipients were placed in metabolic cages at day 4 and 14 after transplantation allowing urine collection and adequate measurement of glomerular filtration rate. Focussing on inflammation, reactive oxygen species production, proximal tubule damage, proteinuria, histology, and renal function after transplantation we could not show any relevant difference between IGL-1 and UW-CSS. Furthermore, the combination of 15-min warm ischemia and by 24-h cold ischemia did not result in life sustaining kidney function after transplantation, irrespective of the used solution. In the present experiment, static CS preservation of ischemically damaged rat kidneys in either IGL-1 or UW-CSS rendered equal results after transplantation.

Introduction

To date, static cold storage (CS) using the University of Wisconsin solution (UW-CSS) is the most frequently used kidney preservation method. It consists of a rapid vascular wash-out allowing cooling of the organ, removal of blood and equilibration between CS solution and tissue [1]. The increasing awareness, however, that ischemia/reperfusion injury does significantly affect outcome after transplantation has stimulated research of preservation damage and the development of new preservation solutions [2,3]. A new and now clinically available preservation solution is Institut Georges Lopez-1 (IGL-1), developed by the Lyon group in 1997 [4–9]. In contrast to UW-CSS, IGL-1 has an extracellular composition and contains polyethylene glycol (PEG) instead of hydroxyethyl starch (HES). PEG is a synthetic colloid that reduces lipid peroxidation and has immune-modulating properties and, unlike HES, PEG does not cause red blood cell (RBC) aggregation [5,7,10–14]. Recommended wash-out volumes are comparable with UW-CSS while the pricing of IGL-1 is approx. 50% of UW-CSS. Both, the extracellular composition and the use of PEG in IGL-1 could be an important advantage over UW-CSS, especially in the donation after cardiac death (DCD) setting [15].

The aim of these transplant experiments was to compare the efficacy of IGL-1 in preserving ischemically damaged rat kidneys to UW-CSS. In a DCD rat model, kidneys were explanted after 0- or 15-min warm ischemia followed by 0- or 24-h static CS preservation in IGl-1 or UW-CSS. After preservation, kidneys were transplanted in an isogenic recipient directly followed by a bilateral native nephrectomy.

Animals, materials and methods

Animals

Inbred male Lewis rats, weighing 250–300 g, obtained from Harlan (Zeist, the Netherlands), where used as kidney donors and recipients. All experimental procedures were approved by the Committee for Animal Experiments of the University of Groningen. All animals demonstrated normal renal function before the start of the study. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Experimental design

The model used was a modification of an established rat kidney transplantation model including bilateral native nephrectomy [16]. Warm ischemia time (WIT) of 0 and 15 min were studied. Cold ischemia times (CIT) were either 0 h, i.e. only wash-out, or 24 h. Donor kidneys ($n = 7$/group) were recovered immediately or after 15 min following cardiac arrest and subsequently preserved for 24 h using either IGL-1 or UW-CSS. Eight experimental groups were studied:

Group 1: 0 min WIT and 0 h CS with IGL-1;
Group 2: 0 min WIT and 0 h CS with UW-CSS;
Group 3: 15 min WIT and 0 h CS with IGL-1;
Group 4: 15 min WIT and 0 h CS with UW-CSS;
Group 5: 0 min WIT and 24 h CS with IGL-1;
Group 6: 0 min WIT and 24 h CS with UW-CSS;
Group 7: 15 min WIT and 24 h CS with IGL-1; and
Group 8: 15 min WIT and 24 h CS with UW-CSS.

DCD donor procedure

After induction of isoflurane anaesthesia, donors were heparinized with 250-IU heparin through the penile vein. The left kidney, renal vessels and ureter were isolated via a midline incision. After opening the thoracic cage, cardiac arrest was induced by manual cardiac tamponade for 5 min [17]. A ligature was placed superior to the left renal artery to prevent flushing of the right kidney, liver and intestine. The explanted left kidney was routinely flushed with 2.5 ml of 0.9% NaCl at 37 °C immediately followed by 2.5-ml IGL-1 or UW-CSS solution at 4 °C by means of a 20-G needle inserted at the aortic bifurcation. The left kidney was removed including patches of aorta and caval vein.

Preservation

Kidneys in groups 5–8 were submerged in 25 ml of preservation solution at 0–4 °C during 24 h in a falcon tube (Greiner, Bio-One B.V., Alphen aan den Rijn, the Netherlands). Kidneys in groups 1–4 were immediately transplanted with a cold ischemic time ranging from 23 to 28 min.

Recipient procedure

To allow stress-free and undisturbed blood sampling, recipient animals received a permanent jugular vein cannula with subcutaneous tunnelling of the cannula to a head attachment apparatus 1 week prior to transplantation [18]. After complete recovery, demonstrated by a return to precannulation bodyweight, animals were eligible for transplantation. Time between jugular vein cannulation and transplantation did not exceed seven days. The microsurgical transplantation technique used in this study was a modification of the technique described by Lee [19]. Prior to reperfusion, the graft was flushed with 1 ml of 0.9% NaCl at 4 °C to wash out preservation solution. The renal vessels were anastomosed end-to-side to the recipients aorta and caval vein using 9-0 prolene suture (Johnson & Johnson, Brussels, Belgium) and the ureter was anastomosed end-to-end to the recipients ureter using 10-0 prolene sutures (Johnson & Johnson, Brussels, Belgium). The vascular clamps were released immediately after the vascular anastomosis was completed, with a mean WIT ranging from 20 to 25 min. At this point both native kidneys were removed.

Blood collection

Blood samples (0.4 ml) were taken daily until day 7 and on day 14 after transplantation. Fluid replacement was obtained using 0.4 ml 0.9% NaCl. Subsequently, 0.08 ml 65% poly vinyl pyrolidone solution with 5000 IE heparin/ml was inserted in the catheter lumen to prevent clotting. After centrifugation, the plasma was collected and stored at −80 °C until further analysis.

Urine collection

Rats were housed for 24 h in individual metabolic cages, with access to drinking water, on day 4 and 14 after transplantation. Urine volume was determined gravimetrically and subsequently stored at −80 °C until further analysis.

Journal compilation © 2008 European Society for Organ Transplantation **21** (2008) 473–482

Tissue preparation and biochemical analysis

Sacrifice

At day 14 rats were anesthetized with isoflurane followed by cannulation of the aorta and a 5-ml blood sample was subsequently taken. The kidney graft was perfused *in situ* with 10 ml 0.9% NaCl at 4 °C to obtain optimal tissue morphology. After removal of the kidney, it was divided in three pieces. Both upper and lower poles were snap frozen in liquid nitrogen. The middle pole was subsequently halved and stored in 4% formalin or embedded (Tissue-Tek, Zoeterwoude, the Netherlands) and snap frozen in liquid nitrogen.

Microscopic techniques

Tissue was collected, fixated in 4% formalin, subsequently paraffin embedded and cut into 3-μm-thick sections. Light microscopy (20× magnification) of haematoxylin and eosin (HE) and periodic acid-Schiff (PAS) stained sections were used to demonstrate changes in morphology.

Immunohistochemistry

To assess the extent of tubular and interstitial injury in the different groups, immunostaining for α-smooth muscle cell actin (prefibrotic changes; α-smooth muscle antigen (α-SMA), clone 1A4, Sigma St Louis, MO, USA), osteopontin (marker of tubular injury; OPN, clone MPIIIB10, Developmental Hybridoma Studies, Iowa City, IA, USA) and macrophages [ectodysplasin-1 (ED-1), Serotec, Oxford, UK] was performed. As previously described, deparafinized sections (3 μm) were subjected to heat-induced antigen retrieval by overnight incubation in 0.1 M Tris–HCl buffer (pH 9.0) at 80 °C [20]. Endogenous peroxidase was blocked for 30 min with 0.3% H_2O_2 in PBS. Primary antibodies (Table 1), diluted in 1% BSA/PBS, were incubated for 60 min at room temperature. Binding was detected using sequential incubations (30 min, Table 1) with appropriate peroxidase-labeled secondary antibodies (DakoCytomation) diluted in PBS with 1% BSA and 1% normal rat serum. Peroxidase activity was determined using 3,3′-diaminobenzidine tetrachloride for 10 min. Relevant sections were counterstained with haematoxylin.

Measurements in blood and urine

Plasma creatinine levels were determined using a routine clinical laboratory test (CREA plus, Roche, Mannheim, Germany) which has been validated for measurements of rat serum creatinine concentrations [21]. Urinary creatine levels were determined by the Jaffé method (Sigma-Aldrich Chemie b.v., Zwijndrecht, the Netherlands). thiobarbituric acid reactive substances (TBARS) in urine were analyzed as an indication of increase in lipid peroxidation by reactive oxygen species (ROS) after reperfusion [22]. Malondialdehyde binds to thiobarbituric acid and the subsequently formed TBARS were extracted in a butanol layer, measured with a fluorescence spectrophotometer at 485/590 nm (Beun de Ronde FL 600, Abcoude, the Netherlands). Urinary protein content was assayed via the method of Lowry *et al.* [23]. Activity of brush border enzymes alanine aminopeptidase (AAP) and lysosomal

Table 1. Antibodies used for immunohistochemistry and primers for RT-PCR.

Specificity	Primary antibody	Supplier	Dilution primary antibody	Secondary/tertiary antibodies
α-Smooth muscle antigen	Monoclonal mouse anti-SMA clone 1A4	Sigma, St Louis, USA	1:10 000	Rampo/Garpo 1:100
Osteopontine	Monoclonal clone MPIIIB10	Developmental Hybridoma Studies, Iowa City, USA	1:250	Rampo: 1:50 Garpo: 1:100
Ectodysplasin-1	Monoclonal	Serotec, Oxford, UK	1:750	Rampo/Garpo 1:100

Primer	Forward	Reverse	Amplicon length (bp)
IL-18	5′-CAACCGCAGTAATACGGAGCATA-3′	5′-CAGGCGGGTTTCTTTTGTCA-3	62
ß-actine	5′-GGAAATCGTGCGTGACATTAAA-3′	5′-GCGGCAGTGGCCATCTC-3′	75
α-Smooth muscle antigen	5′-GAGAAAATGACCCAGATTATGTTTGA-3′	5′-GGACAGCACAGCCTGAATAGC-3′	74
GAPDH	5′-GTATGACTCTACCCACGGCAAGTT-3′	5′-GATGGGTTTCCCGTTGATGA-3′	79
E-selectin	5′-GTCTGCGATGCTGCCTACTTG-3′	5′-CTGCCACAGAAAGTGCCACTAC-3′	73
Collagen III	5′-AGTTCTAGAGGATGGCTGCACTAAAC-3′	5′-TCTCATGGCCTTGCGTGTT-3′	81
Intracellular adhesion molecule-1	5′-CCAGACCCTGGAGATGGAGAA-3′	5′-AAGCGTCGTTTGTGATCCTCC-3′	251
TNF-α	5′-AGGCTGTCGCTACATCACTGAA-3′	5′-TGACCCGTAGGGCGATTACA-3′	67

Rampo, peroxidase-conjugated rabbit antimouse antibody; Garpo, peroxidase-conjugated goat antirabbit antibody.

enzyme *N*-acetyl-β-D-glucosaminidase (NAG) in urine were measured using colorimetric assays [24]. NAG was measured using a modified enzyme assay according to Findlay [25] at pH 4.25 using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as a substrate. AAP was detected with the modified enzymatic assay of Pfleiderer [26] using alanine-*p*-nitroanilide as a substrate.

Measurement of renal damage

Focal glomerular sclerosis (FGS) was semiquantitively scored (scale 0-400) in PAS-stained sections and expressed as the mean score of 50 glomeruli/kidney. FGS was scored positive when mesangial matrix expansion and adhesion of the visceral epithelium to Bowman's capsule were simultaneously present. A score of 1 was given when 25% of the glomerulus was involved, 2 for 50%, 3 for 75% and 4 for 100%. The theoretical maximum score is 400 [27]. Interstitial fibrosis (IF) was scored similarly in 30 interstitial fields. A score of 0 was given when no IF was present in a field, 1 for 0–25%, 2 for 25–50%, 3 for 50–75% and 4 for 75–100% of the field showing IF. IF was defined as expansion of the interstitial space, with or without the presence of atrophied and dilated tubules and thickened tubular basement membranes [28]. Computerized morphometry was used to quantify immunohistochemical data [20].

RNA isolation and quantitative PCR for inflammation, injury and fibrosis

Real-Time Quantitative RT-PCR analysis of interleukin-18 (IL-18), Tumor necrosis factor-alfa (TNFα), E-selectin, intracellular adhesion molecule-1 (ICAM-1), Collagen-type III and α-SMA gene expression was performed to detect inflammation, endothelial damage, tubular injury or fibrosis 14 days after transplantation. Amplification primers (Table 1) were designed with PRIMER EXPRESS software (Applied Biosystems) and validated in a 6-step twofold dilution series. RNA was extracted from snap frozen tissue using TRIzol (Invitrogen, Breda, the Netherlands). Total RNA was treated with DNase I, Amp Grade (Invitrogen, Breda, the Netherlands). cDNA synthesis was performed from 1-μg total RNA using T_{11}VN oligo's and M-MLV Reverse Transcriptase, according to supplier's protocol (Invitrogen). Amplification and detection were performed with the ABI Prism 7900-HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using emission from SYBR green (SYBR Green master mix, Applied biosystems). All assays were performed in triplicate. After an initial activation step at 50 °C for 2 min and a hot start at 95 °C for 10 min, PCR cycles consisted of 40 cycles of 95 °C for 15 sec and 60 °C for 60 sec. Gene expression was normalized with the mean of β-actin mRNA content and calculated relative to controls. Results were finally expressed as $2^{-\Delta CT}$ (CT threshold cycle).

Statistics

Data are expressed as mean ± SEM. Area under the creatinine curve was determined using SIGMAPLOT software (Systat Software, Point Richmond, CA, USA). Statistical significance of differences between groups was assessed by Kruskall–Wallis followed by the Mann–Whitney *U* test. Differences were considered significant at a level of $P < 0.05$.

Results

No significant differences in anastomosis times were observed between groups (Table 2).

Survival

After a challenge of 15-min WIT in combination with 24-h CIT (15-24 groups), independent of the used solution all animals died within three days post-transplant. Death was caused by renal failure demonstrated by increasing serum creatinine levels (Fig. 1). Therefore, on animal welfare grounds, no further attempts were made to complete this group and inclusion was stopped after $n = 4$ for both UW-CSS and IGL-1 groups. Survival in all other groups was 100%.

Renal function

Post-transplant renal function data are presented in Fig. 1 and Table 2. Kidneys subjected to both 15-min WIT and 24-h CIT (15-24 groups) were severely damaged resulting in an irreversible rise of serum creatinine for both IGL-1 and UW-CSS kidneys. Serum creatinine levels at day 1 and day 2 and area under the creatinine curve were significantly higher ($P < 0.05$) in kidneys subjected to 24-h CIT (0-24 groups) compared to control kidneys (0-0 groups). Renal dysfunction in this group was further demonstrated by significant ($P < 0.05$) higher urine volumes at day 4 and 14 post-transplantation compared to controls. (Table 2). There was no difference between IGL-1 and UW-CSS. Serum creatinine levels of kidneys subjected to 15-min WIT (15-0 groups) were numerically higher compared to control kidneys but this did not reach statistical significance.

GFR was calculated using 24-h urine volume and urinary and serum creatinine levels (Table 2). On day 4, there was no significant difference between the GFR although a trend was seen toward a lower GFR in the 0-24 groups. After 14-days, GFR was higher compared to

Table 2. Clinical parameters and damage markers.

WIT (min) – CIT (h)	Group 1 0-0 IGL	Group 2 0-0 UW	Group 3 15-0 IGL	Group 4 15-0 UW	Group 5 0-24 IGL	Group 6 0-24 UW	Group 7 15-24 IGL	Group 8 15-24 UW
Anastomosis time (minutes mean ± SEM)	28 ± 1	30 ± 2	30 ± 1	27 ± 1	30 ± 2	26 ± 1	26 ± 1	25 ± 0
Peak serum creatinine (μmol/l mean ± SEM)	101 ± 28	105 ± 21	138 ± 32	130 ± 32	269 ± 77*	218 ± 23*	522 ± 17*†‡	489 ± 24*†‡
GFR day 4 (ml/min mean ± SEM)	2.1 ± 0.2	1.8 ± 0.3	1.9 ± 0.2	1.8 ± 0.3	1.5 ± 0.4	1.1 ± 0.2	NA	NA
GFR day 14 (ml/min mean ± SEM)	2.3 ± 0.2	2.3 ± 0.4	2.7 ± 0.3	2.6 ± 0.5	3.1 ± 0.5§	3.7 ± 0.6§	NA	NA
Urine volume day 4 (ml/24 h mean ± SEM)	14 ± 1	19 ± 3	16 ± 4	19 ± 2	24 ± 6*	31 ± 4*	NA	NA
Urine volume day 14 (ml/24 h mean ± SEM)	13 ± 1	17 ± 1*	13 ± 2	14 ± 2	23 ± 4*	26 ± 3*	NA	NA
Proteinuria (mg/l/24 h mean ± SEM)	79.2 ± 3.4	68.9 ± 4.2	85 ± 13.9	96.9 ± 18.9	205.1 ± 41.2*†	132.6 ± 23.7*	NA	NA
Urinary AAP day 4 (U/l mean ± SEM)	2.3 ± 0.7	1.8 ± 0.9	5.4 ± 1.7	0.4 ± 0.4†	5.9 ± 2.4	1.9 ± 1.4	NA	NA
Urinary AAP day 14 (U/l mean ± SEM)	6.1 ± 0.9§	6.4 ± 1.8§	5.4 ± 0.6	7.5 ± 1.5§	15.4 ± 7.6	8.6 ± 4.2	NA	NA
Urinary *N*-acetyl-β-d-glucosaminidase (NAG) day 4 (U/l mean ± SEM)	0.33 ± 0.02	0.32 ± 0.04	0.49 ± 0.06*	0.42 ± 0.05	0.64 ± 0.07*	0.56 ± 0.04*	NA	NA
Urinary NAG day 14 (U/l mean ± SEM)	0.42 ± 0.09	0.32 ± 0.04	0.35 ± 0.05	0.39 ± 0.04	0.44 ± 0.07	0.58 ± 0.07*	NA	NA
Urinary thiobarbituric acid reactive substances day 4 (μm/24 h)	90.1 ± 6.4	78.6 ± 5.8	90.6 ± 9.2	107.3 ± 22.2	92.5 ± 13.4	111.9 ± 34.5	NA	NA

NA, not applicable.
*$P < 0.05$ vs. 0-0; †$P < 0.05$ vs. 15-0; ‡$P < 0.05$ vs. 0-24; §$P < 0.05$ vs. day 4.

day 4 for all groups but only statistically significant for the 0-24 group. No difference between the two solutions in renal function was observed.

Proximal tubule damage

Detection of NAG and AAP activity in urine allowed an assessment of injury to the proximal tubule at 4 and 14 days after transplantation (Table 2). Urinary concentrations of AAP were significantly higher in IGL-1 preserved kidneys at day 4 compared to UW-CSS. NAG levels measured at day 4 were significantly increased in 15-0 and 0-24 groups, compared to controls. No difference in NAG levels could be demonstrated between IGL-1 and UW-CSS.

ROS formation

Measurement of TBARS in urine at day 4 allowed detection of ROS production (Table 2). No difference in TBARS levels were demonstrated between IGL-1 and UW-CSS.

Proteinuria

Urinary protein content was measured on day 4 and 14 after transplantation. On day 4, there was no difference between 15-0 groups and controls. 0-24 groups showed, however, a twofold significant ($P < 0.05$) increase in urinary protein concentrations compared to controls (Table 2). 10 days later proteinuria was more profound in all groups compared to day 4 measurements. Again, 0-24 groups demonstrated the most severe proteinuria. No difference in proteinuria between IGL-1 and UW-CSS was seen.

Immunohistochemical Assessment of Infiltrating Cells

To detect any differences in immunoprotection of both solutions infiltrating ED-1-positive macrophages were identified by immunohistochemical analysis 14 days after transplantation (Fig. 2). A distinction was made between interstitial and glomerular infiltration. Interstitial analysis showed significant differences between groups in the number of ED-1-positive cells with more infiltration in

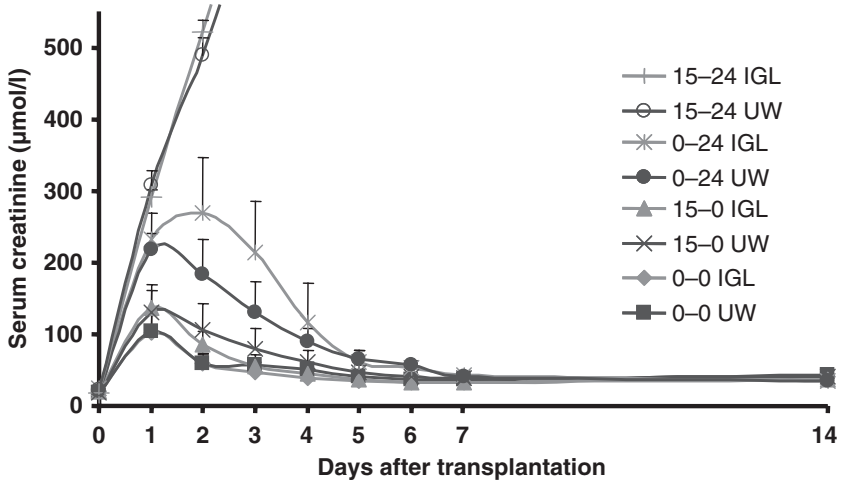

Figure 1 Post-transplant serum creatinine concentration in donation after cardiac death rats after preservation with Institut Georges Lopez-1 or University of Wisconsin cold storage solution.

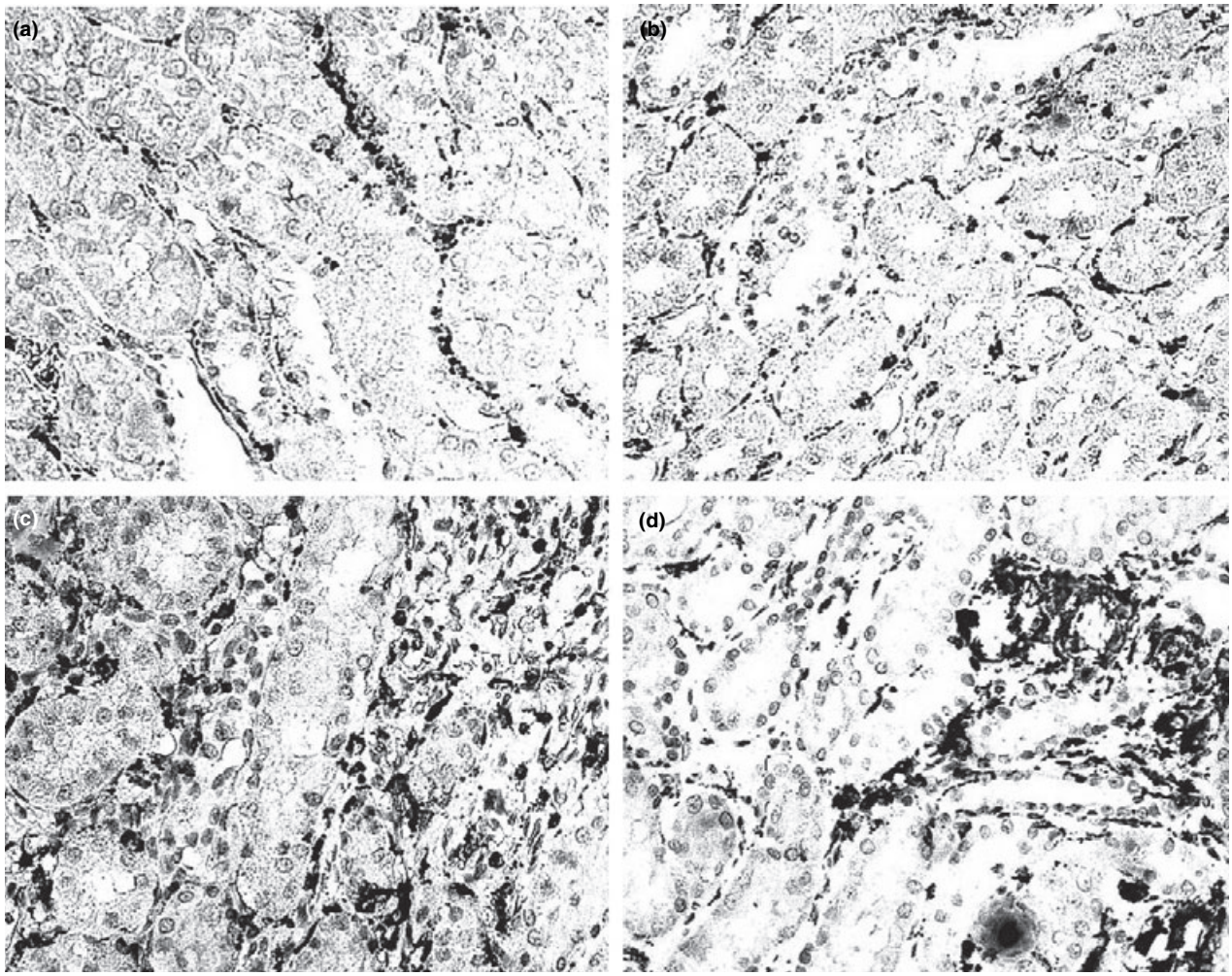

Figure 2 Representative section of kidney specimens after two weeks immunostained for ectodysplasin-1 (ED-1) (arrows) in 0-0 IGL (a), 0-0 University of Wisconsin cold storage solution (UW-CSS) (b), 0-24 IGL (c) and 0-24 UW-CSS (d) preserved grafts. Significant more ED-1 staining was observed in both 0-24 groups compared to both 0-0 groups ($P < 0.05$). No difference between Institut Georges Lopez-1 and UW-CSS could be detected.

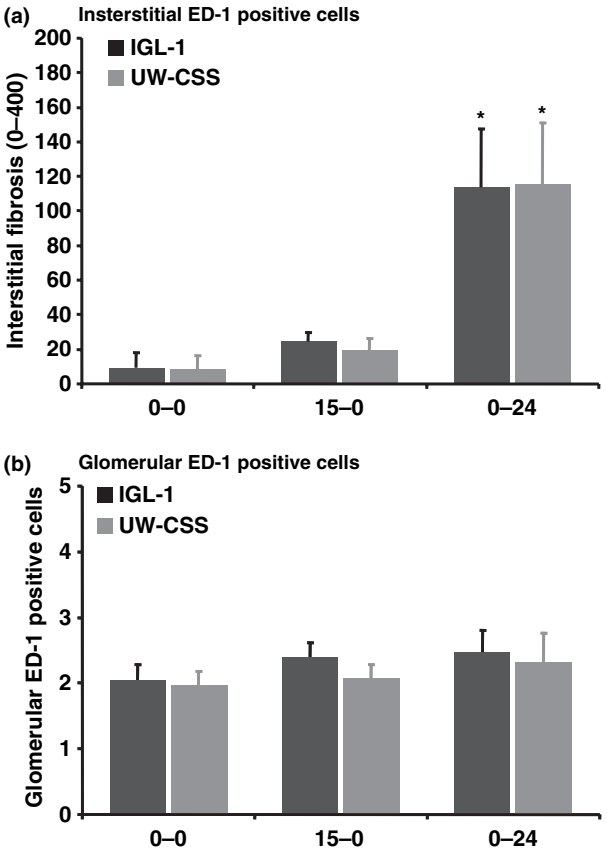

Figure 3 Ectodysplasin-1 positive cells in interstitium (a) and glomeruli (b) 14 days after transplantation using Institut Georges Lopez-1 or University of Wisconsin cold storage solution $*P < 0.05$.

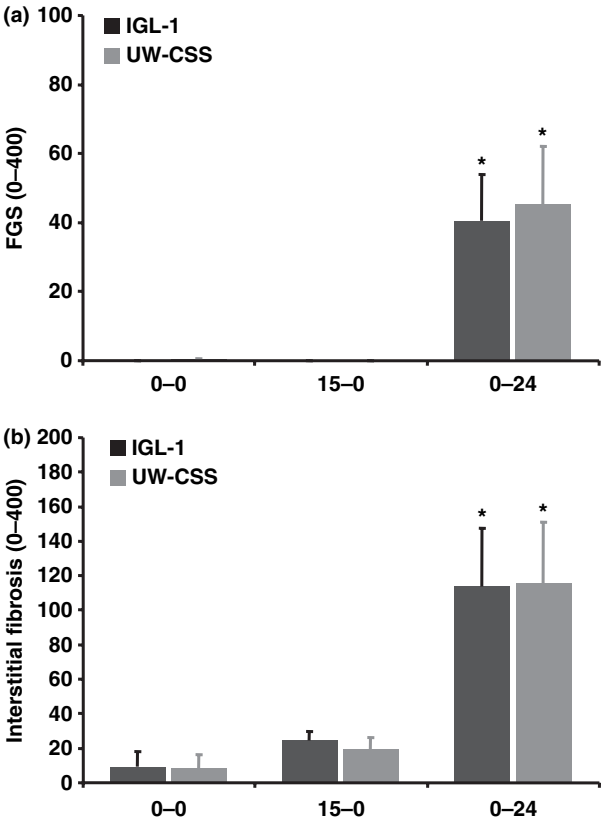

Figure 4 Focal gomerulosclerosis (a) and interstitial fibroris (b) given in arbitrary units 14 days after transplantation using Institut Georges Lopez-1 or University of Wisconsin cold storage solution $*P < 0.05$.

15-0 and 0-24 groups compared to controls (Fig. 3a). Glomerular infiltration was similar in all groups (Fig 3b). In both glomerular and interstitial infiltration of macrophages no difference could be demonstrated between IGL-1 and UW-CSS.

Renal damage

There was a significant increase in FGS and IF in both 0-24 groups compared to controls. Both IGL-1 and UW-CSS preservation resulted in the same amounts of FGS and IF (Fig. 4a,b).

Real-Time Quantitative *Taq*man RT-PCR analysis of IL-18, TNFα, E-selectin, ICAM-1 and α-SMA gene expression did not differ between groups. Computerized morphometry showed the same pattern.

Discussion

In the present study, we have used a Lewis–Lewis rat kidney transplant model with and without warm and cold ischemia to compare the efficacy of IGL-1 and UW-CSS in terms of functional recovery from ischemia/reperfusion injury. As recipient animals were bilateral nephrectomized at time of transplantation, our model is able to selectively study preservation effects of IGL-1 and UW-CSS. Our study shows equivalence of the new IGL-1 solution compared to UW-CSS in rat kidney transplantation.

Several *ex vivo* experiments that have looked at static CS preservation of rat kidneys with (protoypes of) IGL-1 reported increased viability versus UW-CSS using an isolated perfused kidney (IPK) model for functional assessment [4,29]. In the present study, however, we were not able to demonstrate any significant advantage of IGL-1 over UW-CSS. Although the IPK model offers an opportunity to control and manipulate renal function and has been well established in transplantation and pharmacology research, it has important shortcomings. Most relevant in the context of preservation experiments is the absence of whole blood reperfusion. In a transplant model, blood reperfusion aggravates the hypothermic-induced structural damage [2,30]. We therefore speculate that the extra damage in this transplantation experiment

nullified the potential beneficial effects of IGL-1 as demonstrated previously in IPK models.

With our study we also demonstrated that kidney function is negatively affected by 15-min WIT and 24-h CIT. A combination of both 15-min WIT and 24-h CIT did not result in life sustaining post-transplant kidney function. After four consecutive failures, inclusion in these groups was stopped. In the literature only a few studies combine both WIT and 24-h CIT in a rat model with direct bilateral nephrectomy. A study from Yin *et al.* [31], however, combining both 20-min WIT and 24-h static CS using UW-CSS confirms our results with 0% survival in a Lewis rat model. For the remaining groups the first GFR measurements was performed three days after transplantation allowing full postoperative recovery before putting the animals in metabolic cages. Although the area under the creatinine curve was significantly bigger in the 0-24 groups compared to controls, GFR measurements at day 4 did not show a significant difference. Assessing from the serum creatinine values, however, kidney function had already recovered by that time. Overall we were not able to demonstrate any functional differences between IGL-1 and UW-CSS.

Both warm and cold ischemia have drastic and duration-dependent effects on proximal renal tubule cells. Maintaining their integrity is crucial for early graft function as proximal tubule cells play a critical role in electrolyte, water and solute reabsorption from the glomerular filtrate. Therefore, we were interested in comparing the preservation efficacy of both IGL-1 and UW-CSS with regards to the proximal tubule [32–34]. Measuring both urinary AAP and NAG levels as proximal tubule specific damage markers we could demonstrate that both 15-min WIT and 24-h CIT indeed result in significant proximal tubule injury compared to controls. Although AAP levels were significantly higher in IGL-1 compared to UW-CSS preserved kidneys after 15-min WIT this was not the case with NAG levels. AAP reflects damage to the brushborder of the proximal tubule while urinary NAG is pointing toward intracellular, lysosomal damage. We therefore speculate that temporal differences in reconstitution of the brushborder rather than structural differences have caused this marked difference between IGL-1 and UW-CSS. The extent of proteinuria at day 4 and 14 after transplantation was most severe in 0-24 groups irrespective of the preservation solution. Proteinuria progressed in all groups over the course of the experiment.

Based on compositional differences some specific advantages of IGL-1 over UW-CSS could be expected. The extracellular composition of IGL-1, with a low potassium content, is believed to favour wash-out efficacy resulting in better tissue penetration of the preservation solution. In this study we choose to preflush the donor kidney with 0.9% NaCl before flushing it with either IGL-1 or UW-CSS to study preservation capacity without a possible blunting effect of differences in wash-out. We could not demonstrate any advantage of the extracellular IGL-1 over the intracellular UW-CSS.

Furthermore, IGL-1 differs from UW-CSS with regards to the used colloid. The feasibility of HES as a colloid in UW-CSS has been extensively debated. HES prevents interstitial edema but also increases viscosity. Analyzing the effect of HES on RBCs, several authors have shown an increased RBC aggregability in both human and rat whole blood when large molecular sized HES is present [10,12]. PEG in IGL-1 does not have aggregating effects on RBCs and could have immune-modulating properties. In contrast to Hauet *et al.* [7] who found a marked reduction of inflammatory injury when using a PEG based solution compared to UW-CSS we could not detect any differences in macrophage infiltration. There are three possible explanations for the different results. First, we have used an isogenic rat transplant model versus the pig autotransplantation model of Hauet *et al.* Second, our data on infiltration in the present study was obtained 14 days after transplantation, whereas Hauet *et al.* [35] used biopsies taken after seven days. Therefore, it is possible that we have missed a transient macrophage infiltration. At an earlier time-point, 4-day post-transplant, we did measure urinary TBARS levels as indicator for increased lipid peroxidation by ROS. Apart from ischemia/reperfusion injury, leukocyte infiltration is an important generator of ROS production. We could, however, not detect any differences between IGL-1 and UW-CSS when studying downstream postinfiltration effects such as urinary TBARS levels at day 4. Furthermore, FGS and IF at day 14 post-transplant did not differ between the two solutions. Third and probably most important, the molecular weight of PEG in IGL-1 is 35kDa whereas Hauet *et al.* [36] used a 20-kDa PEG in their preservation solution. Therefore, the size of high-molecular weight PEG could be critically important to sterically avoid T-cell activation via immunological synapses. We speculate that PEG 35 kDa is too large to interfere in these synapses and limit T-cell activation. When comparing PEG 35 kDa to PEG 20 kDa in a porcine proximal tubular epithelial cell preservation experiment PEG 35 kDa was more potent than PEG 20 kDa in preserving ATP content, reducing LDH release and limiting oxidative stress [36]. To have the best of both worlds, both PEG 20 kDa and PEG 35 kDa should be present in future preservation solutions.

This study has some limitations. We could not study the combination of 15 min of WIT and 24-h CIT in more detail because all animals died of renal failure shortly

after transplantation. Therefore we are developing a rat kidney transplantation model with isolated cannulation of the ureter. This will allow selective functional assessment of the transplanted kidney, while a native kidney can remain *in situ* to sustain adequate dialysis. As, in our model, serum creatinine levels revealed that adequate kidney function restored within one week after transplantation, future studies need evaluation at earlier timepoints. After two weeks most of the ischemia/reperfusion injury has already been repaired, making it difficult to pinpoint differences between groups.

By using several specific damage markers it was possible to partly unravel the consequences of ischemia/reperfusion injury. Thereby, the present paper contributes to a better understanding of static CS preservation of kidney grafts. Although IGL-1 was expected to have some advantages over UW-CSS based on compositional differences, we were not able to demonstrate any differences between IGL-1 and UW-CSS in this rat transplantation model.

Acknowledgements

The authors would like to thank IGL (Le Pontet, France) for providing preservation solutions and financial support for this study. This work was funded in part by an unrestricted educational grant from Institut Georges Lopez, Le Pontet, France.

Authorship

MHJM designed the study and wrote the paper; PJO, microsurgeon, operated all animals; HvG, pathologist, reviewed the histology; JJZ, performed the biochemical analyses; JJW-B, performed the immunohistochemistry; TAS, performed and analyzed the RT-PCRs; RJP, designed the study; HGD, designed the study.

References

1. Marshall V. Preservation by simple hypothermia. In: Collins GM, Dubernard JM, Land W, Persijn GG, eds. *Procurement, Preservation and Allocation of Vascularized Organs*. Kluwer Academic Publisher, Dordrecht/Boston/London, 1997: 115–29.
2. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007; **83**: 1289.
3. Kay MD, Hosgood SA, Harper SJ, *et al.* Static normothermic preservation of renal allografts using a novel nonphosphate buffered preservation solution. *Transpl Int* 2007; **20**: 88.
4. Badet L, Ben Abdennebi H, Petruzzo P, *et al.* Effect of IGL-1, a new preservation solution, on kidney grafts (a pre-clinical study). *Transpl Int* 2005; **17**: 815.
5. Faure JP, Hauet T, Han Z, *et al.* Polyethylene glycol reduces early and long-term cold ischemia-reperfusion and renal medulla injury. *J Pharmacol Exp Ther* 2002; **302**: 861.
6. Hauet T, Han Z, Doucet C, *et al.* A modified University of Wisconsin preservation solution with high-NA+ low-K+ content reduces reperfusion injury of the pig kidney graft. *Transplantation* 2003; **76**: 18.
7. Hauet T, Goujon JM, Baumert H, *et al.* Polyethylene glycol reduces the inflammatory injury due to cold ischemia/reperfusion in autotransplanted pig kidneys. *Kidney Int* 2002; **62**: 654.
8. Hauet T, Mothes D, Goujon JM, Carretier M, Eugene M. Protective effect of polyethylene glycol against prolonged cold ischemia and reperfusion injury: study in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 2001; **297**: 946.
9. Badet L, Petruzzo P, Lefrancois N, *et al.* Kidney preservation with IGL-1 solution: a preliminary report. *Transplant Proc* 2005; **37**: 308.
10. Morariu AM, Vd Plaats A, Oeveren V, *et al.* Hyperaggregating effect of hydroxyethyl starch components and University of Wisconsin solution on human red blood cells: a risk of impaired graft perfusion in organ procurement? *Transplantation* 2003; **76**: 37.
11. Ben MI, Rosello-Catafau J, Franco-Gou R, *et al.* Preservation of steatotic livers in IGL-1 solution. *Liver Transpl* 2006; **12**: 1215.
12. van der Plaats A, 't Hart NA, Morariu AM, *et al.* Effect of University of Wisconsin organ-preservation solution on haemorheology. *Transpl Int* 2004; **17**: 227.
13. 't Hart NA, der van PA, Leuvenink HG, *et al.* Determination of an adequate perfusion pressure for continuous dual vessel hypothermic machine perfusion of the rat liver. *Transpl Int* 2007; **20**: 343.
14. Faure JP, Petit I, Zhang K, *et al.* Protective roles of polyethylene glycol and trimetazidine against cold ischemia and reperfusion injuries of pig kidney graft. *Am J Transplant* 2004; **4**: 495.
15. Yamauchi JI, Richter S, Vollmar B, Menger MD, Minor T. Warm preflush with streptokinase improves microvascular procurement and tissue integrity in liver graft retrieval from non-heart-beating donors. *Transplantation* 2000; **69**: 1780.
16. Howden B, Rae D, Jablonski P, Marshall VC, Tange J. Studies of renal preservation using a rat kidney transplant model. Evaluation of citrate flushing. *Transplantation* 1983; **35**: 311.
17. Bellemare S, Boucher A, Dandavino R, *et al.* Standardization of a non-heart-beating model in the rat for studying the mechanisms of renal cell death associated with cardiac arrest and preservation of the kidney. *Transplant Proc* 2000; **32**: 500.
18. Steffens AB. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 1969; **18**: 4.

19. Lee S. An improved technique of renal transplantation in rat. *Surgery* 1966; **61**: 771.
20. van Timmeren MM, Bakker SJ, Vaidya VS, *et al.* Tubular kidney injury molecule-1 in protein-overload nephropathy. *Am J Physiol Renal Physiol* 2006; **291**: F456.
21. Mazzachi BC, Peake MJ, Ehrhardt V. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. *Clin Lab* 2000; **46**: 53.
22. Fraga CG, Tappel AL. Damage to DNA concurrent with lipid peroxidation in rat liver slices. *Biochem J* 1988; **252**: 893.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265.
24. van den Eijnden MM, Leuvenink HG, Ottens PJ, *et al.* Effect of brain death and non-heart-beating kidney donation on renal function and injury: an assessment in the isolated perfused rat kidney. *Exp Clin Transplant* 2003; **1**: 85.
25. Findlay J, Levvy GA, Marsh CA. Inhibition of glycosidases by aldonolactones of corresponding configuration. 2. Inhibitors of beta-*N*-acetylglucosaminidase. *Biochem J* 1958; **69**: 467.
26. Pfleiderer G. *Particle Bound Aminopeptidase from Pig Kidney. Methods in Enzymology*. London: Academic Press, 1970: 514–21 pp.
27. Kramer AB, Ricardo SD, Kelly DJ, Waanders F, van Goor H, Navis G. Modulation of osteopontin in proteinuria-induced renal interstitial fibrosis. *J Pathol* 2005; **207**: 483.
28. Kramer AB, van der Meulen EF, Hamming I, van GH, Navis G. Effect of combining ACE inhibition with aldosterone blockade on proteinuria and renal damage in experimental nephrosis. *Kidney Int* 2007; **75**: 417.
29. Ramella SG, Hadj-Aissa A, Barbieux A, *et al.* Evaluation of a high sodium-low potassium cold-storage solution by the isolated perfused rat kidney technique. *Nephrol Dial Transplant* 1995; **10**: 842.
30. Harper S, Hosgood S, Kay M, Nicholson M. Leucocyte depletion improves renal function during reperfusion using an experimental isolated haemoperfused organ preservation system. *Br J Surg* 2006; **93**: 623.
31. Yin M, Buurman WA, Daemen JW, Kootstra G. The PAF antagonist TCV-309 reduces graft PMN infiltration and enhances early function of 24-hour-preserved rat kidneys with long warm ischemia. *Transplantation* 1996; **61**: 1443.
32. Sheridan AM, Bonventre JV. Cell biology and molecular mechanisms of injury in ischemic acute renal failure. *Curr Opin Nephrol Hypertens* 2000; **9**: 427.
33. Ashworth SL, Sandoval RM, Hosford M, Bamburg JR, Molitoris BA. Ischemic injury induces ADF relocalization to the apical domain of rat proximal tubule cells. *Am J Physiol Renal Physiol* 2001; **280**: F886.
34. Bartels-Stringer M, Kramers C, Wetzels JF, Russel FG, Groot H, Rauen U. Hypothermia causes a marked injury to rat proximal tubular cells that is aggravated by all currently used preservation solutions. *Cryobiology* 2003; **47**: 82.
35. Fialkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radic Biol Med* 2007; **42**: 153.
36. Dutheil D, Rioja-Pastor I, Tallineau C, *et al.* Protective effect of PEG 35,000 Da on renal cells: paradoxical activation of JNK signaling pathway during cold storage. *Am J Transplant* 2006; **6**: 1529.

