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Comparative efficacy of renal preservation solutions to limit functional impairment after warm ischemic injury

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In kidney transplantation, cold storage is the dominant modality used to prolong organ viability ex *vivo,* but is inevitably followed by a period of warm ischemia. Preservation fluids limit tissue damage during the ischemic period, but there is little information on the influence of preservation fluids on the physiologic consequences of warm ischemia alone, or on the comparative ability of such preservation fluids to limit warm ischemic injury. In this study, warm ischemia was induced in rat kidneys by crossclamping the left renal pedicle for 45 min with contralateral nephrectomy. The ischemic kidneys were flushed with Euro-Collins (EC), hyper osmolar citrate (HOC), University of Wisconsin (UW), or phosphate buffered sucrose (PBS)140 solution. Over a period of 2 h after reperfusion, urine and blood samples were collected and physiological parameters related to the function of the postischemic kidneys were assessed. The data show that postischemic renal function can be influenced by the choice of preservation fluid. Essentially, the continued use of EC as a renal preservation solution finds little support in these data, and, while HOC and UW solutions were better able to limit the decline in renal function after warm ischemia than EC, the solution most able to limit functional impairment after warm ischemia was PBS140. This analysis compares the efficacies of the commonly used preservation solutions and could form the basis for future solid-organ transplant studies that may ultimately allow us to propose best-practice guidelines and an optimum platform for improved preservation solutions.

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The process of organ procurement and implantation constitutes a severe physical stress on solid organs used in transplantation. To minimize the effects of organ damage and ischemia/reperfusion injury (I/RI), organ donation requires both *in situ* flush with a cold preservation solution and hypothermic storage at 0–4°C. This is inevitably followed by a shorter but potentially more hazardous period of warm ischemia during implantation. A third and potentially greater injury may be endured by the oxidative burst that occurs upon subsequent reperfusion. More recently, with the increasing use of asystolic donors, a variable period of warm ischemia is inevitable prior to organ procurement and hypothermic preservation. Such 'pure' warm ischemia, if prolonged, can make subsequent organ function unpredictable. The occurrence of these forms of tissue injury, prior to any allorecognition, is also thought to promote the immunogenicity of the transplanted tissue and aggravate immune-mediated processes that, if unchecked, result in organ rejection.

During ischemia, energy-dependent active transport mechanisms involving Na^+/K^+ and Ca^{2+}/Mg^{2+} adenosine triphosphatases are inhibited,[1] which leads to a steady influx of Na^+, Cl^- and Ca^{2+} into the cell, with subsequent osmotic influx of water inducing cellular edema. Ischemia also results in lowered pH and an accumulation of toxic products of anaerobic respiration, for example, lactate and hypoxanthine, that contribute to free radical damage upon reperfusion of the organ with recipient blood.[2,3] This process is exacerbated in warm conditions and can lead to severe tissue damage. It is well known that severe I/RI leads to delayed graft function (DGF) post-transplant.[4–6] Such DGF has been linked to poorer prognosis.[7–9] In a clinical study, 1- and 5-year graft survival was significantly reduced from 95 and 81% in early functioning grafts to 87 and 70% in delayed functioning grafts, respectively.[10] In addition, significant associations have been established between DGF and acute rejection,[10] as well as long-term outcomes including graft survival.[11] A meta-analysis examining the modes of preservation linked DGF and graft half-life, and concluded that DGF could contribute to a 20% reduction in 10-year graft survival compared to the immediately functioning renal allografts.[12]

Therefore, the number of functioning graft years lost worldwide as a consequence of poor organ preservation may be significant.

In a previous study, we used isolated proximal renal tubules to assess the ability of the core components of preservation solutions to modulate cellular edema during cold and warm ischemia.[13] That study provided evidence of significant increases in cellular edema due to warm ischemia in the presence of the commonly used preservation solutions, and confirmed other reports that the warm ischemic insult results in more severe tissue damage than cold ischemia alone.[14,15] In that study, such warm ischemic changes were significantly reduced in the University of Wisconsin (UW) solution and phosphate buffered sucrose (PBS)140-preserved kidney compared to the Euro-Collins (EC) and hyper osmolar citrate (HOC)-preserved kidneys. This earlier study, though, did not examine the functional consequences that the choice of preservation solution may lead to. In the subsequent analysis we have assessed the ability of the different preservation solutions to influence acute post-ischemic renal function. The aim of the study is to determine which solution is most capable of limiting the impairment of renal function that is a common feature in the transplanted kidney immediately following ischemia. Clinical and experimental studies have indicated that improvements in organ preservation will have significant benefits for long-term outcomes. Hence, an understanding of the functional consequences of ischemic injury, and the optimization of organ preservation, may permit better use of the scarce resource of donor organs.

RESULTS

Summary of survival and urine production

Warm ischemia for 45 min was a severe test of renal ischemic tolerance. This was reflected in the overall results in terms of survival (Table 1), urine quality (osmolality above that of plasma) and the rate of urine production (Figure 1a and b). In the group of kidneys that were not flushed prior to ischemia ($n=5$), four rats died soon after reperfusion and serum showed a very high potassium concentration (15.8 ± 1.9 mM/l^{-1}); no further data were obtained from this group. In the second control group, where the kidneys were flushed with 0.9% saline prior to ischemia ($n=7$), only one of the five surviving rats demonstrated a moderate degree of concentrating ability in its urine, two of six rats did so in the EC group, and four of six animals did so in the HOC group. In PBS140 ($n=7$) and UW groups ($n=6$) there were no deaths within the 2-h reperfusion period. These data showed that PBS140-preserved kidneys produced the highest mean osmolar concentration above plasma, and a lower flow rate, among those groups of kidneys that recovered urine flow after ischemia. Therefore, PBS140-preserved kidneys demonstrated the best urine production among these groups.

Table 1 | Overall summary of rat survival and quality of urine production in the post-ischemic period in various experimental groups

Groups	Number of animals	Deaths postischemia		No urine	Dilute urine[a]
		1 h	2 h		
No flush	5	4		1	
Saline	7	2		1	3(2[a]+1[b])
EC	6			1	3(1[a]+2[b])
HOC	6		1		1
PBS140	7				
UW	6				1

EC, Euro-Collins; HOC, hyper osmolar citrate; PBS, phosphate buffered sucrose; UW, University of Wisconsin.
[a]Isosthenuria: urine osmolality of $\pm5\%$ within plasma osmolality.
[b]Near isosthenuria: Urine osmolality only slightly (5–10%) above the plasma osmolality.

Assessment of renal function

Various analyses were undertaken to assess postischemic renal function. A terminal blood sample was taken from the heart at the end point of the experiment and analysed for solute concentration (Table 2). Serum potassium was generally high, and significantly higher in the no-flush group ($P<0.05$). Serum creatinine was high in the saline and EC

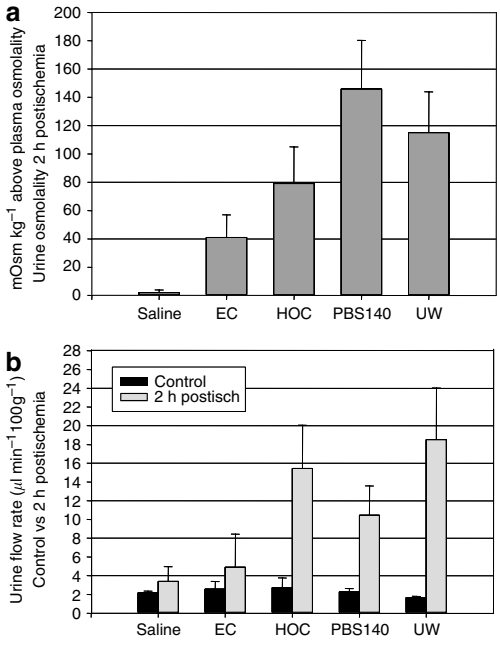

Figure 1 | Assessment of postischemic urine production. (**a**) There was a significant ($P<0.05$) drop in urine osmolality in the postischemic period in all groups from the mean normal level of 300 mOsm kg^{-1} body weight. While the saline and EC groups exhibited isosthenuria and near isosthenuria respectively, the kidneys in the HOC, PBS140, and UW groups showed progressive concentration of urine. The second-hour urine osmolality in the PBS140 and UW groups was significantly higher than that in both the saline and the EC groups ($P<0.05$). The urine output (**b**) in the HOC, PBS140 and UW groups exhibited initial postischemic polyuria. At 2 h postischemia, the urine flow rate in these groups was significantly higher than their respective preischemic control and the second hour urine volume in saline control ($P<0.05$). The urine flow rate in the EC group was not significantly different from the saline control group in this study, but this indicated severe renal damage.

Table 2 | Plasma solute concentrations at the end of the experiment (terminal serum) in animals that survived the experimental protocol

Parameters	Experimental groups					
	No flush (n=5)	Saline (n=7)	EC (n=6)	HOC (n=6)	PBS140 (n=7)	UW (n=6)
Sodium (mM l^{-1})	137±2.9	147±1.1	147±0.5	147±1.9	146±0.8	145±1.1
Potassium (mM l^{-1})	15.8±1.9*	9.2±1.1	7.4±1.0	11±1.6	8.1±0.7	9.1±0.8
Chloride (mM l^{-1})	107±2.2	113±1.8	107±1.1	106±1.6	112±1.7	107±0.9
Bicarbonate(mM l^{-1})		21±2.4	23±1.5	22±2.2	23±1.5	22±2.0
Glucose (mM l^{-1})		3.6±0.5	5.2±1.1	4.0±1.3	4.6±0.4	3.5±1.0
Urea (mM l^{-1})		18±1.9	16±2.0	15±1.2	17±2.0	16±1.2
Creatinine (μM l^{-1})		180±35	151±12	125±17	123±11	110±19

EC, Euro-Collins; HOC, hyper osmolar citrate; PBS, phosphate buffered sucrose; UW, University of Wisconsin.
*$P<0.05$.

groups, but did not reach statistical significance against the kidneys flushed with HOC, UW, or PBS140.

Inulin clearance, as an indicator of glomerular filtration rate (GFR), fell significantly in the postischemic period in all groups. The second-hour postischemic inulin clearance in the saline group (control) was only 5% of the preischemic control and was significantly higher in HOC, PBS140, and UW groups (40, 51, and 61% respectively, $P<0.05$) compared to the control. In the EC group, the second-hour inulin clearance was only 15% of preischemic levels and not significantly different from the saline group (Figure 2a). The urinary excretion of osmoles fell in the postischemic period in both the saline and the EC group to 58% and 88% of preischemic levels, respectively (Figure 2b), whereas the second-hour osmolar excretion rate in the HOC, PBS140, and UW groups was higher than that in the preischemic controls (368%, 208%, and 497%, respectively). The excretion rate of sodium (Figure 2c) in the postischemic period was not significantly higher than the preischemic levels in the saline and EC groups, but was higher in the HOC, PBS140, and UW groups. The potassium excretion rate (Table 3), on the other hand, was significantly lower in the saline group but not significantly lower in the EC group. For the HOC, PBS140, and UW groups, potassium excretion was either similar to (PBS140) or higher than (HOC, UW) that in preischemic controls, although this did not reach statistical significance.

The percentage reabsorption of water and of sodium (Table 3) fell significantly in the postischemic period in all groups compared to the preischemic controls.

Osmolar clearance, solute-free water reabsorption and free water clearance (Table 3) were also calculated to assess the concentrating ability of the kidney. Solute-free water reabsorption dropped significantly ($P<0.05$) in the postischemic period in saline and EC groups. In the HOC, PBS140, and UW groups, there was no significant change in solute-free water reabsorption at 2 h postischemia. Solute-free water reabsorption in the second hour was significantly higher than saline in the HOC, PBS140, and UW groups, and again significantly higher than EC in the UW group.

Analyses for urinary glucose (Figure 3a) and protein excretion rates (Figure 3b) were performed and showed that the glucose excretion rate was significantly higher in the EC group, probably owing to the presence of glucose in EC itself. The HOC group demonstrated a significantly higher protein excretion rate in the second hour postischemia. This was also reflected in a significantly high protein:creatinine ratio for HOC compared to saline, EC, PBS140, or UW ($P<0.05$, Figure 3c).

Assessment of tissue injury

GST was measured in the urine to assess postischemic tubular damage. A raised αGST level and excretion rate indicated proximal tubular damage whereas, a raised πGST and excretion rate indicated distal tubular damage. The control (preischemic) αGST excretion rate was below 0.5 ng^{-1} min^{-1} 100 g^{-1} and that of πGST was below 0.25 ng^{-1} min^{-1} 100 g^{-1}. Both α- and πGST were significantly raised in all the groups in the postischemic period, indicating ischemic injury in these kidneys (Figure 3d and e). However, a statistical comparison could not include the saline and EC groups because of insufficient numbers of surviving animals. The excretion rate of αGST was significantly lower in the PBS140 group compared to the HOC and UW groups at 2 h postischemia ($P<0.05$), suggesting evidence of reduced tissue injury.

As an indicator of fluid retention in preserved kidneys, and hence a marker of edema, the kidneys were weighed at the endpoints in this study. The right kidney (control) was weighed following nephrectomy, just after reperfusion of the left kidney. The percentage change in weight was calculated against the weight of the right kidney (Figure 4a), assuming both kidneys were equal in weight at the start of the experiment. There was a significant weight gain in the saline, EC and HOC flushed kidneys (15, 40, and 33%, respectively) compared to PBS140 (weight loss of 2.5%). Some weight gain was also noted in UW flushed kidneys (11%), although this did not reach statistical significance. The kidneys in the no-flush group also showed a weight increase of 12%. The relationship between wet kidney weight and inulin clearance (C_{in}) was also calculated (Figure 4b). Irrespective of the flush solution used, a greater increase in weight of the left kidney was associated with a lower C_{in}.

At the end of the experiment the left kidney was removed, weighed and sectioned longitudinally. The sections were

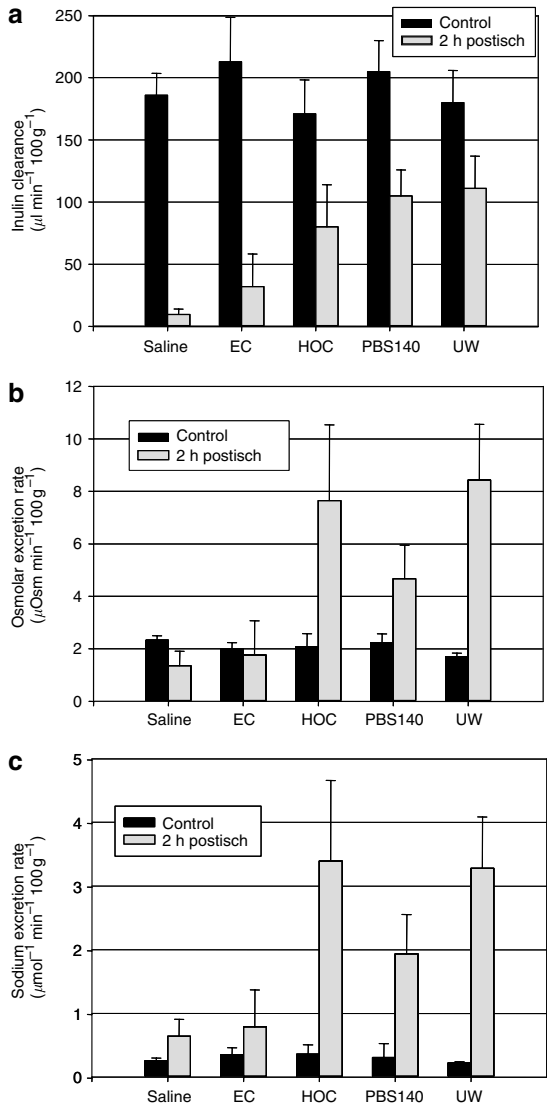

Figure 2 | Assessment of post ischemic renal function. Inulin clearance may be used as an indicator of GFR. (**a**) This was significantly reduced at 2 h postischemia compared to preischemic values in all groups ($P < 0.05$), either expressed directly or as a percentage of preischemic values (not shown). GFR measured by inulin clearance is linked to urine flow, of which there was very little in the saline and EC groups. GFR was higher in the HOC, UW, and PBS140 groups, although there were not significant differences within these groups in postischemic inulin clearance. (**b**) Osmolar excretion rate and (**c**) sodium excretion rate were also lower in the saline and EC groups compared to the HOC, UW, and PBS140 groups, which exhibited significantly higher osmolar and sodium excretion rates compared to preischemic values ($P < 0.05$).

photographed for macroscopic examination. The kidneys in the no-flush group appeared black/brown in color on the surface, and showed severe outer medullary congestion on longitudinal section (Figure 4c). Most kidneys in the EC group appeared visibly swollen. On section, varying degrees of medullary congestion were noted in most kidneys in the saline group and in four of six kidneys in the EC group. This appearance almost always correlated with the production of dilute urine. Patchy medullary congestion was also noted in three HOC flushed kidneys, although these produced relatively concentrated urine. The kidneys flushed with PBS140 and UW macroscopically appeared clean and healthy with little or no medullary congestion. On histological analysis, the kidneys in the saline- and EC-perfused groups demonstrated severe cortical and medullary pathology (Figure 5a–d). The HOC-perfused kidneys exhibited less severe pathology (Figure 5e and f), but tissue was most protected if the organ was perfused with either UW or PBS140 (Figure 5g–j), consistent with the recorded functional data.

DISCUSSION

This study investigated the functional response of the kidney to experimental warm ischemia in the presence of preservation fluids. The experimental design excluded cold ischemia, as this is less damaging than warm ischemia, so that the ability of preservation fluids to limit functional impairment after warm ischemia alone could be investigated. The survival data in this study indicated that 45 min of unilateral renal pedicle crossclamping with contralateral nephrectomy was a severe test of local and systemic tolerance to renal ischemia. Animal survival after renal warm ischemia was an outcome related to: (i) degree of tissue damage, (ii) severity of acute renal failure induced by warm ischemia, and (iii) ability of preservation solutions to attenuate such warm ischemic tissue damage. The deaths within the period of study were coincident to high plasma potassium levels leached from the ischemic tissue upon reperfusion, hyperkalemia through increased potassium release from damaged cells and/or reduced renal excretion of potassium after the ischemic insult. The protocol used was similar to other studies reporting the effects of experimental renal ischemia.[16,17]

The acute effects of I/RI are normally felt over a longer time period (1–3 days) and involve a number of nonimmune and immune mechanisms, for example, the action of free radicals, complement activation,[18] and neutrophil infiltration,[19] that influence events acutely upon reperfusion, or over time. Mechanisms of I/RI occur subsequent to organ preservation; hence such injury is principally influenced by the ability of preservation fluids to limit ischemic-induced edema, the prime function of a preservation fluid. Such edema itself results in direct tissue damage and the prolongation of ischemia through impaired microcirculatory blood flow.[20] This 2-h study sought to compare the efficacy of preservation fluids using acute postischemic renal function as an indicator of their effectiveness.

Overall, the data supported our previous work,[13] and that of others,[21] which showed that EC was least able to control ischemic damage. The use of EC was coincident to the greatest increase in kidney weight during reperfusion. The presence of glucose in EC as the impermeant agent does not limit warm ischemic induced edema as cell membranes, although impermeable to glucose in hypothermic storage, are not so under warm conditions.[22] In addition, Na^+ linked

Table 3 | Calculations of renal function measured pre- and postexperimental renal ischemia and reperfusion

Groups	Period	Osmolar clearance (μl min^{-1} 100 g body wt^{-1})	Free water clearance (μl min^{-1} 100 g body wt^{-1})	Solute-free water reabsorption (μl min^{-1} 100 g body wt^{-1})	Potassium excretion (μM min^{-1} 100 g body wt^{-1})	% reabsorption of water	% reabsorption of sodium
Saline	−1(7)	7.28±0.52	−5.10±0.38	5.10±0.38	0.272±0.03	99±0.1	99±0.2
	2(4)	4.25±1.76	0.00±0.06	0.00±0.06	0.030±0.01	64±7.4	76±6.5
EC	−1(6)	6.31±0.78	−3.73±0.44	3.73±0.44	0.197±0.03	99±0.5	98±0.7
	2(5)	5.66±4.18	−0.75±0.65	0.75±0.65	0.091±0.06	72±12.1	70±12.9
HOC	−1(6)	6.48±1.62	−3.77±1.1	3.77±1.1	0.203±0.05	99±0.4	99±0.5
	2(5)	24.09±9.17	−5.55±2.3	5.55±2.3	0.233±0.09	68±7.9	62±7.3
PBS140	−1(7)	7.06±1.09	−4.81±0.8	4.81±0.8	0.221±0.04	99±0.3	99±0.3
	2(7)	14.93±4.29	−4.49±1.3	4.49±1.3	0.216±0.06	90±2.0	87±2.8
UW	−1(6)	5.36±0.47	−3.69±0.5	3.69±0.5	0.215±0.02	99±0.2	99±0.1
	2(6)	26.77±6.80	−7.88±2.3	7.88±2.3	0.430±0.11	75±7.7	72±7.5

Periods are either 1 h before induction of ischemia (−1) and represent normal readings, or 2 h after reperfusion (2). Numbers in parenthesis indicate the number of completed observations for the given period.
EC, Euro-Collins; HOC, hyper osmolar citrate; PBS, phosphate buffered sucrose; UW, University of Wisconsin.

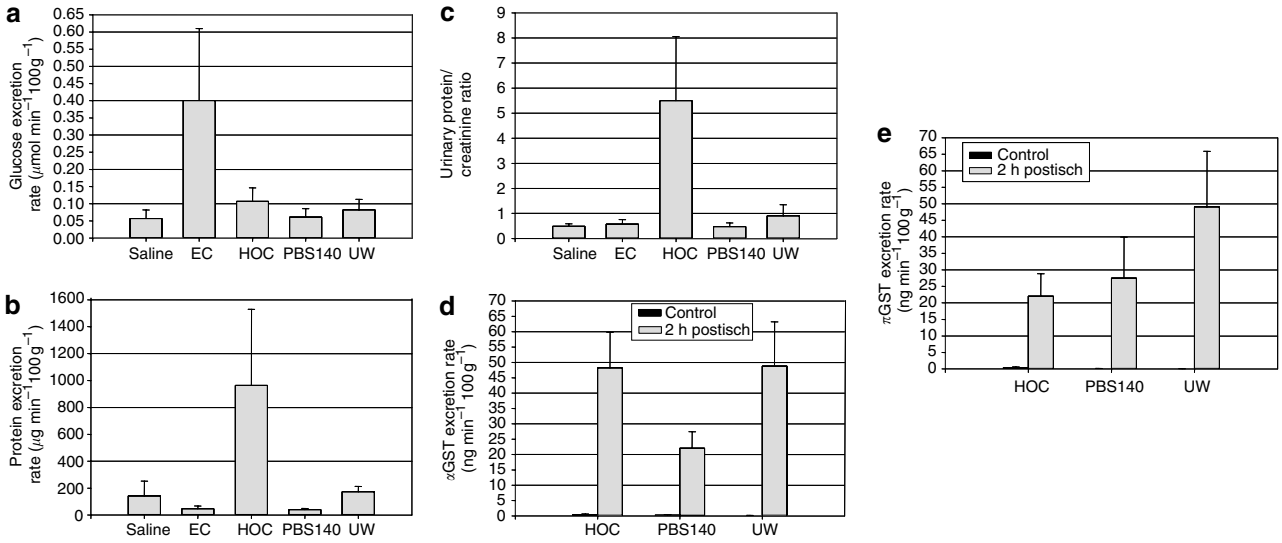

Figure 3 | Assessment of urinary markers of renal damage. (**a**) Glucose excretion in the EC group was significantly higher than that detected in any of the other groups which showed low levels of urinary glucose. Glucose is a constituent of EC that probably accounted for the glucose excretion data in the EC flushed group. (**b**) Data for urinary protein showed low urinary protein in all groups except HOC. (**c**) This was also reflected in the expression of urinary protein/creatinine ratio. (**d, e**) Levels of urinary α- and πGST were not detected in the saline and EC groups because of the lack of urine production in this group, (**d, e**) but data for HOC, UW and PBS140 clearly show highly significant increases ($P<0.001$) in αGSt and πGST, indicative of tubule damage.

glucose transporters that permit glucose influx are not ATP dependent,[1] and hence not limited by ischemia itself. Such edema, and its pathological consequences, presumably contributed to the nonfunction of EC-perfused kidneys in this study, which exhibited a low inulin clearance, low osmolar excretion rate and poor urine output compared to kidneys perfused with HOC, UW, or PBS140. A cumulative assessment of the data in this study could find that the EC-perfused kidneys were not functioning during this experiment, in contrast to the kidneys perfused with HOC, UW, or PBS140. Such an assessment is supported by the histological analyses that showed most severe cortical and medullary pathology in the EC group.

During recovery from acute renal failure, patients pass through a polyuric phase where urine volume is greater than normal, but GFR remains severely depressed;[23] this occurs after clinical renal transplantation.[24] That inulin clearance was lower than normal in the HOC, UW, and PBS140 groups, but excretion time was not affected, could be consistent with other reports that only a proportion, or a minority, of nephrons contribute to renal function following warm ischemia.[25] Such a hypothesis would be indicative of damage

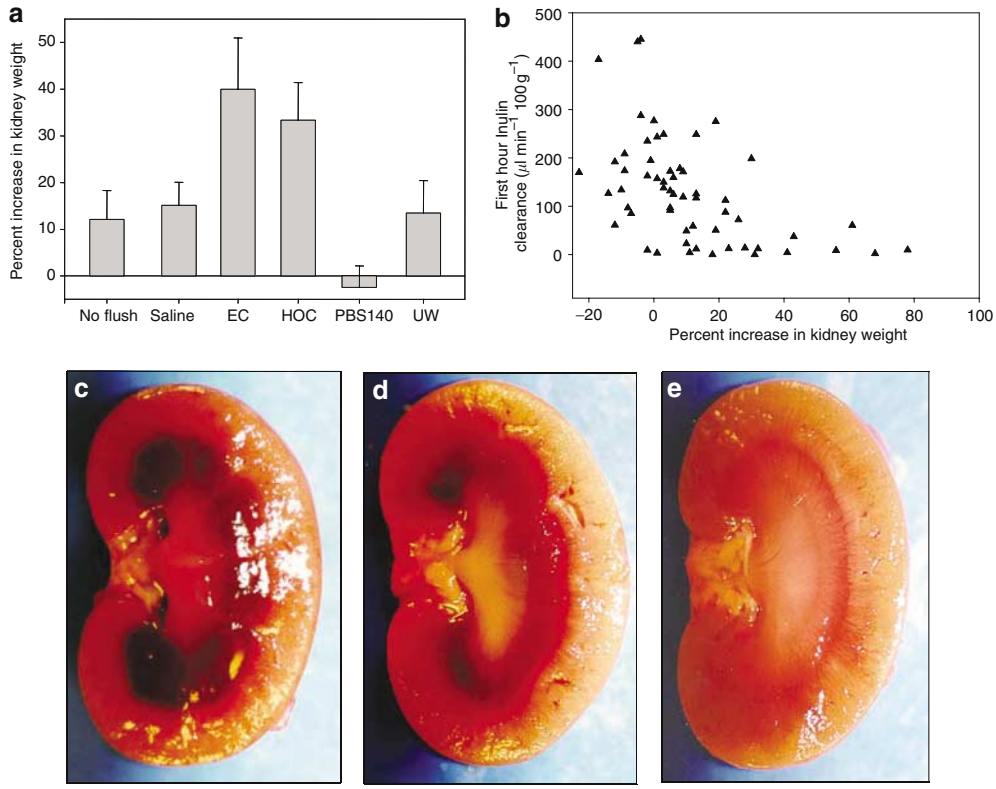

Figure 4 | Assessment of indices of tissue injury. (**a**) Changes in kidney weight showed that there was a significant increase in all groups of perfused organs except PBS140, indicative of retained fluid or cellular edema in these organs. PBS140-perfused tissues were not significantly different in weight than normal kidney controls. (**b**) An analysis of inulin clearance related to change in kidney weight showed that low GFR was associated with higher weight, suggesting that renal function was impaired by edema, irrespective of the preservation fluid used. (**c–e**) Macroscopic examination of the organs in this study showed that groups associated with poor function demonstrated gross medullary congestion, which was less marked in the better-performing groups and near normal in those organs with the best postischemic renal function, that is (**c**) Severe medullary congestion seen without preischemic flush or with saline flush. (**e**) Mild to moderate medullary congestion seen with EC or, occasionally, with HOC flush. (**d**) Normal appearance after PBS140 & UW flush.

to transporting tubular epithelium, the extent or distribution of which, in this study, appeared to be related to the preservation solution used, although glomerular and tubular functions were difficult to assess independently, as tubule damage affects the measurement of GFR. However, as ischemic reduced filtration limits the secretion of inulin by glomeruli, and the damaged tubules also become leaky to inulin, thus also limiting inulin excretion, a higher inulin clearance was still indicative of reduced ischemic damage. Such back-leakage of tubular fluid is associated with structural injury in renal ischemia, and is dependent on the duration, and hence severity, of ischemia.[26] In this setting, therefore, a higher inulin secretion and resulting higher inulin clearance indicated reduced ischemic damage to both glomerular and tubular structures.

Our previous study showed that tubule edema was least in PBS140-perfused kidneys[13] and data on kidney weight show that there was very little weight change (−2.5%) in the PBS140 group of this study, as opposed to +40 and +11% in the HOC and UW groups, respectively. This implied that HOC was less effective in limiting tissue weight gain, which the histological observations would indicate was predominantly due to medullary congestion, in the reperfused kidney than UW or PBS140.

The measurement of urinary αGST and πGST indicated tubule damage.[27] Interpretation of this data could imply that proximal tubules were more susceptible to ischemic damage than distal tubules and showed that UW preserved kidneys produced higher levels of GST than either the HOC or the PBS140 groups. Although counterintuitive to the proposition that UW is the 'gold standard' solution, the data indicated more tubular damage in the UW group than in the HOC or PBS140 groups, and perhaps adds to the interpretation of the solute excretion and absorption data, where the sodium excretion rate was higher in the UW group than in the PBS140 group. High urinary sodium is also indicative of tubulo-interstitial damage[28] and may have been higher in the UW group if organ perfusion was less efficient owing to the highly viscous nature of UW, reported as problematic in experimental liver transplantation.[29] This concurs with the data in this study, indicating that the best renal function was found overall in the PBS140 group. The histological analysis clearly demonstrated improved medullary architecture in the UW and PBS140 groups compared to HOC. However,

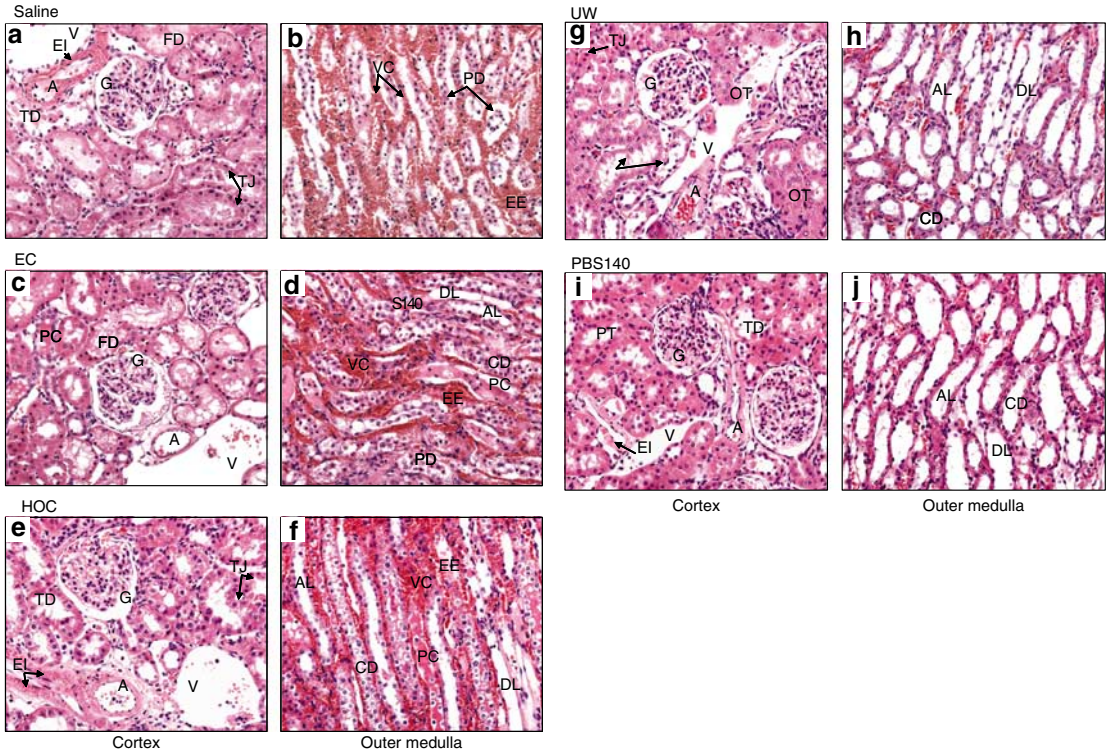

Figure 5 | Terminal histology of kidneys flushed with different fluids. Histological analysis was performed on tissues harvested at the termination of each experiment. The arterioles of the saline flushed kidneys (**a**) exhibited widespread subendothelial infiltration of erythrocytes (EI), while proximal tubules demonstrated feathery degeneration of cytoplasm (FD) and widespread separation of epithelial tight junctions (TJ). In places, tubule degeneration (TD) was evident with cellular material and cell casts present in tubular lumen. (**b**) Medullary arrays of descending and ascending limbs of Loops of Henle (LoH) as well as collecting ducts are difficult to differentiate owing to parenchymal detachment (PD), while the vessels of the vasa recta were congested with erythrocytes. (**c, d**) Extravasation of erythrocytes (EE) into renal lumenal spaces was also evident. In the EC group, there was widespread feathery degeneration (FD) of proximal tubules. Arterioles (A) and venules (V) appeared patent, but some degree of tissue shrinkage permitted expansion of extracellular spaces between tubules and vessels. Protein casts were present in distal tubules. In the medulla, there was some degree of parenchymal detachment (PD) in the collecting ducts (CD), and the descending limbs (DL) and ascending limbs (AL) of LoH contained protein casts (PC). Vascular congestion (VC) of the vasa recta was widespread. (**e, f**) In the HOC group, erythrocyte infiltration (EI) was again a feature in the cortical arterioles. There was also expansion of extracellular spaces as well as a moderate incidence of TJ separation. Elements of the medullary arrays were identifiable with protein casts (PC) in the collecting ducts (CD) vascular congestion in the vessels of the Vasa Recta but with clear descending and ascending limbs of LoH. (**a, h**) Kidneys of the UW flushed group showed degeneration of tight junctions (TJ) in places, as well as moderate occurrence of edematous tubules (OT). Some signs of tubular degeneration were seen (arrows), but erythrocyte infiltration was not a feature in this group. Medullary arrays appeared clear with only minor indications of congestion. (**i, j**) The PBS140 flushed group exhibited well-maintained architecture, with only minor occurrence of tubular degeneration or erythrocyte infiltration of vascular smooth muscle cells. Medullary arrays appeared clear of congestion, but showed signs of some dilation.

potential exists for the cortical edema that was a feature of the UW-perfused kidneys to influence the functional impairment, and indices of tubulo-interstitial injury, recorded in the UW perfused kidneys after ischemia and reperfusion.

A global overview of functional data in this study showed that PBS140-preserved kidneys performed closer to normal than either the HOC- or UW-preserved kidneys (Figure 6). While PBS140-perfused kidneys produced less urine output in terms of urine flow rate and urine volume than the UW group, the solute concentration above plasma was highest in this group, indicating better kidney function. The PBS140-perfused kidneys appeared to be more capable of regulating the solute concentration of urine, and hence fluid volume in the host. It is this vital parameter that is most indicative of improved preservation in PBS140-treated kidneys compared to UW perfused kidneys and may, in part, have been a reflection of some degeneration of proximal tubule tight junctions in the UW group. Although the incidence of this was low, this was not a feature of PBS140-preserved kidneys.

Together with our previous report,[13] and others,[30,31] evidence is emerging that the choice of preservation fluid impacts upon postischemic renal function. Our data suggest that PBS140-based solutions may offer better organ preservation. However, renal tubule intolerance to sucrose has been described after chronic exposure to dietary sucrose in diabetic rats,[32] and, more recently, similar pathology was found in patients given intravenous immunoglobulins suspended in sucrose solutions, used in the treatment of immunological and hematological disorders.[33] Therefore, high levels of circulating sucrose, where there is pre-existing

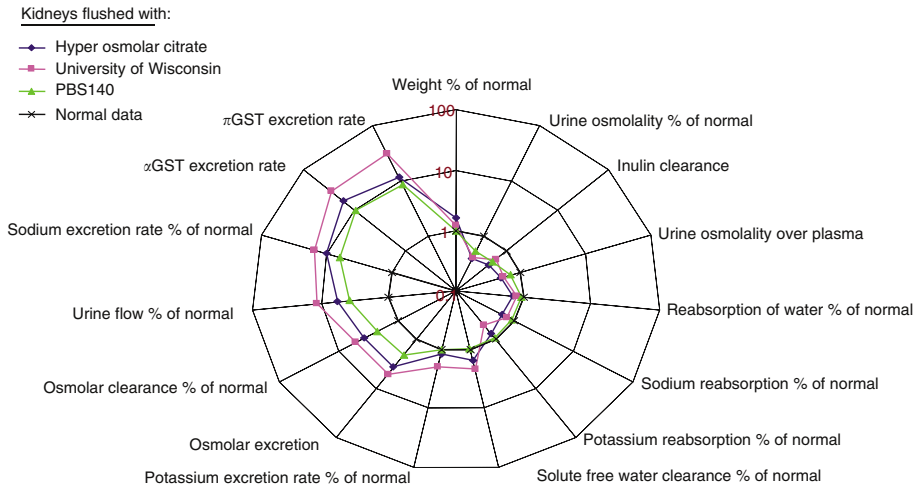

Figure 6 | Comparison of preservation solutions to influence postischemic renal function. The $\log_{10}$ webplot compares the mean data from the HOC, UW, and PBS140 groups against mean preischemic kidney data. Normal control data means are expressed as 1, and experimental mean data were plotted as values relative to normal for all parameters except α- and μGST, which are practically zero in the normal kidney. For these two data sets, the actual experimental values are plotted for the HOC, UW, and PBS140 groups. The closer the experimental values are to the normal value, for each parameter, the better the function of the experimental kidneys and the less severe the tissue injury. This overview of data shows that, for most of the parameters measured, PBS140-perfused kidneys maintained their renal function closer to normal, and the indices of tubular injury were less than in the kidneys in the HOC and UW groups. Taken together, this plot demonstrates that PBS140-preserved kidneys were less damaged than kidneys in the other groups.

chronic disease or renal insufficiency, may exert a nephrotoxic effect. There appear to be no data showing that transient exposure to sucrose-based preservation solutions may adversely affect renal tubules in healthy kidneys in a similar manner, and no data are consistent with such a notion in the current study.

In sum, our previous report,[13] and the current study find little support for the continued use of EC in kidney preservation. It is also reasonable to conclude the ability of HOC to prevent tissue injury is at best limited in prolonged ischemia (i.e. in excess of 20–24 h). The data in this study therefore have important implications for ensuring evidence-led best practice in organ transplantation. Improved organ preservation has the capacity to improve transplant outcomes in the short and long term.[34] Adoption of improved standards in preservation by organ retrieval teams will assist improved outcomes across transplant centers, especially so within organ-sharing schemes, where the receiving center may have no influence on the choice of preservation solution used.

MATERIALS AND METHODS

Animals and anesthesia

Virus-antibody free male Wistar rats, 250–300 g, were purchased for this study (Charles River, Surrey, UK). UK Home Office Guidelines (Scientific Procedures Act, 1986) were strictly adhered to throughout this work. A mixture of halothane and oxygen was used for rapid induction of anesthesia and followed by intraperitoneal injection of 120–150 mg kg^{-1} body weight of inactin (sodium 5-ethyl-5-(1-methyl)-2-thiobarbitone; Promonta Corp., Hamburg, Germany) to maintain anesthesia. The animal was placed on a heated dissection table throughout the experiment and a rectal thermometer was used to monitor body temperature which was maintained between 36 and 38°C throughout the experiment.

Table 4 | Characteristics of organ preservation solutions used in the study

Core attribute	EC	HOC	UW	PBS140
Impermeant	Glucose	Mannitol	Raffinose	Sucrose
pH	7.0	7.1	7.4	7.0
Buffer system	K^+ phosphate	Citrate	K^+/Na^+ hydroxide	Na^+ phosphate
Tonicity	355	400	325	300 (isotonic)

EC, Euro-Collins; HOC, hyper osmolar citrate; PBS, phosphate buffered sucrose; UW, University of Wisconsin.

Surgery and experimental protocol

After anesthesia, the left jugular vein was cannulated with a thin bore (pp10) polyethylene catheter attached to a 20 ml syringe mounted on an infusion pump, and a continuous intravenous infusion was set up at 6 ml h^{-1} (100 μl min^{-1}) using saline-bicarbonate solution (125 mmol NaCl–25 mmol $NaHCO_3$). The infusion solution also contained 37 MBq l^{-1} of [^{3}H] inulin (Amersham Biosciences, Little Chalfont, UK) for the purpose of calculating inulin clearance. A priming dose of 185 kBq [^{3}H] inulin dissolved in 1 ml of infusion solution was given as bolus, immediately after cannulation of the jugular vein. The right carotid artery was then cannulated with a short length of pp10 polyethylene tubing connected via a three-way port to a pressure transducer (Elcomatic Ltd, Glasgow, UK), which facilitated blood sampling (for inulin count).

After midline laparotomy, the left kidney was dissected free of the Gerrota's fascia along its entire circumference. Both ureters were cannulated using pp50 polyethylene tubing and tied with 3/0 silk. The abdomen was then closed using interrupted 3/0 silk sutures. After a 1-h equilibration period, urine was collected separately from both kidneys for a further hour to provide preischemic control data. The abdomen was then re-opened and the aorta clamped above and below the left renal artery. The aorta was injected with 0.5 ml of flush fluid at a controlled rate over 10 s (3 ml min^{-1}) and a clamp

was applied to the left renal pedicle. The whole process of renal flush was completed within 1 min. Urine was collected from the right kidney throughout the period when the left kidney was ischemic. After 45 min, the clamp was removed from the left renal pedicle, allowing reperfusion of the left kidney. At this point, a right nephrectomy was performed and the weight of the right kidney was recorded. The abdomen was closed using interrupted 3/0 silk sutures, and urine was collected. After the experiment, the animal was killed by exsanguination, for serum biochemistry.

The experiments were divided into three main groups:

Group I No flush: Left kidney was not flushed prior to ischemia.
Group II Saline flush: Left kidney was flushed with 0.9% saline priorto ischemia.
Group III Flush with one of the existing preservation solutions, PBS140, UW, EC, and HOC (Table 4).

Measured parameters

Measurements were made on each individual urine and serum sample.

1. *Urine flow rate* ($\mu l\,min^{-1}\,100\,g^{-1}$) was estimated by measuring urine overtime.

$$V = \frac{\text{Urine volume for } 1h \times 100}{60 \times \text{wt. of the rat}} (\mu l min^{-1} 100 g^{-1})$$

2. *Osmolality* ($mOsm\,kg^{-1}$) of urine and terminal serum was determined by cryoscopy (Camlab Roebling Osmometer, Cambridge, UK), which was standardized using a standard solution of 300 $mOsm\,kg^{-1}$ of H_2O.
3. *Urine and plasma electrolytes*. All urine samples and terminal serum samples were analyzed for Na^+, K^+, and Cl^- estimation. Na^+ and K^+ concentrations ($mM l^{-1}$) were determined using a flame photometer (Corning-EEL, model 480, Corning, NY, USA). Chloride concentration was estimated using a silver-silver chloride, electrometric titration (Corning-EL chloride analyser, model 925). Urea, creatinine, and glucose were estimated at the chemical pathology laboratory at St James's University Hospital, Leeds, UK. Urine pH and protein were estimated semiquantitatively using multi-stix reagent strips (Miles Inc., Pittsburgh, PA, USA).
4. *Urinary α- and πGST* (glutathione *S*-transferase (GST) ($\mu g\,l^{-1}$)) were estimated as markers of tubular damage using an enzyme-linked immunosorbent assay technique as per kit instructions (Biotrin International, Dublin, UK).
5. Tritiated [^{3}H]inulin count was obtained in 10-μl aliquots of all serial urine and plasma samples. In all, 10 μl of urine or plasma was dissolved in 5 ml of scintillation cocktail (Triton-x + xylene) and placed in a liquid scintillation spectrometer. Each sample was counted twice for 10 min and the mean of the two counts was calculated as number of counts per 10 min.
6. Derived values and calculations: Various parameters of renal function were calculated using strandard formulae.[35] The indices analysed were:
   1. Inulin clearance (C_{in})
   2. Osmolar clearance (C_{osm})
   3. Osmolar excretion rate (V_{osm})
   4. Solute filtered load (sodium, potassium, chloride, urea, creatinine)
   5. Solute excretion rate (Na^+, K^+, Cl^-, urea, creatinine, and glucose)
   6. Protein excretion rate
   7. Absolute reabsorption of water
   8. Absolute reabsorption of solutes (sodium, potassium, chloride, urea, creatinine)
   9. Percent reabsorption of water
   10. Percent absorption of solute
   11. Solute-free water reabsorption
   12. Urinary protein/creatinine ratio

Histological analysis

Kidney tissue was harvested at the end of the experiment, fixed in formalin and wax embedded prior to sectioning and staining with hemotoxylin and eosin using standard methods.

Statistics

Results. were standardized by calculating and expressing the results as per 100 g body wt of the rat. The results are presented as mean $\pm$s.e.m. for each group. The significant differences between two groups were calculated by Student's *t*-test. Significant differences among groups for a given time period were determined by one-way analysis of variance and by applying Dunnett's or Tukey's test. Where the numbers were found to be not normally distributed, a Kruskall–Wallis test was applied. Significance was defined as $P < 0.05$.

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