

Normothermic *Versus* Hypothermic *Ex Vivo* Flush Using a Novel Phosphate-Free Preservation Solution (AQIX) in Porcine Kidneys

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Background. The initial flush of an organ is important to remove any cellular components from the microcirculation before storage. The aim of this study was to assess graft function after an *ex vivo* warm flush with a novel non-phosphate buffered preservation solution AQIX RS-I (AQIX) compared with a traditional cold flush.

Methods. Porcine kidneys were either warm-flushed with AQIX RS-I at 30°C, or cold-flushed at 4°C with University of Wisconsin solution (UW) or hyperosmolar citrate (HOC) preservation solution at a pressure of 100 cmH₂O ($n = 6$). Renal function was measured *ex vivo* by perfusing the organs with autologous blood at 37°C on an isolated organ perfusion system.

Results. The AQIX group flushed significantly quicker than the cold stored groups (22 ± 1.8 versus UW 4.9 ± 1.6 versus HOC 10 ± 1.6 mL/min/100g; $P = 0.001$) and gained less weight than the UW group (19 ± 2.9 versus UW 30 ± 3.4 versus HOC $21\% \pm 7.7\%$; $P = 0.025$). The AQIX group also had superior acid-base homeostasis. Functional results, histologic analysis, and ADP: ATP levels were comparable between the groups.

Conclusion. Flushing kidneys with AQIX at 30°C cleared the renal microcirculation of blood more rapidly without any detrimental effects when compared to traditional cold flushing with UW or HOC at 4°C. Warm initial flushing has potential to be developed as part of normothermic renal preservation techniques. © 2010 Elsevier Inc. All rights reserved.

Key Words: kidney; normothermic preservation; hypothermic preservation.

INTRODUCTION

Hypothermic static storage of organs for transplantation yields good functional results and is a simple and cost-effective technique. As such, it continues to be the preferred method of organ preservation in the UK [1, 2]. The initial flush of an organ is important to remove blood, any cellular components that may have clumped, and to perfuse the microcirculation and, hence, the interstitium, with a solution that is designed to impair cellular swelling. The various preservation solutions currently in use are flushed cold, the aim being to slow metabolism down quickly in the face of a potentially damaging anaerobic environment. In keeping with renewed interest in normothermic preservation [3–5], some authors have assessed the role of a warm pre-flush and have reported beneficial results. For example, reduced hepato-cellular damage has been demonstrated in rat livers [6], and improved microperfusion in liver grafts by combining a warm flush with streptokinase [7].

The use of a warm flush is a rational attempt to achieve more rapid clearance of blood from the microcirculation. Warm preservation solution may reduce vasoconstriction and prevent stiffening of the cell membranes in both endothelial cells and the cellular components of the blood. This may then ameliorate the no-reflow phenomenon, which is an important mechanism in ischemia-reperfusion injury.

We have previously published work using a novel normothermic preservation solution, AQIX (AQIX Ltd, London, UK), showing that it has the capability to maintain acid-base balance after warm flush and 2 h of warm ischemia [8].

AQIX has been designed as a universal normothermic organ preservation solution that would reflect

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TABLE 1

Constituents of AQIX, Hyperosmolar Citrate (HOC) and University of Wisconsin (UW) Preservation Solutions

Components	AQIX	HOC	UW
Sodium	110	84	
Potassium	5.0	80	
Citrate		54	
Magnesium Sulphate	0.45	41	
Sodium bicarbonate	25.0		
BES	5.0		
D-glucose	10.0		
Calcium chloride	1.25		
Glycerol	0.11		
L-glutamate	0.30		
L-glutamine	0.40		
L-aspartate	0.02		
L-carnitine	0.05		
Choline chloride	0.01		
TPP (cocarboxylase)	40.0 nmol/L		
Insulin	28.0 mIU		
Potassium citrate		8.6 g/L	
Sodium citrate		8.2 g/L	
Mannitol		33.8 g/L	
Magnesium sulphate		10.0 g/L	
Poly (0-2 hydroxyethyl starch)			50 g/L
Lactobionic acid			105
Potassium hydroxide			100
Sodium hydroxide			27
Adenosine			5
Allopurinol			1
Potassium dihydrogen phosphate			25
Magnesium sulphate			5
Raffinose			30
Glutathione			3
Ph	7.27	7.1	7.4
Osmolarity	286 mOsmol/L	486 mOsmol/L	320mOsmol/L

physiologic ionic concentrations, osmolarity, and ion conductivity, thereby maintaining the ionized status of the cell membrane and the function of enzymatic and receptor moieties. The osmolarity of AQIX is similar to that of serum at 286 mOsmol/L, and its ionic concentrations have been chosen to maintain it as an isosmotic solution. The ionic conductivity is also comparable to human serum, namely, 12.6 mS cm⁻¹.

AQIX is a non-phosphate buffered solution that utilizes a combination of the bicarbonate system ($\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$) and Good's BES as its buffer which, because of its ideal pK_a value, allows the pH to be maintained within a range of 7.2–7.46 over a temperature range of 4–37°C, thereby facilitating the transition from hypothermic to normothermic and vice versa. A comparison of its constituents with traditional solutions is displayed in Table 1.

AQIX is capable of carrying oxygen in solution or by incorporating red blood cells during machine

preservation. Initial studies using AQIX were focused on isolated, small animal tissues and organs preserved and perfused in an organ bath system using oxygenated AQIX at 15–37°C. Such studies demonstrated functional viability of rat jejunum for 9 d, rat colon for 5 d, and rabbit uterus for 7 d [9]. The aim of the present study was to assess the effectiveness of an initial warm flush using AQIX solution, compared with a traditional cold flush using cold hyperosmolar citrate solution (HOC) or University of Wisconsin solution (UW), in porcine kidneys. These kidneys were then given a short preservation period and subsequently reperfused under normothermic conditions during which assessment of function was made.

METHODS

Kidney Retrieval

Large white pigs (60–70 kg) were sacrificed by electrocution followed by exsanguination and approximately 1 L of blood was collected into a sterile receiver containing 25,000 units of heparin (Multiparin; CP Pharmaceuticals, Wrexham, UK). The kidneys were surgically removed with minimal warm ischaemic time and immediately flushed with 400 mL of preservation solution at a hydrostatic pressure of 100 cmH₂O. The time taken for the total of 400 mL of solution to run into the kidney (the flush time) was recorded to the nearest minute along with the exact warm ischaemic interval. Perfusate flow rates were calculated and expressed as mL/min/100 g of kidney. All kidneys were then preserved by cold storage on ice for 2 h to allow transfer to the laboratory.

Three different preservation techniques were compared ($n = 6$ kidneys per group): AQIX flush at 30°C; HOC flush at 4°C; UW flush at 4°C. For warm flushing, the AQIX solution was prewarmed in a temperature regulated water-bath and maintained at 30°C during transport in an insulated organ retrieval carrier; temperature was monitored with a temperature probe.

Isolated Organ Perfusion

The isolated organ perfusion system has been described in detail previously [10], but in brief consists of a centrifugal blood pump, a heat exchanger, a venous reservoir, and a membrane oxygenator. The circuit hardware includes a speed controller, a flow transducer, a pressure transducer, and a temperature probe. Two infusion pumps and a urinometer were incorporated into the system.

The circuit was primed with 500 mL Ringer's solution containing mannitol 10 mg (Baxter Healthcare, Norfolk, UK), dexamethasone 10 mg (Organon Labs Ltd., Cambridge, UK), cefuroxime 750 mg (Bri-tannia Pharmaceuticals Ltd., Surrey, UK), and 12 mL 8.4% sodium bicarbonate (Fresenius Kabi, Warrington, UK); 500 mL of heparinized whole blood was added to the circuit after priming and allowed to circulate at a temperature of 37°C.

The perfusate was also supplemented with a nutrient solution (Nutriflex B; Braun, Sheffield, UK) to which 100 units of insulin (Actrapid; Novo Nordisk, Denmark and UK) and 25 mL 8.4% sodium bicarbonate (Fresenius Kabi) were added. The nutrient solution was infused at a rate of 20 mL/h. A vasodilator, sodium nitroprusside 25 mg, (Mayne Pharma PLC, Warwickshire, UK) was administered during the first hour of reperfusion at 25 mL/h, after which 5% glucose solution (Baxter) was infused at 7 mL/h. Ringer's solution (Baxter) was used to accurately replace urine output. Creatinine (Sigma-Aldrich, Steinheim, Germany) was added to the perfusate to achieve an initial circulating concentration of 1000 μmol/L.

Experimental Protocol

Pairs of kidneys retrieved from the same animal were flushed by the same technique and then simultaneously perfused with autologous blood, using two separate isolated organ perfusion circuits. The renal artery, vein, and ureter were cannulated, and any residual preservation solution was removed by flushing the kidneys with 90 mL of plasma substitute (Gelofusine; B. Braun, Sheffield, UK) at 4°C. The kidneys were weighed immediately pre- and post-perfusion using a balance accurate to 1 g; the weight change was recorded.

Kidneys ($n = 6$ per group) were perfused for 6 h at a mean arterial pressure of 55 mmHg. Renal blood flow (RBF) and mean arterial pressure (MAP) were continuously recorded and intra-renal vascular resistance calculated (MAP/RBF). The addition of 1000 μmol creatinine to the circuit enabled an accurate measurement of renal function to be made. Serum and urine samples were taken hourly for biochemical analysis and whole blood for hematology. Creatinine clearance ($U_{\text{cr}} \times U \text{ volume}/P_{\text{cr}}$) and fractional excretion of sodium $[(U_{\text{t}} \times U \text{ flow})/(\text{GFR} \times P_{\text{t}}) \times 100]$ were calculated and serum creatinine levels recorded hourly. Blood gases were measured for the calculation of oxygen consumption in mL/min/g $[(\text{PaO}_2 \text{ art} - \text{PaO}_2 \text{ ven}) \times \text{flow rate}/\text{weight}]$ and acid-base balance. Arterial pH was measured prior to perfusing the kidneys, then at 1, 3, and 6 hourly intervals for assessment of acid-base homeostasis.

Needle core biopsies were taken pre-perfusion and 6 h post-perfusion, fixed in 4% formalin, dehydrated, and embedded in paraffin wax. Sections were cut at 4 μm , then stained with hematoxylin and eosin for evaluation using light microscopy. Eight morphologic parameters (tubular dilation, epithelial flattening, epithelial shredding, tubular debris, vacuolation, red blood cell presence, condensed tubular nuclei, and glomerular shrinkage) were each scored over five fields using a semiquantitative scale (0 normal; 1 mild; 2 moderate; 3 severe).

Biopsies were also used to assess ADP:ATP ratios as a measurement of cellular viability both pre- and post-perfusion. This was performed using an adenylate nucleotide ratio assay kit (Cambrex BioScience, Berkshire, UK), whose detection is based on the bioluminescent measurement of ATP using the enzyme luciferase. This enzyme catalyzes the formation of light from ATP and was measured using a luminometer.

Needle core biopsies were attached to cork blocks using tissue-Tek medium, immediately submerged in liquid nitrogen and transferred to cryotubes for storage at -80°C . Six sections of 10 μm thickness were cut from each core using a cryostat and placed in an Eppendorf tube pre-cooled to -20°C ; 600 μL of nucleotide releasing reagent was added to the sections and the sample vortexed and stored at 4°C. A 180 μL aliquot of this sample was brought to room temperature (the remainder of the sample was kept at 4°C for replicate analysis).

Twenty μL of nucleotide monitoring reagent was added to the aliquot and the sample placed on a luminometer (1250 Bio Orbit, Turku, Finland). An immediate voltage reading (A) on a chart recorder (LKB 2210, Bromma, Sweden) was noted. A second reading (B) was taken after 10 min. The sample was then removed, 20 μL of ADP converting reagent added, and the sample placed back into the luminometer. A final reading (C) was taken after 5 min.

Statistical Analysis

Values are presented as mean \pm SD. Continuous variables, such as serum creatinine, were plotted against time, and the area under the curve for individual perfusion experiments calculated using Microsoft Excel software (Reading, UK). Mean areas under the curve were then compared using the Kruskal-Wallis test, and post testing was performed with Dunn's test for serum creatinine, creatinine clearance, renal blood flow, and urine output. The Kruskal-Wallis test was also used for comparison of mean pressure, temperature, pH, oxygen consumption, weight gain, and intra-renal vascular resistance (mean values presented \pm SD). A P value ≤ 0.05 was taken as statistically significant.

RESULTS

The mean (SD) warm ischaemic time during retrieval for all kidneys was 6.4 ± 1.0 min, and there were no significant differences between groups ($P = 0.335$). Warm flushing with AQIX at 30°C achieved higher perfusate flow rates than HOC or UW at 4°C (22.0 ± 1.8 versus 10.0 ± 1.6 versus 4.9 ± 1.6 mL/min/100 g, respectively, $P = 0.001$). There were no significant differences in renal blood flow, intra-renal vascular resistance, or oxygen consumption between the three groups (Table 2). Weight gain post-perfusion was greatest in the UW group, and this was significantly higher than the warm AQIX group but not the HOC group ($P = 0.025$).

Acid Base Homeostasis

The arterial pH fell in all three groups over the 6 h perfusion period with the steepest decline observed in

TABLE 2

Comparison of Perfusion Parameters Following Initial Flushing of Porcine Kidneys with HOC, UW, or AQIX Solutions (Mean \pm SD)

Parameters	HOC	UW	AQIX (30°C)	P value
WIT (min)	7.1 \pm 1.0	6.2 \pm 1.5	6.2 \pm 0.4	0.164
Flush (mL/min/100 g)	10 \pm 1.6	4.9 \pm 1.6*	22 \pm 1.8*	0.001*
Arterial pressure (mmHg)	54 \pm 0.7*	59 \pm 2.6*	57 \pm 3.9	0.008*
Temperature (°C)	37.3 \pm 0.5	37 \pm 0.6	37.3 \pm 0.3	0.200
Renal blood flow (mL/min/100 g)	50 \pm 10	56 \pm 22	48.4 \pm 19	0.770
Intra-renal vascular resistance (mmHg/mL/min/100 g)	0.52 \pm 0.1	0.8 \pm 0.4	0.5 \pm 0.2	0.520
O ₂ Consumption (mL/min/g)	31 \pm 6.3	33.7 \pm 15.1	25.3 \pm 12.8	0.395
Weight Gain (%)	21 \pm 7.7	30 \pm 3.4*	19 \pm 2.9*	0.025
pH	7.2 \pm 0.1	7.2 \pm 0.12	7.3 \pm 0.1	0.458
Bicarbonate (mmol/l)	14.6 \pm 2.9	15.3 \pm 4.3	17.8 \pm 5.9	0.699

Renal Blood flow, intra-renal vascular resistance, oxygen consumption, pH and bicarbonate levels after 6 h of reperfusion. Values are mean \pm SD. Kruskal-Wallis Test with post test.

* $P < 0.05$ between groups.

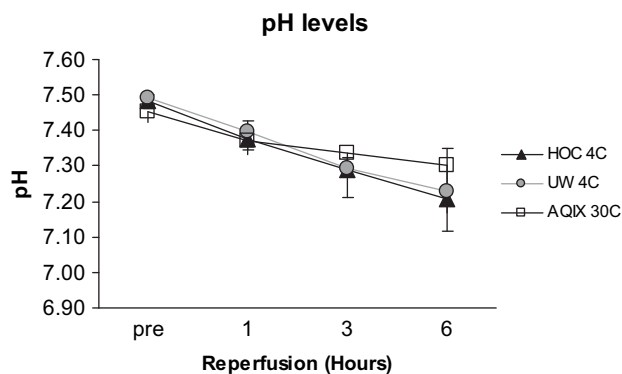


FIG. 1. Blood pH levels during 6 h of isolated organ perfusion after flushing with HOC, UW, AQIX solutions.

the HOC group (pre-perfusion pH 7.49 ± 0.1 versus 7.21 ± 0.1 after 6 h perfusion; Fig. 1). The warm AQIX group demonstrated superior acid-base homeostasis, with near-normal arterial pH at the end of the perfusion period (7.3 ± 0.1). Bicarbonate levels were also better in the warm AQIX group (falling from a mean of 22.9 mmol/L pre-perfusion to 17.8 mmol/L after 6 h versus 25.4 to 14.6 mmol/L for HOC and 25.6 to 15.3 mmol/L for UW; Table 2).

Functional Parameters

Renal function during the 6 h perfusion period was comparable among the three study groups, with no significant differences in serum creatinine levels, creatinine clearance, urine output, or renal blood flow (Table 3).

ATP Levels

The ADP:ATP ratio levels were highest in the pre-perfusion biopsies, reflecting the ischemic damage sustained during the cold storage period. Levels were numerically highest in the UW group but there were no significant differences between the three groups ($P = 0.55$; Fig. 2). The ADP:ATP ratio decreased after 6 h of perfusion in all three groups, showing recovery of cellular function. The HOC group had a lower

post-perfusion ADP:ATP ratio than the UW group ($P < 0.05$), but there were no statistically significant differences between the warm AQIX group and either HOC or UW. The pre-perfusion to post-perfusion change in ADP:ATP ratio was numerically higher in the HOC group but did not reach statistical significance ($P = 0.78$).

Histology

The HOC-flushed group showed most evidence of damage in pre-perfusion biopsies with significantly more tubular dilatation, epithelial flattening, and tubular debris than the warm AQIX and the cold UW groups ($P < 0.05$; Table 4). Mild glomerular shrinkage was also evident with HOC. The warm AQIX group demonstrated a mild degree of tubular dilatation and epithelial shredding. The only pre-perfusion histologic changes seen in the UW group was mild epithelial shredding.

Post-perfusion, the only significant difference between the groups was the presence of more glomerular shrinkage in the warm AQIX group compared with both the HOC and UW groups ($P = 0.05$; Fig. 3A–F).

DISCUSSION

This study shows that initial flushing with AQIX perfusate at 30°C leads to a more rapid clearance of blood from porcine kidneys compared with HOC or UW solutions at the more conventional temperature of 4°C . This improved rate of initial organ perfusion did not lead to an improvement in post-ischemic renal function; however, the additional period of normothermia with the use of AQIX did not have any deleterious effect on renal function. Histologically, there were no significant differences between the groups after 6 h of normothermic reperfusion. Furthermore, there were no differences in post-perfusion ADP:ATP ratios between the 30°C AQIX group and the two cold flush groups.

Porcine kidneys that were of similar weight to adult human kidneys were used in this study, as they are

TABLE 3

Renal Function, Renal Blood Flow, and Urine Output After Flushing with HOC, UW, or Warm (30°C) AQIX Solution

	HOC	UW	AQIX (30°C)	P value
SCr ($\mu\text{mol/L}\cdot\text{h}$)	2404 ± 596	2887 ± 865	2333 ± 1066	0.581
CrCl ($\text{mL}/\text{min}/100\text{g}\cdot\text{h}$)	14.4 ± 12	17.2 ± 9.8	11.6 ± 11	0.568
RBF ($\text{mL}/\text{min}/100\text{g}\cdot\text{h}$)	226 ± 55	215 ± 69	172 ± 51	0.262
Urine flow (mL/h)	536 ± 221	410 ± 189	309 ± 189	0.394

Values are mean \pm SD area under the curve (AUC) for each parameter vs time. (Kruskal-Wallis test with post test).

SCr, serum creatinine; CrCl, Creatinine clearance; RBF, renal blood flow.

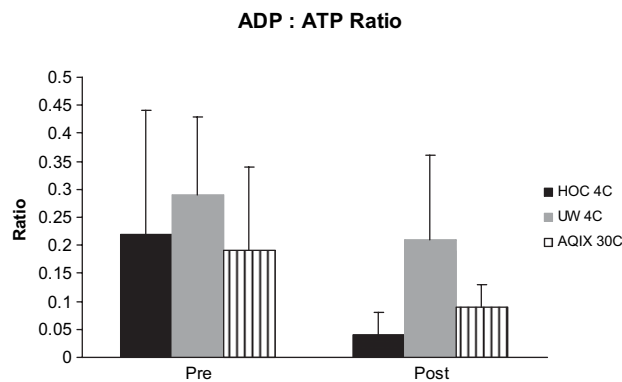


FIG. 2. Renal cortical ADP: ATP Ratios pre-perfusion and 6 h post-isolated perfusion. Values are mean \pm SEM.

known to have anatomical and physiologic characteristics that more closely resemble the human situation than small animal models [11]. The isolated organ perfusion system was designed using state-of-the-art cardiopulmonary bypass technology, and was successful in yielding reproducible results within each of the three study groups. Whilst this appears to be a valid model for studying post-ischemic renal injury and has been used to assess the effects of warm and cold ischemic injury relevant to the transplant setting [12], the system does not reproduce exact normal physiologic conditions. Kidneys were perfused by nonpulsatile flow at a relatively low mean perfusion pressure. Another limitation of the study is that the kidneys were subjected to quite short periods of warm and cold ischemia prior to reperfusion with blood. These ischemic times are

clinically relevant as they are similar to those to which human live donor kidneys are exposed, but future studies should include longer cold ischemic times, which reflect the clinical deceased donor transplant situation. Extended warm times could also be added to the model in order to study the effects of initial warm flushing in deceased donor kidneys.

AQIX was designed as a universal organ preservation and perfusate solution that would reflect physiologic serum ionic concentrations, osmolarity, and ion conductivity, thereby maintaining the ionized status of the cell membrane and the function of enzymatic and receptor moieties under *ex vivo* conditions. Acid-base homeostasis is a key function of the kidney that is necessary for the maintenance of optimum enzyme function. In this study, the HOC and UW flushed groups developed profound acidosis in comparison with the warm-flushed AQIX group, which maintained an almost normal pH throughout the 6 h perfusion period. AQIX utilizes the $\text{NaHCO}_3/\text{pCO}_2$ buffer system in combination with Good's buffer BES rather than the traditional phosphate-buffered solutions, which are known to inhibit glycolysis and oxidative phosphorylation [13]. Inorganic phosphate has also been associated with the release of reactive oxygen species [14]. BES maintains a stable pH and has been shown to give superior metabolic effects in hypothermic preservation of porcine hearts with higher ATP levels, ATP:ADP ratios, and conservation of phosphofructokinase compared with other buffering agents when included in a modified UW solution [15].

TABLE 4

(A) Pre-Perfusion Biopsy Scores; (B) Post-Perfusion Biopsy Scores

(A) Pre-perfusion biopsy scores	HOC	UW	AQIX	P Value
Tubular dilation	2 \pm 0.4	0 \pm 0.41	1 \pm 0.82	0.05
Epithelial flattening	2 \pm 0.8	0 \pm 0.52	0 \pm 0.52	0.001
Epithelial shredding	1 \pm 0.0	1 \pm 0.0	1 \pm 0.41	Ns
Tubular debris	1 \pm 0.0	0 \pm 0.5	0 \pm 0.52	0.05
Vacuolation	0 \pm 0.0	0 \pm 0.0	0 \pm 0	Ns
Condensed tubular nuclei	0 \pm 0.5	0 \pm 0.5	0 \pm 0	Ns
RBC presence	0 \pm 0.0	0 \pm 0.4	0 \pm 0	Ns
Glomerular shrinkage	1 \pm 0.4	0 \pm 0.0	0 \pm 0.82	Ns
(B) Post-perfusion biopsy scores	HOC	UW	AQIX	P Value
Tubular dilation	2 \pm 0.0	3 \pm 0.5	3 \pm 0.0	Ns
Epithelial flattening	2 \pm 0.75	2 \pm 0.55	3 \pm 0.5	Ns
Epithelial shredding	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	Ns
Tubular debris	1 \pm 0.0	1 \pm 0.5	1 \pm 0.55	Ns
Vacuolation	0 \pm 0.5	0 \pm 0.4	0 \pm 0.4	Ns
Condensed tubular nuclei	1 \pm 0.8	1 \pm 0.6	1 \pm 0.4	Ns
RBC presence	1 \pm 0.55	1 \pm 0.55	0 \pm 0.4	Ns
Glomerular shrinkage	1 \pm 0.0	0 \pm 0.5	2 \pm 0.6	0.05

Biopsies were scored over five fields, assessing the morphologic changes; score 0 = normal, 1 = mild damage, 2 = moderate damage, 3 = severe damage. The Kruskal-Wallis test with post test was used to compare the groups (HOC, UW, and AQIX). Values are mean \pm SD.

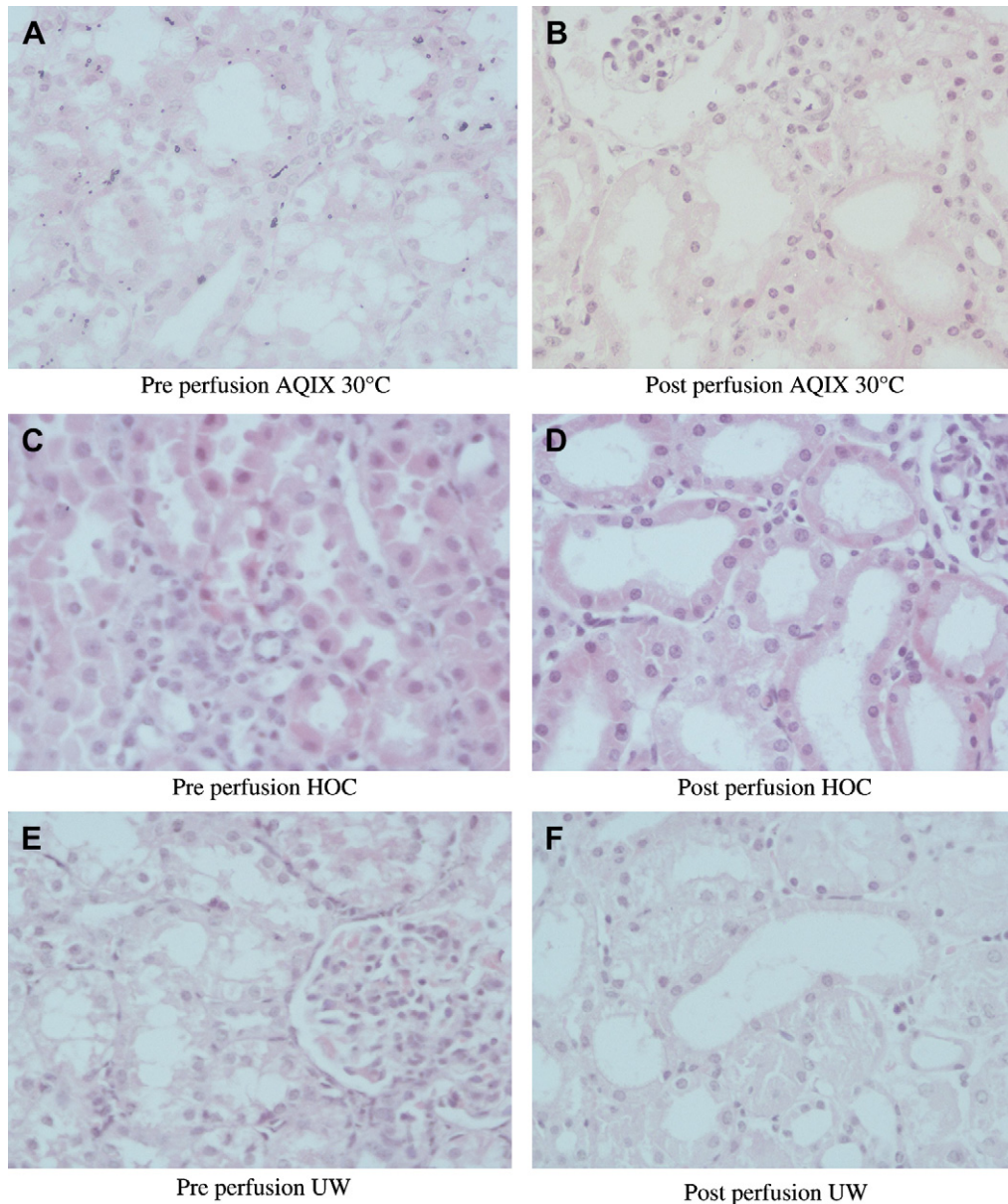


FIG. 3. Pre- and post-perfusion biopsies in the AQIX, HOC, and UW groups. Nikon Light-microscope magnification $\times 40$.

The AQIX group displayed a significantly lower percentage weight gain compared with the UW group. Weight gain is an important index of the cellular edema, which develops during anaerobic cold storage and leads to functional impairment. The degree of cellular swelling is related to the length of storage and the composition of the preservation solution used [16–18], and relationships between cell swelling, histologic changes, and cell death have also been observed [19, 20].

In metabolically active cells, ATP levels are maintained constantly, but ischemic cell death is associated with severe ATP depletion and the ratio of ADP:ATP can therefore be used as an indicator of cell viability.

ATP depletion is also important as the breakdown products of its degradation, such as hypoxanthine, result in free radical production, and subsequent reperfusion injury [21]. Despite reducing cellular metabolism, hypothermic organ preservation still results in depletion of ATP levels and a corresponding increase in ADP levels. This was apparent in our study, where the ADP:ATP ratios were relatively high after only 2 h of cold storage. Following normothermic reperfusion, this ratio decreased in all three groups suggesting good recovery of cellular function. This effect was more marked in the HOC and AQIX groups compared to UW. Some authors have assessed mechanisms of reducing ATP depletion by the use of

a period of re-warming during storage [21, 22], or oxygen persufflation [23]. Others have modified the preservation solution with substrates that promote ATP regeneration, such as inorganic phosphate [24] and histidine-lactobionate-raffinose-based solution [25]. It is clear from the results presented here that a period of normothermic machine preservation using autologous blood has potential for the resuscitation of high energy phosphate metabolites in ischemically damaged kidneys [26].

The use of an initial warm flush prior to cold preservation has previously shown improved survival of rat livers flushed with warm UW compared with cold UW, possibly because warm UW has a significantly lower viscosity [27]. Other authors have demonstrated improved function by combining a warm pre-flush with streptokinase in rat donation after cardiac death (DCD) donor livers and kidneys [28, 29] rather than warm pre-flush alone.

The three strategies used in this study all have potential advantages and disadvantages. HOC solution has a low viscosity, which would facilitate flushing, but may be less cytoprotective than UW solution. UW is much more viscous and so flows more slowly, but is the gold-standard hypothermic preservation solution for a number of organs. Warm AQIX flushes kidneys at the quickest rate as a result of its low viscosity and its use at 30°C, but this temperature increases the first warm ischemic time. The comparable post-ischemic renal function in the three groups suggests that these positive and negative influences have balanced each other out to some extent. Further studies are needed to assess the influence of prolonged cold ischemic storage to mimic the clinical situation.

The findings of this study suggest that an initial warm flush with AQIX solution clears the renal microcirculation more effectively than cold flushing with conventional preservation solutions. An initial warm flush is a new paradigm in organ preservation, and part of an overall goal of complete normothermic preservation, which removes the deleterious effects of cold preservation, allows organ resuscitation, and assessment of function prior to transplantation. Further work is required before this technique can be considered for introduction to clinical practice.

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