

ORIGINAL ARTICLE

Static normothermic preservation of renal allografts using a novel nonphosphate buffered preservation solution

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Summary

The aim of this study was to assess the viability and function of renal allografts under normothermic conditions using a novel nonphosphate buffered preservation solution AQIX®RS-I. Porcine kidneys were flushed at 30 °C with AQIX®RS-I at 100 mmHg pressure after 5–10 min warm ischaemic time and stored statically at either 4 °C or 30 °C for 2 h ($n = 6$ per group). Assessment of renal function by physiological and biochemical parameters was performed by perfusing the organs with autologous blood at 37 °C, with an initial circulating serum creatinine concentration of 1000 $\mu\text{mol/l}$ on an isolated organ perfusion system for 6 h. Although the hypothermic group demonstrated overall superior renal function, the normothermic stored kidneys displayed a statistically comparable acid-base balance (7.37 ± 0.15 vs. 7.3 ± 0.09 , $P = 0.24$). Furthermore, renal function was still evident after 6 h perfusion with increasing oxygen consumption, renal blood flow and reduced renal vascular resistance. The effectiveness and versatility of AQIX®RS-I as a preservation solution under both normothermic and hypothermic conditions has been demonstrated. Renal viability was maintained after 2 h static normothermic storage. This study provides a foundation for further analysis utilizing normothermic preservation.

Objective

Throughout the UK, hypothermic static storage of organs prior to reperfusion and transplantation is the preferred method of preservation. The rationale behind this is the simplicity, cost-effectiveness, portability and proven good allograft results using such methods [1,2]. Whilst the cold environment slows metabolism, anaerobic metabolism continues to occur, albeit at a slower rate, the end-products of which contribute to the generation of oxygen free radicals and hence ischaemia-reperfusion injury. It is known that cellular oedema and functional impairment develop during anaerobic cold storage, the extent of which is related to the length of storage and the composition of the preservation solution used [3–5]. Cold storage does not allow assessment of viability markers as it renders metabolic studies difficult to interpret. Thus some

marginal organs may be discarded unnecessarily, or transplanted and have primary nonfunction.

The expanding mismatch between supply and demand for renal allografts has prompted development of preservation techniques to try and help expand the donor pool and improve allograft function. Normothermic preservation is not a new concept but has seen a recent revival in renewed interest [6–8]. The potential beneficial roles include maintaining an organ at physiological conditions thus avoiding the harmful effects of an anaerobic environment, viability assessment allowing use of marginal organs and possible *ex vivo* manipulation.

The aim of this study was to assess the viability and function of renal allografts under normothermic conditions using a novel nonphosphate buffered normothermic preservation solution AQIX®RS-I (Res-Del International Ltd., London, UK).

Table 1. Composition of AQIX.

Sodium	110
Potassium	5.0
Magnesium	0.45
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 (co-carboxylase)	40.0 nmol/l
Insulin	28.0 mIU

All units in mmol/l unless specified.

AQIX solution

AQIX is a novel nonphosphate buffered preservation solution that has been designed for use with any organ at any temperature. It utilizes $\text{NaHCO}_3/\text{pCO}_2$ as its buffer in combination with Good's buffer Zwitterionic buffer (BES). The osmolarity is 286 mOsmol/l, similar to serum and its ionic concentrations have been chosen to maintain it as an isosmotic solution. The ionic conductivity of 12.6 mS/cm is comparable with human serum. The composition of AQIX is displayed in Table 1.

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 and warm flushed at 30 °C with 250 ml of AQIX@RS-I at a hydrostatic pressure of 100 cmH_2O after 5–10 min warm ischaemic time. Flush times were measured using a stopwatch and both warm ischaemic time and flush time were recorded. Perfusate flow rates were calculated and expressed as millilitre per minute per 100 g of kidney. The kidneys were then stored statically in AQIX solution either on ice at 4 °C or 30 °C for 2 h ($n = 6$ per group) during transportation back to the laboratory. The AQIX solution was prewarmed in a temperature regulated water-bath prior to flushing and maintained at 30 °C during transport in an insulated organ retrieval carrier with warmed solution bags. The temperature was monitored with a temperature probe.

Experimental protocol

The renal artery, vein and ureter were dissected and cannulated. The kidneys were weighed using a balance accurate to 1 g, both immediately pre- and postperfusion and weight change recorded. Kidneys were then perfused with autologous blood at 37–39 °C, on an isolated organ perfusion system for 6 h as an *ex vivo* method of transplantation. Creatinine was added to the system giving an initial circulating serum concentration of 1000 $\mu\text{mol/l}$ allowing an accurate measurement of renal function to be made.

The kidneys were perfused at a set mean arterial pressure (MAP) with renal blood flow (RBF), and MAP being continuously recorded and intra-renal vascular resistance calculated (MAP/RBF). Serum and urine samples were taken hourly for biochemical analysis and whole blood for haematological analysis. Creatinine clearance ($U_{\text{cr}} \times U \text{ volume}/P_{\text{cr}}$) ((urinary creatinine \times urinary volume)/plasma creatinine) and fractional excretion of sodium ($(U_{\text{t}} \times U \text{ flow})/(GFR \times P_{\text{t}}) \times 100$) were calculated and serum creatinine levels recorded hourly. Blood gases were measured for the calculation of oxygen consumption in millilitre per minute per gram [($\text{PaO}_2 \text{ art} - \text{PaO}_2 \text{ ven}$) \times (flow rate/weight)]. pH measurements were taken from arterial samples prior to perfusing the kidneys, then at 1, 3 and 6 hourly intervals for assessment of acid-base homeostasis. Statistical analysis was carried out using the Mann–Whitney test with a P -value of <0.05 taken as significant.

Needle core biopsies were taken preperfusion and 6 h postperfusion to assess the ADP:ATP ratio as a marker of cellular viability. This was performed using the adenylate nucleotide ratio assay kit (Cambrex Bio Science, Rockland, ME, USA), whose detection is based upon the bioluminescent measurement of ATP using the enzyme luciferase. This enzyme catalyses the formation of light from ATP and is measured using a luminometer.

Needle core biopsies were attached to cork blocks using tissue-tek, immediately submersed 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 precooled to -20 °C, and 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 microlitre 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 was noted on a chart recorder (2210 LKB, Kipp, Zonen, Delft, Holland) and a second reading 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 was taken after 5 min.

Isolated organ perfusion

The isolated organ perfusion system (IOPS) was designed using commercially available clinical grade cardiopulmonary technology (Medtronic, Watford, UK) and consisted of a centrifugal blood pump (550 Bio-pump, Medtronic, Watford, UK), a heat exchanger (Grant, GD120, Cambridge, UK), a 5-l venous reservoir (Medtronic) and a minimax plus membrane oxygenator (Medtronic). The circuit hardware included a speed controller, a TX50P flow transducer, a pressure transducer and a temperature probe (Cole-Parmer, London, UK). Two PC-2 Gemini infusion pumps (Alaris, Basingstoke, UK) and a urinometer (Bard, Crawley, UK) were incorporated into the system.

The circuit was primed with 500 ml of Ringer's solution containing mannitol 10 mg (Baxter, Healthcare, Norfolk, UK), dexamethasone 10 mg (Organon Labs Ltd, Cambridge, UK), cefuroxime 750 mg (Britannia Pharmaceuticals Ltd, Surrey, UK) and 12 ml of sodium bicarbonate solution (8.4%; Fresenius Kabi, Warrington, UK). Five hundred millilitre 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 insulin (Actrapid; Novo Nordisk, Denmark, UK) and 25 ml of sodium bicarbonate solution (8.4%; 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.

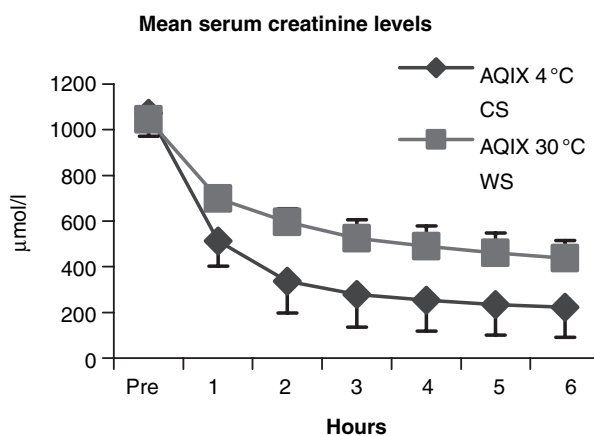


Figure 1 Mean serum creatinine levels.

Results

The warm flushed/hypothermically stored group demonstrated overall superior renal function, with significantly better oxygen consumption ($P = 0.0015$), RBF ($P = 0.0043$), percentage creatinine fall ($P = 0.0043$; Fig. 1), and lower renal vascular resistance ($P = 0.015$) after 6 h perfusion (Table 2). Total urine output was greatest in the cold stored group (692 ± 230 ml vs. 257 ± 118 ml, $P = 0.0043$) and percentage weight gain lower (12 ± 9 ml vs. $30.3 \pm 9\%$, $P = 0.017$). However the normothermally stored group displayed a statistically comparable acid-base balance (pH of 7.37 cold stored vs. 7.3 warm stored, $P = 0.24$) after 6 h of perfusion. Furthermore, renal function was still evident at 6 h perfusion despite a warm time of over 2 h. Whilst not reaching significance, both groups displayed improvement during the 6 h perfusion period with increasing oxygen consumption ($P = 0.23$ at 4 °C, $P = 0.34$ at 30 °C) RBF ($P = 0.26$ at 4 °C, $P = 0.47$ at 30 °C) and reduced renal vascular resistance ($P = 0.57$ at 4 °C, $P = 0.9$ at 30 °C; Table 2).

The ADP:ATP ratio levels were highest in the pre-perfusion biopsies, reflecting the ischaemic damage sustained during the storage period (Fig. 2). There was no significant difference in the preperfusion ADP:ATP ratio

Table 2. Functional results. Values are mean \pm SD.

Functional parameters	AQIX 4 °C storage		AQIX 30 °C storage	
	1 h	6 h	1 h	6 h
pH	7.4 \pm 0.03	7.37 \pm 0.15	7.37 \pm 0.03	7.3 \pm 0.09
Bicarbonate, mmol/l	22.7 \pm 1.6	21.8 \pm 6.8	20 \pm 1.1	17.6 \pm 4.2
O ₂ consumption, ml/min/g	37.3 \pm 9.6	47.3 \pm 12.1	23.8 \pm 5.2	28.7 \pm 6.5
% creatinine fall	52 \pm 11.1	84 \pm 10	33 \pm 4.3	58 \pm 7.9
GFR, ml/min/100 g	6 \pm 4.7	1.1 \pm 0.9	0.9 \pm 0.7	0.5 \pm 0.29
RBF, ml/min/100 g	63.6 \pm 15.1	79.3 \pm 179	40.3 \pm 7.3	48 \pm 11.3
RVR, mmHg/ml/min	0.5 \pm 0.15	0.4 \pm 0.09	0.84 \pm 0.32	0.73 \pm 0.26

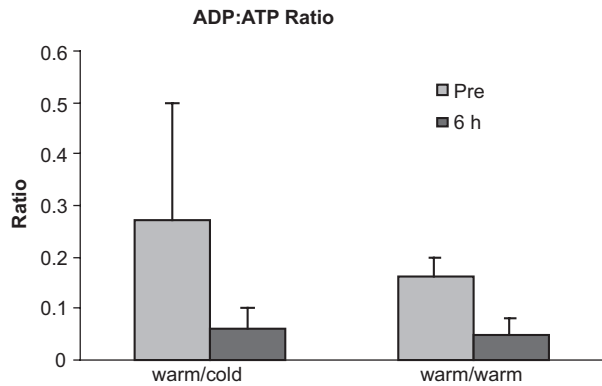


Figure 2 ADP:ATP ratio.

($P = 0.54$) between the two groups. After 6 h perfusion, the ratio had improved in both groups showing recovery of cellular function, with no significant difference observed between the groups ($P = 0.71$).

Discussion

This study has revealed promising results for AQIX®RS-I as a preservation solution. Renal allografts functioned well after a warm flush and hypothermic storage. The rationale behind the warm flush is that it should in theory achieve a more rapid flush of erythrocytes from the microcirculation without causing vasoconstriction. Without the vasoconstrictive effect, it is likely that there is a better clearance from the microcirculation. The initial flush is important to remove blood, cellular components that may have clumped, and perfuse the microcirculation with a composition that is designed to impair cellular swelling. Reduced hepato-cellular damage has been demonstrated in warm-flushed rat livers [9] and improved microperfusion in liver grafts by combining a warm flush with streptokinase [10].

Most encouraging was the maintenance of acid-base homeostasis, a key function of the kidney and necessary for the maintenance of conditions conducive for enzyme action. pH was normal in the cold stored group after 6 h assessment and near normal in the warm stored group, which had had 2 hours of un-impaired anaerobic metabolism. Furthermore, the recovery of cellular function as demonstrated by the improvement in the ADP:ATP ratio highlights the potential of AQIX as a preservation solution.

Porcine kidneys that were of similar weight to adult human kidneys were used in this study, as they are known to have anatomical and physiological characteristics that more closely resemble the human situation than small animal models [11]. The isolated organ perfusion system was successful in yielding reproducible results

making it a valid model for studying post-ischaemic renal injury as previously demonstrated in other papers [12,13].

There are limitations of the study such that the kidneys were subjected to quite short periods of warm and cold ischaemia prior to reperfusion with blood. Future studies should include longer cold ischaemic times, which reflect the clinical transplant situation. Extended warm times could also be added to the model in order to study the effects of initial warm flushing in nonheart-beating kidneys. Future studies will also compare AQIX with other commonly used solutions such as University of Wisconsin and hyperosmolar citrate solution. The IOPS system was reliable, but arguably does not reproduce normal physiological conditions such as that of an auto-transplant model.

Whilst overall those kidneys stored normothermically did not function as well as hypothermic storage, renal viability was maintained after 6 h perfusion. To fully assess the potential benefit of normothermic preservation, warm machine perfusion during transport back to the laboratory prior to viability assessment should be utilized. The logistics of this are somewhat complex, making a period of cold preservation a likely prerequisite prior to viability assessment. The above results are encouraging both for the use of a warm flush and of AQIX® RS-I as a preservation solution.

References

1. Stubenitsky BM, Booster MH, Nderstigt AP, Kievit JK, Jacobs RW, Kootstra G. Kidney preservation in the next millennium. *Transpl Int* 1999; **12**: 83.
2. UK Transplant. *Transplant Update*. UK: www.uktransplant.org.uk, 2005.
3. Van Wagenveld BA, Reinders ME, van Gulik TM, Gelderblom HC, Frederiks WM, Wanders WM. Warm flush at 37 °C following cold storage attenuates reperfusion injury in preserved rat livers. *Transpl Int* 1998; **11**: 38.
4. 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.
5. Lindell S, Ametani M, Belzer FO, Southard JH. Hypothermic perfusion of rabbit livers: effect of perfusate composition (Ca and Lactobionate) on enzyme release and tissue swelling. *Cryobiology* 1989; **26**: 407.
6. Askenasy N, Navon G. Intracellular volumes and membrane permeability in rat hearts during prolonged hypothermic preservation with St Thomas and University of Wisconsin solutions. *J Mol Cell Cardiol* 1998; **30**: 1329.
7. Sundberg R, Ar'rajab A, Ahren B, Bengmark S. The functional effects of suppression of hypothermia-induced cell

- swelling in liver preservation by cold storage. *Cryobiology* 1991; **28**: 150.
8. Imber CJ, St Peter SD, Lopez de Cenarruzabeitia I, Pigott D, James T, Taylor R. Advantages of Normothermic Perfusion over cold storage in liver preservation. *Transplantation* 2002; **73**: 701.
 9. Stubenitsky BM, Booster MH, Nederstigt AP, Kievit JK, Jacobs RW, Kootstra G. Kidney preservation in the next millennium. *Transpl Int* 1999; **12**: 83.
 10. McLaren AJ, Friend PJ. Trends in Organ Preservation. *Transpl Int* 2003; **16**: 701.
 11. Sachs DH. The pig as a potential xenograft donor. *Vet Immunopathol* 1994; **43**: 185.
 12. Harper SJF, Hosgood S, Kay MD, Nicholson ML. Leucocyte depletion improves renal function during reperfusion using an experimental isolated haemoperfused organ preservation system. *Br J Surg* 2006; **93**: 623.
 13. Hosgood S, Harper SJF, Kay MD, Bagul A, Waller H, Nicholson ML. Effects of arterial pressure in an experimental isolated haemoperfused porcine kidney preservation system. *Br J Surg* 2006; **93**: 879.