

Audit Trial Report I: AQIX® RS-I solution

Comparative study with conventional hypothermic preservation solutions

Aim

Our aim was to explore kidney preservation techniques under normothermic conditions using AQIX ® RS-I solution. Renal viability was then assessed by measuring functional parameters when reperfused with oxygenated autologous blood with a circulating creatinine concentration of 1000µmmol/L at 37°C on an isolated organ perfusion system (IOPS).

Retrieval

Large white pigs (60 – 70kg) were sacrificed by electrocution followed by exsanguination and the blood collected into a sterile container containing 25,000 units of heparin (Multiparin ; CP Pharmaceuticals, Wrexham, UK). The kidneys were surgically removed and immediately flushed with 400ml of RS-I solution at 100cm hydrostatic pressure at 30°C then transported on ice or at 30°C to the laboratory. After minimal storage time, the renal artery and vein were dissected and cannulated with appropriated sized renal cannula and the ureter cannulated with a 10fr urinary catheter (Pennine, Derby, UK). Kidneys were then placed on the Isolated Organ Perfusion System (IOPS) for a period of 6 hours and renal function assessed.

Circuit

The Isolated Organ Perfusion Systems (IOPS) consisted of a pulmonary by-pass system, (Medtronic, Watford, UK), incorporating a centrifugal blood pump (550 Bio-pump), speed controller, TX50P flow transducer and pressure transducer. A heat exchanger (Grant, GD120, Cambridge, UK), temperature probe (Cole-parmer, London, UK) and two PC- 2 Gemini infusion pumps (Alaris, Basingstoke, UK) were added to the system. The disposable circuit consisted of a 5L venous reservoir container (Medtronic),

polyvinylchloride tubing 1/4 and 3/16 inch (Medtronic), minimax plus membrane oxygenator (Medtronic), and a urine meter (Bard, Crawley, UK) (Fig 1).

The circuit was primed with a solution containing Ringer solution 500ml, mannitol 10mg (Baxter, healthcare, Norfolk, UK), dexamethasone 10mg, (Organon labs Ltd, Cambridge, UK) and cefuroxime 750mcg (Britannia pharmaceuticals Ltd, Surrey, UK). 15ml sodium bicarbonate 8.4% (Fresenius kabi, Warrington, UK) was added to the blood to meet physiological conditions. 500ml 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) infused at 20ml/hr to which 100 units insulin (Actrapid : Novo Nordisk, Denmark, UK) and 25ml sodium bicarbonate 8.4% (Fresenius kabi) was added. A vasodilator, Sodium Nitroprusside 25 mg, (Mayne pharma PLC, Warwickshire, UK) was administered during the first hour of reperfusion at 25ml/hr. 5% glucose solution (Baxter) was infused at 7ml/hr once the sodium nitroprusside had been discontinued. Ringers solution (Baxter) was used to replace urine output ml for ml. The perfusate was 'spiked' with creatinine (Sigma, Germany) to bring the initial circulating concentration to 1000µmol/L.

Isolated organ perfusion system (IOPS)

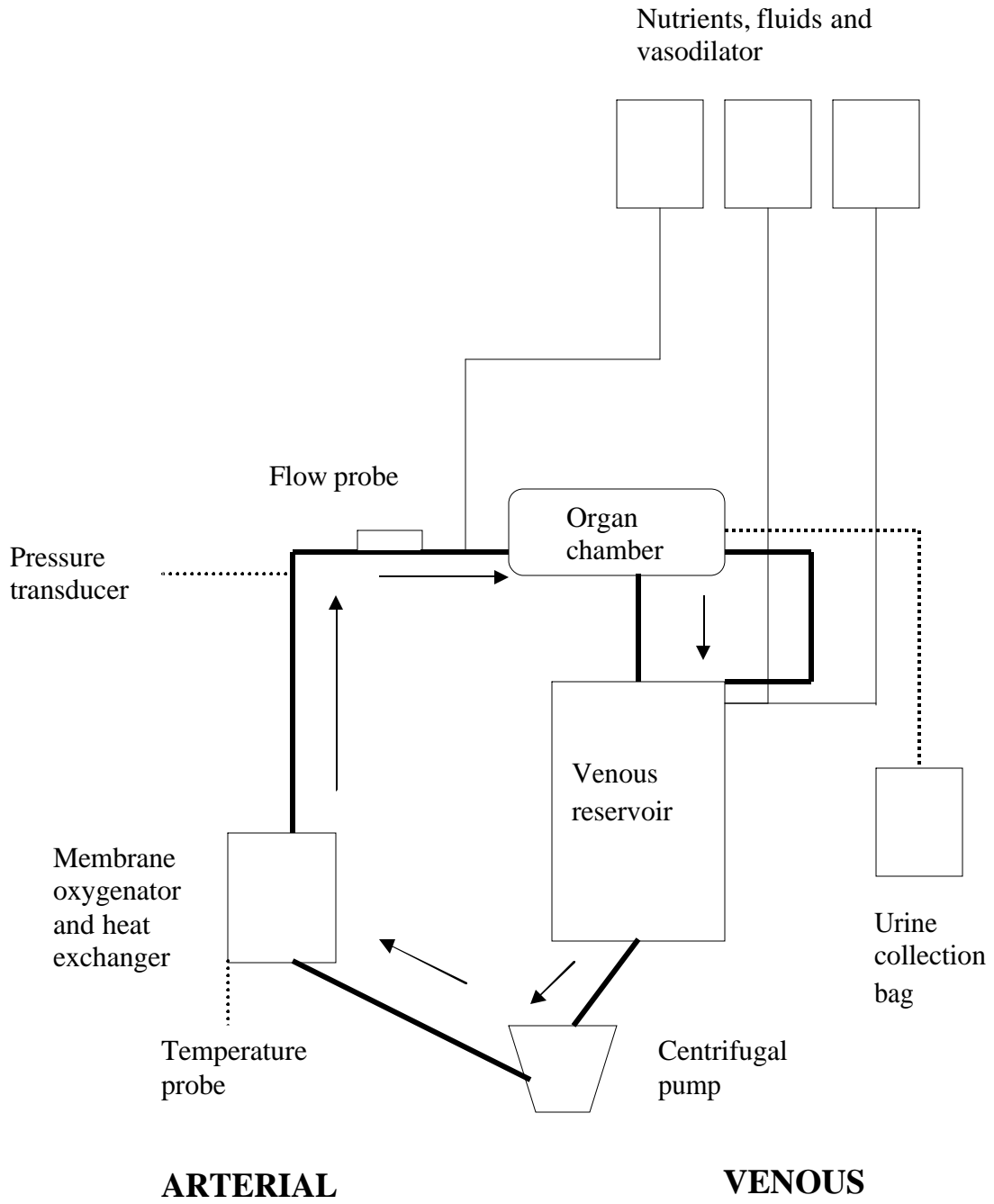


Figure 1: Diagram of the Isolated Organ Perfusion System (IOPS) outlining the direction of blood flow.

Experiments

Initially 6 experiments were carried out using RS-I solution under different conditions. The perfusion pressure was altered during normothermic assessment to determine optimal perfusion of the kidney after different storage conditions 4°C and 30°C.

12 kidneys were then assessed using RS-I under two different conditions.

- A) 30°C flush with RS-I solution then stored on ice at 4°C during transportation for approximately 2 hours (n=6).
- B) 30°C flush with RS-I solution then stored at 30°C during transportation for approximately 2 hours (n=6).

The 2 groups were then compared with two control groups, using a standard cold preservation solution, hyperosmolar citrate solution (Soltran®) and phosphate buffered UW® solution, infused and stored at 4°C under the same protocol (n=6).

Statistical analysis was carried out using the Kruskal-Wallis test all values are the mean ± SD.

Functional parameters

The renal blood flow, pressure and resistance ($R = P / F$) were continually monitored and recorded. Biochemistry analysis was carried out on arterial blood and urine samples hourly and the following metabolic and functional measurements calculated from the values. Creatinine clearance: estimated GFR, ($U_{cr} \times U \text{ flow} / P_{cr}$).

Area under the creatinine curve

Oxygen consumption ml/min/100g { $(PaO_2 \text{ art} - PaO_2 \text{ ven})$ } x flow rate/weight,

Fractional excretion of sodium: (t) referring to the substance.

$FE = (U_t \times U \text{ flow}) / (GFR \times P_t) \times 100.$

Results

The warm ischaemic time was kept to a minimum with no significant difference between the groups. Flushing the kidneys with RS-I at 30°C significantly improved the rate at which blood was flushed from the kidneys immediately after retrieval (Table 1).

Parameters	AQIX ®RS-I 4°C storage (n = 6)	AQIX ® RS-I 30°C storage (n = 6)	Soltran flush 4°C storage (n = 6)	UW Flush 4°C storage (n=6)	P value
WIT	6.3 ± 0.58	6.2 ± 0.26	7.1 ± 0.97	6.2 ± 1.47	0.2109
Flush ml/min/100g	20.6 ± 2.26	26.1 ± 2.9	10 ± 1.63	4.9 ± 1.63	0.0001
Arterial pressure mmHg	74.1 ± 1.44	68.2 ± 4.99	54 ± 0.71	59.1 ± 2.58	0.0002
Temperature	37.5 ± 0.17	37.3 ± 0.42	37.3 ± 0.48	37 ± 0.56	0.3156

Table 1: Parameters

After 6 hours of perfusion the AQIX ® RS-I cold stored kidneys demonstrated overall improved function compared to the other 3 groups. AQIX ® RS-I normothermic stored kidneys produced less urine than the other groups however, they demonstrated comparable metabolic function to the `Soltran` and `UW` groups and showed improved handling of the acid base balance (Table 2) (figures 1-5).

Functional parameters After 6 hours perfusion	AQIX ®RS-I 4°C storage (n=6)	AQIX ®RS-I 30°C storage (n=6)	Soltran flush 4°C storage (n=6)	UW Flush 4°C storage (n=6)	P value
pH	7.37 ± 0.15	7.30 ± 0.09	7.21 ± 0.1	7.23 ± 0.12	0.1468
Bicarbonate	21.8 ± 6.83	17.6 ± 4.24	14.6 ± 3.08	15.3 ± 4.26	0.154
Base excess	-4.7 ± 9.16	-10 ± 5.9	-9.7 ± 5.90	-13.5 ± 6.4	0.248
Serum K ⁺ levels	5.83 ± 0.34	8.01 ± 1.22	7.73 ± 1.21	8.23 ± 1.27	0.003
O ₂ consumption ml/min/g	47.3 ± 12.11	28.7 ± 6.53	31 ± 6.26	33.7 ± 15.1	0.059
% weight gain	12.7 ± 9	30.3 ± 9.3	21.2 ± 7.7	29.7 ± 3.44	0.0109
Total urine output ml	692 ± 230	257 ± 118	536 ± 221	410 ± 153	0.0103
RBF ml/min/100g	79.3 ± 17.89	48 ± 11.28	50 ± 10.16	55.5 ± 21.9	0.0214
RVR mmHg/ml/min	0.4 ± 0.09	0.73 ± 0.26	0.52 ± 0.09	0.8 ± 0.43	0.0173

Table 2: Functional parameters after 6 hours of perfusion.

Oxygen consumption

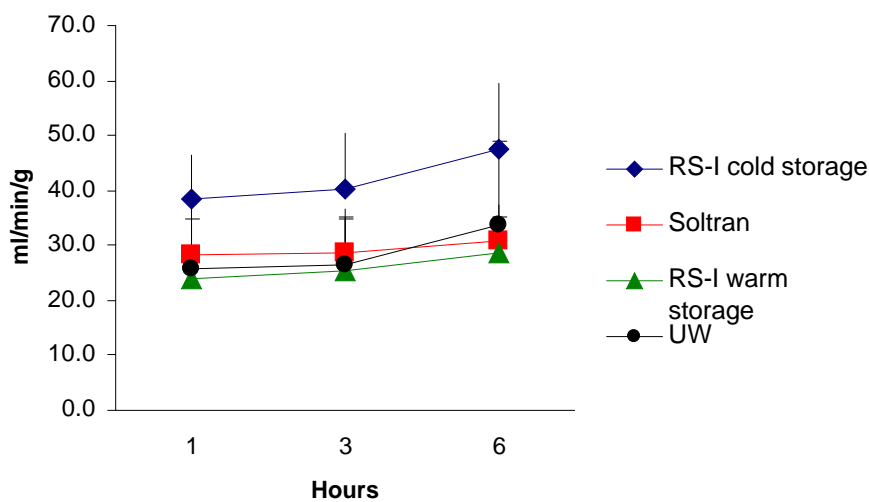


Figure 1: Oxygen consumption over 6 hours of perfusion.

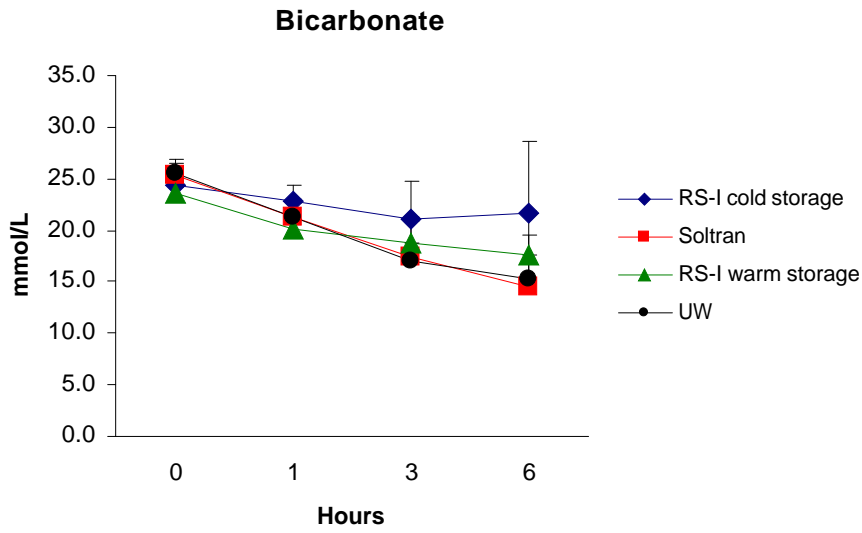


Figure 2: Bicarbonate levels over 6 hours of perfusion

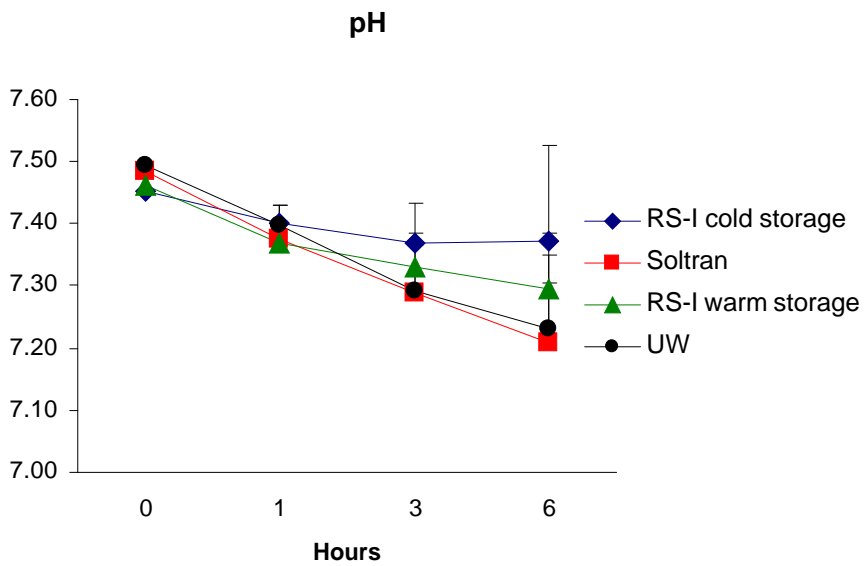


Figure 3: pH levels over 6 hours of perfusion.

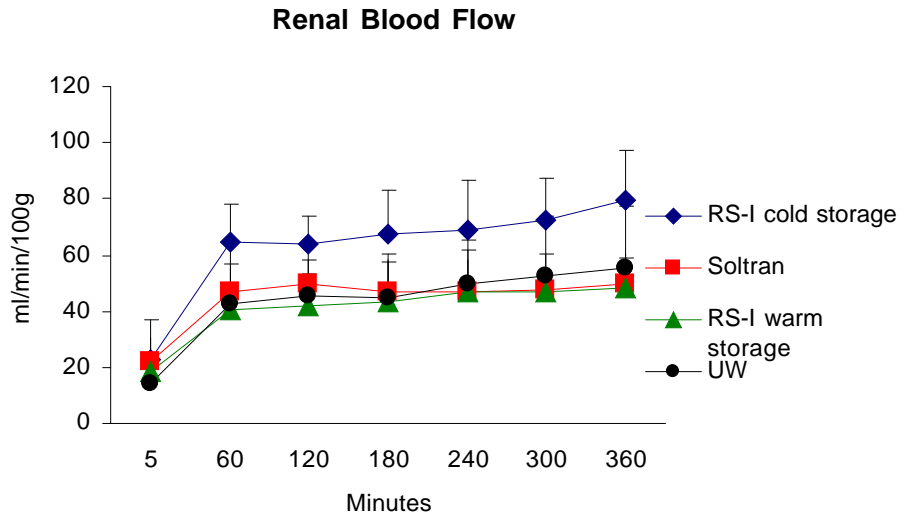


Figure 4: Renal blood flow ml/min/100g over 6 hours.

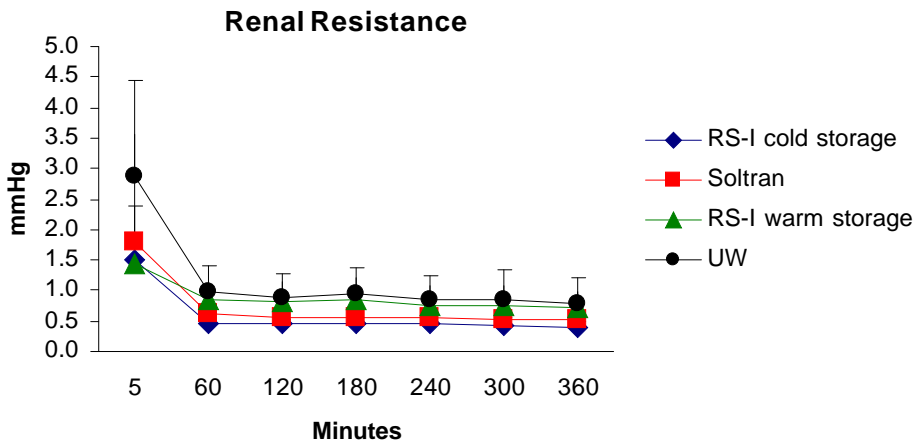


Figure 5: Renal vascular resistance over 6 hours of perfusion.

Serum creatinine levels fell the greatest in AQIX® RS-I cold stored kidneys and AQIX® RS-I normothermic stored kidneys fell the least. Final serum creatinine levels in the `UW` and `Soltran` groups were comparable. Creatinine clearance was low in all groups but again, group AQIX® RS-I cold stored kidneys demonstrated improved clearance

during the first 2 hours of perfusion before falling to a similar level as the 'UW' and 'Soltran' groups . (Table 3), (Figures 7 and 8)

Functional parameters After 6 hours perfusion	AQIX ®RS-I 4°C storage (n=6)	AQIX ®RS-I 30°C storage (n=6)	Soltran flush 4°C storage (n=6)	UW Flush 4°C storage (n=6)	P value
Serum creatinine levels µmol/L	173 ± 105	437 ± 88.4	237 ± 112	278 ± 151	0.0171
% Creatinine fall	84 ± 10	58 ± 7.9	76 ± 13.9	73 ± 14	0.0253
Area under the creatinine curve	2028 ± 548	3509 ± 379	2404 ± 595	2887 ± 865	0.0114
CrClml/min/100g	1.1 ± 0.9	0.5 ± 0.29	0.9 ± 0.31	1.5 ± 0.8	0.0172

Table 3: Functional parameters after 6 hours of perfusion

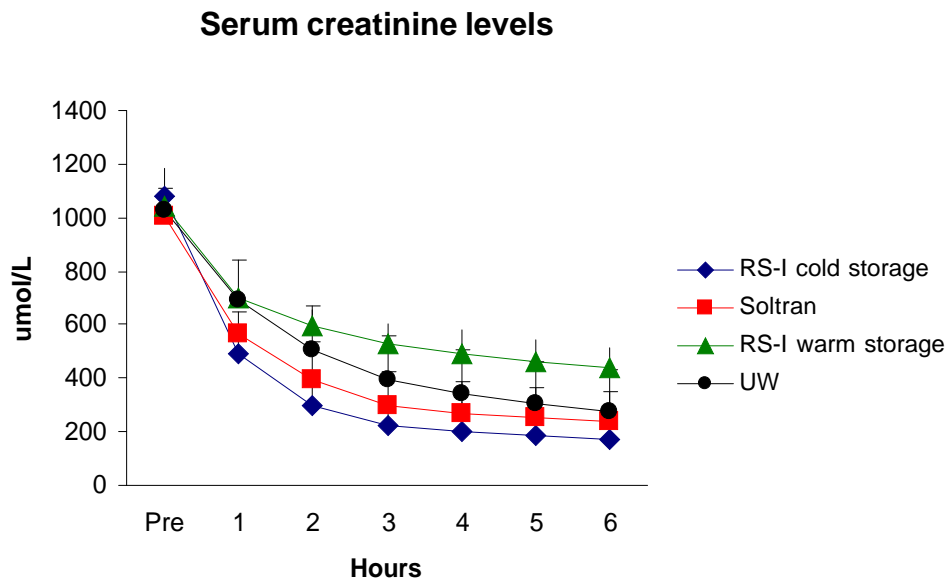


Figure 6: Serum creatinine levels over 6 hours of perfusion.

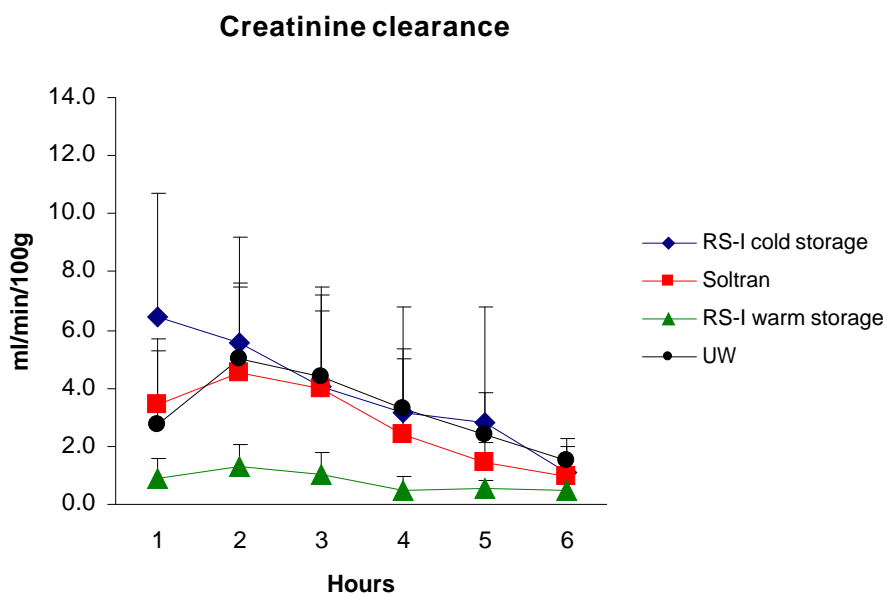


Figure 7: Creatinine clearance over 6 hours of perfusion.

Conclusion

This series of experiments demonstrates that using AQIX® RS-I as a normothermic flush followed by cold, static storage was superior to conventional cold flush using Soltran®) or UW® solutions and to normothermic storage in AQIX® RS-I. Although in general kidneys stored at 30°C in AQIX® RS-I solution did not function as well as the other groups they were comparable to the conventional hypothermic flushed and stored kidneys and they demonstrated an improved handling of renal acid base balance.

Discussion

This study provides a foundation for further analysis utilizing normothermic preservation with AQIX® RS-I. Further studies would aim to improve the normothermic conditions by machine perfusion with the addition of oxygen and red blood cells, potentially extending the storage period to that which is clinically applicable.

The versatility of AQIX® RS-I as a preservation solution has been demonstrated in this study, however, we are unsure of the effects of normothermically flushing the kidneys immediately after retrieval. Clearly using AQIX® RS-I solution in this way then cooling the kidney had no detrimental effects but we have not established the significance of this.

We propose to carry out a further group of experiments using AQIX® RS-I solution at 4°C [see LGH RS-I Report II & III] to provide an accurate comparison the 'Soltran' and 'UW' groups and also to establish the significance of normothermically flushing the kidneys after retrieval.

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Abstracts

Submitted to The American Transplant Society, Seattle, May 2005:

M. D. Kay, S. A. Hosgood, S. J. F. Harper, D. Rees, M. L. Nicholson. *Normothermic versus hypothermic ex vivo flush. A comparison of a novel phosphate-free preservation solution and a standard hypothermic method in renal allografts.*

Abstracts & Poster presentations: British Transplant Society, Belfast, April 2005:

M. D. Kay, S. A. Hosgood, S. J. F. Harper, D. Rees, M. L. Nicholson. *Static normothermic preservation of renal allografts using a novel non-phosphate buffered preservation solution.*

M. D. Kay, S. A. Hosgood, S. J. F. Harper, D. Rees, M. L. Nicholson. *Normothermic versus hypothermic ex vivo flush. A comparison of a novel phosphate-free preservation solution and a standard hypothermic method in renal allografts.*