

Audit Trial Report III: AQIX® RS-I solution

Assessment of a novel non-phosphate, pH buffered preservation solution, AQIX® RS-I, under hypothermic and normothermic static storage conditions.

Recent research has demonstrated favourable results with normothermic preservation, with the ability to resuscitate the organ and the elimination of the detrimental affects of hypothermic conditions.

Aim

Our aim was to investigate the use of a novel non-phosphate buffered commercially available solution, AQIX® RS-I solution, under traditional hypothermic and normothermic conditions that could be applied in clinical practice without the additional costs of current experimental normothermic conditions.

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 AQIX® RS-I. at 100cm hydrostatic pressure at 4°C then transported on ice or at 30 °C to the laboratory. After minimal storage time of 2 hours, the renal artery and vein were dissected and cannulated with appropriated sized renal cannula and the ureter cannulated. Kidneys were then placed on the Isolated Organ Perfusion System (IOPS), perfused with autologous blood at 38°C at a controlled arterial pressure for a period of 6 hours and renal function assessed.

Experiments (n=6)

Group 1: RS-I 4°C flush followed by 4°C storage

Group 2: RS-I 30°C flush followed by 4°C storage

Group 3: RS-I 30°C flush followed by 30°C storage

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 { $(P_{aO_2} \text{ art} - P_{aO_2} \text{ ven})$ } x flow rate/weight,

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

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

Potassium Clearance $(U_{K^+} \times U \text{ flow} / P_{K^+})$.

Results

There was no significant difference between the warm ischaemic time sustained by the kidneys in all three groups and no difference in the mean kidney weight. The time taken to flush the kidneys immediately after retrieval was significantly longer when AQIX® RS-I was used at 4°C, group 1. The mean arterial pressure was significantly lower in group 1 during the assessment period on the IOPS (table 1).

	Group 1	Group 2	Group 3	p Value
WIT minutes	6.3 ± 0.4	6.3 ± 0.6	6.2 ± 0.3	0.93
Flush ml/min/100g	7.5 ± 1.6	20.6 ± 2.3	26 ± 2.9	0.001
Kidney weight grams	233 ± 17.5	259 ± 9.5	224 ± 39	0.13
Mean arterial pressure (mmHg)	58 ± 1.5	74 ± 1.4	69 ± 4.2	0.002

Table 1: Parameters.

Haemodynamics.

Renal blood flow followed a similar pattern in all groups increasing sharply during the first hour with Group 2, AQIX® RS-I 30°C flush followed by 2 hours static cold storage reaching a higher level. The blood flow steadily increased in groups 2 and 3. However, the blood flow in group 1 remained a constant level after the initial rise during the first hour. Renal vascular resistance was significantly higher in group 1 throughout the perfusion period compared to the other two groups following an irregular pattern after an initial fall over the first hour. After a fall in the resistance in group 2 and 3, group 3 from a higher level than group 2 the level remain constant but group 3 was significantly higher throughout the perfusion period. The percentage of weight gained after 6 hours of perfusion was significantly lower in group 2 with groups 1 and 3 gaining a similar amount (table 2), (figures 1 and 2).

	Group 1	Group 2	Group 3	p Value
RBF ml/min/100g	22.9 ± 17.4	79 ± 17.9	48 ± 11.3	0.001
RVR mmHg	1.7 ± 1.1	0.4 ± 0.1	0.73 ± 0.3	0.008
Weight gain %	22.2 ± 5.4	12.7 ± 9	30 ± 9	0.01

Table 2: Haemodynamics after 6 hours of perfusion.

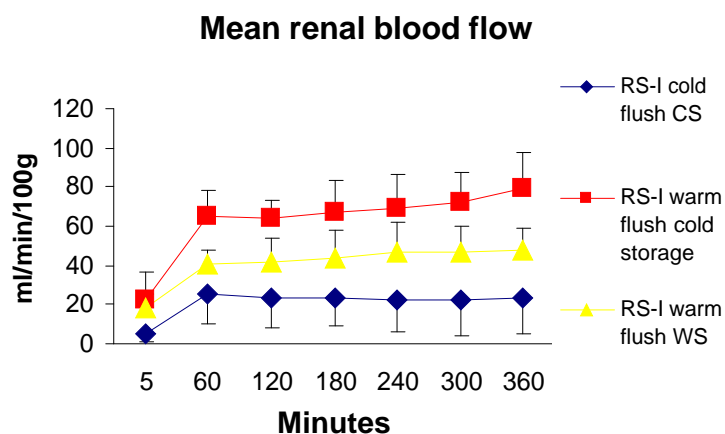


Figure 1: Mean renal blood flow over 6 hours

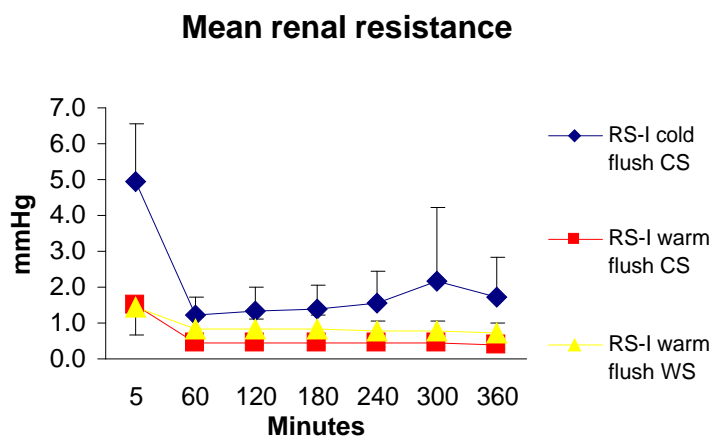


Figure 2: Mean renal vascular resistance.

Acid base balance

There were no significant differences in pH, bicarbonate levels and base excess after 6 hours between group 2 and 3, although group 2 did demonstrate more normalized

values. Group 1 showed significantly poorer handling of acid base balance in all of the parameters measured with a steady decline throughout the perfusion period compared to groups 2 and 3 (table 3) (figures 3 and 4).

	Group 1	Group 2	Group 3	p Value
pH	7.16 ± 0.1	7.37 ± 0.2	7.3 ± 0.1	0.02
Bicarbonate $\mu\text{mol/L}$	12.8 ± 2.6	21.8 ± 6.8	17.6 ± 4.2	0.03
Bass excess	-17.2 ± 4.1	-4.7 ± 9.2	-10 ± 5.9	0.02

Table 3: Acid base balance after 6 hours of perfusion.

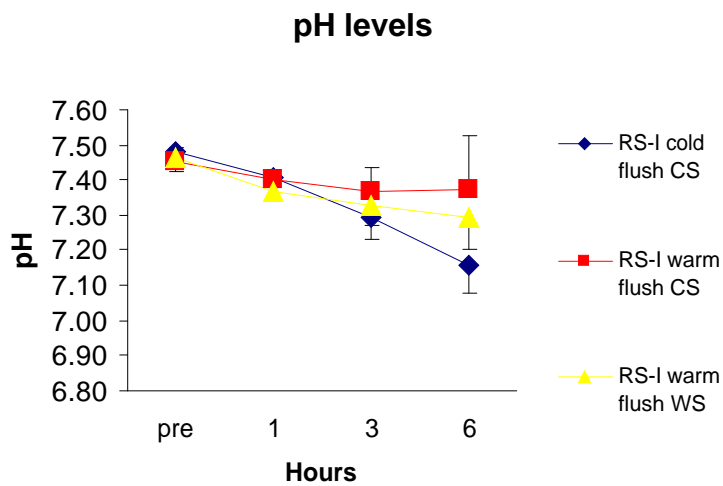


Figure 3: Mean pH levels over 6 hours

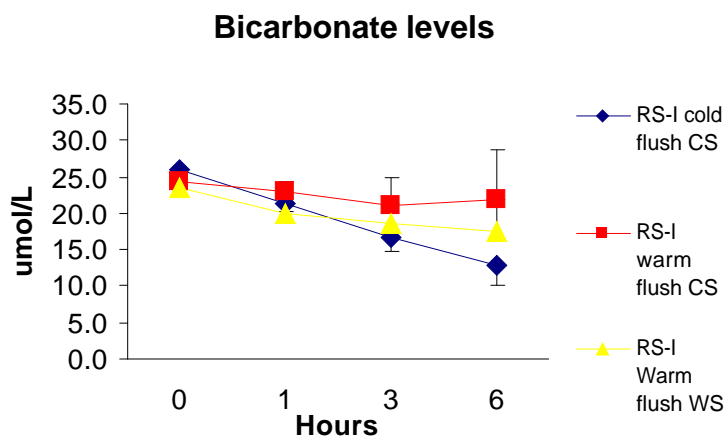


Figure 4: Mean bicarbonate levels

Functional parameters

The most dramatic fall in serum creatinine levels from 1000 μ mol/L was seen in group 2 with a drop of 84% compared to group 1 and 3, 51 and 58% respectively. This significantly improved function in group 2 was also reflected in creatinine clearance, area under the creatinine curve and urine output compared to groups 1 and 3 which performed similarly (table 4), (figures 5 and 6).

At 6 hrs	Group 1	Group 2	Group 3	p Value
Cr μmol/L	493 \pm 186	173 \pm 105	437 \pm 79	0.01
% Cr fall	51 \pm 16	84 \pm 10	58 \pm 8	0.01
CrCl ml/min/100g	0.48 \pm 0.5	1.1 \pm 0.9	0.5 \pm 0.3	0.17
AUC Cr	3676 \pm 896	2028 \pm 548	3509 \pm 379	0.01
Urine output total mls	289 \pm 195	691 \pm 230	257 \pm 118	0.001

Table 4: Functional parameters after 6 hours of perfusion.

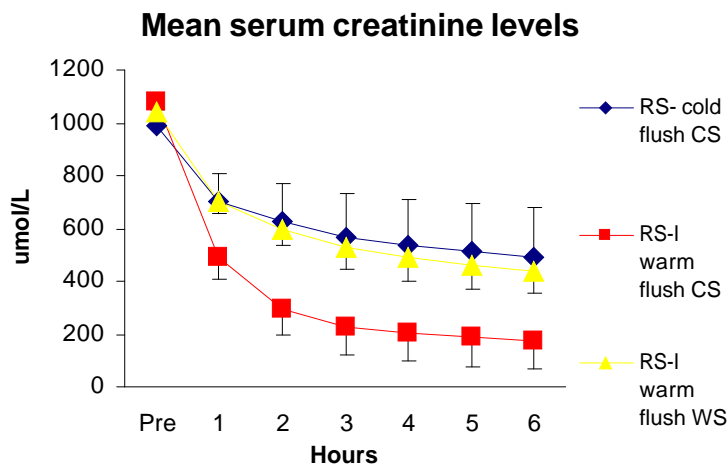


Figure 5: Mean serum creatinine levels over 6 hours.

Creatinine Clearance

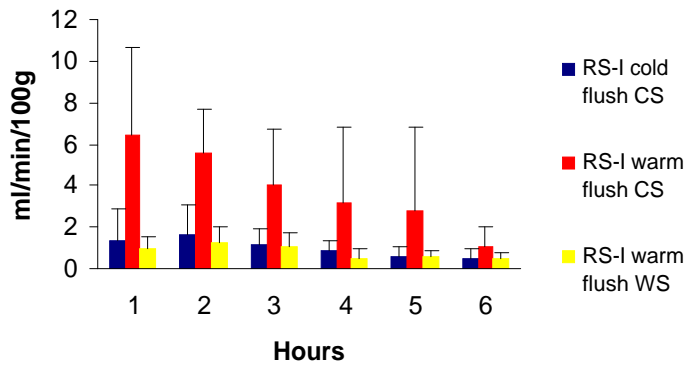


Figure 6: Mean creatinine clearance.

Metabolism

Sodium reabsorption was within normal physiological parameters during the first 4 hours in group 2 before falling to a comparable level as groups 1 and 3 which maintained equally poor sodium handling with high levels of excretion over the 6 hours. Potassium clearance was significantly higher in group 2 over the first 4 hours of perfusion before falling to a similar level as group 1 and 3 which maintained a steady but lower clearance throughout the perfusion period. Serum potassium levels gradually increased in groups 1 and 3 from 5.4, 5.7 to 9.6, 9.2mmol/L respectively compared to group 2 where levels remained static 5.6 to 6.4mmol/L [p = 0.009]. In contrast, the serum sodium levels rose in group 2 from 152 to 169mmol/L whereas in groups 1 and 3 they remained constant. Oxygen consumption was also overall significantly better in group 2 and group 3 significantly better than group 1 throughout the perfusion period. (table 5) (figures 7-9)

At 6 hrs	Group 1	Group 2	Group 3	p Value
Na + excretion	48.6 ± 27.9	35 ± 22.6	37 ± 18.7	0.59
K+ cl ml/min	1.4 ± 1.3	2.8 ± 2.2	1.3 ± 0.7	0.22
Oxygen consumption ml/min/g	12.8 ± 10.7	47 ± 12.1	28.7 ± 6.5	0.05

Table 5: Renal metabolism after 6 hours of perfusion.

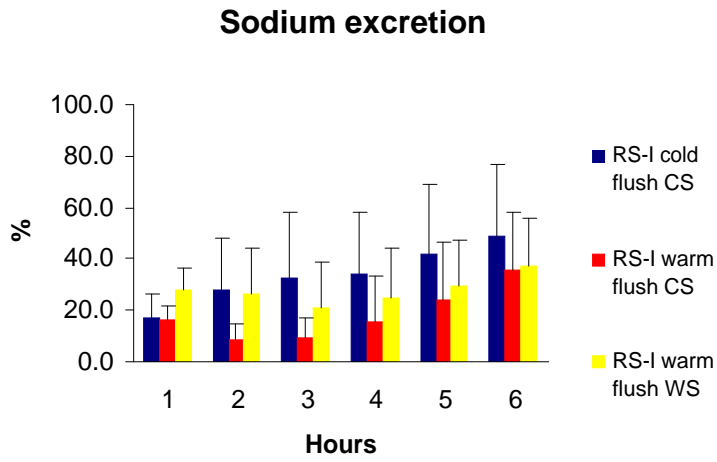


Figure 7: Mean sodium excretion.

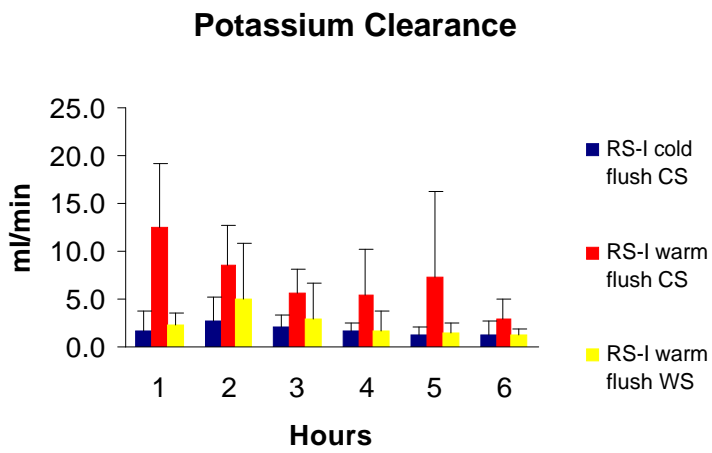


Figure 8: Mean potassium clearance

Mean oxygen consumption

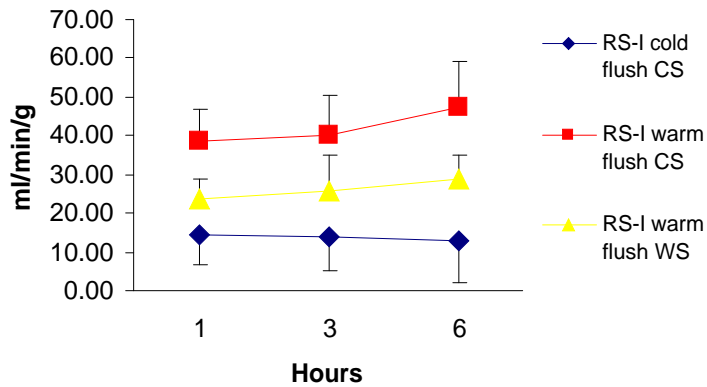


Figure 9: Mean oxygen consumption

Conclusion

AQIX® RS-I solution when used normothermically to initially flush the blood out of minimally, ischaemically damaged kidneys and then stored statically on ice for two hours proved to be the optimal condition for renal preservation under simplistic conditions. Kidneys demonstrated good renal function and handling of acid base balance when assessed on an isolated organ perfusion system. Normothermically flushed then statically stored kidneys maintained a level of renal viability and demonstrated good regulation of acid base balance. However, as a hypothermic flush and static cold storage solution AQIX ® RS-I demonstrated a poor level of renal viability across all functional parameters.

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