

AQIX[®] RS-I solution Audit Trial TR-1

AQIX[®] RS-I solution:

The Investigation between AQIX[®] RS-I and hypothermic storage conditions. HTK, Soltran and Machine perfusion (KPS-1) solutions in long-term preservation using a porcine kidney model

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A Comparative Study on the Effectiveness of AQIX RS-I solution vs HTK vs Soltran vs machine perfusion with KPS-1 solution in the hypothermic preservation of NHBD porcine kidneys

Aim

The aim of this study is to determine if isolated porcine kidneys preserved in AQIX® RS-I solution was an improved or equivalent method of preservation in comparison to HTK, Soltran or machine perfusion (KPS-1) solution following 18 hours of 'cold' storage. Renal viability will be assessed by measuring functional parameters when re-perfused with oxygenated autologous blood with a circulating creatinine concentration of 1000µmmol/L at 37°C in an isolated organ perfusion system (IOPS).

EXPERIMENTAL PROTOCOLS

Organ Retrieval

Large white pigs (60 – 70kg) will be 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 blood will be stored in CPDA-1 bags at 4°C.

The kidneys will then be surgically removed after 10 minutes of warm ischemia and the left and right kidney flushed with 400ml of either (1):AQIX® RS-I @ +30°C followed by a short flush with AQIX® RS-I @ +4°C , (2): HTK @ 4°C, (3): Soltran @ 4°C, or (4): Soltran @ 4°C at 100cm hydrostatic pressure respectively. Groups 1-3 will be transported on ice to the laboratory and stored at 4°C. Group (4) kidneys will be connected to the lifeport organ preservation system (Organ Recovery Systems) immediately after flushing and then perfused with KPS-1 solution @ 4 - 6°C After the hypothermic storage period of 18 hours, the renal artery and vein will be dissected and cannulated with appropriated sized renal cannulae and the ureter cannulated. Kidneys will then be flushed with Gelofusine at 4 °C (B.Braun, UK) to

remove the preservation solution. Kidneys will then be placed in the Isolated Organ Perfusion System (IOPS) and perfused normothermally with oxygenated autologous blood at a controlled arterial pressure for a period of 3 hours and renal function assessed as previously described (Kay et al., 2005; 2006).

Organ Perfusate and Perfusion Circuit

The Isolated Organ Perfusion Systems (IOPS) will consist of a pulmonary by-pass system, (Medtronic, UK), incorporating a centrifugal blood pump (550 Bio-pump), speed controller, TX50P flow transducer and pressure transducer. A heat exchanger (Grant, GD120, UK), temperature probe (Cole-Parmer, UK) and two PC- 2 Gemini infusion pumps (Alaris, UK) are added to the system. The disposable circuit consists 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, UK) (Fig. 1).

The circuit will be primed with:

- 500cm³ Ringers solution (Baxter Healthcare, Norfolk, UK)
- 500cm³ Whole autologous blood
- mannitol - 5g (Baxter, Healthcare, Norfolk, UK)
- 14cm³ sodium bicarbonate 8.4%
- Cefuroxime - 750mcg (Britannia Pharmaceuticals Ltd, Surrey, UK).

The perfusate will also be supplemented with 5% glucose infused at a rate of 7cm³/hr (Baxter healthcare UK), a nutrient solution 20cm³/hr (Nutriflex B;B. Braun) to which 100units insulin and 25 cm³ 8.4% sodium bicarbonate are added. Creatinine (Sigma, Germany) will be added to bring the initial circulating concentration to 1000µmol/L.

Ringers' solution (Baxter healthcare, Norfolk, UK) will be used to replace urine output cm³ for cm³.

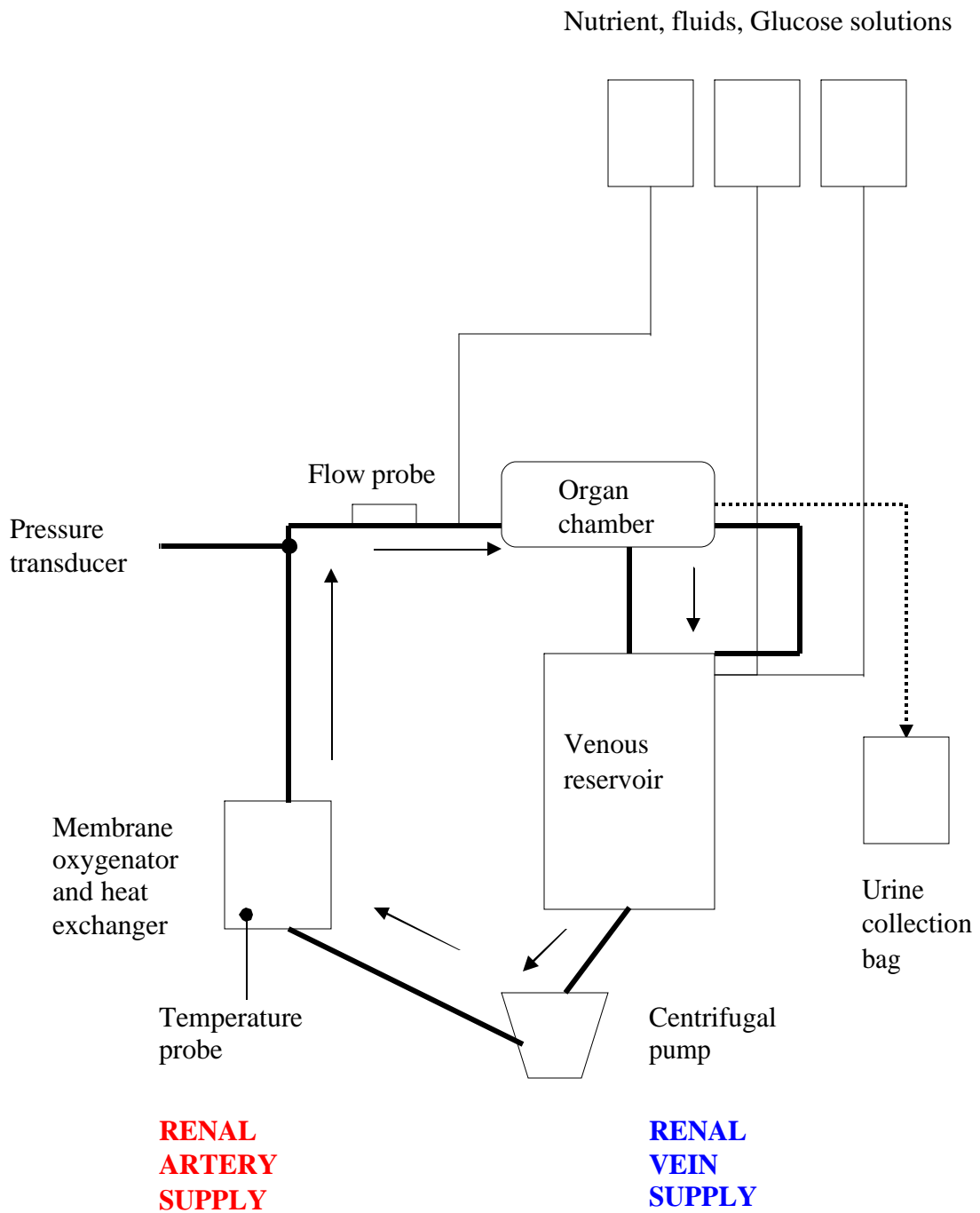


Figure 1: Diagram of the Isolated Organ Perfusion System (IOPS) indicating the direction of blood flow.

Analyses

Functional parameters

The renal blood flow, pressure and resistance ($R = P / F$) will be continually monitored and recorded. Biochemistry analysis will be carried out on arterial blood and urine samples hourly and the following metabolic and functional measurements calculated from the recorded values as previously described (Kay et al., 2006);

Creatinine clearance: estimated GFR, $(U_{cr} \times U \text{ flow} / P_{cr})$. [Area under the creatinine curve]

Oxygen consumption: ml/min/100g $\{ (PaO_{2art} - PaO_{2ven}) \}$ x flow rate/weight,

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

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

Acid base balance (pH, Bicarbonate, Base excess) *Potassium Clearance*: $(U_{K+} \times U \text{ flow} / P_{K+})$.

Biopsy samples

Tissue samples will be collected: pre storage, post storage and post reperfusion:

Histology evaluation

Oxidative damage: Lipid, protein, DNA

Apoptosis: Caspase 3 activity, apoptag staining

ADP:ATP

Plasma samples

Arterial sample will be taken pre and post reperfusion, centrifuged and the plasma stored at -80°C

Von Willebrand factor (vWF) by enzyme-linked immunosorbent assay

Oxidative damage: Lipid, protein

Urine samples

Urine samples will be collected after 1 and 3 hours of reperfusion and stored at -80°C .

Urinary N-acetyl glucosaminidase (NAG), a well recognized marker for renal tubular damage, will be measured using a colourimetric assay.

Results

Haemodynamics

There was no significant difference in the renal blood flow between any of the groups during reperfusion (Table 1, Figure 1). Intra renal resistance was higher in the Soltran group compared to the KPS-1 (CP) group ($P = 0.024$; Table 1). No significant difference was found in oxygen consumption between the groups after 1 or 3 hours of reperfusion (Table 1.)

	AUC RBF	AUC IRR	1hr O² consumption	3hr
Soltran	326 ± 70	9.12 ± 4.3*	29.2 ± 7.6	23.7 ± 11.9
KPS-1	600 ± 319	3.9 ± 1.7*	44.2 ± 27.9	35.1 ± 18.9
HTK	482 ± 129	5.6 ± 1.9	33.3 ± 11.2	38.2 ± 6.8
AQIX	375 ± 110	6.2 ± 3.0	22.3 ± 7.8	27.7 ± 8.4
P value	0.166	0.024	0.373	0.142

Table 1: Haemodynamics throughout reperfusion (mean ± SD).

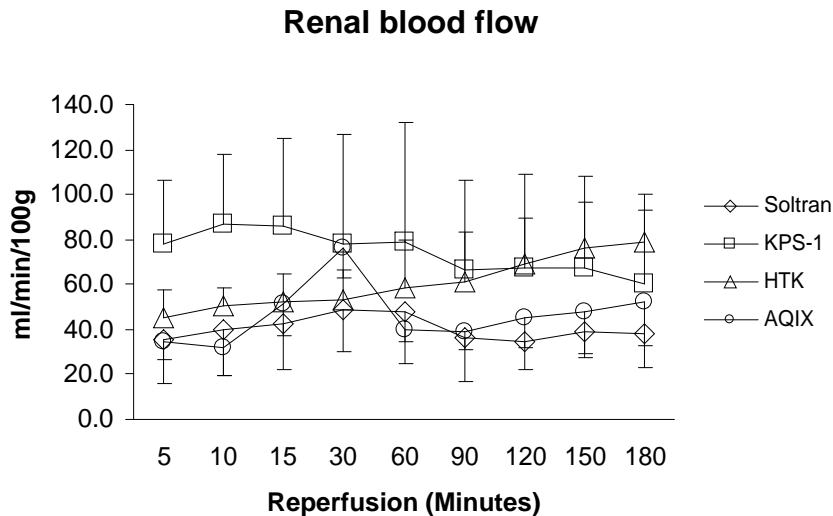


Figure 1: Renal blood flow during reperfusion.

Function

Serum creatinine fall was highest in the KPS-1 (CP) group compared to the Soltran and AQIX group (P = 0.004; Table 2). Creatinine clearance although low in all groups was significantly higher in the KPS-1 (CP) group throughout reperfusion compared to the AQIX group (P = 0.001; Table 2). Fractional excretion of sodium was significantly increased in the AQIX group compared to the Soltran and KPS-1 (CP) groups, and higher in the HTK group compared to the KPS-1 (CP) group (P = 0.002; Table 2). The total urine output was significantly lower in the AQIX group compared to the KPS-1 (CP) group (P = 0.008; Table 2).

	AUC: Cr	AUC: CrCl	AUC: Na⁺ excret	Total urine output
Soltran	2156 ± 401*	2.2 ± 1.7	116.6 ± 37.5*	302 ± 210
KPS-1	1354 ± 300*¶	9.8 ± 7.3*	24.9 ± 28.3¶†	463 ± 175.7*
HTK	2162 ± 121	2.1 ± 1.8	155.8 ± 12.3¶	367 ± 204.4
AQIX	2306 ± 141¶	0.4 ± 0.1*	177.5 ± 10*†	122 ± 42.7*
P value	0.004	0.001	0.002	0.008

Table 2: Renal functional parameters throughout reperfusion (mean ± SD).

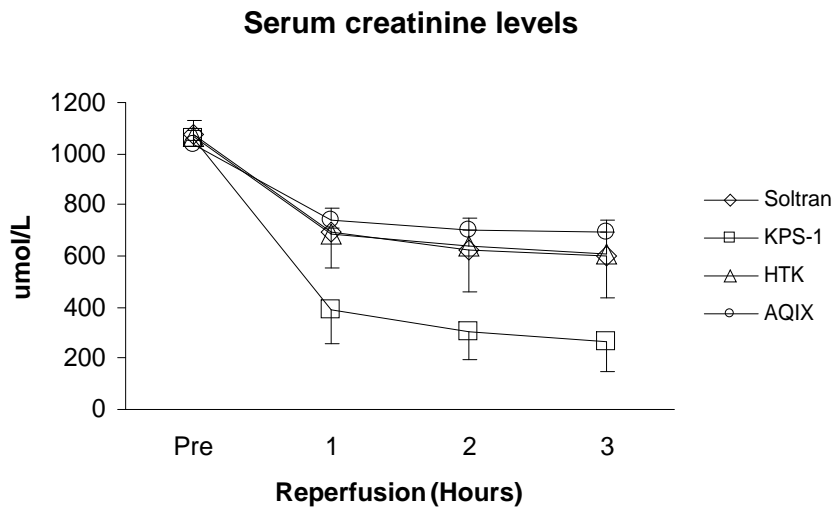


Figure 2: Serum creatinine levels during reperfusion

Acid base balance

pH levels fell throughout perfusion in all groups with no significant difference after 3 hours of reperfusion (Table 3; P = 0.337). There was also no significant difference in bicarbonate levels after reperfusion (Table 3; P = 0.528). Serum potassium levels increased in all groups with a significant difference in the Soltran group compared to the KPS-1 (CP) group (Table 3; P = 0.001).

	Pre pH	3hr pH	Pre Bicarb	3hr Bicarb	Pre K ⁺	3hr K ⁺
Soltran	7.51 ± 0.07	7.37 ± 0.07	23.9 ± 2*	21.62 ± 3.7	5.5 ± 0.1*	10.0 ± 0*
KPS-1	7.42 ± 0.1	7.33 ± 0.11	20.5 ± 1.2*¶	22.02 ± 6.9	5.0 ± 0.2¶†	5.62 ± 1.2*
HTK	7.46 ± 0.1	7.32 ± 0.1	23.2 ± 1.1¶	20.4 ± 2.5	5.9 ± 0.2*¶	8.9 ± 0.6
AQIX	7.46 ± 0.1	7.28 ± 0.05	22.8 ± 1.1	19.2 ± 1.9	5.6 ± 0.1†	9.3 ± 0.4
P value	0.078	0.337	0.007	0.528	0.001	0.001

Table 3: Acid base balance after 3 hours of reperfusion (mean ± SD)

Cellular enzymes

Serum levels of Aspartate aminotransferase (AST) increased in all groups and were significantly higher in the AQIX group compared to the Soltran and KPS-1 (CP) groups (Table 4; P = 0.003). Levels of Lactate dehydrogenase (LDH) also rose throughout reperfusion with significantly higher levels again in the AQIX group compared to the Soltran and KPS-1 (CP) groups (Table 3; P = 0.003).

	Pre AST	3hr AST	AUC AST	Pre LDH	3hr LDH	AUC LDH
Soltran	13 ± 1.7	125 ± 40.1	239 ± 70.4*	213 ± 70	382 ± 28.2*	980 ± 78.3*
KPS-1	10 ± 2.3	96 ± 32.2*¶	168 ± 37.6¶	182 ± 36.5	358 ± 83.9¶	875 ± 215¶
HTK	15 ± 2.9	481 ± 173*	533 ± 182	204 ± 25.4	400 ± 89.2	1103 ± 193.6
AQIX	11 ± 1.5	581 ± 123¶	947 ± 95*¶	207 ± 25.4	710 ± 72.2*¶	1516 ± 83.5*¶
P value	0.005	0.001	0.003	0.446	0.001	0.003

Table 4: Serum levels of AST and LDH pre and post reperfusion and AUC.

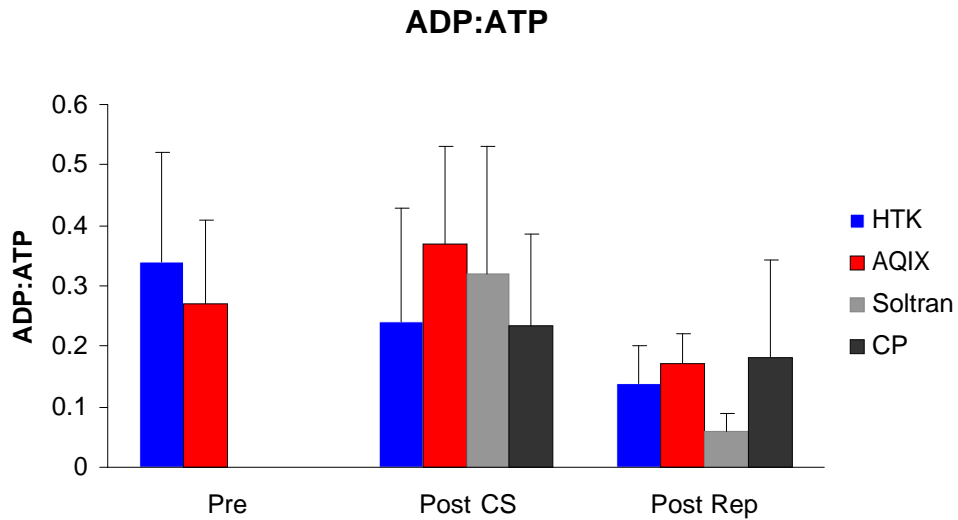


Figure 3: ADP: ATP ratio pre cold storage post cold storage and post reperfusion. There was no significant difference in the ratio between groups pre or post cold storage ($P = 0.527$). There was a significant difference between the AQIX®RS-I and Soltran group post reperfusion ($P = 0.037$) and a significant fall in the ratio post reperfusion in the Soltran group compared to the post cold storage ($P = 0.013$).

Histology Pre cold storage

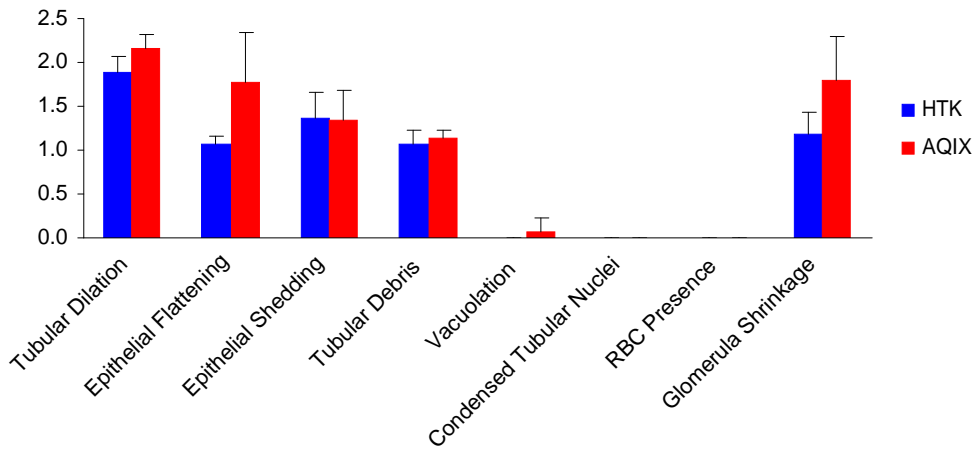


Figure 4: Pre cold storage histology. There was no significant difference between groups.

Histology Post cold storage

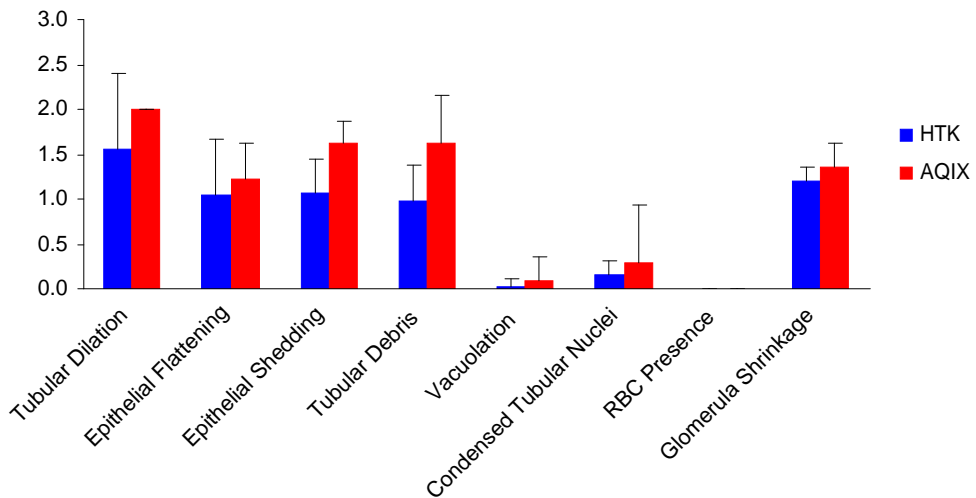


Figure 5: Post cold storage histology. There was no significant difference between groups.

Histology Post reperfusion

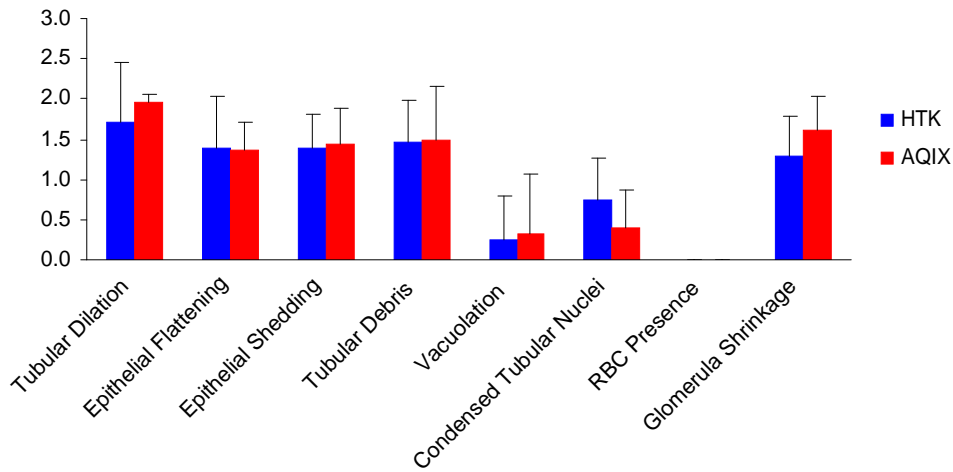


Figure 6: Post reperfusion histology: There was no significant difference between groups post reperfusion. There was a significant increase in tubular dilation pre cold storage and post reperfusion in the AQIX®RS-I group (P=0.017) and a significant increase in condensed tubular nuclei in the HTK group pre cold storage and post reperfusion (P=0.007).

Summary

During 3 hours of isolated organ reperfusion, kidneys flushed and stored in AQIX[®] RS-I demonstrated equivalent levels of renal blood flow and intra renal resistance to that of standard static hypothermic techniques using either Soltran or HTK and to that of cold machine perfusion with KPS-1 solution.

AQIX[®] RS-I demonstrated impaired renal and tubular function compared to cold machine perfusion with KPS-1 solution.

AQIX[®] RS-I showed equivalent handling of the acid base balance compared to the other groups.

Serum levels of AST and LDH measures of cellular damage were increased in the AQIX[®] RS-I group compared to that of cold machine perfusion with KPS-1.

After reperfusion ATP levels increased in all groups. However, only significantly in the Soltran group. The ADP: ATP ratio was significantly lower in the Soltran group compared to the AQIX[®] RS-I group post reperfusion.

No significant changes in histology were observed after cold storage but there was a significant increase in tubular dilation in the AQIX[®] RS-I group and condensed tubular nuclei in the HTK group.

Conclusion

Flushing warm ischaemically damaged kidneys with AQIX[®] RS-I at 30° C followed by AQIX[®] RS-I at 4°C then storing them on ice for 18 hours in this model did not prove to be an equivalent or superior method of preservation than the current preferred method of cold machine perfusion with KPS-1 solution for non heart beating donor kidneys.

However, the results of using AQIX[®] RS-I under these static conditions were comparative to that of static cold storage with HTK solution.