

## **AQIX CARDIOPLEGIA: a study to determine the efficacy of Aqix (RS-C) cardioplegia in comparison to that of St Thomas' Hospital cardioplegia.**

### **BACKGROUND**

Cardioplegic solutions are used to arrest and protect the heart during cardiac surgery. The predominant method used to achieve this is by elevating the extracellular potassium concentration that results in a depolarisation of the resting membrane potential of the myocardium and prevents activation of the voltage-dependent Na-channel. This will prevent initiation of the action potential and induce rapid arrest. The Na-channel inactivation threshold is around -65 mV and the level of potassium needed to depolarise the membrane potential to this level is about 10 mM; however, the Ca-channel is activated at a threshold potential of -40 mV and this occurs at approximately 30 mM potassium. Thus, there is a relatively safe concentration of potassium that can be used in a cardioplegic solution to achieve arrest during cardiac surgery and, conventionally, these concentrations vary between 15-25 mM. However, at these levels of depolarisation, the activation and inactivation profiles of sodium and calcium channels are such that 'window' currents occur that result in non-inactivating channel activity allowing a constant flux of sodium and calcium into the cell, leading to sodium and calcium overload and resulting in ischaemic and reperfusion injury [1].

A potentially beneficial alternative to depolarisation is to arrest the heart in a 'polarised' state, in which the arrest occurs at a membrane potential that is closer to the resting membrane potential. This can be achieved by using sodium channel blockers or potassium channel openers as arresting agents and use of these agents have shown beneficial effects when compared to hyperkalaemic solutions [2-6]. An alternative option is to arrest hearts with a calcium channel blocker, and this should also arrest the heart in a polarised manner. Calcium channel blockers have been used previously as arresting agents during cardiac surgery, but they do not washout easily and the heart tends to remain depressed for relatively long periods at a time when heart function is normally decreased as a result of ischaemia and cardiopulmonary bypass [7]. An alternative to a drug-based calcium channel blocker would be to use an agent such as magnesium, which competes with calcium for the rapidly exchangeable sarcolemmal binding sites involved in excitation-contraction coupling [8]. It has been shown that the efficacy of magnesium is species-specific with the rat being very sensitive, and the rabbit being relatively insensitive [8]. Magnesium has previously been used as the basis of the Kirsch solution [9] at a concentration of 160 mM but in combination with 11 mM procaine (a sodium-channel blocker).

Aqix® RS-1 is a new perfusion solution that is formulated to prevent the deleterious effects of free inorganic phosphate ions present in other conventionally used perfusion solutions. Aqix uses the natural bicarbonate/pCO<sub>2</sub> buffering system and has no phosphate buffering compounds in the formulation. It also contains amino acids (L- glutamate, L- glutamine and L- aspartate), substrates (D- glucose, glycerol, choline chloride and carnitine) and co- factors (thiamine pyrophosphate (cocarboxylase) and insulin). As a consequence, the Aqix perfusion solution is reported to maintain organs for up to 10-times longer in isolated perfusion than other phosphate buffered solutions, such as Krebs Henseleit solution.

Aqix has also been used as a cardioplegic solution by the addition of 25 mM magnesium sulphate to the basic Aqix solution (Aqix RS-C). Studies have demonstrated that, using continuous infusion of this solution in rat hearts subjected to ischaemic durations up to 6 hours, recovery was still measurable. In addition, using bolus infusions, recovery was detectable after 3 hours of ischaemia. Hence, it would appear that the Aqix solution may be beneficial for isolated rat heart perfusion and that Aqix cardioplegia may exert more beneficial protection on the ischaemic rat heart than other cardioplegic solutions.

We propose to investigate the properties of Aqix in our standard isolated rat heart perfusion system in comparison to our standard Krebs Henseleit solution. In addition, we will compare the protective properties of Aqix cardioplegia (Aqix RS-C) in comparison to the St Thomas' Hospital No 2 solution (Plegisol: STH2) that is used in cardiac surgery throughout the world.

## **METHODS**

### **Animals.**

Adult male Wistar rats (240-300 g body weight) will be used (Bantin and Kingman, Hull, United Kingdom). All animals will receive humane care in accordance with the "Guidance on the Operation of the Animals (Scientific Procedure) Act of 1986" published by Her Majesty's Stationery Office, London, United Kingdom, and studies will be approved by the institutional ethics committee. Rats will be anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and anticoagulated with heparin (1000 IU/kg i.v.).

### **Heart isolation, perfusion and perfusion medium.**

Hearts will be immediately excised from the anaesthetised rat and immersed in cold (4°C) buffer, as appropriate. The aorta will be rapidly cannulated, and the heart perfused in the Langendorff mode (at a constant pressure of 75 mmHg and at 37°C). The left atrial appendage is removed and a deflated ultrathin intraventricular balloon, constructed from cling film over the tip of a 20-gauge cannula and made to match the internal dimensions of the left ventricle, is introduced through the mitral valve into the left ventricle. The balloon is attached to a pressure transducer, the calibrated output from which is recorded on an Apple Macintosh computer (Apple Computer Inc, Cupertino, CA, USA) using the PowerLab system (ADInstruments Ltd, Hastings, East Sussex, UK). The intraventricular balloon is gradually inflated with water to give a stable left ventricular end-diastolic pressure (LVEDP) of 3.0 to 8.0 mmHg, and this isovolumic state is maintained throughout the rest of the protocol. All hearts will be subjected to an equilibration period of aerobic perfusion for 20 min, and baseline readings of left ventricular systolic pressure (LVSP: in mmHg), left ventricular end-diastolic pressure (LVEDP: in mmHg), heart rate (beats/min) and coronary flow rate (ml/min) will be measured. Left ventricular developed pressure (LVDP) is calculated as the LVSP minus LVEDP. Coronary flow rate is measured by timed collection into a measuring cylinder of the coronary effluent exiting the heart.

The perfusion medium will be either a modified Krebs Henseleit bicarbonate buffer (KHB) with the following composition (mmol/L): NaCl, 118.5; NaHCO<sub>3</sub>, 25.0; KCl, 4.8; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 1.4; and glucose, 11.0, or the Aqix perfusion buffer (as detailed). The KHB is prepared daily, filtered through a 5-µm poresize cellulose nitrate membrane filter before use, and continuously gassed with a mixture of 95% O<sub>2</sub>:5% CO<sub>2</sub> to give a pH of 7.4 at 37°C. Aqix will also be prepared daily from the pre-prepared 10X concentrate (100 ml) by dilution with de-ionised water to make up 1000 ml of solution and this will also be filtered through a 5µm poresize cellulose nitrate filter before use. Separate perfusion systems will be established for Aqix perfusion and KHB perfusion to ensure that there is no contamination of the Aqix solution with inorganic phosphate from KHB.

### **Exclusion criteria.**

Hearts not satisfying pre-assigned exclusion criteria at the time of the baseline readings (after 20 min of aerobic perfusion) will be excluded from study. The acceptable ranges for LVDP, heart rate, and coronary flow rate are: >100 mmHg, >220 beats/min, and 8-16 ml/min, respectively.

## **Proposed Protocols**

### **1. Stability and long-term function**

Hearts will be perfused as detailed above for periods of 6 hours and heart function will be measured throughout. This will allow comparison between basic function characteristics of hearts perfused in Aqix and KHB. As indicated above, it has been suggested that perfusion in Aqix maintains organ function up to 10x longer than other perfusion solutions.

### **2. Comparison of cardioprotection using Aqix RS-C or STH2**

#### **2.1. Normothermia**

##### **2.1.1. Single bolus infusion**

After an equilibration period when control pre-ischaemic function will be measured, hearts will be arrested with a single 2 minute infusion of the appropriate cardioplegic solution (at 37°C) and then subjected to varying durations of normothermic (37°C) ischaemia to establish recovery profiles for the respective cardioplegia solutions. Various combinations can be envisaged: hence, study (i) will investigate KHB perfusion with either Aqix RS-C or STH2 prior to ischaemia; study (ii) will investigate Aqix RS-1 perfusion with either Aqix RS-C or STH2 prior to ischaemia. Hearts will be reperfused for 60 minutes and the recovery profiles throughout this period will be assessed.

##### **2.1.2. Multiple infusions**

Depending on the duration of ischaemia that is established in the above study, cardioplegia will be infused every 20(?) minutes to determine the efficacy of multiple infusions of the respective cardioplegic solutions. Similar combinations will be used as described above. Hearts will be reperfused for 60 minutes and the recovery profiles throughout this period will be assessed.

#### **2.2. Hypothermia**

##### **2.2.1. Multiple infusions**

After an equilibration period when control pre-ischaemic function will be measured, hearts will be arrested with a 2 minute infusion of the appropriate cardioplegic solution (at 15°C) and subjected to ischaemic durations of 2-3 hours (this will need to be established) with re-infusion of cardioplegia every 30 minutes. Hearts will be reperfused for 60 minutes and the recovery profiles throughout this period will be assessed.

### **3. Working heart preparations**

Following the above studies, consideration will be given to whether to conduct studies in the 'working' mode. This allows the heart to function as a pump and to measure the aortic flow and cardiac output. This is a more physiological measure of heart function, and is more clinically relevant for cardioplegia studies. It also establishes clinically relevant differences between cardioplegic solutions.

### **4. Additional studies**

These will develop as the studies progress, and with discussion between the two parties involved (ie. St Thomas' group and Aqix group). For example, initial comparative studies have suggested a slight benefit with Aqix RS-C over STH2; the differences between the solutions could be investigated such that the magnesium concentration could be increased in STH2, or reduced in Aqix RS-C, to determine whether this is the cardioprotective factor that is inducing these differences.

## Additional measurements

During Langendorff perfusion, contracture parameters will be measured. These will be: time to onset of contracture, time to peak contracture, peak contracture. In addition, release of creatine kinase will be measured using an assay preparation purchased from Randox (Northern Ireland) throughout the reperfusion period.

## PRELIMINARY RESULTS

### Stability Studies

We have compared the function and stability of isolated rat hearts when perfused for extended (6 hour) periods. The profile of left ventricular developed pressure (LVDP) throughout 6 hours of continuous perfusion with either KHB or Aqix is shown in Figure 1. In both perfusates, the function deteriorates relatively rapidly over the initial 90 minutes of perfusion but then reaches a plateau phase where function is maintained relatively constant for the remaining perfusion period. During this later period, there is a trend for the LVDP to deteriorate slightly more rapidly with KHB perfusion but this may be a reflection of the slightly slower rate of deterioration during the initial 90 minutes of perfusion. At the end of 6 hours of perfusion, the function in the 2 groups of hearts is identical.

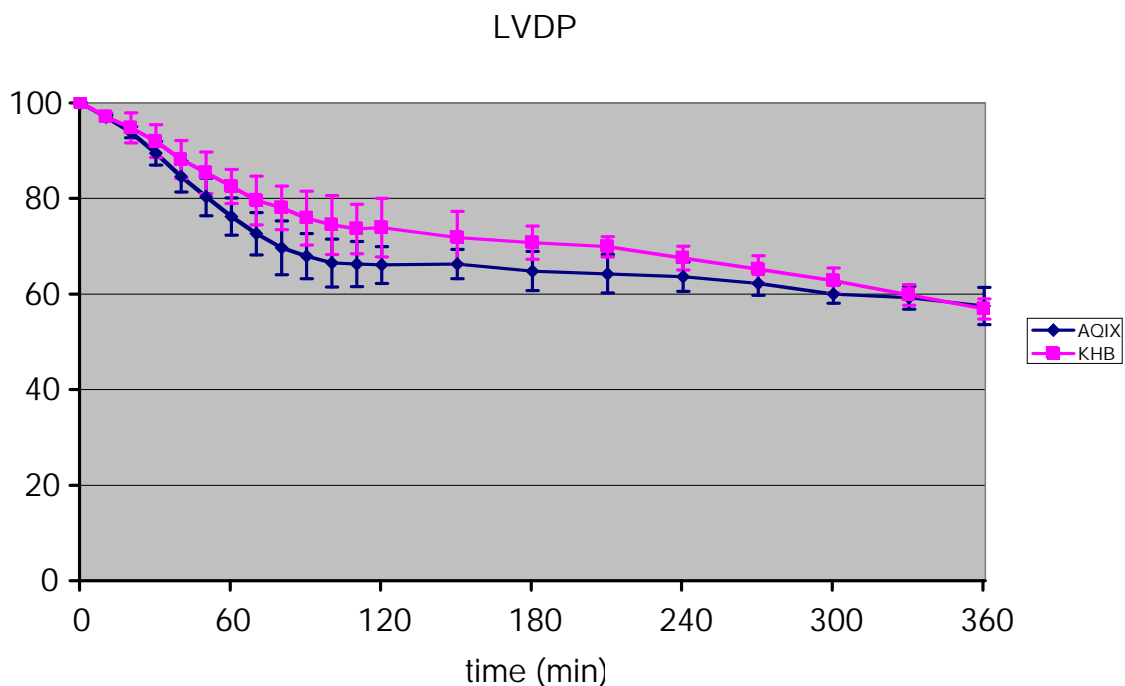


Figure 1: Left ventricular developed pressure in hearts perfused with either Krebs Henseleit buffer (KHB) or Aqix during continuous perfusion for 6 hours.

The effect of continuous perfusion with either KHB or Aqix on left ventricular end-diastolic pressure (LVEDP) is shown in Figure 2. As with LVDP, the 2 groups are very similar; LVEDP increases very slowly over the initial 4 hours of perfusion, but then increases more rapidly but at a similar rate for both perfusates. This shows that the myocardial stiffness increases towards the end of the perfusion period.

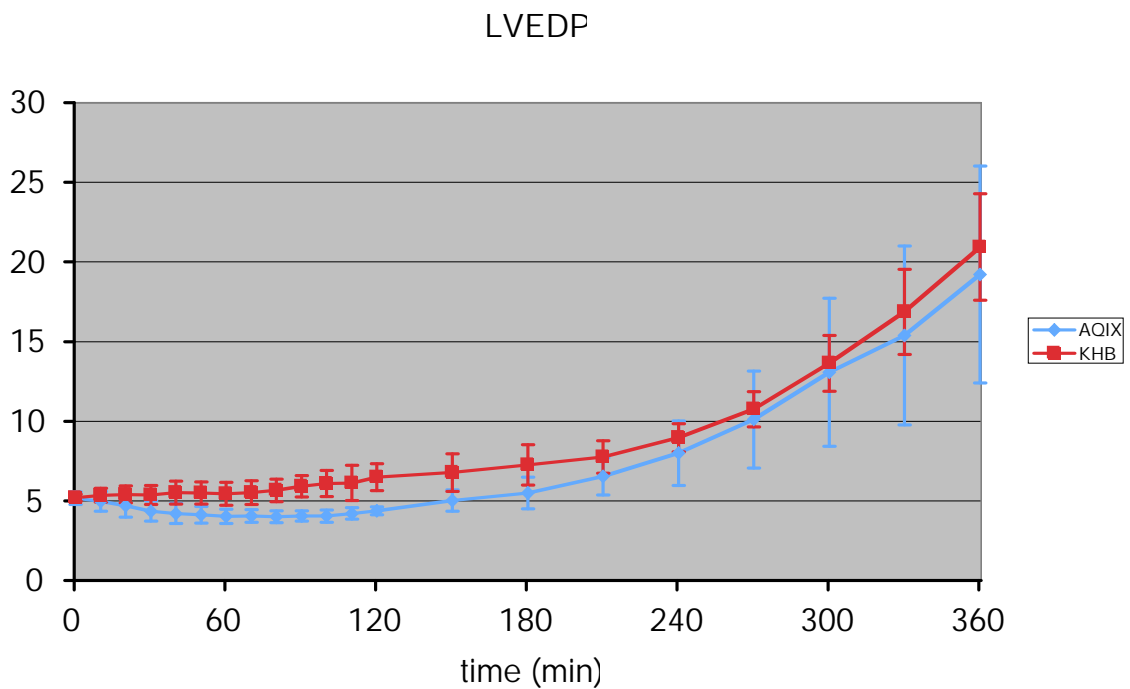


Figure 2: Left ventricular end-diastolic pressure (LVEDP) in hearts perfused with either Krebs Henseleit buffer (KHB) or Aqix during continuous perfusion for 6 hours.

### Comparative recovery studies

These are currently being conducted. Initial results suggest that Aqix RS-C may provide slightly improved recovery at longer durations of ischaemia that that of STH2, but these are still underway and so no definitive results can be shown at this stage.

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