Warm blood hyperkalaemic reperfusion (‘hot shot’) prevents myocardial substrate derangement in patients undergoing coronary artery bypass surgery

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Abstract

Objective: A significant metabolic derangement occurs in the ischaemic-reperfused heart of patients undergoing coronary artery bypass surgery using cold blood cardioplegia. The aim of the present study was to investigate whether this effect could be reversed by complementing cold blood cardioplegia with a short terminal exposure of warm blood hyperkalaemic cardioplegia (‘hot shot’). Methods: Thirty-five patients undergoing primary elective coronary revascularisation were randomized to one of two different techniques of myocardial protection. In the cold blood group \( n = 17 \) myocardial protection was induced using antegrade hyperkalaemic cold blood cardioplegic solution. In the hot shot group \( n = 18 \) this was supplemented with a short exposure to hyperkalaemic warm blood cardioplegia prior to removal of the cross clamp. Intracellular substrates (ATP and amino acids) were measured in left ventricular biopsies collected 5 min after institution of cardiopulmonary bypass, after 30 min of ischaemic arrest and 20 min after reperfusion. Results: Biopsies taken at the end of the period of myocardial ischaemia, when compared to control, did not show any significant change in the intracellular concentration of ATP (from 2.71 ± 0.32 to 2.43 ± 0.37 \( \mu \)mol/g wet weight for cold blood group and from 2.6 ± 0.3 to 2.5 ± 0.34 \( \mu \)mol/g wet weight for hot shot group) or total free intracellular amino acids pool (from 33.0 ± 1.4 to 30.0 ± 1.4 \( \mu \)mol/g wet weight for cold blood group and from 34.0 ± 1.4 to 34.5 ± 2.3 \( \mu \)mol/g wet weight for hot shot group). Upon reperfusion, however, there was a significant fall in ATP (23.7 ± 1.6 \( \mu \)mol/g wet weight amino acids, \( P < 0.05 \)) and in amino acids (1.53 ± 0.24 \( \mu \)mol/g wet weight, \( P < 0.05 \)) in the group receiving only cold blood cardioplegia but not in the hot shot group (2.27 ± 0.27 \( \mu \)mol/g wet weight ATP and 30.5 ± 1.6 \( \mu \)mol/g wet weight amino acids). Conclusions: The data suggest that warm blood hyperkalaemic reperfusion hot shot prevents myocardial metabolic derangement seen during coronary artery surgery. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Cold blood cardioplegia; Hot shot; Coronary surgery

1. Introduction

We have recently demonstrated that a significant metabolic derangement occurs in the ischaemic-reperfused heart of patients undergoing coronary artery bypass surgery using cold blood cardioplegia [1,2]. In these hearts, a fall in the intracellular amino acid pool and ATP in left ventricular biopsies was demonstrated. This is likely to influence metabolic and functional recovery as several of these substrates are essential for normal cellular function [1–3].

A short period of warm blood terminal cardioplegic perfusion ‘hot shot’ following cold blood cardioplegic arrest, has been shown to improve metabolic and functional recovery on reperfusion [4]. The beneficial effects of a hot shot are thought to include improved delivery of oxygen to the arrested myocardium after a period of relative ischaemia facilitated by coronary vasodilatation associated with warm perfusion [4–6]. In addition, washout of the products of anaerobic metabolism while arrest is maintained results in better preservation of ATP [4,5].

This study investigated the effect of warm blood hyperkalaemic reperfusion on the intracellular concentration of amino acids, ATP and lactate during ischaemic arrest and reperfusion in patients undergoing routine coronary artery surgery. The postoperative release of troponin I, a sensitive marker of myocardial injury [7], was also measured.
2. Materials and methods

Thirty-five patients (30 males, mean age 59.7 ± 2.0 years) with a left ventricular ejection fraction greater than 50%, undergoing primary elective coronary revascularisation were randomized to one of two different techniques of myocardial protection. In the control group (n = 17) myocardial protection was induced using antegrade administration of hyperkalaemic cold blood cardioplegic solution (blood and St. Thomas’ I cardioplegic solution 4:1 with extra K⁺ added to give a 20 mM K⁺ concentration). In the hot shot group (n = 18) the same blood cardioplegia was used as in group one with the addition of a short exposure to this hyperkalaemic but warm cardioplegia prior to removal of the cross clamp. The study was approved by the hospital ethics committee and patients informed consent obtained.

Anaesthetic technique was standardized for all patients. Thiopentone (1–3 mg/kg) was used for induction with 3–5 μg/kg fentanyl, and volatile agents were delivered in 50% air–O₂ mixture for maintenance. Propofol (3 mg/kg/h) was given as an infusion during cardiopulmonary bypass and neuromuscular blockade was achieved by 0.1–0.15 mg/kg Pancuronium Bromide. Alpha stat acid-base management was adopted. Initial anticoagulation was accomplished with 3 mg/kg body weight of heparin and was supplemented as required in order to maintain an active clotting time of 480 s or above. All operations were performed using cardiopulmonary bypass with ascending aortic cannulation, two-stage venous cannulation, and moderate systemic hypothermia (32°C).

The cardioplegic solution was administered under pressure into the aortic root as a 1 l bolus (10–12°C) at the start of the ischaemic period. Infusions of 300 ml were repeated at 30-min intervals or earlier if electrical activity returned. In the group receiving the hot shot, an additional 500 ml of warm blood hyperkalaemic solution was infused at 37°C over 2 min into the aortic root at a pressure of 50 mm Hg before aortic unclamping.

Distal coronary anastomoses were completed during a single period of aortic cross-clamping. Proximal anastomoses were completed on a beating heart using an aortic partial occlusion clamp.

2.1. Collection of ventricular biopsy

Myocardial biopsy specimens (4–10 mg wet weight) were taken from the apex of the left ventricle using a ‘Tru-cut’ needle (Baxter Healthcare Corporation, IL, USA). The first biopsy was taken 5 min after institution of cardiopulmonary bypass. The second biopsy was taken after 30 min of ischaemic arrest and the third after 20 min of reperfusion. Each specimen was immediately frozen in liquid nitrogen until processing analysis of amino acids, ATP and lactate. All the biochemical analyses were performed by an investigator blind to the techniques used.

2.2. Amino acids, ATP and lactate

The procedure used to extract free amino acids, ATP and lactate was similar to that described previously [1,2]. In brief, frozen biopsy specimens were crushed under liquid nitrogen and the resultant powder was extracted with perchloric acid. The extracts were centrifuged at 1500 × g for 10 min at 4°C. The supernatant was neutralized and the ATP content measured using a bioluminescent assay [8]. Aliquots of extracts that were left after determination of amino acids and ATP, were also taken for lactate determination. Lactate was measured using a plasma lactate determination kit from Sigma Diagnostics (Sigma, Poole, UK).

Amino acids in the extracts from both groups, were determined according to the Waters Pico-Tag method as reported elsewhere [1,2,9]. Essentially, 100 μl of the extract was dried using vacuum centrifugation (Savant SV160, Farmingdale, NY, USA). Free amino acids were derivatized using phenylisothiocyanate. The phenylisothiocarbamyl derivatized amino acids were separated by HPLC using a 30 cm Pico-Tag column (Millipore Corporation, Milford, MA, USA) with two Waters delivery pumps (A and B) at a constant flow of 1 ml/min with the following gradient: 100% A for 13.5 min, 97% A for 10.5 min, 94% A for 6 min, 91% A for 20 min, 66% A for 12.5 min and 0% A for 4 min. The solvents used were: (A) 132 mM Na acetate, 470 mM triethylamine, pH 6.4 and 6% acetonitrile; (B) 60% acetonitrile. Derivatized amino acids were detected at 254 nm (46°C) using a Waters 486 detector. Quantitative and qualitative analysis was carried out using amino acid standards (Sigma, Dorset, UK) and the acquired data was processed using the Millenium 2000 software supplied by Waters–Millipore (Watford, UK).

Chemicals needed to derivatize amino acids and separate them were obtained from Waters–Millipore.

2.3. Troponin I

Recent years have witnessed an increased use of myocardial troponin I as marker of myocardial injury [7]. In contrast to other markers of myocardial injury (e.g. CK-MB, myoglobin) which can originate from cardiac and skeletal muscle, myocardial troponin I is almost exclusively released from damaged heart cells. Determination of blood concentration of troponin I was conducted prior to surgery and at 4, 12, 24 and 48 h postoperatively. The analysis was carried out using an Access machine and kits provided by Sanofi Diagnostics Pasteur (Guilford, UK).

2.4. Data collection and analysis

Data were expressed as the mean ± SE unless otherwise stated. The myocardial tissue concentration of amino acids and ATP was used to approximate the intracellular concentration of these metabolites. Myocardial biopsies contain little fat and connective tissue. The extracellular space
(blood vessels) constitutes only a small proportion of myocaridal tissue and the low concentration of amino acids and ATP in the blood makes the effect of the extracellular space negligible. Total troponin release was defined as the area under the serum concentration curve and was calculated by the trapezium rule using Microsoft Excel as the area under the curve and was present in very small amounts and therefore could not be easily detected. Data are the mean ± SD.

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**significantly different from biopsies 1 and 2 (**P=0.05); † Significantly different from biopsies 1 and 3 (**P=0.05).

### 3.2. Changes in amino acids during ischaemia and reperfusion

At the end of the ischaemic period, there was no change in the intracellular concentration of the free intracellular amino acid pool regardless of the cardioplegic technique used (Fig. 1). However after 20 min reperfusion following ischaemic arrest with cold blood cardioplegia, there was a significant and marked fall in the concentration of the free intracellular amino acid pool (Fig. 1). This loss of amino acids was completely prevented in the hot shot group (Fig. 1). The intracellular free amino acid pool is largely made up of taurine and the non-essential amino acids (constituting more than 90% of the pool). Table 2 shows the changes in these amino acids during ischaemia and on reperfusion for both groups. With the exception of alanine which showed a significant increase, there was no significant change in the intracellular concentration of individual amino acids after bypass surgery using cold blood cardioplegia with or without reperfusion with warm blood hyperkalaemic cardioplegia. The intracellular amino acid pool includes 15 essential and non-essential amino acids as well as the non-protein amino acid taurine. The rest of the protein amino acids were used (Fig. 1). However after 20 min reperfusion following ischaemic arrest with cold blood cardioplegia, there was a significant and marked fall in the concentration of the free intracellular amino acid pool (Fig. 1). This loss of amino acids was completely prevented in the hot shot group (Fig. 1). The intracellular free amino acid pool is largely made up of taurine and the non-essential amino acids (constituting more than 90% of the pool). Table 2 shows the changes in these amino acids during ischaemia and on reperfusion for both groups. With the exception of alanine which showed a significant increase, there was no significant change in the intracellular concentration of individual amino acids after bypass surgery using cold blood cardioplegia with or without reperfusion with warm blood hyperkalaemic cardioplegia. The intracellular amino acid pool includes 15 essential and non-essential amino acids as well as the non-protein amino acid taurine. The rest of the protein amino acids were present in very small amounts and therefore could not be easily detected. Data are the mean ± SD.

### 3. Results

#### 3.1. Clinical outcome

There were no deaths in the series. The clinical information is presented in Table 1. The two groups were similar with respect to sex, age, preoperative ventilator function, extent of coronary disease, ischaemic and cardiopulmonary bypass time, incidence of perioperative myocardial infarct, ITU or hospital stay.

### Table 1

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Blood cardioplegia</th>
<th>Blood cardioplegia + hot shot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.9 ± 6.8</td>
<td>60.7 ± 8.4</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>15/2</td>
<td>15/3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Previous MI</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>61.3 ± 8.4</td>
<td>62.4 ± 4.8</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>3.18 ± 0.85</td>
<td>3.13 ± 0.69</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>91.6 ± 18.2</td>
<td>88.9 ± 19.3</td>
</tr>
<tr>
<td>Ischaemic time (min)</td>
<td>43.6 ± 12.9</td>
<td>43.6 ± 10.5</td>
</tr>
<tr>
<td>Perioperative MI</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ITU stay (h)</td>
<td>10 ± 1.2</td>
<td>11 ± 0.9</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

Data are the mean ± SD.

### Table 2

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Cardioplegia cold blood (n = 17)</th>
<th>Cardioplegia blood + hot shot (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ischaemia</td>
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<tr>
<td>Glutamine</td>
<td>9.09 ± 0.40</td>
<td>8.42 ± 0.52</td>
</tr>
<tr>
<td>Taurine</td>
<td>9.47 ± 0.42</td>
<td>8.10 ± 0.41</td>
</tr>
<tr>
<td>Glutamate</td>
<td>7.68 ± 0.46</td>
<td>6.59 ± 0.43</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.98 ± 0.11</td>
<td>2.54 ± 0.17†</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.33 ± 0.15</td>
<td>1.07 ± 0.10</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.38 ± 0.02</td>
<td>0.34 ± 0.02</td>
</tr>
</tbody>
</table>

Changes in the intracellular concentrations of amino acids (μmol/g wet weight) in ventricular biopsies collected from patients undergoing coronary artery bypass surgery using cold blood cardioplegia with or without reperfusion with warm blood hyperkalaemic cardioplegia. Data are the mean ± SEM. *Significantly different from biopsy 1 (**P=0.05); **significantly different from biopsies 1 and 2 (†P=0.05); † Significantly different from biopsies 1 and 3 (**P=0.05).
ischaemic arrest with cold blood cardioplegia. Similar trends were also seen in the hot shot group, although the increase in alanine was not statistically significant (Table 2). However, upon reperfusion following arrest with cold blood, a significant fall in the intracellular concentrations of glutamate, glutamine, taurine, aspartate and asparagine was seen. Alanine fell significantly back to its resting level. A similar pattern of change was also seen for alanine after reperfusion following arrest with cold blood and a hot shot. However in the hot shot group, there was preservation of the other individual amino acids, although a small decrease was evident for glutamate, aspartate, taurine and glutamine (Table 2).

3.3. Changes in biochemical markers of myocardial injury

There was no significant fall in ATP concentration (Fig. 2) after the ischaemic period using cold blood cardioplegia with or without hot shot. On reperfusion, however, there was a marked significant fall in ATP after arrest using cold blood but not when the arrest was terminated with a hot shot reperfusion (Fig. 2).

During anaerobic metabolism when the heart utilizes glutamate for energy production, there is a fall in tissue glutamate with a corresponding rise in alanine [10,11]. In the brain and the myocardium, the tissue alanine/glutamate ratio has been used as a marker of ischaemia [1,12]. Fig. 3 shows that an increase in the alanine/glutamate ratio occurred as a result of ischaemia irrespective of whether a hot shot was employed or not. However, upon reperfusion this ratio remained significantly high in the cold blood group but fell to levels that were not significantly different from control after a hot shot.

The increase in alanine/glutamate ratio provides evidence for anaerobic metabolism during ischaemic arrest and on reperfusion. The changes in lactate, another marker of anaerobic metabolism are shown in Fig. 4. A trend to increase in tissue lactate was evident during ischaemia which seemed to decline on reperfusion in both groups. The changes were not statistically significant. The reason for this could be due to the smaller number of biopsies used for lactate determination.

The release of myocardial troponin I as a marker of reperfusion damage was also measured. The estimated total release (area under the curve) was used. This was done on Excel software using the Trapezium rule. The release of troponin I was reduced when using cold blood followed by a hot shot, although the difference did not reach statistical significance (Fig. 5).

4. Discussion

Reperfusion with warm blood hyperkalaemic cardioplegic solution has been used experimentally and clinically to limit myocardial damage after global and regional ischae-
The changes in ATP, glutamate and aspartate may not result in significant utilization of the endogenous amino acids. It is plausible however that the addition of exogenous amino acids like glutamate may significantly improve the levels of endogenous amino acids. This may in turn elevate intracellular ATP to higher levels than control, thus leading to better cellular repair and recovery. This we have found to be the case in isolated perfused guinea-pig heart (Suleiman et al., unpublished data).

The changes in ATP, glutamate and aspartate may not provide a complete picture of the cellular changes that occur as a result of reperfusion with warm blood cardioplegia. For example the preservation of ATP levels after ischaemia suggests that anaerobic metabolism may be minimal. However there was a clear trend for lactate to accumulate which was also associated with an increase in the alanine/glutamate ratios, suggesting the occurrence of anaerobic metabolic activity during ischaemic arrest. Evidence for anaerobic metabolic activity continues on reperfusion in both groups but was less marked after reperfusion with warm blood cardioplegia.

Preservation of other amino acids (glutamine, taurine and asparagine) after reperfusion with warm blood cardioplegia is significant as these amino acids are important for a number of cellular activities. Glutamine is important as a nitrogen donor for the biosynthesis of a number of compounds such as nucleotides and amino acids [13]. Furthermore, muscular glutamine has been shown to increase protein synthesis and decrease protein degradation and regulate glycogen metabolism [13–15]. On the other hand taurine affects cellular calcium homeostasis and taurine deficiency is associated with the development of cardiomyopathy [2, 16, 17]. As for asparagine, little is known of its myocardial cellular activity; in addition it is present at a much lower concentration than other amino acids. It may be important however for producing aspartate which is essential for normal cellular function.

In conclusion, this work has shown that terminal warm blood hyperkalaemic reperfusion after cold blood cardioplegic arrest, preserves intracellular substrates, reduces metabolic stress and reperfusion damage in the hearts of patients undergoing coronary artery bypass graft surgery. The clinical implications of these findings need further investigation.

Acknowledgements

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References


