Intermittent antegrade hyperkalaemic warm blood cardioplegia supplemented with magnesium prevents myocardial substrate derangement in patients undergoing coronary artery bypass surgery

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Abstract

Objective: The influence of the addition of magnesium on myocardial protection with intermittent antegrade warm blood hyperkalaemic cardioplegia in patients undergoing coronary artery surgery was investigated and compared with intermittent antegrade warm blood hyperkalaemic cardioplegia only.

Methods: Twenty-three patients undergoing primary elective coronary revascularization were randomized to one of two different techniques of myocardial protection. In the first group, myocardial protection was induced using intermittent antegrade warm blood hyperkalaemic cardioplegia. In the second group, the same technique was used except that magnesium was added to the cardioplegia. Intracellular substrates (ATP, lactate 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: There were no significant changes in the intracellular concentration of ATP or free amino acid pool in biopsies taken at the end of the period of myocardial ischaemia. However, the addition of magnesium prevented the significant increase in the intracellular concentration of lactate seen with intermittent antegrade warm blood hyperkalaemic cardioplegia. Upon reperfusion there was a significant fall in ATP and amino acid concentration when the technique of intermittent antegrade warm blood hyperkalaemic cardioplegia was used but not when magnesium was added to the cardioplegia. Conclusions: This work shows that intermittent antegrade warm blood hyperkalaemic cardioplegia supplemented with magnesium prevents substrate derangement early after reperfusion. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Intermittent antegrade warm blood hyperkalaemic cardioplegia; Magnesium; Coronary surgery; Amino acids; ATP; Lactate

1. Introduction

Intermittent antegrade warm blood hyperkalaemic cardioplegia (IAWBC) has been recently proposed [1–3] as an improved method of myocardial protection when compared to intermittent antegrade cold blood cardioplegia during open heart surgery. The reduction in mortality and morbidity associated with the use of IAWBC was attributed to the prevention of myocardial oxidative stress [1,2].

IAWBC utilizes potassium as the only additive. This arrests the heart by partially depolarizing the cardiac myocyte membrane but at the same time may facilitate opening of the L-type calcium-channels [4]. The direct result of this is calcium loading and therefore potential cellular damage [5,6]. Magnesium is known to block the L-type calcium-channels and therefore prevent the rise in intracellular calcium during ischaemia [4,7,8] and is therefore likely to reduce energy demands and preserve intracellular metabolites. There are therefore strong theoretical reasons to support the addition of magnesium to this cardioprotective strategy.

To investigate whether the addition of magnesium to IAWBC improves myocardial protection, the intracellular concentrations of ATP, amino acids and lactate were monitored in left ventricular biopsies taken from patients undergoing routine coronary artery bypass surgery.
2. Methods

Twenty-three patients (19 males, mean age ± SD: 59.7 ± 9.6 years) with a left ventricular ejection fraction greater than 50%, undergoing primary elective coronary artery bypass surgery were randomized to one of two techniques of myocardial protection: (i) intermittent antegrade warm blood hyperkalaemic cardioplegia (IAWBC) or (ii) intermittent antegrade warm blood hyperkalaemic cardioplegia supplemented with magnesium sulphate (IAWBC + Mg). 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 mg/kg fentanyl, and volatile agents were delivered in 50% air–O₂ mixture for maintenance. Propofol (3 mg/kg per h) 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 and two-stage venous cannulation. Target systemic temperatures were between 34°C and 37°C.

The cardioplegic solution was delivered and prepared as described by Calafiore et al.[1,2]. Blood was taken directly from the oxygenator via a 1/4-inch tubing and was infused at 34–37°C into the aortic root by means of a roller pump. A syringe pump containing 50 ml KCl (2 mmol/ml) (IAWBC) or KCl + MgSO₄ (40 ml of 2 mmol/ml KCl + 10 ml of 2 mmol/ml MgSO₄) (IAWBC + Mg) was connected to the 1/4-inch tubing to deliver the cardioplegic solution. Following induction of ischaemic arrest with dose 1, subsequent dosages (2, 3 etc.) were administered on completion of each distal coronary anastomosis (Table 1). All the distal coronary anastomoses were completed during a single period of aortic cross-clamping. Proximal anastomosis were completed on a beating heart using an aortic partial occlusion clamp.

Three myocardial biopsy specimens (4–14 mg wet wt.) were taken from the apex of the left ventricle using a 14 gauge TWX 11.4 cm Cannula ‘Trucut’ needle (Baxter Healthcare, Deerfield, IL). The first biopsy was taken 5 min after institution of cardiopulmonary bypass. The second biopsy was taken after 30 min of ischaemic time, and the third after 20 min of reperfusion. Each specimen was immediately frozen (in the operating theatre) in liquid nitrogen until processing for amino acid, ATP and lactate analysis. All biochemical analyses were performed by an investigator blind to the technique used.

2.2. Amino acids, ATP and lactate

The procedure followed to extract free amino acids, ATP and lactate was similar to that described previously [9]. In brief, frozen biopsy specimens were crushed under liquid nitrogen and the resultant powder (taken as wet wt.) 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 [10]. Lactate was measured using a serum lactate determination kit from Sigma Diagnostics (Sigma, Poole, UK). As the kit was designed for measuring a much higher concentration of lactate compared to the biopsy specimen, only percentage change in lactate is shown in the results section (all samples were paired).

Amino acids in the extracts from both groups were determined according to the Waters Pico–Tag method as reported previously [9]. Essentially, 100 µl of the extract was dried using vacuum centrifugation (Savant SV160, Farmingdale NY). Free amino acids were derivatized using phenylisothiocyanate. The phenylthiocarbamyl derivatized amino acids were separated by HPLC using a 30 cm Pico–Tag column (Millipore, Milford, MA) 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 for A: 132 mM sodium acetate, 470 ml/l triethylamine (pH 6.4),

Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>Blood (ml/min)</th>
<th>KCl (ml/h)</th>
<th>Duration (min)</th>
<th>K⁺ (mEq/l)</th>
<th>Mg⁺ (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>push 2 ml, then 150</td>
<td>2</td>
<td>18–20</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>120</td>
<td>2</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>90</td>
<td>2</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>60</td>
<td>3</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>60</td>
<td>4</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>10</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*These are the concentrations of blood potassium [K] or magnesium [Mg] obtained by infusion of these electrolytes at the corresponding flow rates, and they are independent of the intrinsic blood potassium or magnesium concentrations of each patient.
and 6% acetonitrile. Solvent B was 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 Millennium 2000 software supplied by Waters Millipore (Watford, UK). Chemicals needed to derivatize amino acids and separate them were obtained from Waters Millipore (Watford, UK).

2.3. Data collection and analysis

Values are expressed as mean ± standard error of mean (SEM) unless otherwise stated. Statistical analysis was carried out using repeated measures ANOVA and Bonferroni multiple comparisons test (intragroup analysis) and Mann–Whitney test (intergroup analysis) using InStat and statview packages provided on a Macintosh PC. The correlation matrix was calculated and the significance determined using Fisher’s r to z. The level of statistical significance was taken as 5%.

3. Results

3.1. Clinical outcome

There were no deaths in the series. The clinical information is presented in Table 2. The two groups were similar with respect to sex, age, preoperative ventricular function, extent of coronary disease, ischaemic and cardiopulmonary bypass time, ITU and hospital stay.

3.2. Changes in cellular metabolites during ischaemia and reperfusion

Following ischaemic arrest using either IAWBC or IAWBC + Mg, there was a modest decline in the intracellular concentration of ATP (12% and 16% respectively) (Fig. 1). After 20 min reperfusion the ATP levels decreased markedly and significantly in the IAWBC group but not in the IAWBC + Mg group (48% and 20%, respectively, Fig. 1).

In contrast to the changes in ATP, there was a rise in tissue lactate levels after ischaemic arrest when using IAWBC or IAWBC + Mg. However this was statistically significant for the IAWBC group only. On reperfusion there was a trend for lactate to decrease but remained relatively higher in the IAWBC group (Fig. 2).

Following the period of ischaemia prior to reperfusion, there was no change in the intracellular concentration of the total free intracellular amino acid pool compared to the control biopsy: 36.1 ± 2.2 to 35.0 ± 1.9 μmol/g wet weight, respectively, for the IAWBC group and 34.2 ± 1.8 to 34.8 ± 1.9 μmol/g wet weight, respectively, for the IAWBC + Mg group (Fig. 3). The intracellular free amino acid pool is largely made up of the principal free amino acids, glutamine, glutamate, taurine, aspartate, alanine and asparagine, which constitute more than 90% of the pool. Although there was no overall change in the amino acid pool, individual variations were observed (Tables 3 and 4). A significant increase in the intracellular concentration...
Table 3  
Changes in the intracellular concentrations of amino acids (μmol/g wet wt.) in ventricular biopsies collected from patients undergoing coronary artery bypass surgery using IAWBC in the absence of magnesium (n = 11). The P-values using repeated measures analysis of variance (Bonferroni multiple comparisons test) are shown.
dence for glutamate utilization for energy production is provided by the finding that alanine accumulated, resulting in an increase in alanine/glutamate ratio (Fig. 4). The addition of magnesium prevented a significant rise in lactate. However, alanine rose significantly resulting in an increase in the alanine/glutamate ratio. These observations suggest that the inclusion of magnesium in the cardioplegic solution results in relatively lower anaerobic metabolic activity during ischaemic arrest.

The relatively minor changes seen during ischaemia were markedly accentuated after 20 min of reperfusion with a significant fall in ATP and the free intracellular amino acid pool seen only in the IAWBC group. Amino acids are important for normal cellular function [9,15] and can be utilized for energy production and therefore help the heart during ischaemia/reperfusion. A preservation in these amino acids, as seen in the IAWBC + Mg group, may facilitate recovery following cardiac surgery. Consistent with improved myocardial preservation in IAWBC + Mg group is the finding that ATP and individual amino acids (with the exception of glutamate) were significantly preserved.

A comparison between the two groups further supports the suggestion that the inclusion of magnesium in the IAWBC prevents substrates derangement. With the exception of ATP and taurine, the rest of the substrates were significantly higher in the IAWBC + Mg group after reperfusion.

The data suggest that IAWBC + Mg prevents metabolic derangement on reperfusion. The cardioprotective effect of magnesium is likely to be due to effects on calcium transport. Ataka et al. [4] have found that hyperkalaemic cardioplegia without magnesium does not prevent the rise in intracellular calcium during ischaemia. Hyperkalaemic cardioplegic solutions partially depolarize the membrane and may open the L-type calcium-channels. Elevated intracellular calcium levels will activate a variety of cellular enzymes and transport systems as well as influencing mitochondrial function [5,6,16]. This will lead to increased cellular energy demands, some of which can be met by using amino acids like glutamate (which can also be produced from glutamine) and aspartate [4,9,15]. Magnesium, by reducing calcium loading during ischaemia and reperfusion [4,7,8], will also reduce energy demands and preserve intracellular metabolites.

From the data presented above, it would seem that the addition of magnesium to the protocol of IAWBC is justified, since it preserves intracellular metabolites and reduces metabolic stress in hearts of patients undergoing coronary surgery. Further work is needed to establish whether these changes are translated into better clinical outcome.

Acknowledgements

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Table 4
Changes in the intracellular concentrations of amino acids (μmol/g wet wt.) in ventricular biopsies collected from patients undergoing coronary artery bypass surgery using IAWBC in the presence of magnesium (n = 12). The P-values using repeated measures analysis of variance (Bonferroni multiple comparisons test) are shown.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>IAWBC</th>
<th>Statistical significance</th>
<th>IAWBC + Mg</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ischaemia</td>
<td>Reperfusion</td>
<td>Control vs. ischaemia</td>
</tr>
<tr>
<td>Glutamine</td>
<td>9.3 ± 0.9</td>
<td>9.8 ± 1.0</td>
<td>8.6 ± 0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Taurine</td>
<td>9.0 ± 1.0</td>
<td>9.0 ± 0.8</td>
<td>7.9 ± 0.6</td>
<td>ns</td>
</tr>
<tr>
<td>Glutamate</td>
<td>9.6 ± 0.5</td>
<td>8.3 ± 0.7</td>
<td>7.5 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.07 ± 0.15</td>
<td>3.46 ± 0.24</td>
<td>2.60 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.20 ± 0.11</td>
<td>1.07 ± 0.10</td>
<td>0.99 ± 0.14</td>
<td>ns</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.30 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.28 ± 0.02</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, Not significant.

Fig. 4. Alanine/glutamate ratio calculated for biopsies taken from patients undergoing coronary artery bypass surgery using IAWBC (n = 11) or IAWBC + Mg (n = 12). Biopsy 1 was collected 5 min after institution of cardiopulmonary bypass (ob), biopsy 2 after ischaemic arrest (cb) and biopsy 3 after 20 min of reperfusion (hb). The ratio was first calculated for individual patients and then the mean ± SEM was used in the figure. P-values using repeated measures ANOVA (Bonferroni multiple comparison) test are also shown.
References


