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.

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 (IWHBC) or (ii) intermittent antegrade warm blood hyperkalaemic cardioplegia supplemented with magnesium sulphate (IWHBC+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) 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 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) (IWHBC) or KCl + MgSO4 (40 ml of 2 mmol/ml KCl + 10 ml of 2 mmol/ml MgSO4) (IWHBC + 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.

<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.

2.1. Collection of ventricular biopsies

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 phenylisothiocarbamyl 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),
and 6% acetonitrile. Solvent B was 60% acetonitrile. Deri-
vatized 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 Millen-
nium 2000 software supplied by Waters Millipore (Watford,
UK). Chemicals needed to derivatize amino acids and sepa-
rate 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 car-
rried 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 informa-
tion 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 intracel-
lar 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 mmol/g wet weight,
respectively, for the IAWBC group and 34.2 ± 1.8 to
34.8 ± 1.9 mmol/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 2 |
|-----------------|-----------------|-----------------|
|               | IAWBC           | IAWBC + Mg      |
| Number         | 11              | 12              |
| Age (years)    | 57.2 ± 13.8     | 62.7 ± 5.6      |
| Sex (M/F)      | 10/1            | 9/3             |
| Diabetes       | 0               | 2               |
| Hypertension   | 5               | 1               |
| Previous MI    | 5               | 5               |
| Ejection fraction (%) | 65.0 ± 9.2        | 59.1 ± 7.6      |
| No. of grafts  | 2.72 ± 0.7      | 2.75 ± 0.4      |
| CPB time (min) | 66.5 ± 24.8     | 80.0 ± 14.3     |
| Ischaemic time (min) | 43.4 ± 13.2    | 44.7 ± 5.6      |

Values are expressed as mean ± SD.
of alanine was seen in both groups. In the IAWBC group, the increase in alanine was associated with a significant fall in glutamate. A small non-significant fall in glutamate was also seen in the IAWBC + Mg group.

In contrast to ischaemic arrest, after 20 min reperfusion, there was a marked and significant fall in the intracellular concentration of the amino acids pool in the IAWBC group (Fig. 3). This was due to a fall in glutamine, glutamate, taurine, aspartate and asparagine (Table 3). When IAWBC + Mg was used there was no significant change in the intracellular free amino acids pool (Fig. 3). The increase in the intracellular concentration of alanine seen after ischaemia in both groups was significantly reversed after 20 min reperfusion.

Tissue alanine/glutamate ratio has been used as a marker of ischaemic stress and during anaerobic metabolism, there is a fall in tissue glutamate with a corresponding rise in alanine [9,11–13]. Fig. 4 shows that a significant increase in the alanine/glutamate ratio occurred as a result of ischaemic arrest using IAWBC or IAWBC + Mg, although the increase was lower in the later. On reperfusion the increase in the alanine/glutamate ratio was maintained in the IAWBC group but tended to fall in the IAWBC + Mg group (Fig. 4). If this ratio is indeed a true measure of metabolic stress, then the alanine/glutamate ratios should correlate with ATP or lactate levels. This indeed was the case as there was a negative correlation between ATP and the alanine/glutamate ratio (P < 0.005, Fisher’s r to z), but the correlation was positive between lactate and alanine/glutamate ratio (P < 0.05).

A comparison between the two groups (unpaired analysis using Mann–Whitney test) did not show any differences in the concentration of metabolites in the first biopsy (control). After 30 min of ischaemia the concentration of aspartate was significantly lower (P < 0.05) in the IAWBC group. On reperfusion the concentration of aspartate, glutamate, asparagine, glutamine and amino acid pool was significantly lower in the IAWBC group compared to the IAWBC + Mg group.

4. Discussion

In this work we documented the changes in the intracellular concentrations of ATP, intracellular amino acid pool and lactate in the ischaemic-reperfused myocardium of patients undergoing coronary artery surgery using the technique of IAWBC, with or without the addition of magnesium.

The finding that normothermic arrest using IAWBC was associated with a modest fall in tissue ATP and in the total free intracellular amino acid pool (Fig. 1), supports an early suggestion that this technique is associated with mild ischaemia [1,2]. However, during this period there was a significant rise in tissue lactate which is consistent with anaerobic metabolism. This is further supported by the fall in the amino acid glutamate, known to be utilized for energy production by the ischaemic myocardium [11,12,14].

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

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>IAWBC</th>
<th>Statistical significance P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ischaemia</td>
</tr>
<tr>
<td>Glutamine</td>
<td>10.2 ± 1.0</td>
<td>9.9 ± 0.8</td>
</tr>
<tr>
<td>Taurine</td>
<td>10.6 ± 1.1</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td>Glutamate</td>
<td>9.6 ± 0.5</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.18 ± 0.24</td>
<td>3.52 ± 0.25</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.0 ± 0.1</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.32 ± 0.03</td>
<td>0.32 ± 0.03</td>
</tr>
</tbody>
</table>

ns, Not significant.
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 Control</th>
<th>IAWBC Ischaemia</th>
<th>IAWBC Reperfusion</th>
<th>Statistical significance</th>
<th>Control vs. ischaemia</th>
<th>Control vs. reperfusion</th>
<th>Ischaemia vs. reperfusion</th>
</tr>
</thead>
<tbody>
<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>
<td>ns</td>
<td>ns</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>
<td>ns</td>
<td>ns</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>
<td>&lt;0.001</td>
<td>ns</td>
<td>&lt;0.01</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>
<td>ns</td>
<td>ns</td>
<td>ns</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>
<td>ns</td>
<td>ns</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>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, Not significant.

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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References


