Aspartate improves recovery of the recently infarcted rat heart after cardioplegic arrest

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Abstract

Background: We have previously shown that aspartate improves the tolerance of normal hearts to cardioplegia. The aim of this study was to investigate whether aspartate is also beneficial in the recently infarcted heart. Methods: Myocardial infarction was produced in rats by left coronary artery ligation. Twenty hours later their hearts were perfused on an isolated working rat heart apparatus and underwent cardioplegic arrest for 30 min at 37°C with or without 20 mM aspartate in the cardioplegic solution (n = 11 per group). Functional recovery and myocardial high energy phosphate levels were measured at the end of arrest and after 30 min of reperfusion. Results: There was no difference in pre-arrest pump function between the untreated and aspartate-treated groups. However, after reperfusion the aspartate group generated more power (3.4 ± 0.2 mJ/s per g) than the untreated group (2.5 ± 0.3 mJ/s per g; P < 0.05) such that the percentage recovery of pre-arrest power in the aspartate group (67.7 ± 3.5%) was greater than in the untreated group (53.6 ± 4.9%; P < 0.05). The aspartate group also showed increases in aortic flow and myocardial oxygen consumption compared to the untreated group (P < 0.05). There were no between-group differences in high energy phosphate levels at the end of arrest or after reperfusion. Conclusion: Aspartate improves functional recovery of the recently infarcted heart during cardioplegic arrest, and therefore has potential as a useful adjunct to myocardial protection in patients with recent myocardial infarction undergoing cardiac surgery. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Cardioplegic solutions; Aspartate; Metabolism; Myocardial infarction-surgery

1. Introduction

Coronary bypass surgery in patients operated on within 48 hr of Q-wave myocardial infarction is associated with a higher operative mortality than surgery in such patients operated upon after this time [1]. Similarly, coronary bypass within 1 week of myocardial infarction is associated with more post-operative complications than surgery in post-infarction patients operated upon later in their recovery [2]. An important contributing factor to these unfavourable results may be the adverse response to cardioplegic arrest of the surviving, but stressed, non-infarcted areas of the myocardium. In the first few days after major acute myocardial infarction, the non-infarcted myocardium, remote from the infarct, manifests depressed contractility [3] and biochemical perturbations including decreased levels of glycogen [4] and high energy phosphates [5,6]. Thus, in the presence of recent infarction the surviving myocardium, when subjected to a period of cardioplegic arrest with impaired contractile function and depleted energy stores, is highly vulnerable to ischemic injury.

We, and others, have shown that metabolic supplementation with aspartate in the cardioplegic solution improves the tolerance of normal hearts to global ischemia at normothermia or hypothermia, and that aspartate is more effective than glutamate in this regard [7,8] although other investigators have not shown this effect in neonatal myocardium [9] or under hypothermic conditions [10]. Likely mechanisms...
of action are prevention of ischemia-induced depletion of tricarboxylic acid cycle intermediates and stimulation of the malate-aspartate shuttle. Whether supplementation of cardioplegia with aspartate would be effective in the recently infarcted heart has not been previously investigated. We therefore set out to study, in the rat, the effect of aspartate added to the cardioplegic solution in the setting of recent myocardial infarction.

2. Methods

2.1. Production of myocardial infarction

Anaesthesia was induced in male Munich Wistar rats, weighing 280–380 g, by intra-peritoneal injection of pentobarbitone (15 mg/kg), methohexitone (60 mg/kg) and atropine (0.5 mg/kg). After endotracheal intubation, mechanical ventilation was commenced. A thoracotomy was performed through the left 4th intercostal space and the left coronary artery was ligated. Coronary occlusion was verified by discolouration of the distal myocardium. The thoracotomy was closed and the animal allowed to recover. In pilot studies in 15 rats this procedure produced an infarct of predictable size, 35.5 ± 1.81% of the left ventricle, measured by weighing after nitroblue tetrazolium delineation of the infarct [11]. All animals were treated in accordance with the Code of Practice for Animal Experimentation of the National Health and Medical Research Council of Australia.

2.2. Isolated working heart preparation

Twenty hours after coronary occlusion, under halothane anaesthesia, the heart was rapidly excised and immersed in Krebs Henseleit solution at 4°C. On an isolated working rat heart apparatus [12] the aorta was cannulated and retrograde perfusion commenced for a 15 min stabilisation period with oxygenated (PO2 500 mmHg) Krebs Henseleit bicarbonate buffer at 37°C. During this period, left atrial cannulation was carried out to convert the heart to the working mode. Hearts were then randomly assigned to either the aspartate cardioplegia or the non-aspartate cardioplegia (untreated) group.

2.3. Experimental time sequence

To simulate the events of an open heart operation, the following experimental time sequence of five time periods was employed. (1) Pre-arrest non-working period of 15 min retrograde aortic perfusion at 100 cm H2O in the non-working mode. (2) Pre-arrest working period of 15 min with a left atrial pressure (LAP) of 15 cm H2O and an aortic pressure of 100 cm H2O. (3) Normothermic cardioplegic arrest was induced by ceasing atrial perfusion and infusing a potassium cardioplegic solution for 1 min at 100 cm H2O, with or without 20 mM sodium aspartate. The heart was immersed in the cardioplegic solution and arrest was maintained for 30 min at a myocardial temperature of 37°C. (4) Non-working reperfusion period of 15 min in a retrograde manner with warm, oxygenated perfusate at 37°C and 100 cm H2O. (5) Post-arrest working period of 15 min under the same conditions as in the pre-arrest working period.

At the end of each working period, cardiac function was measured under the standard loading conditions (LAP, 15 cm H2O; aortic column, 100 cm H2O). The power output (rate of performing external work) of the heart was calculated using the formula:

\[ W_p = P_{dev} \times CO \times 0.0022 \]

where \( W_p \) is the power (rate of performing work) in mJ/s, \( P_{dev} \) is the developed pressure in mmHg (systolic aortic pressure minus left atrial pressure) and \( CO \) is the cardiac output in ml/min (aortic flow plus coronary flow). A conversion factor for SI units (0.0022) is included. Five hearts were excluded because they showed very low initial power (less than 2.7 mJ/s).

To determine oxygen consumption, oxygen tension was measured in samples collected from the inflow line (arterial sample) and from the pulmonary artery (venous sample). Oxygen consumption per gram was calculated using the formula [13]:

\[ MV_O_2 = \frac{(P_{O_2} - P_{O_2}) \times 0.024 \times CF \times 1000}{760 \times W} \]

where \( MV_O_2 \) is oxygen consumption in \( \mu \)l/min per g, \( P_{O_2} \) and \( P_{O_2} \) are partial pressures of oxygen in mmHg in the arterial and venous samples respectively, 760 is the barometric pressure in mmHg, \( CF \) is coronary flow in ml/min, and \( W \) is wet heart weight in grams.

Myocardial efficiency was calculated by dividing power (mJ/s per g), by \( MV_O_2 \) (\( \mu \)l/min per g) and expressing the result as a percentage of the expected energy equivalent of complete oxygen combustion, which is 20.97 J/ml of O2 [14]:

\[ \text{Myocardial efficiency (\%)} = \frac{W_p \times 60 \times 100}{MV_O_2 \times 20.97} \]

2.4. Cardioplegic solutions

For the untreated group the cardioplegic solution was modified Ringer’s solution containing 20 mM KCl, 127 mM NaCl and 2 mM CaCl2 at pH 7.4, oxygenated with a mixture of 95% O2 and 5% CO2. In the aspartate group, 20 mM sodium aspartate was added to the solution and the amount of NaCl was decreased to keep the sodium concentration and osmolarity unchanged.

2.5. Tissue sampling and biochemical analysis

The non-infarcted region of each heart (including the
right ventricle) was rapidly freeze-clamped with tongs precooled in liquid nitrogen either at the end of cardioplegic arrest or at the end of reperfusion. Tissue samples were stored in liquid nitrogen. Before assay the frozen tissue was pulverized in a mortar with liquid nitrogen and extracted with ice-cold perchloric acid. Tissue extracts were neutralised and used for spectrophotometric enzymatic assays of ATP, ADP, AMP, phosphocreatine (PCr) and aspartate using standard enzymatic techniques [15] on a Cobas Bio autoanalyser (Roche Diagnostic Systems).

2.6. Statistics

All results are expressed as the mean ± SEM. For biochemical data unpaired t-tests were used to compare aspartate-treated with untreated values. Since the physiological measurement values were not normally distributed, non-parametric tests were used: the Wilcoxon signed rank test for pre- versus post-arrest values, and the Mann–Whitney rank sum test for aspartate-treated versus untreated. P < 0.05 was considered statistically significant.

3. Results

3.1. Physiological measurements

There were no significant differences in any of the pre-arrest measurements between the untreated and aspartate-treated groups (Table 1). In the untreated group cardioplegic arrest produced highly significant decreases in aortic flow, systolic aortic pressure, power, oxygen consumption and efficiency. In the aspartate-treated group, cardioplegic arrest produced similar decreases in power, aortic flow and efficiency but not in aortic pressure or oxygen consumption. After reperfusion, the aspartate group generated more power (3.4 ± 0.2 mJ/s per g) than the untreated group (2.5 ± 0.3 mJ/s per g) (P < 0.05) such that the percentage recovery of pre-arrest power was greater in the aspartate group (67.7 ± 3.5%) than in the untreated group (53.6 ± 4.9%) (P < 0.05) (Fig. 1). There were similar improvements in the aspartate group in absolute values for aortic flow and myocardial oxygen consumption compared to the untreated group (P < 0.05). Percentage recovery of aortic flow after arrest also tended to be greater in the aspartate group than in the untreated group (P = 0.053) (Table 1). There was no difference between the two groups in coronary blood flow or efficiency either before or after arrest (Table 1).

3.2. Biochemical measurements

At the end of cardioplegic arrest myocardial total adenine nucleotide levels (Table 2) were low in both groups compared to values obtained from normal rat hearts placed on the same apparatus and subjected to the same period of normothermic global ischemia under identical conditions in our laboratory [7]. Ischemia caused breakdown of ATP to ADP and AMP with a reduction in energy charge. After reperfusion ATP levels returned toward normal in both groups by reconstitution from ADP and AMP. Phosphocreatine was low at the end of ischemia with a return to normal after reperfusion. There were no between-treatment differences in high energy phosphates either at the end of ischemia or after reperfusion. At the end of cardioplegic arrest there was a higher myocardial level of aspartate in the aspartate-treated group, 54.7 ± 9.6 µmol/g dry wt, than in the untreated group 10.4 ± 5.6 µmol/g dry wt. (P < 0.01), a difference not seen after reperfusion.

4. Discussion

This study shows that recovery of infarcted rat hearts is improved after cardioplegic arrest by the addition of aspartate to the cardioplegic solution. This improvement is of a similar magnitude to our previous observations in normal rat hearts [7]. Despite the improved function, aspartate supplementation was not associated with increases in static levels of myocardial high energy phosphates either at the end of arrest or after reperfusion.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Pre-arrest</th>
<th>Post-arrest</th>
<th>% Recovery</th>
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<tr>
<td></td>
<td>Untreated</td>
<td>Aspartate treated</td>
<td>Untreated</td>
</tr>
<tr>
<td>Aortic flow (m/minute)</td>
<td>19.0 ± 1.6</td>
<td>22.5 ± 1.4</td>
<td>5.7 ± 1.8**</td>
</tr>
<tr>
<td>Systolic aortic pressure (mmHg)</td>
<td>97.8 ± 1.4</td>
<td>97.1 ± 1.4</td>
<td>90.2 ± 2.4*</td>
</tr>
<tr>
<td>Power (mJ/s per g wet wt.)</td>
<td>4.0 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>2.5 ± 0.3**</td>
</tr>
<tr>
<td>Coronary flow (m/minute)</td>
<td>12.7 ± 0.5</td>
<td>12.6 ± 0.7</td>
<td>12.3 ± 0.4</td>
</tr>
<tr>
<td>MVO₂ (µmol/min per g wet wt)</td>
<td>139.3 ± 7.6</td>
<td>145.6 ± 6.6</td>
<td>117.3 ± 6.8**</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>9.3 ± 0.5</td>
<td>9.9 ± 0.3</td>
<td>5.8 ± 0.5*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>247 ± 8.0</td>
<td>267 ± 8.0</td>
<td>229 ± 9.0</td>
</tr>
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*Post-arrest versus pre-arrest: *P < 0.01, **P < 0.001.
†Aspartate treated versus untreated: *P < 0.05.
4.1. Importance of metabolic correction for infarcted hearts prior to cardioplegia

We, and others, have shown that there are many metabolic disturbances in the non-infarcted zone of the recently infarcted heart, in particular, depletion of high energy phosphates [4–6]. We have previously shown in the rat and the dog that, compared with normal hearts, these energy-depleted, recently infarcted hearts show reduced recovery from cardioplegic arrest [4,6,16,17]. We have shown that infarcted hearts enter cardioplegic arrest with depleted energy stores in the surviving myocardium, and that this may compromise recovery from arrest. Thus, patients with post-infarction cardiogenic shock or failed angioplasty, who require cardiac surgery, may benefit from metabolic support to minimize metabolic disturbances in the myocardium and improve post-operative recovery.

The original metabolic supplement for infarcted hearts was glucose-insulin-potassium (GIK). However, conflicting data on the use of GIK for acute myocardial infarction have discouraged its widespread use as metabolic support for this and other cardiac problems [18]. The efficacy of warm induction and warm reperfusion ('hot shot') with blood cardioplegia, supplemented with the amino acid glutamate, in patients with post-infarction cardiogenic shock undergoing coronary bypass has been shown in a non-randomised patient series [19]. However, how much of the favourable effect in this high risk group of patients was attributable to the 'hot shot' itself and how much to the effect of glutamate supplementation was not clear.

Another approach to metabolic supplementation for infarcted hearts is the use of the pyrimidine precursor, orotic acid. In experimental studies [4,16,17] in recently infarcted rat or dog hearts undergoing cardioplegic arrest, orotic acid therapy produced a powerful cardioprotective effect. Treatment with orotate after recent infarction and before urgent surgery, followed by aspartate supplementation of the cardioplegic solution is a logical combination of two forms of metabolic supplementation, and is a therapy that we use in patients with recent infarction undergoing coronary bypass surgery at the Alfred Hospital.

4.2. Mechanisms of aspartate action

There are anaplerotic, anaerobic, and aerobic mechanisms of aspartate action [7] all of which can be shown to operate under certain conditions [20].

The anaplerotic action of aspartate postulated by Russell and Taegtmeyer [21] means that the deficiencies of the tricarboxylic acid (TCA) cycle intermediates known to occur after ischemia can be immediately met within the mitochondria by replenishment of TCA cycle intermediates [22] via transamination of exogenous aspartate to oxaloacetate and conversion to malate [21]. Thus, during ischemia in aspartate-treated hearts the TCA cycle intermediates are well maintained, so that the TCA cycle is prepared to function more efficiently during reperfusion, i.e. ‘priming the pump’. This mechanism is tightly linked with stimulation of the energy-producing reactions in mitochondria coupled to succinate formation and thus with enhancement of anaerobic ATP production [23].

During reperfusion, aspartate may act aerobically by stimulating the operation of the malate-aspartate shuttle and TCA cycle [24]. At the end of cardioplegic arrest, in the present study, the aspartate level was 5-fold higher in the treated group. Presumably this increment reflects a rise of aspartate level both in the extracellular space and in the intracellular fluid. Thus, aspartate would have been able to affect aerobic intracellular metabolism in the myocardium and improve post-operative recovery.

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by transamination of aspartate in the cytoplasm and the resulting amino acid group is transferred to pyruvate to form alanine via an intermediate reaction involving glutamate [22]. However, we found no increase in myocardial ATP at the end of ischemia in the aspartate-treated group. This is in accord with other observations in our laboratory using NMR spectroscopy showing that, although aspartate improved recovery after ischemia, there was no aspartate-induced increase in ATP during ischemia [25]. Thus, an anaerobic action of aspartate is unlikely in the present study.

4.3. Critique of the study

The concentration of aspartate of 20 mM was chosen on the basis of two previous studies of normal rat hearts, one from our laboratory [7] and another from that of Choong [8] showing a marked protective effect of aspartate in the cardioplegic solution at this concentration. In this study we have shown the beneficial effect of aspartate supplementation for cardioplegia in recently infarcted hearts. To bring out differences in recovery between aspartate-supplemented and non-supplemented groups we employed incomplete protection by using a simple cardioplegic solution containing potassium alone and without hypothermia. We did not include a non-infarcted group in the present study. However, in our previous study of normal rat hearts [7] the beneficial effect of aspartate-supplemented cardioplegia (50% recovery of function versus 32% in the control group) was of a similar magnitude to that found in the present study.

The procedure of coronary ligation in our hands produces an infarct of very predictable size [6,16,17]. In the current study a pilot group of coronary ligations produced infarcts of 35.5 ± 1.8% of ventricular weight. In the two groups of the main study, infarct size could not be measured because the majority of the non-infarcted myocardium was removed by freeze clamping for biochemical analysis. However, there was no significant difference in function between the aspartate group and the untreated control group (both reduced to 30% of that in non-infarcted rat hearts in our previous study (16 mJ/s per g) [7]), indicating that infarct sizes were also not different between the two groups.

Such a large infarct places a severe stress on the non-infarcted area (60%) of the left ventricle which is thus called upon to maintain the entire cardiac output. This, in turn, would place stress on metabolic pathways for energy production, pathways which would be likely to benefit from appropriate metabolic supplementation.

Theoretically, aspartate involvement in energy metabolism should result in measurable elevations in myocardial high energy phosphates. However, in the present study, despite their improved functional recovery, aspartate-treated hearts did not exhibit enhanced cellular energy state as evidenced by raised tissue content of adenine nucleotides, phosphocreatine and increased energy charge either at the end of arrest or after reperfusion (Table 2). This can be explained in part by the relatively small ATP depletion after arrest and reperfusion, to 73% of normal in the control group, which may not have been sufficiently severe to bring out the effect of aspartate on myocardial levels of high energy phosphates. Additionally, a small increment of ATP produced by aspartate might be rapidly utilised for more effective myofibril contraction and thus not be detectable in static tissue levels of ATP. Furthermore it is known that myocardial metabolite contents do not necessarily reflect the rate of turnover of metabolic cycles which may be both effectively triggered and coupled by aspartate transamination.
5. Conclusion

We have shown that aspartate protects the recently infarcted heart during cardioplegic arrest and is at least as beneficial in recently infarcted hearts as in normal hearts. Although the mechanism of action of aspartate in the ischemic myocardium was not addressed in this study, we did find that aspartate improved functional recovery by augmenting myocardial energy production mainly during early post-ischemic reperfusion. Given the fact that patients with recent infarction are a high risk group for cardiac surgery, we recommend, and routinely use metabolic therapy with aspartate in our clinical practice in these patients.

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References