The myocardial protective effect of adenosine as an adjunct to intermittent blood cardioplegia during open heart surgery

Ruifang Liu a, Jialin Xing a, Na Miao a, Weiran Li a, Wei Liu a, Yong-Qiang Lai b, Yi Luo b, Bingyang Ji a, *

a Department of Cardiopulmonary Bypass, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, PR China
b Department of Cardiac Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, PR China

Received 15 February 2009; received in revised form 17 May 2009; accepted 16 June 2009; Available online 15 August 2009

Abstract

Background: Although adenosine (ADO) has been shown to have beneficial effects against tissue injury after myocardial ischaemia, the controversy still remains regarding the optimal timing, dose, temperature, method of ADO administration and duration of exposure to the drug. This study investigates the cardioprotective effect of exogenous ADO pretreatment as an adjunct to 1 mmol l⁻¹ ADO cold (12 °C) blood cardioplegia during heart valve replacement surgery. Materials and methods: Thirty patients with rheumatic heart valve disease undergoing heart valve replacement operations were randomly assigned to two groups: group C (n = 15) and group A (n = 15). Patients in group C were the control group and received antegrade cold (12 °C) high-potassium ([K⁺] = 20 mol l⁻¹) institute blood cardioplegia. The patients in group A received 10-min 100 μg kg⁻¹ min⁻¹ ADO pretreatment before application of the aortic cross-clamp and antegrade 1 mmol l⁻¹ adenosine high-potassium ([K⁺] = 20 mol l⁻¹) cold (12 °C) blood cardioplegia. Clinical outcomes were observed before, during and after the operation. Plasma level markers of myocardial damage: cardiac Troponin I (cTnI), creatine kinase (CK-MB) and inflammatory factors (interleukin (IL)-6 and IL-8) were obtained from serial venous blood samples after induction, 5 min after cross-clamp of aorta, 10 min after clamp-off, 1 h after return to ICU and postoperatively 24 h and 48 h. Right atrial samples were harvested before cross-clamp and after clamp-off. Results: Heart valve replacement was successful in all patients. There were no differences regarding operative parameters in the two groups. Time to arrest (during cardioplegia perfusion electrocardiography (ECG) change to a line) was shorter in group A compared to group C (19.9 ± 4.6 s vs 29.3 ± 10.6 s; p = 0.03). Group A also had lower cTnI and IL-8 levels (p = 0.03) at 10 min after aortic declamping, and lower IL-6 (p = 0.04) at 24 h postoperatively as well. Ultrastructural changes were lighter in group A than group C after clamp-off. Compared to group C, post-reperfusion biopsies in group A displayed only slight overall ultrastructural changes, and scored significantly better on mitochondrial damage (group A 2.23 ± 0.65 vs group C 2.85 ± 0.66) (p = 0.04). Conclusion: Compared with simple cold blood cardioplegia in heart valve replacement patients, ADO pretreatment as an adjunct to 1 mmol l⁻¹ ADO cold blood cardioplegia may reduce cTnI, IL-6 and IL-8 release, resulting in reduced myocardial injury in ultrastructure after surgery.

Keywords: Adenosine; Cardiopulmonary bypass; Myocardial protection; Ischaemia reperfusion injury

1. Introduction

Myocardial ischaemia and reperfusion injury after heart surgery is frequently observed and is associated with increased morbidity and mortality. Inadequate myocardial protection is the key issue in myocardial dysfunction after cardiopulmonary bypass (CPB) procedure. Although new methods and strategies of myocardial protection have been developed and adopted in clinical work, the optimal method of myocardial protection is still uncertain. In our previous study, the results showed the cardioprotective effect of ischaemia preconditioning (IPC) on the myocardium [1]. However, the limitation of IPC with a clamp in clinical practice still needs to be cautioned, since reports exist showing that the risk of stroke is increased during clamp ‘on or off’ if there is calcification in the ascending aorta [2]. Recently, there has been an attempt to find a pharmacologic way to create the IPC effect with adenosine (ADO) instead of cross-clamping the aorta. Several experimental studies have now demonstrated that A₁, A₂a and A₃ ADO receptors are involved in the endogenous cardioprotective response as their activation reproduces the infarct-limiting effect of ischaemic preconditioning, whereas protection is lost when the receptors are blocked [3]. It was also well documented that heart surgery with CPB induced a cascade of events resulting in systemic inflammatory response syndrome (SIRS)
The leakage of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) [5], interleukin (IL)-6 and IL-8 [6], is related to the postoperative myocardial dysfunction. It is believed that an inflammatory response plays an important role in myocardial ischaemia and reperfusion injury. More evidences have shown that ADO could reduce cardiac TNF-α production following ischaemia and reperfusion [7]. ADO may inhibit the release of the pro-inflammatory cytokines (IL-6 and IL-8) involved in the response to ischaemia and reperfusion [8]. This may represent a novel anti-inflammatory property of ADO by which it could reduce inflammatory response and limit reperfusion injury. The present prospective, randomised study was designed to investigate the effect of combination ADO pretreatment before application of the aortic cross-clamp and antegrade 1 mmol l\(^{-1}\) ADO high-potassium cold (12 °C) blood cardioplegia on myocardial recovery and inflammatory response in patients undergoing heart valve replacement operations.

## 2. Materials and methods

### 2.1. Patient selection

The study was approved by the Beijing Anzhen Hospital, Beijing, China. Before surgery, every patient participating in this investigation gave informed written consent. Thirty patients with rheumatic heart valve disease receiving valve replacement surgery were prospectively randomised into control group (group C) and ADO group (group A). Patients with co-morbidities of coronary heart disease, severe non-cardiac diseases, hypertension, diabetes mellitus, congenital heart disease or who were undergoing a repeat operation were excluded as well.

### 2.2. Operation method

All patients were operated on by the same surgical team in the Beijing Anzhen Hospital, including surgeons, an anaesthesiologist and a perfusionist. The cardiologists in the intensive care unit (ICU) were blinded to group allocation in this investigation.

### 2.3. Anaesthesia and monitoring

The left radial artery and the right internal jugular vein were catheterised for haemodynamic monitoring. The electrocardiogram and temperature were also monitored. Anaesthesia was induced by intravenous administration of fentanyl citrate and vecuronium bromide and was maintained with intravenously administered propofol and inhalation of isoflurane. No inhaled anaesthetic was used throughout the procedure. Electrocardiogram and temperature were also monitored. Anaesthesia was induced by intravenous administration of fentanyl citrate and vecuronium bromide and was maintained with intravenously administered propofol and inhalation of isoflurane. No inhaled anaesthetic was used throughout the procedure. In addition, fentanyl was infused at a rate of 10 μg kg\(^{-1}\) min\(^{-1}\) and vecuronium at a dose of 0.05 mg kg\(^{-1}\) before CPB. During CPB, fentanyl was infused at a rate of 10 μg kg\(^{-1}\) min\(^{-1}\) and vecuronium at a dose of 0.05 mg kg\(^{-1}\) before CPB. During CPB, fentanyl was infused at a rate of 10 μg kg\(^{-1}\) min\(^{-1}\) and vecuronium at a dose of 0.05 mg kg\(^{-1}\). Anaesthesia was maintained by intermittent low-potassium ([K\(^+\)] = 10 mmol l\(^{-1}\)) cold (12 °C) blood institutional cardioplegia without enrichment.

### 2.4. Surgery procedure and CPB management

The surgical techniques were standardised in all cases. Following routine mid-sternotomy, CPB was started under full heparinisation (4 mg kg\(^{-1}\) heparin) and when ACT reached 480 s. The heart–lung machine (Jostra, Munich, Germany) with roller pump and affinity NT oxygenators (Medtronic, Minneapolis, MN, USA) was used; tubing pack with cardioplegia delivery (blood:crystalloid = 4:1) system (Kewei, Dongguan, China), and an arterial filter (Medos, Beijing, China) were employed. Continuous non-pulsatile CPB was adjusted at a flow rate of 2.4—2.6 L min\(^{-1}\) m\(^{-2}\) at 30 °C. Mean arterial pressure was maintained at 50—80 mmHg during CPB.

### 2.5. Myocardial protection

Fifteen patients in the control group (group C) received regular institutional high-potassium ([K\(^+\)] = 20 mmol l\(^{-1}\)) cold (12 °C) blood cardioplegia. Fifteen patients in the ADO group (group A) received a 10 min 100 μg kg\(^{-1}\) min\(^{-1}\) ADO pre-treatment immediately before application of the aortic cross-clamp and antegrade 1 mmol l\(^{-1}\) ADO high-potassium cold (12 °C) blood cardioplegia after clamp-on. All patients received routine blood cardioplegia delivered through the antegrade route. The two groups had cardioplegia arrest maintained by intermittent low-potassium ([K\(^+\)] = 10 mmol l\(^{-1}\)) cold (12 °C) blood institutional cardioplegia without enrichment, which was re-infused every 30 min. The route of delivery was exclusively antegrade. The initial dose of cardioplegia was 20 ml kg\(^{-1}\) of body weight, and each subsequent dose was half of the initial dose.

### 2.6. Laboratory assay

Plasma level markers of myocardial damage, cardiac Troponin I (cTnI), creatine kinase (CK-MB) and inflammatory factors (IL-6 and IL-8), were obtained from serial venous blood samples after induction, 5 min after cross-clamp of aorta, 10 min after clamp-off, 1 h after return to the ICU and postoperatively 24 h and 48 h. All samples were anticoagulated with ethylenediaminetetraacetic acid, immediately cooled in 4 °C and centrifuged within 30 min (4000 \(\times\) g for 10 min); plasma was transferred to polypropylene test tubes and stored at –70 °C until assay. The IL-6 and IL-8 levels in the plasma were determined using a commercially available enzyme-linked immunosorbent assay. The concentrations of cTnI and CK-MB were measured by a specifically developed immunoenzymometric assay that has been described in our previous study [1]. The IL-6 and IL-8 levels were measured using an enzyme-linked immunosorbent assay that has been described in a previous trial [9].

### 2.7. Ultrastructure analysis of myocardium samples

Samples of the right atrium were obtained before clamp-on and after clamp-off from a total of eight patients randomly selected from either group. Samples were immediately fixed in neutral-buffered 10% formalin, embedded in paraffin and cut in 5-mm sections for histological analysis. Ultrathin sections (15 for each biopsy) were mounted on copper grids, stained with uranyl acetate and lead citrate and examined under a JEOL-1200 Japan, electron microscope. Electron micrographs were taken systematically at 5000 \(\times\) magnification to permit comparative evaluation of pre-clamp-on and post-clamp-off biopsy.
samples from both groups. Images were analysed by two experienced investigators unaware of the sequence of sampling and of patient clinical data. Mitochondrial damage was scored, assigning a numerical value of 0 through 4 to each mitochondrion, according to the degree of morphologic alterations [10]. Grading scale was 0 = normal; 1 = initial swelling (separation of cristae, decreased matrix density); 2 = more marked swelling than in grade 1; 3 = massive swelling with architectural disruption; and 4 = findings as in grade 3, plus rupture of inner and outer mitochondrial membranes. The average obtained from two observers was expressed for each grade as a percentage of the total number of mitochondria counted per sample. Approximately 20 mitochondria per sample were graded.

2.8. Statistical analysis

Statistical analysis was performed using software (SPSS for Windows, Version 10.0; SPSS, Inc., Chicago, IL, USA). The Mann–Whitney U-test was used to distinguish demography differences between the groups. Continuous variables were analysed by analysis of variance (ANOVA) for repeated measures. The preoperative values were taken as co-variables, and changes in preoperative values were assessed. Statistical significance was attributed to p-values <0.05. All results are expressed as mean ± standard deviation (SD).

3. Results

3.1. Preoperative parameters

The major preoperative variables were similar in both the groups (Table 1). There were no significant differences between the mean values of age, sex ratio, body weight, left ventricular ejection fraction, the New York Heart Association class, and preoperative medication used between the two groups.

3.2. Intra-operative data and surgery outcome

There were no operative deaths (to 1 month postoperatively) in either group. No differences were observed in either group with regard to postoperative incidences of major complications. In the operative data, there was no significant difference between the two groups in CPB time, clamp time, total amount of cardioplegic solution and the lowest temperature during bypass and auto-resuscitation. However, time to arrest (during cardiolegia perfusion ECG change to a line) was shorter in group A compared with that in group C (19.9 ± 4.6 s vs 29.3 ± 10.6 s; p = 0.03). Although the time of postoperative mechanical ventilation was shorter in group A than in group C, the difference did not reach statistical significance (19.6 ± 9.6 h vs 15.1 ± 3.8 h; p > 0.05). ICU stay was similar for both the groups. Essential data on operation and postoperative recovery are presented in Table 2.

3.3. CK-MB and cTnI

Before induction, cTnI and CK-MB in both groups were baseline. After CPB, the levels of cTnI and CK-MB in both groups increased indicating myocardial injury. Compared with group C, group A had lower cTnI (p = 0.03) at 10 min after aortic declamping. There was no difference between the groups in CK-MB at any point in time (Figs. 1 and 2).

---

**Table 1**

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>Group A (N = 15)</th>
<th>Group C (N = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>49.8 ± 12.7</td>
<td>48.0 ± 12.2</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/7</td>
<td>7/8</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>63.4 ± 8.8</td>
<td>62.9 ± 13.4</td>
<td>NS</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.72 ± 0.3</td>
<td>1.69 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>CTR</td>
<td>0.56 ± 0.07</td>
<td>0.58 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61.5 ± 7.2</td>
<td>60.3 ± 11.7</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA</td>
<td>3.3 ± 0.5</td>
<td>3.1 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>MVR</td>
<td>8</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>MVP</td>
<td>4</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>AVR</td>
<td>3</td>
<td>2</td>
<td>NS</td>
</tr>
</tbody>
</table>

BSA: body surface area; CTR: ratio of cardiac/thoracic; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; MVR: mitral valve replacement; MVP: mitral valve plasty; AVR: aortic valve replacement; NS: not significant.

---

**Table 2**

<table>
<thead>
<tr>
<th>Operative data and postoperatively data</th>
<th>Group A (N = 15)</th>
<th>Group C (N = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB time (min)</td>
<td>94 ± 9</td>
<td>91 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Clamp time (min)</td>
<td>62 ± 22</td>
<td>65 ± 28</td>
<td>NS</td>
</tr>
<tr>
<td>Time to arrest (s)</td>
<td>19.9 ± 4.6</td>
<td>29.3 ± 10.6</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Lowest rectal temperature (°C)</td>
<td>31.2 ± 1.4</td>
<td>31.5 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Auto-resuscitation</td>
<td>8/15</td>
<td>11/15</td>
<td>NS</td>
</tr>
<tr>
<td>Doses of dopamine, μg kg⁻¹ min⁻¹ (24 h)</td>
<td>433.3 ± 176.7</td>
<td>474.0 ± 313.6</td>
<td>NS</td>
</tr>
<tr>
<td>IABP support during or after operation</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Intubation time (h)</td>
<td>15.1 ± 3.8</td>
<td>19.6 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>ICU stay (h)</td>
<td>20.3 ± 4.6</td>
<td>22.8 ± 4.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

CPB: cardiopulmonary bypass; IABP: intra-aortic balloon pumping; ICU: intensive care unit; NS: no significant.

---

Fig. 1. Cardiac troponin (cTnI) concentration time courses in group A and group C.
3.4. IL-6 and IL-8

Plasma levels of IL-6 and IL-8 increased after reperfusion, compared with baseline. Compared with group C, group A had lower IL-8 levels ($p = 0.03$) at 10 min after aortic declamping and lower IL-6 ($p = 0.04$) at 24 h postoperatively as well (Figs. 3 and 4).

3.5. Electron microscopy studies and quantitative analysis

Biopsies taken before clamp-on showed, as expected, that the large majority of mitochondria in both the groups had minimal signs of injury. Normal or just slightly altered structure (grades 0—1) characterised the mitochondria in both the groups, and there was no difference in the scores of mitochondria between the two groups (group A 1.175/C0 0.81 vs group C 1.143/C0 0.43; $p = 0.06$) before clamp-on. Mitochondrial injury developed during post clamp-off, as reflected by the decrease of the proportion of mitochondria showing normal morphology, and the concomitant increase of the number of mitochondria showing higher scores in both the groups. In this case, the two groups showed distinct differences (group A 2.23 ± 0.65 vs group C 2.85 ± 0.66; $p = 0.04$) (Fig. 5).

4. Discussion

The results of the present study have shown that using a combination 10-min 100 μg kg$^{-1}$ min$^{-1}$ ADO pretreatment with antegrade 1 mmol L$^{-1}$ ADO cold (12 °C) blood high-potassium cardioplegia resulted in less cTnI and inflammatory factors (IL-6 and IL-8) release, and scored significantly better on mitochondrial damage. These results confirmed previous clinical reports [11—17] that ADO is a cardioprotective agent during cardiac surgery.

In the literature, there is considerable experimental evidence to prove that ADO reduces both myocardial stunning and infarct size in different species [18,19]. Although the exact mechanism underlying the cardioprotective effect of ADO is not clear, it has been demonstrated that A$_1$, A$_2a$ and A$_3$ ADO receptors are involved in the endogenous cardioprotective response as their activation reproduces the infarct-limiting effect of IPC [20]. ADO A$_1$ receptors and possibly A$_3$ receptors are also known to confer protection through inhibitory G-protein-coupled pathways, which in...
some instances have been linked to the opening of sarcolemmal ATP-sensitive K⁺ channels [21]. Moreover, ADO A₁ receptor activation has been linked to new cardioprotective targets, including the mitochondria. ADO’s ability to activate the A₁ receptor subtype, slow the sinoatrial nodal pacemaker rate, delay atrioventricular nodal impulse conduction and reduce atrial contractility all contribute to arresting the heart.

However, the controversy over the benefits of using ADO as a cardioprotective agent during clinical setting still remains in the literature, because of the varied ADO administration protocols that were applied in different clinical experimental designs. According to the ADO administration protocols listed in Table 3 [11—17,22—25], several factors, including the timing, dose, concentration of ADO administration, temperature of the blood cardioplegia and duration of exposure to the drug, may have an impact on the final results. In our pilot study, we found the dose with a 10-min 100 μg kg⁻¹ min⁻¹ pretreatment could be well tolerated by the patients; and antegrade 1 mmol L⁻¹ ADO high-potassium ([K⁺] = 20 mol L⁻¹) cold (12 °C) blood cardioplegia could arrest the heart faster than regular blood cardioplegia. In the pilot study, ADO was not tolerated well by the patients during the post-reperfusion period. Owing to safety concerns, therefore, we applied the protocol in this present study. It has been demonstrated that ADO acts on adenosine A₁ receptors to increase the potassium permeability of atrial and sinus node tissues, resulting in an outward potassium flux that hyperpolarises the membrane potential and causes both inhibition of atrial activity and atrioventricular block. This could be responsible for a more rapid arrest of ventricular contraction than potassium alone, which is in agreement with our results in this study.

The present results are also in line with the previous indications that ADO treatment exerts a myocardial protective effect against ischaemic reperfusion injury, as combining ADO pretreatment with 1 mmol L⁻¹ ADO cold blood cardioplegia resulted in lower release of cTnI at 10 min after clamp-off, and a better mitochondria score. Superior arrest and protection may also be related to the coronary vasodilatory properties of ADO, resulting in an increased coronary artery blood flow during reperfusion, which may be beneficial not only for increasing oxygen and substrate delivery but also for enhancing the washout of toxic products of ischaemia. ADO pretreatment reduces experimental myocardial infarct size, and there is additional evidence that reperfusion ADO treatment may also reduce infarct size by its ability to reduce platelet and neutrophil adherence to coronary endothelium [20]. For safety considerations, although we did not apply post-ADO treatment, we still found that IL-6 and IL-8 in group C were higher than in group A post-operatively at 24 h and 10 min after clamp-off, respectively, it still could imply that ADO has a potential impact on ischaemia—reperfusion injury with simple pretreatment. Moreover, the cytokine assays applied have an inherent sensitivity limit. Cytokines are characterised by tight gene control, short duration of action and an autocrine or paracrine rather than an endocrine mode of action, as opposed to hormones, and thus affect only the immediate environment. Systemic plasma cytokine levels may thus not properly reflect local cytokine production.

5. Limitation

Although we found significantly different results between group A and group C in terms of myocardial injury, there are still a few limitations on this study: (1) Because of our research budget, we did not use the Swan—Ganz catheter to monitor the haemodynamic data; therefore, we were limited in recording changes between the two groups in ventricular
function. (2) We did not apply ADO post-treatment after reperfusion, because ADO was not tolerated well by the patients in the pilot study. Although there were some differences between the two groups, we did not find any significant differences in terms of clinical data postoperatively. This may be because the groups were small and the patients were at low risk.

6. Conclusion

The present studies have demonstrated that ADO has the potential to enhance the efficacy of cardioplegic arrest. Compared with simple cold blood cardioplegia in heart valve replacement patients, ADO pretreatment as an adjunct to 1 mmol L \(^{-1}\) ADO cold blood cardioplegia may reduce cTnI, IL-6 and IL-8 release, resulting in less myocardium injury in ultrastructure after surgery.

References


