Adenosine-Supplemented Blood Cardioplegia Attenuates Postischemic Dysfunction After Severe Regional Ischemia

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Background—Various studies have reported that the administration of adenosine (ADO) in cardioplegia reduces myocardial ischemic injury, but this timing may not utilize ADO’s potential against myocardial reperfusion injury. This study tested the hypothesis that ADO-supplemented blood cardioplegia (BCP) or ADO administered during reperfusion reduces postischemic dysfunction after severe regional ischemia.

Methods and Results—After 75 minutes of left anterior descending coronary artery occlusion, total cardiopulmonary bypass was initiated; cold (4°C) antegrade BCP (8:1 blood:crystalloid) was delivered every 20 minutes for the first 3 doses, and 27°C BCP was delivered for the terminal infusion. Dogs (n=6 per group) received unsupplemented BCP, ADO (100 μmol/L/L) supplemented in all infusions of BCP (ADO-CP), or ADO (100 μmol · L⁻¹ · L⁻¹) supplemented only in the terminal infusion of BCP followed by intravenous ADO (140 μg · kg⁻¹ · min⁻¹) infusion for the first 30 minutes of reperfusion (ADO-R). Postischemic regional systolic shortening was significantly greater in the ADO-R group (5±2.0%) than in the BCP group (−3±1.0%), but not in the ADO-CP group (2±0.2%). Postischemic regional diastolic stiffness in the area at risk during end reperfusion was lower with ADO-R (1.8±0.3%) than with ADO-CP (2.7±0.3%) or BCP (4.4±0.5%). Infarct size was reduced in the ADO-CP (29±2%) and ADO-R (21±2%) groups compared with the BCP group (42±4%). Edema in the myocardial area at risk was decreased in the ADO-CP (82±0.2%) and ADO-R (80±0.4%) groups compared with the BCP group (86±0.7%). Adherence of fluorescently labeled neutrophils (PMNs) to postischemic coronary artery endothelium was attenuated by ADO-R (55±2 PMNs/mm²), but not by ADO-CP (114±5 PMNs/mm²), compared with BCP (118±3 PMNs/mm²).

Conclusions—The results show that BCP supplemented with ADO reduces infarct size, preserves postischemic systolic and diastolic regional function but does not attenuate coronary artery endothelial dysfunction unless administered during reperfusion. (Circulation. 1999;100[suppl II]:II-376–II-383.)

Key Words: adenosine ■ cardioplegia ■ myocardial protection

Current myocardial protective strategies during coronary artery bypass grafting (CABG) for high risk surgical patients, such as patients with congestive heart failure, low ejection fraction, cardiogenic shock, an acute myocardial infarction, or transplant patients, are evolving and still controversial. The endogenous autacoid, adenosine (ADO), has been shown to have a broad spectrum of physiological cardioprotecive effects when delivered as a pretreatment agent (chemical preconditioning),¹ during ischemia (cardioplegic arrest),²⁻³ or during reperfusion.⁴⁻⁵

The cardioprotective effects exerted during pretreatment and ischemia involve, in part, metabolic changes and hyperpolarization of ATP-sensitive potassium (KATP) channels,⁶ mediated through A₁ receptor mechanisms.⁷ The cardioprotective mechanisms exerted during reperfusion involve the inhibition of neutrophil-mediated injury, primarily by A₂a receptor processes.⁵⁻⁸

Historically, cardioprotective strategies used to attenuate ischemia-reperfusion injury following cardiac surgery have concentrated on the reduction in ischemic injury during the cardioplegic arrested interval. Strategies designed to increase myocardial ischemic tolerance, which may consequently lead to a reduction in reperfusion injury, have focused on the alteration of cardioplegia constituents (eg, glutamate, aspartate, ADO), the temperature of cardioplegia delivery (warm versus cold cardioplegic infusion), and the method of cardioplegia delivery (antegrade versus retrograde and intermittent versus continuous infusion). In a canine model, Hudspeth et al² investigated ADO (400 μmol · L⁻¹ · L⁻¹) as an adjunct to a standard hypothermic, hyperkalemic blood cardioplegia (BCP) in hearts subjected to 30 minutes of normothermic global ischemia before 60 minutes of total cardiopulmonary bypass. ADO-enriched BCP reversed postischemic systolic...
dysfunction when compared with unsupplemented BCP, and this protection was inhibited with the nonspecific ADO antagonist, 8-p-sulfophenyltheophylline. However, ADO used as an adjunct to cardioplegia solutions may not utilize the purine’s potential to inhibit reperfusion injury.

Because ADO has potent inhibitory effects against reperfusion injury, particularly in reducing neutrophil activation and neutrophil-mediated damage, the present study tested the hypothesis that ADO administered as an adjunct to hypothermic cardioplegic arrest or solely during reperfusion would attenuate postcardioplegic myocardial and endothelial injury. Specifically, this study investigates the reduction in ischemia-reperfusion injury following cardiopulmonary bypass by pharmacologic interventions when delivered during the early reperfusion period.

Methods

Surgical Procedure

The dogs were handled in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). The Institutional Animal Care and Use Committee of Emory University approved the study protocol.

Eighteen heartworm-free adult dogs of either sex were anesthetized with sodium pentobarbital (20 mg/kg) and endotracheally intubated. Anesthesia was supplemented with fentanyl citrate (0.3 µg·kg⁻¹·min⁻¹) and diazepam (0.03 µg·kg⁻¹·min⁻¹) administered intravenously as needed to maintain deep anesthesia. Each dog was ventilated with a volume-cycled respirator using oxygen-enriched room air. Serial arterial blood gases were measured to maintain the arterial oxygen tension >100 mm Hg. Arterial carbon dioxide tension was maintained between 30 and 40 mm Hg by adjustment of the ventilatory rate, and pH was adjusted between 7.35 and 7.45 with intravenous sodium bicarbonate. A rectal temperature probe was inserted to measure core body temperature. The right femoral artery and vein were cannulated with polyethylene catheters for arterial blood sampling and for fluid administration.

After median sternotomy, the superior and inferior vena cava were looped with umbilical tapes, the aygous vein ligated, and the heart suspended using a pericardial cradle. Millar catheter-tipped pressure transducers (Millar Instruments) were placed in the proximal aorta and in the left ventricular cavity. A 1-cm portion of the left anterior descending (LAD) coronary artery distal to the first diagonal branch was dissected and loosely encircled with 2-0 silk suture, which was loosened before the second cardioplegic infusion. After 60 minutes of cardiopulmonary bypass, aortic perfusion and reperfusion was continued in the working state for 30 minutes. The experiment was terminated with a bolus of intravenous sodium bicarbonate to maintain the pH between 7.35 and 7.45.

Experimental Protocol

The dogs were randomly assigned to 1 of 3 groups (n=6 in each group) based on the drug regimen used. One group received standard BCP without ADO supplementation. In the second group, BCP was supplemented with 100 µmol·L⁻¹·L⁻¹ ADO (ADO-CP). The third group received ADO (100 µmol·L⁻¹·L⁻¹) only in the terminal warm cardioplegic infusion and as an intravenous infusion (140 µg·kg⁻¹·min⁻¹) for 30 minutes beginning 10 minutes before release of the aortic cross-clamp (ADO-R).

Data Acquisition

Analog hemodynamic and cardiodynamic data were sampled by a personal computer using an analog-to-digital converter (Data Translation). The data were captured, stored, and analyzed using SPECTRUM cardiovascular acquisition and analysis software (Wake Forest University, Winston-Salem, NC). Measurements were taken before coronary artery occlusion (baseline), after 75 minutes of LAD occlusion, 30 minutes of empty beating reperfusion, and every 30 minutes during the 90 minutes of reperfusion in the working state. Hemodynamic and cardiodynamic data were averaged, and output was obtained from no fewer than 10 cardiac cycles. Percent systolic shortening, segmental work, and the characteristics of segmental stiffness were determined as previously described.

Cardiac Microdialysis for Purine Concentration

A microdialysis probe (CMA/20 Microdialysis AB) was implanted to a mid-myocardial depth in the myocardial AAR and was continuously perfused with Krebs-Henseleit buffer at a rate of 2.0 µL/min. Dialysate samples were collected continuously throughout the experimental protocol, and purine concentration in the dialysate was analyzed by high performance liquid chromatography as previously described.

Plasma Creatine Kinase Activity

Arterial blood samples for measuring creatine kinase activity were analyzed spectrophotometrically (CK-10 kit, Sigma Diagnostics) and protein concentration (Sigma Diagnostics). Creatine kinase activity was expressed as international units per gram of protein.

Determination of AAR and Infarct Size

After postexperimential excision of the heart, the myocardial AAR (Unisense blue dye) and infarct size (1% triphenyltetrazolium chloride [TTC, Sigma Chemical]) were determined as previously
The AAR was calculated as the sum of the weights of the infarcted and necrotic tissue within the AAR, divided by the weight of the left ventricle (AAR/LV) and expressed as a percentage. The infarct size (area of necrosis, AN) was calculated as the weight of necrotic tissue divided by the weight of the left ventricle (AN/LV) or the area of risk (AN/AAR) and expressed as a percentage.

**Determination of Myocardial Edema**

Postexperimental myocardial tissue samples weighing ~0.3 grams were taken from the nonischemic zone and from the nonnecrotic and necrotic areas of the ischemic zone were blotted, weighed, and desiccated for 48 hours. Percent myocardial water content was defined as: (weight-dry weight)/(weight)×100.

**Cardiac Myeloperoxidase Activity**

Tissue samples weighing ~0.4 grams were taken from the nonischemic zone and from the nonnecrotic and necrotic areas of the AAR for spectrophotometric analysis of myeloperoxidase activity as an assessment of neutrophil accumulation in myocardium as described previously.12 Myeloperoxidase activity was described as the rate of hydrogen peroxide degradation–induced color change per min per 100 mg tissue.

**Neutrophil Isolation**

Arterial blood was withdrawn immediately after femoral artery cannulation, and neutrophil isolation was performed using the Ficoll-Paque (Sigma Chemical) density gradient technique previously described.8 The isolated cell preparation contains >95% fluorescently labeled neutrophils (PMNs) and cell viability is >90% (trypan blue exclusion).3

**Neutrophil Adherence to Coronary Artery Endothelium (Basal Endothelial Function)**

The adherence of unstimulated neutrophils to canine epicardial coronary arteries was assessed using isolated neutrophils labeled with Zynaxis PKH26 vital fluorescent dye (Zynaxis Cell Science) as previously described.8 The isolated cell preparation contains >95% fluorescently labeled neutrophils (PMNs) and cell viability is >90% (trypan blue exclusion).3

**Statistical Analyses**

The data were analyzed by 1-way ANOVA or repeated measures 2-way ANOVA of group, time, and group-time interactions. If significant interactions were found, Tukey’s or Student-Newman-Keuls post hoc multiple comparisons tests were applied to locate the sources of differences. P<0.05 was considered significant; mean±SEM are reported.

**Results**

**Myocardial Temperature and Delivery of BCP Solution**

The second cardioplegia infusion was significantly less than the induction volume in each group, whereas the terminal cardioplegia infusion volume was greater than intermittent cardioplegia volumes (Table 1). There were no group differences in BCP delivery within each time point.

Myocardial temperature was not statistically different among BCP (11±1°C), ADO-CP (12±2°C), or ADO-R (11±1°C) groups during the 3 cold BCP infusions, and there were no group differences during the terminal infusion (27±1°C, 31±1°C, and 29±1°C, respectively) (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Blood Cardioplegia Delivery Volume and Myocardial Temperature During Cardioplegic Arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCP volume, mL</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>ADO-I</td>
</tr>
<tr>
<td>ADO-R</td>
</tr>
<tr>
<td>Myocardial temperature, °C</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>ADO-R</td>
</tr>
</tbody>
</table>

BCP delivery volume and myocardial temperature during the 60-min cardioprotective arrest interval. Values are mean±SEM. At each interval, statistically significant differences in blood cardioplegia delivery volumes or myocardial temperature among groups were not achieved.

**Interstitial Dialysate Purines**

Interstitial dialysate ADO in the AAR increased during the first 20 minutes of ischemia and then decreased thereafter to baseline concentrations (Figure 1A). During hypothermic cardioplegia, dialysate ADO concentrations remained near baseline levels in all groups, even when 100 μmol/L ADO was included. There was a slight increase in dialysate ADO concentration in all 3 groups during the initial 10 minutes of reperfusion, which tended to taper to baseline concentrations. There was a similar temporal profile in dialysate inosine concentrations (Figure 1B). There were no group differences at any time in dialysate ADO or inosine. Similar profiles were observed with hypoxanthine and xanthine without group differences (data not shown). In addition, there were no group differences in total purines during infusion of cardioplegia or during reperfusion independent of presence of ADO (Figure 1C).

**Hemodynamic Parameters**

Heart rate was significantly increased after ischemia and during reperfusion compared with baseline in all 3 experimental groups (Table 2). Mean arterial pressure in all 3 groups was significantly less during reperfusion compared with baseline and ischemia time points (Table 2). Left ventricular end-diastolic pressure (LVEDP) was not statistically significant in time-time or group-time relationships (Table 2). There were no statistical differences in heart rate, mean arterial pressure or left ventricular end-diastolic pressure among groups.

**Cardiodynamic Function**

Hearts administered unsupplemented BCP exhibited poor recovery of percent systolic shortening in the AAR throughout reperfusion (Table 3). Although not statistically significant, systolic shortening in the ADO-CP group (2±0.2%) showed a trend toward improved recovery at the end of reperfusion compared with the BCP group (−3±1%). In contrast, when ADO was added only to the last cardioplegic infusion and administered during initial reperfusion (ADO-R), recovery of systolic shortening at the end of reperfusion...
was significantly greater than the BCP group (5±2% versus
−3±1%, respectively). Although systolic shortening in the
ADO-R hearts was greater than that in the ADO-CP group
(5±2% versus 2±0.2%, respectively) at the end of reperfu-
sion, this did not reach statistical significance (P=0.275).

Segmental work (mm Hg×mm) showed similar changes as
seen with systolic shortening (Table 3). Segmental work in
BCP hearts recovered poorly throughout reperfusion. In
contrast, hearts which received ADO-CP showed improved
recovery of segmental work throughout reperfusion, with
significantly greater segmental work at 90 minutes of working
beating reperfusion compared with the BCP group (13±7
versus −13±5, respectively). In the ADO-R group, the
recovery of segmental work at 90 minutes of reperfusion
was also significantly greater than the BCP group (35±6 versus
−13±5, respectively). There was a trend toward greater
segmental work in the ADO-R compared with ADO-CP
hearts (35±6 versus 13±7, respectively), but this did not
reach statistical significance (P=0.07).

In the BCP group, the β-coefficient (a measure of diastolic
segmental stiffness) was increased by approximately 11-fold

### AAR and Infarct Size

The area placed at risk by the LAD coronary artery occlusion,
expressed as a percentage of the left ventricular mass (AAR/
LV), was comparable among the BCP (30±2%), ADO-CP
(27±2%), and ADO-R (29±1%) groups. When necrosis was
expressed as a percentage of left ventricular mass (AN/LV),
necrotic areas were significantly smaller in ADO-CP (8±0.5%,
P=0.008) and ADO-R (6±1%, P<0.001) groups relative to
the BCP group (13±1%). The infarct size, expressed as a per-
centage of the AAR (AN/AAR, Figure 2), was reduced by 30% in
ADO-CP treated hearts compared with the BCP group. In hearts
treated with ADO in the terminal cardioplegic infusion and
during reperfusion (ADO-R), infarct size was significantly
reduced compared with that observed in the BCP group. Al-
though there was a strong trend for infarct size in ADO-R to be
smaller than in the ADO-CP group, this did not reach statistical
significance (P=0.12).

### Plasma Creatine Kinase Activity

There was no significant difference in plasma creatine kinase
activity at baseline among the 3 groups (Figure 3). All 3
groups showed a modest and similar increase in plasma
creatine kinase activity following LAD occlusion. There was
a significant reduction in creatine kinase activity at the end
of reperfusion for the ADO cardioplegia group compared with
the BCP group. In addition, the ADO-R group also showed a

### Table 2: Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischemia</th>
<th>W30min</th>
<th>W60min</th>
<th>W90min</th>
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<tbody>
<tr>
<td>HR, bpm</td>
<td>85±12*</td>
<td>121±14</td>
<td>156±6</td>
<td>150±13</td>
<td>147±15</td>
</tr>
<tr>
<td>ADO-I</td>
<td>108±14*</td>
<td>158±18</td>
<td>182±11</td>
<td>166±7</td>
<td>165±6</td>
</tr>
<tr>
<td>ADO-R</td>
<td>92±6*</td>
<td>158±9</td>
<td>151±9</td>
<td>155±7</td>
<td>153±8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>Control</td>
<td>86±8</td>
<td>74±3</td>
<td>61±3†</td>
<td>60±3†</td>
</tr>
<tr>
<td>ADO-I</td>
<td>99±8</td>
<td>71±2</td>
<td>65±4†</td>
<td>61±4†</td>
<td>55±4†</td>
</tr>
<tr>
<td>ADO-R</td>
<td>83±11</td>
<td>90±6</td>
<td>54±3†</td>
<td>54±2†</td>
<td>55±3†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>Control</td>
<td>6±1</td>
<td>7±1</td>
<td>6±1</td>
<td>6±1</td>
</tr>
<tr>
<td>ADO-I</td>
<td>6±1</td>
<td>6±1</td>
<td>6±1</td>
<td>5±1</td>
<td>5±1</td>
</tr>
<tr>
<td>ADO-R</td>
<td>5±1</td>
<td>5±1</td>
<td>6±0.3</td>
<td>4±0.4</td>
<td>4±1</td>
</tr>
</tbody>
</table>

LVEDP indicates left ventricular end-diastolic pressure;
Baseline, before normothermic LAD coronary artery occlusion;
Ischemia, at the end of 75 minutes of LAD ischemia; W30min, W60min, and W90min, reperfusion of the heart after 75 minutes of LAD ischemia and 60 minutes of cardioplegic arrest at 30, 60, and 90 minutes, respectively, during the working beating state. Values are mean±SEM. At each interval, statistically significant differences in
HR, MAP, and LVEDP were not achieved.
*P<0.05 vs other time points in same group; †P<0.05 vs baseline and ischemic time points in same group.

at 90 minutes of working beating reperfusion compared with
baseline values, indicating an increase in stiffness in the AAR
(Table 3). In contrast, hearts treated with ADO-CP showed a
significant (40%) reduction in β-coefficient at the end of
reperfusion. Hearts administered ADO-R also had a signifi-
cant (60%) reduction in myocardial stiffness. There were no
significant differences between either ADO groups.
significant reduction in creatine kinase release compared with the BCP group. There were no significant differences between either ADO groups. The attenuation in creatine kinase release in the ADO groups is consistent with the reduction in infarct size.

Cardiac Myeloperoxidase Activity
Myeloperoxidase activity in the nonischemic zone was low and comparable among the 3 groups (Figure 4). In the nonnecrotic AAR, myeloperoxidase activity was the greatest in the BCP and ADO-CP hearts. In contrast, hearts in the ADO-R group had significantly less (82% and 76%) myeloperoxidase activity in the ischemic nonnecrotic AAR compared with BCP and ADO-CP groups, respectively. In the necrotic AAR, both ADO-CP and ADO-R hearts had a significantly less myeloperoxidase activity compared with the BCP group.

Myocardial Edema
After 2 hours of reperfusion, the unsupplemented BCP group demonstrated the greatest myocardial edema in the transmural AAR with a percent water averaging $86\pm0.7\%$ (Figure 5). In the

![Figure 2](image)

**Figure 2.** Infarct size (AN) expressed as a percent of AAR. Bars represent group means±SEM. *$P<0.05$ vs BCP.

![Figure 3](image)

**Figure 3.** Plasma creatine kinase activity during time course of the experiment. +$P<0.05$, BCP vs ADO-R; *$P<0.05$, BCP group vs ADO-CP and ADO-R groups.

**TABLE 3. Cardiodynamic Function**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischemia</th>
<th>W30min</th>
<th>W60min</th>
<th>W90min</th>
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<tbody>
<tr>
<td>EDL, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11±1</td>
<td>13±1</td>
<td>10±1*</td>
<td>10±1*</td>
<td>11±1*</td>
</tr>
<tr>
<td>ADO-I</td>
<td>12±1</td>
<td>11±2</td>
<td>12±1</td>
<td>12±1</td>
<td>12±1</td>
</tr>
<tr>
<td>ADO-R</td>
<td>11±1</td>
<td>13±0.3</td>
<td>11±0.3</td>
<td>11±0.4</td>
<td>10±0.3</td>
</tr>
<tr>
<td>ESL, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10±1</td>
<td>14±1†</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
</tr>
<tr>
<td>ADO-I</td>
<td>11±1</td>
<td>12±2†</td>
<td>12±1</td>
<td>12±1</td>
<td>12±1</td>
</tr>
<tr>
<td>ADO-R</td>
<td>9±1</td>
<td>14±0.3†</td>
<td>10±1</td>
<td>10±0.5</td>
<td>10±0.5</td>
</tr>
<tr>
<td>% SS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14±2†</td>
<td>-6±1</td>
<td>-3±1</td>
<td>-6±1</td>
<td>-3±1</td>
</tr>
<tr>
<td>ADO-I</td>
<td>13±2*</td>
<td>-10±2</td>
<td>2±0.4‡</td>
<td>1±1‡§</td>
<td>2±0.2‡</td>
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<tr>
<td>ADO-R</td>
<td>17±1*</td>
<td>-7±0.4</td>
<td>5±2‡§</td>
<td>2±2‡§</td>
<td>5±2‡§</td>
</tr>
</tbody>
</table>

EDL indicates end-diastolic length; ESL, end-systolic length; % SS, percent systolic shortening. All other abbreviations as in Table 2. Values are mean±SEM.

* $P<0.05$ vs ischemia time point in the same group; † $P<0.05$ vs other time points in same group; ‡ $P<0.05$ vs baseline and ischemia time points in the same group; § $P<0.05$ vs control within the same time point.
ADO-CP group, there was a significant reduction in myocardial edema in the transmural AAR (82±0.2%) compared with the BCP group. Hearts in the ADO-R group showed significantly less myocardial edema in the transmural AAR (80±0.4%) compared with both the BCP and ADO-CP groups.

**Neutrophil Adherence to Coronary Endothelium**

In the BCP group, neutrophil adherence to coronary endothelium was significantly greater by 242% in the ischemic-reperfused LAD compared with the nonischemic left circumflex coronary artery (LCx) (118±3 PMNs/mm² versus 35±3 PMNs/mm², respectively) (Figure 6). Similarly, neutrophil adherence in the ADO-CP group was significantly greater by 280% in the ischemic-reperfused LAD compared with the nonischemic LCx (114±5 PMNs/mm² versus 30±2 PMNs/mm², respectively). Neutrophil adherence in the ischemic-reperfused LAD coronary segments in the ADO-R group was greater by 77% of that in the nonischemic reperfused LCx segments of the same group (55±2 PMNs/mm² versus 31±1 PMNs/mm², respectively). The adherence to the ischemic-reperfused LAD in the ADO-R group was significantly less than that in the BCP or ADO-CP groups.

**Discussion**

In the present study, we investigated the use of ADO in reducing postcardioplegia injury following normothermic regional myocardial ischemia and cardiopulmonary bypass. The results of this study have shown that ADO administered either as an adjunct to BCP (ADO-CP) or only during reperfusion (ADO-R) showed a reduction in postcardioplegia myocardial stiffness, infarct size, and creatine kinase release compared with an unsupplemented BCP. However, only the hearts given ADO during the reperfusion period showed an improvement in systolic shortening and a significant decrease in both myocardial edema and neutrophil accumulation relative to the group receiving ADO in BCP. Furthermore, only the ADO-R–treated hearts showed attenuated neutrophil adherence to coronary artery endothelium, consistent with a decrease in postischemic endothelial damage. The lack of an increase in interstitial concentrations of ADO during the delivery of ADO-enhanced cardioplegia suggests an intravascular mechanism. This study suggests that a further reduction in ischemia-reperfusion injury following surgical revascularization of regional ischemia may be achieved by interventions aimed directly at the early reperfusion phase.

Although the use of ADO as a cardioprotective agent during nonsurgical myocardial ischemia and reperfusion has been well documented,7 few reports demonstrating the cardioprotective effects afforded by ADO as an adjunct to BCP in a bypass model with jeopardized myocardium have been reported. ADO possesses anti-ischemic effects in that it hyperpolarizes the ventricular myocytes by activation of K_ATP channels,13 which may inhibit calcium influx,14 and augments glucose uptake and glycolytic flux.15 Hudspeth et al2 have previously shown that ADO in BCP following 30 minutes of normothermic global ischemia attenuates postcardioplegia dysfunction in severely injured hearts through receptor-mediated mechanisms. Similarly, in our study, we have shown that ADO-supplemented cardioplegia reduced postcar-
dioplegia dysfunction in myocardium jeopardized by severe regional ischemia. Although ADO administered during the period of cardioplegia may attenuate ischemic mechanisms during the period of arrest, it does not modulate the ischemia (global or regional) preceding cardioplegia, and it may not directly interfere with mechanisms of reperfusion injury set in motion when the cross-clamp is removed (oxygen radical burst, neutrophil-mediated events).

This study suggests that ADO may have limited infarction and preserved postischemic endothelial function (in the ADO-R group) by inhibiting neutrophil actions. The mechanisms by which ADO may directly inhibit neutrophil-mediated injury include the attenuation of neutrophil degranulation, generation of superoxide anions or other oxygen-derived free radicals, the release of inflammatory cytokines, or the expression of CD11/18 complexes. Accordingly, in the present study both ADO groups attenuated neutrophil accumulation in the AAR. This accumulation, which occurs primarily during reperfusion, has been linked to contractile dysfunction and development of necrosis. Other investigators have shown that ADO is protective against reperfusion injury by attenuating neutrophil-related mechanisms. Neutrophils are important participants in ischemic-reperfusion injury, and neutrophil activation and subsequent interaction with the coronary vascular endothelium may lead to a burst of free radicals responsible for endothelial dysfunction, and microvascular injury with blood flow defects (no-reflow phenomenon). In vitro studies by Cronstein and colleagues and Todd et al suggest that the presence of ADO may directly inhibit neutrophil activities and neutrophil-mediated injury to endothelium and myocytes. However, pretreatment with ADO does not significantly attenuate these neutrophil-endothelial interactions. Hence, ADO administered before reperfusion may not directly inhibit neutrophil-mediated injury. The present study, in which ADO was administered at reperfusion, suggests that postcardioplegia injury can be attenuated by inhibiting the neutrophil-endothelial cell interaction after release of the aortic cross-clamp. In contrast, the addition of ADO in the cardioplegic solution in this study did not show a significant reduction in neutrophil adherence to coronary endothelium and did not reduce neutrophil accumulation in postischemic tissue to the same extent as ADO at reperfusion. Limited efficacy in inhibiting neutrophil-mediated injury in the ADO-CP group may be due, in part, to the lack of neutrophils in the AAR during cardioplegic arrest, the effects of hypothermia on reducing ADO interactions with receptors and transduction mechanisms, and/or the delayed expression of adhesion molecules involved in neutrophil-endothelial interactions with cold temperatures.

Although we found that there was a dramatic reduction in ex vivo neutrophil adherence to the LAD endothelium in the group receiving ADO during the terminal BCP infusion and reperfusion, there was no such attenuation of neutrophil adherence in the ADO-BCP group. Whereas ADO has been shown to directly attenuate neutrophil adherence to coronary artery endothelium, the reduced adherence demonstrated in postischemic vessels of the ADO-BCP group is indicative of the loss of the endothelium’s anti-neutrophil function. Osten- sibly, the increased adherence of neutrophils ex vivo in the ADO-BCP group is secondary to endothelial damage and impaired release of antineutrophil autacoids such as nitric oxide and ADO itself.

There are many potential mechanisms for the failure of ADO in hypothermic cardioplegia to protect the endothelium. First, hypothermia may have attenuated the interaction between ADO and its receptor subtypes, or postreceptor signal transduction. Recently, Katayama et al used an isolated perfused rat model, reported that the protective effects of ADO decrease with hypothermia. However, other studies in addition to the present study have shown significant postcardioplegia protection in an in vivo canine model of surgical revascularization with hypothermic BCP; the role of hypothermia in attenuating receptor interactions is not clear. It is not known how warm induction may affect the extent of cardioprotection with ADO-supplemented hypothermic maintenance cardioplegia solutions. Second, the physical presence of ADO in the vascular compartment may be required to attenuate neutrophil-endothelial cell interactions (adherence) that occur before endothelial damage. With ADO-supplemented cardioplegia, pharmacologic concentrations of ADO are absent during the reperfusion phase. Williams et al reported that pretreatment of the coronary vascular endothelium before introduction of neutrophils failed to attenuate neutrophil adherence to thrombin-stimulated endothelium. The requirement for the presence of ADO also assumes that ADO exerts its physiological effects on neutrophils and endothelium in the intravascular space, rather than indirectly, ie, by reducing the severity of ischemia by an interstitial or intramyocyte mechanism. Indeed, Todd et al demonstrated that large molecular weight ADO (polyadenylic acid), which is confined to the intravascular space, reduced postischemic injury by neutrophil-related mechanisms. The lack of elevated ADO and purine levels measured by interstitial dialysis in the present study supports an intravascular effect of ADO. It is possible that the administration of ADO during hypothermic cardioplegia in the present model may not attenuate postischemic neutrophil-related endothelial dysfunction. However, this study suggests that the introduction of ADO during reperfusion, when neutrophils are actively in contact with the coronary vascular endothelium, does attenuate the neutrophil-endothelial cell interactions involved in postischemic injury, which may potentially lead to endothelial dysfunction, contractile dysfunction, and infarct extension.

Current strategies in myocardial protection have advocated the use of cardioplegic solutions as a vector by which to introduce cardioprotective agents targeting specific mechanisms of ischemic-reperfusion injury. However, the effectiveness of this strategy is predicated on the ability of the agent of interest to exert its therapeutic effects during the cardioplegic period or thereafter. The present study has exposed a weakness in this concept of cardioplegia as a therapeutic vector by showing that ADO may not have exerted optimal effects against neutrophil-mediated injury when delivered with hypothermic cardioplegia. However, these anti-neutrophil effects were exerted to a greater extent when ADO was administered in the peri/peri reperfusion period at normothermic temperatures. The limited effects of other antineutrophil therapies such as nitric oxide delivered by...
hypothermic cardioplegic may also be overcome by administering the agent during normothermic reperfusion or with a normothermic terminal infusion (hot shot). The present study supports the immediate reperfusion period as an additional window of therapeutic opportunity in which to interfere with mechanisms of myocardial reperfusion injury.

Acknowledgments

The authors are grateful to Gail Nechtman for assisting in the formatting of the manuscript and to Susan Schmarkey and Jill Robinson for technical support. We are also grateful to Quest Medical, Inc (Allen, Tex) for providing the MPS cardioplegia delivery system and related supplies. Vinod H. Thourani, MD, is a recipient of a Fellowship from the Thoracic Surgery Research Foundation, and Zhi-Qing Zhao, PhD, is a recipient of a Scientist Development Award from the national American Heart Association. The Foundation for its continued support of the research effort.

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References

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_Circulation_. 1999;100:II-376-II-383
doi: 10.1161/01.CIR.100.suppl_2.II-376

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/100/suppl_2/II-376

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