Cardioprotective Effect of Adenosine Pretreatment in Coronary Artery Bypass Grafting*

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Objective: There are several reports of the use of adenosine as a cardioprotective agent during cardiac surgery. Adenosine treatment might affect neutrophils and inflammatory mediators. The present prospective randomized study was designed to investigate the effect of adenosine pretreatment on myocardial recovery and inflammatory response in patients undergoing elective coronary artery bypass surgery.

Design: A prospective, randomized, controlled study.

Setting: Operative unit and ICU in a university hospital in Finland.

Patients: Thirty male patients undergoing primary, elective coronary revascularization.

Interventions: Patients in the adenosine group received a 7-min infusion of adenosine (total, 650 μg/kg) before the initiation of cardiopulmonary bypass.

Measurements: Postoperative creatine kinase (CK)-MB release and hemodynamics were recorded. Perioperative leukocyte and cytokine release were measured.

Results: Adenosine pretreatment resulted in less CK-MB release and an improved postbypass cardiac index. Similar leukocyte counts and cytokine responses were seen in both groups perioperatively. Neutrophil counts were similar between the groups before and after myocardial ischemia when measured simultaneously in arterial and coronary sinus blood.

Conclusions: The present results support the hypothesis that adenosine pretreatment is cardioprotective in humans, but the present dose failed to regulate the inflammatory responses after coronary artery bypass grafting.

(CHEST 2001; 120:860–865)

Key words: adenosine; coronary artery bypass grafting; cytokine responses; myocardial injury

Abbreviations: ANOVA = analysis of variance; CABG = coronary artery bypass grafting; CI = cardiac index; CK = creatine kinase; CPB = cardiopulmonary bypass; CVP = central venous pressure; HR = heart rate; IL = interleukin; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; SAP = systolic arterial pressure; TNF = tumor necrosis factor

Myocardial stunning after heart surgery is frequently observed and is associated with increased morbidity and mortality, especially in patients of advanced age and with decreased myocardial reserve. Although we now possess more data on myocardial cellular function, the problem of inadequate myocardial protection persists. New methods and strategies to enhance cardioprotection are needed, and adenosine is receiving increased attention. A considerable body of experimental evidence shows that adenosine is a cardioprotective agent independently of its well-known vascular smooth-muscle relaxing effects and its antiadrenergic and negative chronotropic and dromotropic properties.1 There are several reports of the use of adenosine as a cardioprotective agent during cardiac surgery. The phenomenon in question appears to be mediated by activation of the A1 receptor coupled to guanine nucleotide inhibitory binding proteins.1,2 Adenosine exhibits a broad spectrum of effects against neutrophil-mediated events and can therefore intervene in the ischemia and reperfusion response, a capacity that may offer therapeutic benefits.3 Adenosine may also trigger a hibernation effect that may be cardioprotective.4

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Supported by a grant from the Medical Research Fund of Tampere University Hospital, the CIMO Foundation of Finland, and the Fund of Tampere Tuberculosis Foundation.

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Clinical Investigations
Coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) initiates a cascade of events resulting in a systemic inflammatory response syndrome. The release of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8 is associated with the development of myocardial dysfunction after CPB. The myocardium is a major source of cytokines after CABG. It is believed that an inflammatory reaction is involved in myocardial ischemia and reperfusion injury. The release of cytokines during cardiac surgery may be deleterious to the heart and other organs. Studies have suggested that the proinflammatory cytokines evoke significant cardiovascular activity in regulating nitric oxide homeostasis and mediating interactions between leukocytes and the endothelium. One animal study has shown that adenosine treatment reduces cardiac TNF-α production following ischemia and reperfusion. Adenosine may inhibit the release of the proinflammatory cytokines (IL-6 and IL-8) involved in the response to ischemia and reperfusion, and enhance IL-10 secretion by stimulated monocytes. This may represent a novel anti-inflammatory property of adenosine by which it could modulate inflammation and limit ischemia and reperfusion injury. To our knowledge, however, there have been no studies investigating cytokine changes in patients undergoing CABG after adenosine pretreatment. The present prospective, randomized study was designed to investigate the effect of adenosine pretreatment on myocardial recovery and inflammatory response in patients undergoing elective coronary artery bypass surgery.

**Materials and Methods**

**Patient Selection**

The investigation was approved by the local ethics committee, and informed written consent was obtained from all patients entering the study. Thirty male patients with multiple-vessel coronary artery disease and stable angina admitted to the hospital for the first time for elective coronary artery bypass surgery were invited to take part. They were randomized into control or adenosine groups between February 2000 and July 2000. Patients with unstable angina, poor left ventricular function (ejection fraction < 30%), valve disease, and patients receiving corticosteroids were deemed not eligible. Patients with aortic cross-clamping time > 120 min and any postoperative complication requiring reexploration were also excluded. No significant differences were noted between the groups in age, body surface area, and disease classification. The demographic data on the 30 patients completing the study are presented in Table 1.

**CPB and CABG**

A standardized anesthesia technique was used with sufentanil, midazolam, and pancuronium. A standard CABG operation was undertaken with one internal thoracic artery and from one to four peripheral vein grafts taken in each case from the lower extremities. The patients received perfusion at a temperature of 32°C with nonpulsatile flow from a membrane oxygenator (D903 AVANT; Dideco; Mirandola, Italy). The circuit was primed with 2,000 mL of Ringer acetate. Cold-blood antegrade-retrograde cardioplegia (6°C to 8°C) was delivered through a device (BDE-Plus; Dideco) that mixed blood with a sanguineous solution in a ratio of 4:1. The potassium concentration of the induction cardioplegia was 21 mmol/L. After each distal anastomosis, additional cardioplegic solution was delivered for 1 min through the vein graft and coronary sinus catheter. Warm-blood retrograde cardioplegia was administered at the end of the cross-clamping. Corticosteroids and aprotinin were not administered perioperatively. After weaning from CPB, pharmacologic therapy with inotropes and/or vasodilators was used to maintain a cardiac index (CI) > 2.0 L/min/m².

**Adenosine Administration**

Routine preoperative medication for anesthesia were the same in both groups. The adenosine group (n = 15) received an infusion of Adenoscan (Sanofi; Winthrop, France) prior to initiation of CPB (after complete cannulation of appropriate vessels and before administration of cardioplegic solution) through a Swan-Ganz catheter to the superior vena cava using a computer-controlled pump infusion system. The initial infusion rate was a 50-µg/kg increment to the dosage of 100 µg/kg/min at the second minute; after this, the infusion lasted for 6 min or until the patient developed a systolic arterial pressure (SAP) < 70 mm Hg. Three minutes after the completion of adenosine infusion, the CPB machine was started. In patients with immediate hypotension (SAP < 70 mm Hg) resulting from adenosine infusion, the infusion was stopped and CPB was initiated at once.

**Sample Collection and Analysis**

Blood samples for cytokine measurement were collected from the radial artery before induction of anesthesia (baseline), and 5 min, 1 h, 4 h, 8 h, and 20 h after reperfusion to the myocardium. All samples were anticogulated with ethylenediaminetetra-acetic acid, immediately cooled in 4°C, and centrifuged within 30 min (4,000g for 10 min); plasma was transferred to polypyrrole test tubes and stored at −70°C until assay. TNF-α, IL-6, IL-8, and IL-10 levels in the plasma were determined by means of a commercially available enzyme-linked immunosorbent assay (Pe-

**Table 1—Clinical Data**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>Adenosine Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age, yr</td>
<td>64.7 ± 8.6</td>
<td>65.9 ± 7.9</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Ejection fraction before operation, %</td>
<td>63.1 ± 9.9</td>
<td>60.1 ± 12.6</td>
</tr>
<tr>
<td>NYHA class II/III</td>
<td>6/9</td>
<td>3/12</td>
</tr>
<tr>
<td>CPB time, min</td>
<td>107.6 ± 21.9</td>
<td>109.9 ± 54.5</td>
</tr>
<tr>
<td>Ischemia time, min</td>
<td>89.6 ± 19.1</td>
<td>90.4 ± 19.7</td>
</tr>
<tr>
<td>Grafts</td>
<td>3.7 ± 1.0</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>Ventilation time, h</td>
<td>11.7 ± 3.3</td>
<td>11.0 ± 3.5</td>
</tr>
<tr>
<td>Inotropic support, yes/no</td>
<td>11/4</td>
<td>10/5</td>
</tr>
<tr>
<td>24-h bleeding, mL</td>
<td>519.3 ± 349.0</td>
<td>493.3 ± 348.7</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or No. NYHA = New York Heart Association.*

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Adenosine-Mediated Myocardial Protection During Cardiopulmonary Bypass

Adenosine has multiple effects on the myocardium and inflammatory response. The purpose here was to assess whether adenosine pretreatment could protect the myocardium from ischemia-reperfusion (I-R) injury and could induce a regulatory inflammatory response during cardiopulmonary bypass (CPB). We hypothesized that adenosine pretreatment during CPB would decrease myocardial injury, as assessed by creatine kinase-MB (CK-MB) levels, and myocardial release of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10), and would result in a regulatory inflammatory response.

**Methods**

15 patients (age 58 ± 10 years) undergoing elective CABG surgery were pretreated with adenosine (CLB; Amsterdam, Netherlands) at the induction of general anesthesia. 10 min after the induction of anesthesia, the patients received a bolus of adenosine (47.87 mg/kg body weight). Thereafter, patients received 400-500 mg/h adenosine for 10 min before the start of CPB. During CPB, patients did not receive adenosine. 15 patients served as control subjects. Myocardial CK-MB levels were measured before CPB and 1 min and 10 min after reperfusion. Cell counts were adjusted for hemodilution. All measurements were performed blind to the study medication.

**Hemodynamic Measurements and Data Collection**

Hemodynamic monitoring comprised measurement of heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output. Derived cardiovascular variables, CI, systemic vascular resistance index, and pulmonary vascular resistance index were calculated from standard formulas. All measurements were based on the thermodilution technique. Hemodynamic measurements and calculations based on the thermodilution technique were collected at four time points: (1) baseline value, after anesthesia induction; (2) 15 min after completion of CPB; (3) 6 h after completion of CPB; and (4) 12 h after completion of CPB.

**Results**

Fifteen patients were pretreated with adenosine, and the other 15 served as control subjects. There was no major complication in any of the 30 patients who completed the study. Essential data on operation and postoperative recovery are summarized in Table 1. Four of the 15 patients who received adenosine could not finish the full dose owing to resultant profound hypotension (SAP < 70 mm Hg) during infusion. Their average (± SD) time for adenosine infusion was 4.63 ± 0.48 min, and the total dose in these cases was 412.5 ± 47.87 µg/kg (63.5% of the full dose).

**Creatine Kinase MB**

Adenosine-treated patients released significantly less creatine kinase (CK)-MB than the control subjects postoperatively (p = 0.007; Fig 1). The maximum CK-MB level was also lower in the adenosine group (41.9 ± 12.3 U/L vs 75.1 ± 50.0 U/L, respectively; p = 0.013), indicating less myocardial injury in patients receiving adenosine when compared to the control subjects.

**Hemodynamics**

Mean SAP decreased and HR increased after the operation. The changes in HR, MAP, central venous pressure (CVP), MPAP, and PCWP were closely identical in both groups (Table 2). CI decreased 15 min after CPB in the control subjects, whereas in the adenosine group it increased. The recovery of CI was better in the adenosine group than in the control subjects (p = 0.039; Fig 2).

**Leukocyte Counts and Cytokines**

Although leukocyte counts were lower in the adenosine group than in the control subjects at all sampling time points, the difference did not reach statistical significance (Fig 3, top, left). Transcoronary neutrophil differences were similar in both groups before the start of CPB (0.06 ± 0.19 × 10^9/L vs 0.05 ± 0.10 × 10^9/L). After 1 min and 10 min of reperfusion, the transcoronary leukocyte difference in the control subjects was higher than in the adenosine group (0.11 ± 0.35 × 10^9/L vs 0.07 ± 0.35 × 10^9/L at 1 min, and 0.18 ± 0.55 × 10^9/L vs 0.12 ± 0.57 × 10^9/L at 10 min, respectively); however, the differences were not statistically significant.

Only traces of TNF-α (lower than the lowest standard, 3.0 pg/mL) were detected in most of the patients after reperfusion (data not shown). Plasma levels of IL-6, IL-8, and IL-10 increased after reperfusion. Similar cytokine responses were seen in both groups. None of the differences in IL-6, IL-8, and IL-10 reached statistical significance (Fig 3, top right, bottom left, bottom right).

**Discussion**

Adenosine has multiple effects on the myocardium and inflammatory response. The purpose here was to assess whether adenosine pretreatment during CPB would decrease myocardial injury, as assessed by creatine kinase-MB (CK-MB) levels, and myocardial release of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10), and would result in a regulatory inflammatory response.
establish whether adenosine would be cardioprotective and its effect on cytokines release after coronary artery bypass surgery. The results confirmed previous reports\textsuperscript{13–15} that adenosine is a cardioprotective agent during cardiac surgery. Adenosine pretreatment resulted in less CK-MB release and an improved postbypass CI after the operations, but the present dose failed to regulate the inflammatory responses after CABG.

A considerable body of experimental evidence\textsuperscript{16} indicates that adenosine pretreatment induces potent endogenous protection against subsequent ischemic stress in the human myocardium. There have also been several reports of the clinical use of adenosine as a cardioprotective agent. Leesar and colleagues\textsuperscript{17} reported that a 10-min intracoronary adenosine preconditioning treatment significantly reduced ST-segment changes during percutaneous transluminal coronary angioplasty. There are also reports\textsuperscript{18} that IV adenosine administered to patients with acute myocardial infarction is well tolerated and may lead to increased salvage of ischemic tissue. Although the exact mechanism underlying the cardioprotective effect of adenosine is unknown, the beneficial effects of this agent appear to be related to the activation of specific adenosine-receptor subtypes, at least three of which (A\textsubscript{1}, A\textsubscript{2a}, and A\textsubscript{3}) may be involved. Experimental findings\textsuperscript{19,20} indicate that adenosine is most effective in protecting the reversibly injured heart when administered prior to ischemia, most likely by activation of cardiac myocyte A\textsubscript{1} and A\textsubscript{3} receptors. Other authors\textsuperscript{21} have reported that adenosine reduces oxygen-derived free radical production by neutrophils, an effect that could minimize the free radical-induced damage believed to occur during reperfusion.

Adenosine is a well-known vasodilator, and infusion of adenosine causes hypotension. The infusion rate of adenosine selected for this study was based on a pilot study\textsuperscript{13} showing that patients tolerate an infusion rate only up to $\text{100}\text{mg/kg/min}$ prior to CPB. Lee and associates\textsuperscript{13} treated seven patients with higher IV adenosine infusion (total, $2,450\text{mg/kg}$) before initiation of CPB, and these patients exhibited improved postoperative ventricular performance and reduced CK release. Two of their patients could not tolerate the full dose, and the investigator had to use a slower infusion rate.\textsuperscript{13} Mentzer and colleagues\textsuperscript{22,23} used a higher dosage in IV adenosine infusion (total, $1,400\text{mg/kg}$ and $2,000\text{mg/kg}$) for 10 min, at which time the aortic cross-clamp was applied, and all patients finished the full protocol; the authors found that adenosine treatment was associated with a lower requirement of dopamine and fewer postoperative complications. Our present protocol resembles that of Lee and associates,\textsuperscript{13} but differs from that of Mentzer and colleagues,\textsuperscript{22,23} in that adenosine

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Perioperative CI in patients undergoing CABG. Data are presented as mean ± SD. Pre = preanesthesia level that is the level for comparisons. Higher CIs were seen in the adenosine group (ANOVA for repeated measurement, p = 0.039).}
\end{figure}

\begin{table}[h]
\centering
\caption{Hemodynamic Data*}
\begin{tabular}{|l|c|c|c|c|}
\hline
Variables & Baseline & 15 min After CPB & 6 h After CPB & 20 h After CPB \\
\hline
HR, beats/min & & & & \\
Control & 59.9 (12.4) & 78.8 (8.8) & 90.3 (14.6) & 84.3 (14.6) \\
Adenosine & 54.7 (8.7) & 75.0 (17.7) & 87.6 (16.1) & 82.3 (11.5) \\
\hline
MAP, mm Hg & & & & \\
Control & 89.4 (8.0) & 73.6 (8.0) & 79.4 (17.7) & 78.4 (15.0) \\
Adenosine & 91.6 (12.5) & 72.6 (11.2) & 84.0 (13.1) & 70.2 (12.4) \\
\hline
MPAP, mm Hg & & & & \\
Control & 18.4 (2.3) & 17.6 (3.4) & 20.4 (4.5) & 20.0 (4.6) \\
Adenosine & 19.1 (4.5) & 22.4 (2.9) & 22.8 (5.4) & 20.8 (4.1) \\
\hline
PCWP, mm Hg & & & & \\
Control & 11.6 (2.6) & 10.6 (2.3) & 9.0 (3.1) & 10.8 (3.1) \\
Adenosine & 12.1 (2.9) & 13.3 (2.0) & 10.8 (3.0) & 10.4 (2.4) \\
\hline
CVP, mm Hg & & & & \\
Control & 7.4 (2.5) & 10.1 (2.1) & 8.9 (2.2) & 8.4 (3.1) \\
Adenosine & 8.3 (2.3) & 10.9 (2.1) & 9.8 (2.4) & 9.1 (2.8) \\
\hline
\*Data are presented as mean (SD); no statistically significant difference was found between the groups.
\end{tabular}
\end{table}
infusion was terminated 3 min before the initiation of CPB, a treatment mode referred to as adenosine preconditioning. Our dosage (total, 650 µg/kg) was lower than the recommended safety dosage in patients with coronary artery disease (140 µg/kg/min for 6 min; total, 840 µg/kg). Even though safety issues limited the dose, the present protocol resulted in improved postbypass cardiac performance and less CK-MB release after the operation.

Ischemia and reperfusion results in contractile dysfunction, necrosis, and vascular injury. Previous studies of intraoperative measurements during CABG have demonstrated that CI remains essentially unchanged or is even lowered immediately after the completion of the revascularization procedure.¹³ In agreement with these, the present results showed that adenosine-pretreated patients had improved recovery in myocardial performance postoperatively, as indicated by faster recoveries of CI. Since the preload of the heart, manifested as CVP and PCWP, was similar in both groups, the improvement in cardiac performance might result from better recovery of contractility.

Adenosine treatment has been shown to reduce experimental myocardial ischemic reperfusion injury in many species. The present results are also in line with previous indications that adenosine pretreatment exerts a myocardial protective effect against ischemic reperfusion injury, as adenosine pretreatment here resulted in lower release of CK-MB during the first 48 h of the recovery period.

Myocardial stunning after CABG is associated with increased morbidity and mortality in patients with severe multivessel disease and with reduced myocardial function. Although the present study with low-risk patients showed no difference in clinical outcome between the groups, the better CI and lower myocardial enzyme release in the adenosine group might be even more important in patients with decreased left ventricular function.

Many studies have demonstrated that adenosine attenuates the adherence of neutrophils to endothelial cells. Bullough and associates showed that adenosine inhibits neutrophil adherence to myocytes. Although higher systemic leukocyte counts and transcoronary neutrophil sequestration were observed in the control subjects as compared to the adenosine group, the present results failed to show significant differences between the groups. Adenosine has a broad spectrum of physiologic effects, which make it suitable as a cardioprotective agent with efficacy in all three windows of opportunity (pretreatment, and during ischemia and reperfusion) and against numerous targets, including the neutrophils. Preischemic adenosine treatment reduces experimental myocardial infarct size, but there is additional evidence to suggest that adenosine treatment during reperfusion may also reduce infarct size, in that it reduces platelet and neutrophil adherence to the coronary endothelium. Intracoronary administration of adenosine after reperfusion has significantly reduced neutrophil and RBC stagnation in capillaries, and was
associated with reduced infarct size and improved regional ventricular function in the ischemic zone. An additional adenosine infusion during reperfusion could be included in future studies to maximize the inhibitory effects of adenosine on neutrophils, which may contribute to reperfusion injury.

The present results failed to demonstrate the effect of adenosine pretreatment on cytokine response after CABG. Possibly the experimental findings under ideal and standardized circumstances may be difficult to repeat and observe in the clinical setting. For safety considerations, the present dose of adenosine pretreatment is lower than the previously reported protocols. Furthermore, the cytokine assays applied have an inherent sensitivity limit. Cytokines are characterized 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. The present study, based on systemic sampling, may have yielded a lower sensitivity of detection than studies examining intracellular cytokine production or cytokine messenger RNA transcription levels in appropriate target tissues. Studies on proper timing and dose of adenosine pretreatment are warranted in order to get benefit of the anti-inflammatory properties of adenosine. In summary, adenosine infusion immediately prior to the initiation of CPB appears to improve myocardial recovery of the human heart after CABG, but the dosage here failed to regulate inflammatory responses after CABG.

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