Reduction of Myocardial Reperfusion Injury by Intravenous Adenosine Administered During the Early Reperfusion Period

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Adenosine influences the function of several cell types thought to be involved in the pathogenesis of myocardial reperfusion injury. We have previously demonstrated that intracoronary administration of adenosine enhances myocardial salvage 24 hours after reperfusion. To determine if these beneficial effects could be obtained during a prolonged period of reperfusion using an intravenous route of administration, 22 closed-chest dogs were subjected to 90 minutes of proximal left anterior descending coronary artery occlusion and 72 hours of reperfusion. Animals randomly received either intravenous adenosine (0.15 mg/kg/min) or an equal volume of Ringer's lactate during the first 150 minutes of reperfusion. The area at risk was defined in vivo with Monastral blue, and infarct size was measured histologically with Mallory's trichrome stain. Serial global and regional ventricular function were determined with contrast ventriculography and analyzed using a computerized radial shortening method. Biopsies were obtained from the central ischemic zone to assess endothelial ultrastructure and capillary obstruction. No significant effects in heart rate or blood pressure were noted during adenosine infusion. Transmural collateral blood flow during ischemia was similar in the groups. Infarct size expressed as a percentage of the anatomical area at risk was significantly less in the adenosine-treated group (35.3±4.3% in controls versus 17.1±4.3% in treated animals, p<0.01). A progressive decrease in transmural blood flow was noted in control animals during reperfusion, resulting in a significant reduction at 3 hours compared with the preocclusion value (0.69±0.11 ml/min/g [at baseline versus 0.45±0.10 ml/min/g at 3 hours, p<0.05]). In contrast, flow in adenosine animals at 3 hours was similar to baseline values (0.91±0.15 ml/min/g at baseline versus 0.98±0.14 ml/min/g at 3 hours, p=NS) and was significantly higher (p<0.05) than the control group. Radial shortening in the ischemic zone was significantly improved at 3 (−2.6±2.8% in controls versus 11.6±3.3% in treated animals, p<0.01) and 72 hours (5.5±3.0% in controls versus 17.3±3.5% in treated animals, p<0.01) after reperfusion in treated animals. Electron microscopy showed reduced neutrophil and erythrocyte plugging of capillaries with relative preservation of endothelial cell structure in the adenosine group. This study demonstrates that intravenous administration of adenosine during the early reperfusion period results in a sustained reduction in myocardial infarct size associated with preservation of regional ventricular function. Although the exact mechanisms remain to be elucidated, results from this study suggest that the beneficial effects of the drug persist for a prolonged period after reperfusion. (Circulation 1991;83:237–247)

Experimental studies have demonstrated that myocardial salvage after reperfusion may be limited by deleterious changes that occur in the microvasculature in the perireperfusion period.1–7 Reperfusion results in a significant impairment of endothelial-dependent and -independent vasodilatory reserve in both large and small vessels after 90 and 120 minutes of regional ischemia.6,7 There is

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extensive disruption of endothelial cells in capillaries of the reperfused bed associated with luminal obstruction by cellular elements, particularly neutrophils.\textsuperscript{3,4} These abnormalities result in a progressive decrease in blood flow to potentially viable myocytes after reperfusion with eventual lethal injury to these cells (reperfusion injury).\textsuperscript{1,3,7,8} Experimental studies support a role for the neutrophil in the pathogenesis of microvascular injury after reperfusion.\textsuperscript{9-13} Neutrophils may reduce microcirculatory flow by mechanically obstructing capillary lumens\textsuperscript{12} or cause extensive endothelial cell disruption through the liberation of cytotoxic substances such as oxygen-derived free radicals and proteolytic enzymes.\textsuperscript{14-17} Activated neutrophils may further reduce blood flow by the release of vasoactive substances such as leukotrienes and platelet activating factor.\textsuperscript{18} Additional limitation of infarct size with neutropenia, anti-inflammatory agents, and antineutrophil agents lend further support to the role of the neutrophil in mediating reperfusion injury.\textsuperscript{5,13,19,20}

Adenosine is an endogenous arteriolar vasodilator present in relatively high concentrations at the time of reperfusion.\textsuperscript{21} It is a metabolic by-product of ATP with numerous properties that may attenuate reperfusion injury, including inhibition of superoxide anion and proteolytic enzyme release by neutrophils,\textsuperscript{22,23} limitation of endothelial–neutrophil interactions,\textsuperscript{24} inhibition of platelet aggregation and thromboxane release,\textsuperscript{25} and augmentation of postreperfusion coronary blood flow.\textsuperscript{26,27} These cardioprotective efforts of adenosine support the hypothesis that it may be a potentially useful agent in limiting myocardial reperfusion injury.

We have previously demonstrated that intracoronary administration of adenosine commencing immediately after reperfusion following 90 minutes of regional ischemia in a canine model substantially reduces infarct size and hastens the recovery of postischemic left ventricular dysfunction ("stunning") when measured 24 hours after reperfusion.\textsuperscript{3} Because some pharmacological interventions may delay the process of myocardial necrosis for 24–72 hours, it is essential that the effects of the drug be determined over a longer reperfusion period. Furthermore, the clinical applicability of intracoronary administration of drugs would be limited to a few patients. Therefore, we investigated the effects of an intravenous adenosine infusion administered during the early peri-reperfusion period on infarct size and regional ventricular function in a closed-chest canine model subjected to 90 minutes of proximal left anterior descending artery (LAD) occlusion and 72 hours of reperfusion. Also, the effects of adenosine on serial myocardial blood flow were measured and correlated with ultrastructural changes in the microvasculature of the reperfused bed.

**Methods**

**Experimental Preparation**

Twenty-six mongrel dogs of either sex weighing 15–26 kg were quarantined for 2 weeks to exclude common canine diseases. Five to seven days before coronary occlusion, a left thoracotomy was performed under general anesthesia (30 mg/kg sodium pentobarbital). The heart was exposed via a pericardiotomy, and a surgical monofilament ligature was placed around the LAD proximal to the first diagonal and enclosed in a polyethylene tube. A Silastic catheter was inserted into the left atrial appendage and filled with heparin. Both the LAD snare and the left atrial line were buried in a subcutaneous pouch, the pericardium was loosely approximated, and the thorax was closed. Animals were administered one dose of penicillin and dihydrostreptomycin (Combicin) and allowed to recover. The entire protocol conformed to the guiding principles set forth by the American Physiological Society.

**Experimental Protocol**

The dogs were reanesthetized with sodium pentobarbital, intubated, and mechanically ventilated with room air to maintain arterial blood gases within the physiological range. Intravenous diazepam (mean dose, 5 mg) and morphine (mean dose, 5 mg) were administered periodically throughout the protocol as required for supplemental anesthesia. Using sterile technique, both femoral arteries and the right femoral vein were exposed and cannulated with a 7F sheath (Cordis, Miami, Fla.). A 7F Gensini (USCI, C.R. Bard, Tewksbury, Mass.) catheter was positioned with its distal tip in the proximal inferior vena cava for the infusion of adenosine or Ringer’s lactate. A 7F pigtail catheter was placed in the right femoral artery and used for monitoring mean and phasic systemic arterial pressure and left ventricular end-diastolic pressure. Electrocardiographic leads I, aVF, and aVL were monitored continuously throughout the protocol (model VR-12, Electronics for Medicine, P.P.G. Biomedical Systems, Pleasantville, N.Y.). The LAD snare and left atrial line were retrieved from the subcutaneous pocket. Before initiating the protocol, the animals were assigned to receive either Ringer’s lactate or adenosine.

Baseline hemodynamic measurements were then obtained. Regional myocardial blood flow was determined with a bolus of 15-μm microsphere injections into the left atrium. Microspheres were labeled with radioisotopes and injected serially (2×10⁶/injection) in the following order: iodine-125, cerium-141, chromium-51, strontium-85, niobium-95, and scandium-46 (3M Company, St. Paul, Minn.). Reference samples were withdrawn from the left femoral artery via the Cordis sheath at a rate of 7.85 ml/min to allow calculation of myocardial blood flow.

Selective injection of contrast into the left coronary artery via a modified Judkin’s catheter confirmed patency of the LAD. Left ventriculography in the right anterior oblique projection with 8–10 ml megilumine diatrizoate (Renografin 76) injected during 1 second through a power injector was performed to confirm normal and uniform wall motion. After each animal received a bolus injection of lidocaine (2
mg/kg) followed by a maintenance infusion at 0.1 mg/kg/min during the occlusion period, the snare was tightened progressively during 5–10 minutes to occlude the proximal LAD and maintained for 90 minutes. Total occlusion was confirmed by selective coronary angiography. Hemodynamic measurements, left ventriculography, and regional myocardial blood flow were repeated after 60 minutes of occlusion. In the treatment group, adenosine (2 mg/ml in 0.9% NaCl) (Sigma Chemical Co., St. Louis) was administered through the Gensini catheter in the inferior vena cava as a continuous infusion at 0.15 mg/kg/min by a Harvard pump beginning 5 minutes before reperfusion and ending 150 minutes after reperfusion. The mean volume administered was 1.5 ml/min for 150 minutes for a total volume of 225 ml (450 mg adenosine). After 90 minutes of LAD occlusion, the snare was gradually released during approximately 3 minutes, followed by selective coronary angiography and determination of regional myocardial blood flow to confirm vessel patency. Hemodynamic measurements and regional blood flow were determined at 1, 2, and 3 hours after reperfusion. Left ventriculography and coronary angiography were again performed at 3 hours of reperfusion after determining regional blood flow. The LAD snare and left atrial catheter were then reimbedded into the subcutaneous pocket, femoral lines were removed, and the vessels were ligated, the animals were weaned from the ventilator, administered antibiotics, and allowed to recover. After 72 hours, the animals were reanesthetized with pentobarbital. Hemodynamic measurements and left ventriculography were performed, and patency of the LAD was confirmed by selective coronary angiography. A left thoracotomy was performed, and the snare was tightened under direct vision. Monastral blue dye (du Pont, Wilmington, Del.) at a dose of 1 mg/kg was injected into the aortic root through a pigtail catheter within 60 seconds after occluding the vessel. This method provides for a more physiological risk region because when infarction occurs, the bed at risk is not being perfused and therefore has a low flow state. Although in vitro techniques allow injection of colored dyes at fixed pressure in all beds, in vivo methods allow administration of a known volume and viscosity of dye in a situation more analogous to the conditions at the time of previous myocardial ischemia. After a lethal dose of pentobarbital and potassium chloride, the heart was rapidly explanted and rinsed with tap water to prevent counterstaining.

**Analysis of Area at Risk and Area of Necrosis**

The hearts were sectioned into six 1-cm segments parallel to the atrioventricular groove from apex to base and photographed with Ektachrome (Kodak) for later determination of the area at risk (unstained by Monastral blue). The slices not used for blood flow (slices 1, 3, 5, and 6) were then dehydrated. All slices were embedded into paraffin (with the slice facing the apex to be used as the cutting surface) by the method described by Reimer and Jennings. Microscopic sections 7 µm thick were cut from the apical end of each slice and stained with hematoxylin and eosin and Mallory's trichrome stain. Using magnified tracings ×5 from each slide with the aid of a microscopic slide projector, the area at risk and area of necrosis (stained purple by Mallory's) were then determined by computerized planimetry of the tracings drawn by an observer blinded to the treatment groups. The ratio of the infarcted area to area at risk for each heart was calculated by a method previously described.

**Analysis of Ventricular Function**

Global and regional ventricular functions were measured by digitization of an end-systolic and end-diastolic cineangiographic frame. Left ventricular ejection fraction was calculated by Simpson's rule. Regional wall motion was assessed with a radial shortening method using customized software as previously described and validated in our laboratory. Radii were constructed from the midpoint of the longitudinal axis connecting the midpoint of the aortic valve plane and the apex of the left ventricle at 10° intervals for a total of 36 radii. Radii that involved the mitral and aortic valves were excluded from analysis. The ischemic zone was defined as the largest number of radii that were akinetic or dyskinetic after 1 hour of occlusion. Ventricular function was also assessed at baseline and 3 and 72 hours after reperfusion.

**Determination of Regional Myocardial Blood Flow**

The second and fourth transverse myocardial sections were used for determination of regional blood flow. Samples were obtained from the endocardium, midmyocardium, and epicardium (0.15–0.55 g) in the nonischemic zone (posterior wall) and the central and lateral regions of the ischemic zone as demarcated in vivo by Monastral blue dye. These samples and arterial reference samples were counted for 5 minutes in a multichannel autogamma scintillation spectrometer (model 5986, Packard Instrument Company, Downers Grove, Ill.). Background contamination and overlapping radioactivity from other isotopes were accounted for using a matrix correction method (Compusphere Software, Packard Instrument Company) and corrected for microsphere loss and tissue edema.

Samples were averaged for each section, and myocardial blood flow was calculated in milliliters per gram wet weight per minute as previously described in our laboratory.

**Light Microscopy**

The first and third sections were stained with hematoxylin and eosin and examined by light microscopy in a blinded manner. The extent of patchy myocardial necrosis, degree of hemorrhage, and extent of contraction band necrosis were evaluated. The acute inflammatory infiltrate within vessels and in the surrounding myocardium (interstitium) and macrophage infiltration was assessed in the ischemic and nonischemic zones. An average of 20 high-power
Table I. Scoring Method of Myocardial and Microvascular Injury

<table>
<thead>
<tr>
<th>Score</th>
<th>Myocyte injury</th>
<th>Endothelial injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal myocardium</td>
<td>Normal endothelium</td>
</tr>
<tr>
<td>1</td>
<td>Reversible injury</td>
<td>Mild endothelial swelling with decreased pinocytic vesicles</td>
</tr>
<tr>
<td></td>
<td>Mild nuclear clumping, mild mitochondrial swelling, prominent I-bands</td>
<td>Moderate endothelial swelling with decreased pinocytic vesicles</td>
</tr>
<tr>
<td></td>
<td>Intracellular edema, moderate nuclear changes, mild-to-moderate mitochondrial swelling with some separation of cristae</td>
<td>Severe endothelial cell swelling and/or membrane bound vesicles, myelin figures</td>
</tr>
<tr>
<td></td>
<td>Marked intracellular edema, vacuoles, moderate mitochondrial swelling with occasional mitochondria demonstrating severe swelling, clearing of mitochondrial matrices, occasional focal clumping of cristae</td>
<td>Endothelial cell protrusions, membrane bound vesicles, myelin figures</td>
</tr>
<tr>
<td>2</td>
<td>Irreversible injury</td>
<td>Red blood cell stacking with and without endothelial cell swelling</td>
</tr>
<tr>
<td></td>
<td>Presence of flocculent densities within mitochondria including the above changes</td>
<td>Platelets and fibrin deposition with and without endothelial cell swelling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocyte adhesion with and without endothelial cell swelling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss or disintegration of endothelium with and without endothelial cell swelling</td>
</tr>
</tbody>
</table>

fields (hpf) (×400) per slide was evaluated. The degree of neutrophil and macrophage infiltration, hemorrhage, and patchiness of the infarct was semiquantitated according to the method of Romson et al, with a score of 4+ being assigned to the more severe infiltrate and a score of 0 assigned when very few or no changes are seen. The extent of contraction band necrosis was quantitated by the method of Tazelaar et al, with rare presence given a score of 1+ and diffuse presence a score of 4+.

Electron Microscopy

Ultrastructural analysis was performed in nine additional animals (four adenosine-treated and five control) who underwent a similar protocol except for termination of the study 3 hours after reperfusion (Table 1). Myocardial biopsy samples were taken within 60 seconds of death from the central ischemic zone (anterior wall) and the nonischemic zone (posterior wall) and divided into endocardial and epicardial halves and fixed in 3% buffered glutaraldehyde for transmission electron microscopy. Tissue was allowed to fix for 1–6 hours and then transferred to 1% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated, and embedded in Epon. Semithin sections were cut, stained with toluidine blue, and examined by light microscopy. The artifact-free areas with the most capillaries were selected for ultrathin section cutting, stained with uranyl acetate lead citrate, and examined with a Zeiss 109 IGF electron microscope (Thornwood, N.Y.). The degree of myocyte and endothelial injury was semiquantitated (Table 1).

Statistical Analysis

Results at defined time points for hemodynamic variables, regional myocardial blood flow, and ventricular function were analyzed with a two-factor analysis of variance in which one factor was treatment and the other was time. If a statistically significant interaction between treatment and time was obtained, further examination of differences between groups was conducted using a two-tailed, unpaired Student’s t test with correction for multiple comparisons. Comparison of other variables such as ventricular weight and infarct size was by unpaired Student’s t test. For analysis of the electron microscopy data, data from multiple samples within the same animal were averaged before performing the t test. The null hypothesis was rejected at the 5% level. All data are given as mean±SEM.

Results

Twenty-six dogs were entered into the study. Three dogs (two control and one adenosine-treated) were excluded due to technical difficulties with the snare. There was one death during reperfusion in the control group and no deaths in the treatment group. Nine animals (five control and four adenosine-treated) required electrical cardioversion for ventricular tachycardia. Data from 22 dogs completing the protocol were analyzed: 13 control animals were randomized to intravenous Ringer’s lactate, and nine animals were randomized to intravenous adenosine.

Laboratory and Hemodynamic Parameters

No significant differences in serial pH or PaO₂ were noted between the two groups throughout the protocol (data not shown). Left ventricular end-diastolic pressure measured 60 minutes after LAD occlusion was significantly higher in the adenosine-treated animals (15.3±1.7 mm Hg in controls versus 23.0±2.0 mm Hg in treated animals, p<0.01, Table 2). Infusion of adenosine was not associated with significant hemodynamic effects. A small increase in heart rate and decrease in systolic and diastolic blood pressure observed during the infusion period did not reach statistical significance. There was no difference in myocardial oxygen demand as assessed by the rate–pressure product between the two groups throughout the protocol.
**TABLE 2. Hemodynamic Measurements**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cont</th>
<th>Ado</th>
<th>Cont</th>
<th>Ado</th>
<th>Cont</th>
<th>Ado</th>
<th>Cont</th>
<th>Ado</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (beats/min)</td>
<td>SYS AP (mm Hg)</td>
<td>DIAS AP (mm Hg)</td>
<td>RPP [(HR×SBP)/1,000]</td>
<td>LVEDP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>122±5</td>
<td>131±6</td>
<td>147±6.1</td>
<td>144±6.9</td>
<td>106±4.1</td>
<td>108±5.2</td>
<td>18.1±1</td>
<td>19.1±1</td>
</tr>
<tr>
<td>1-hour occlusion</td>
<td>115±6</td>
<td>114±4</td>
<td>143±6.6</td>
<td>135±5.4</td>
<td>108±4.9</td>
<td>110±4.6</td>
<td>16.3±1.0</td>
<td>15.3±0.9</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>112±6</td>
<td>122±8</td>
<td>138±5.6</td>
<td>123±7.2</td>
<td>102±6.1</td>
<td>88±7.8</td>
<td>15.8±1.1</td>
<td>14.2±1.1</td>
</tr>
<tr>
<td>1-hour reperfusion</td>
<td>117±9</td>
<td>130±8</td>
<td>141±4.6</td>
<td>132±7.2</td>
<td>105±4.1</td>
<td>95±5.5</td>
<td>16.6±1.5</td>
<td>17.2±1.5</td>
</tr>
<tr>
<td>2-hour reperfusion</td>
<td>123±7</td>
<td>135±9</td>
<td>143±4.5</td>
<td>142±6.9</td>
<td>104±4.5</td>
<td>100±5.2</td>
<td>17.7±1.2</td>
<td>18.2±2.3</td>
</tr>
<tr>
<td>3-hour reperfusion</td>
<td>134±8</td>
<td>125±5</td>
<td>145±5.0</td>
<td>160±5.8</td>
<td>106±5.1</td>
<td>123±4.1*</td>
<td>19.0±1.3</td>
<td>19.7±0.9</td>
</tr>
<tr>
<td>72-hour reperfusion</td>
<td>152±6</td>
<td>128±7*</td>
<td>137±4.7</td>
<td>141±6.9</td>
<td>92±4.3</td>
<td>107±5.8*</td>
<td>20.1±0.6</td>
<td>18.1±1.4</td>
</tr>
</tbody>
</table>

HR, heart rate; SYS AP, systolic arterial pressure; DIAS AP, diastolic arterial pressure; RPP, rate-pressure product; SBP, systolic blood pressure; LVEDP, left ventricular end-diastolic pressure; Cont, control; Ado, adenosine.

Values are given as mean±SEM.

*p<0.05; **p<0.01.

**Effects on Infarct Size**

The left ventricular weight was similar in treated and control groups (55.6±4.0 g in controls versus 55.0±2.9 g in treated animals, p=NS, Figures 1 and 2 and Table 3). Although the area at risk expressed as a percentage of the total left ventricle was significantly larger in the adenosine-treated animals (30.2±1.9% in controls versus 39.1±3.2% in treated animals, p=0.03), a marked and highly significant decrease in myocardial infarct size was demonstrated when expressed as a percentage of the area at risk (35.3±4.3% in controls versus 17.1±4.3% in treated animals, p=0.009). Because area at risk, a baseline predictor of infarct size, was greater in the treatment group and left ventricular mass was similar, infarct size was not significantly different between the two groups when expressed as a percentage of the total left ventricle (10.8±1.5% in controls versus 7.1±1.9% in treated animals, p=0.14). However, analysis of a subset of animals (four adenosine-treated and four controls) with similar areas at risk and collateral blood flows revealed a marked decrease in infarct size when expressed as a percent of the area at risk and when expressed as a percent of the total left ventricle (Table 3). The relation of infarct size expressed as a percentage of area at risk and transmural collateral blood flow in the central ischemic zone 60 minutes into occlusion is shown by linear regression in Figure 2. A weak relation occurred between these variables for both groups. An inverse relation between infarct size and collateral flow was noted; dogs with high collateral blood flow had smaller infarcts. This graph also suggests that adenosine enhanced myocardial salvage independent of the degree of collateral blood flow.

**Effects on Myocardial Blood Flow**

Regional myocardial blood flow in the central ischemic zone was similar in the groups at baseline (Figures 3 and 4). After 60 minutes of occlusion, endocardial blood flow was lower in the adenosine-treated group, although this did not achieve statistical significance (0.06±0.02 ml/min/g in controls versus 0.01±0.01 ml/min/g in treated animals, p=0.08). This trend suggests the treatment group experienced a greater severity of endocardial ischemia; however, transmural blood flow 60 minutes after occlusion was similar. Both groups exhibited reactive hyperemia during the immediate reperfusion period. During the first 2 hours of adenosine administration, a similar enhancement in myocardial blood flow was noted.

![Figure 1](http://circ.ahajournals.org/)

*Figure 1. Bar graph of effect of intravenous adenosine on infarct size (AN) expressed as a percentage of area at risk (AR) and total left ventricle (LV). Despite a greater AR in treated group, AN was significantly reduced in adenosine animals when expressed as a percent of the perfusion bed.*

![Figure 2](http://circ.ahajournals.org/)

*Figure 2. Scatterplot of relation between infarct size (AN) expressed as a percentage of area at risk (AR) and transmural collateral blood flow in central ischemic zone in control and adenosine animals. A weak inverse relation is noted between AN and collateral flow. Adenosine-treated animals tended to have smaller infarcts for any change of flow, suggesting an effect of therapy irrespective of the degree of collateral flow.*
from the subendocardial to the subepicardial region in the ischemic zone. In the control group, a progressive decrease in flow occurred in the endocardial and midmyocardial regions of the central and lateral ischemic zones compatible with the no-reflow phenomenon. In the treated animals, regional blood flow in the inner two thirds of the myocardium was significantly higher than in controls at 3 hours after reperfusion (30 minutes after discontinuation of adenosine infusion) and was similar to the preocclusion value. Regional myocardial blood flows in the lateral ischemic zones were similar to flows in the central ischemic zone throughout the protocol (data not shown). Myocardial blood flow in the nonischemic zone was similar at baseline with the expected augmentation in flow during the adenosine infusion. Flow in the outer two thirds of this zone remained significantly higher 30 minutes after discontinuing adenosine.

**Effect on Ventricular Function**

Baseline ventricular function was similar and both groups demonstrated marked anteroseptal akinesia or dyskinesia during the occlusion period (Figure 5). A significant improvement in anterior wall radial shortening in the adenosine-treated group compared with controls was noted as early as 3 hours (−2.6±2.8% in

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**TABLE 3. Analysis of Infarct Size in a Subset of Four Adenosine and Four Control Animals With Comparable Area at Risk and Collateral Blood Flow**

<table>
<thead>
<tr>
<th>Transmural CBF (ml/min/g)</th>
<th>AR/LV (%)</th>
<th>AN/AR (%)</th>
<th>AN/LV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>Ado</td>
<td>Cont</td>
<td>Ado</td>
</tr>
<tr>
<td>0.05</td>
<td>0.03</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>0.05</td>
<td>0.00</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>0.03</td>
<td>0.05</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>0.04</td>
<td>0.03</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>33±2</td>
<td>32±1</td>
</tr>
</tbody>
</table>

CBF, collateral blood flow; AR, area at risk; LV, left ventricle; AN, area of necrosis; Cont, control; Ado, adenosine. Values are given as mean±SEM.

*\(p=0.005\); †\(p=0.004\).

**FIGURE 3.** Plots of serial regional myocardial blood flow in nonischemic (posterior wall) and central ischemic zones at baseline (BASE), occlusion (OCC), reperfusion (REP), and 1, 2, and 3 hours after reperfusion. A progressive decrease in flow in inner two thirds of myocardium is noted in control animals during first 3 hours of REP. Myocardial blood flow was significantly increased during adenosine infusion (duration, 150 minutes). Note that at 3 hours, flows in endocardial and midmyocardial regions were significantly increased in adenosine group compared with control animals and were similar to BASE values. Persistent vasodilitation in inner two thirds of nonischemic zone is present 3 hours after reperfusion, 30 minutes after discontinuing adenosine.
controls versus 11.0±3.3% in treated animals, p<0.01) and as long as 72 hours after reperfusion (5.5±2.0% in controls versus 17.3±3.5% in treated animals, p<0.01). No significant difference in global ventricular function as measured by ejection fraction was observed in either group throughout the protocol.

**Light Microscopy**

Hearts from 13 control and nine adenosine-treated animals were examined by light microscopy (Table 4). Acute inflammatory infiltration tended to be less in adenosine-treated animals than in controls (intra-vascular: 1.07±0.17 in controls versus 0.77±0.14 in treated animals, p=NS; interstitial: 0.84±0.18 in controls versus 0.66±0.23 in treated animals, p=NS), and no differences were noted between interstitial and intravascular distribution within each animal. Similarly, hemorrhagic infarction was less severe in adenosine animals (1.73±0.36 in controls versus 1.33±0.16 in treated animals, p=NS), and there tended to be greater contraction band necrosis in controls than in adenosine-treated animals. However, because histological parameters were assessed 72 hours after reperfusion, no significant differences in neutrophil infiltration between the two groups were noted. Infarcts were predominantly subendocardial in adenosine-treated animals compared with controls, which had greater degrees of midmyocardial and subepicardial involvement.

**Electron Microscopy**

A total of 36 specimens from five control and four adenosine-treated animals were examined by electron microscopy (Figure 6). An average of 16 capillaries were examined from each animal from the endocardial and epicardial ischemic region and representative capillaries from nonischemic zones at magnification of ×3,000. Both capillaries and myocyte injury were quantitated from electron photomicrographs using established criteria identified in Table 1. Endothelial injury was greatest in the subendocardial region of control animals. Animals treated with adenosine had significantly less endothelial injury in the epicardial region (1.22±0.17 in controls versus 0.43±0.07 in treated animals, p<0.01), and because of the wide sampling variation, they tended to have less endothelial injury in the endocardial region (2.36±0.18 in controls versus 1.39±0.53 in treated animals, p=0.16). No significant differences were noted in myocyte injury in the subendocardial and subepicardial ischemic regions between adenosine and control animals. The nonischemic control areas had minimal changes (data not shown).

**Discussion**

Results of the present study demonstrate that the intravenous administration of 0.15 mg/kg/min adenosine for 150 minutes after reperfusion resulted in a reduction in infarct size, and this was associated with enhanced recovery of regional ventricular function in a closed-chest canine model subjected to 90 minutes of LAD occlusion and 72 hours of reperfusion. Because the adenosine infusion began just before reperfusion, the beneficial effects observed suggest that the drug primarily attenuated deleterious anatomical and biochemical events occurring only in the peri-reperfusion period. Although adenosine is known to have negative chronotropic effects as well as being an endogenous vasodilator, no significant differences were noted in the heart rate and systolic and diastolic blood pressures during the infusion. A significant decrease in infarct size was observed despite a tendency for greater ischemia in the treated group as manifested by a larger perfusion bed at risk and higher left ventricular filling pressure during ischemia. Analysis of a subset of animals with comparable areas at
TABLE 4. Semiquantitative Histological Assessment by Light Microscopy

<table>
<thead>
<tr>
<th>Histological parameter</th>
<th>Control (n=13)</th>
<th>Adenosine (n=9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic infarct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1+</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>8</td>
<td>3</td>
<td>0.34</td>
</tr>
<tr>
<td>Contraction band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1+</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>8</td>
<td>5</td>
<td>0.59</td>
</tr>
<tr>
<td>Inflammation (acute)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intersitial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1+</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>2</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Intravascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1+</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>3</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0+ to 1+</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>2</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>Patchiness of infarct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1+</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2+ to 4+</td>
<td>10</td>
<td>8</td>
<td>0.35</td>
</tr>
</tbody>
</table>

risk and collateral blood flow also demonstrated a striking reduction in infarct size in the adenosine group. The failure to observe differences in ultrastructural parameters of myocyte injury may be related to the site and number of samples obtained. The present study is in agreement with previous observations in our laboratory of a significant reduction in infarct size with selective intracoronary administration of adenosine after 90 minutes of ischemia and 24 hours of reperfusion. Results of the present study extend these observations by demonstrating that intravenous administration of the agent during the early reperfusion period is associated with a similar and permanent enhancement of myocardial salvage.

Delayed Versus Permanent Reduction in Reperfusion Injury

Experimental studies in the canine model suggest that infarct evolution is virtually complete at 24 hours after permanent coronary occlusion. However, pharmacological interventions may delay the process of myocardial necrosis until 24–72 hours in both permanent occlusion and reperfusion models. This phenomenon has been observed with various agents, including calcium channel blockers (verapamil), anti-inflammatory agents (ibuprofen), and the prostacyclin analogue (iloprost). This may be one explanation for the disparate results obtained with the free radical–scavenging enzymes, superoxide dismutase, and catalase because the majority of positive studies occurred in animals killed within 48 hours of reperfusion. Numerous other explanations may be responsible for the inconsistent effects of superoxide dismutase on myocardial reperfusion injury; these have been recently summarized by Engler and Gilpin. Because animals were killed at 24 hours in our previous study with intracoronary adenosine, it is important to confirm whether the drug had sustained effects on myocardial salvage. The present study demonstrates that intravenous adenosine administered during the first 150 minutes of reperfusion results in a persistent reduction in reperfusion injury in the canine model.

Possible Mechanisms of Action

Although the precise mechanism whereby adenosine attenuated reperfusion injury in the study was not addressed, numerous possibilities exist, including vasodilatation of coronary arterioles, reduction of neutrophil activation and adherence to endothelial cells, inhibition of platelet aggregation and thromboxane release, and alteration of intracellular nucleotide and calcium concentrations. Intravenous adenosine produced a twofold to threefold increase in the flow in all layers of the myocardium in both ischemic and nonischemic zones. Flow in the nonischemic zone in treated animals was only slightly elevated compared with flow in control animals 30 minutes after discontinuing adenosine. In contrast, flow in the ischemic zone remained markedly increased compared with control animals, which demonstrated a progressive decrease in endocardial and midmyocardial flows in the ischemic zone during the first 3 hours of reperfusion. This suggests that adenosine attenuated the no-reflow phenomenon, possibly by inhibiting vasospasm, platelet aggregation, and/or vascular obstruction by neutrophil plugs. Our results are entirely consistent with the finding of Engler, who showed that pretreatment with 5-amino-4 imidazole carboxamide-riboside (AICA-riboside), a compound that augments adenosine release from ischemic myocytes, significantly improved regional myocardial blood flow after 60 minutes of regional ischemia in the dog.
The reason for the persistent elevation in blood flow in the nonischemic zone 30 minutes after discontinuation of adenosine is unclear; one possibility is that adenosine suppressed renin release.39Because renin has a half-life of 15–90 minutes, the increased blood flow may be due to reduced circulating renin levels. Other potential mechanisms include changes in sympathetic nerve reflexes in the coronary vasculature or alterations in myocardial oxygen demand.

Numerous studies support a role for the neutrophil in the pathogenesis of myocardial reperfusion injury.5,9,10,19,40 In vitro studies have demonstrated that adenosine inhibits various neutrophil functions, including superoxide anion production, proteolytic enzyme release, neutrophil adherence, and cytotoxicity to cultured endothelial cells.23–25 A possible explanation for the protective effects of adenosine in the study may therefore be related to prevention of neutrophil-mediated microvascular damage. This hypothesis is supported by ultrastructural changes that demonstrated extensive endothelial disruption with plugging of capillary lumina by endothelial cell debris and blood elements in control animals, whereas vascular plugging was infrequently observed in adenosine animals and was associated with relative preservation of endothelial cells. A study by Simpson et al demonstrated that a prolonged infusion of the prostacyclin analogue (iloprost) was required to permanently reduce myocardial reperfusion injury, whereas a relatively short infusion time of adenosine was highly effective in the present study. The reasons for this discrepancy are unknown but may be related to the different cellular effects of the two drugs. The pharmacological effects of adenosine are more complex than iloprost and involve both A1 and A2 receptors.

**Methodological Considerations**

Numerous factors must be considered before these data can be extrapolated to the clinical situation. Abrupt occlusion of a normal canine coronary artery allows for only a crude comparison to humans, in whom infarction occurs due to gradual thrombotic occlusion of a chronically diseased coronary artery. Richard et al have demonstrated that a residual critical stenosis at the time of reperfusion abolishes reactive hyperemia but does not alter neutrophil infiltration 60 minutes after reperfusion or the extent of lethal reperfusion injury. Infarct size is also highly variable in the canine model and is dependent on the extent of collaterals between the LAD and the left circumflex vessels. We evaluated collateral blood flow in all animals and found that the two groups were comparably ischemic. Although the drug was effective over a broad range of collateral flows, producing a ±52% reduction in infarct size for the total group, analysis of a subgroup of animals with severe ischemia (transmural blood flow ≤0.5 ml/g/min) demonstrated an even more striking reduction (±80%) in infarct size in the treated group. This is consistent with other studies demonstrating that reperfusion injury tends to be greater in hearts exposed to more severe ischemia.3,36

Therapeutic doses of lidocaine were used in both groups during occlusion and for the first 30 minutes of reperfusion. There are reports suggesting that large doses of lidocaine may alter neutrophil function and reduce infarct size.42–45 A preliminary report suggested that adenosine was only beneficial in attenuating reperfusion injury when administered with lidocaine.46 However, in previous studies using a similar therapeutic dose of lidocaine, we have shown that significant neutrophil activation occurs during the first 24 hours of reperfusion, as manifested by enhanced neutrophil chemotaxis, and augmented lysozyme degranulation.47 Further studies are necessary to determine whether the beneficial effects of adenosine are dependent on the concomitant administration of lidocaine.

**Implications**

In this era of early reperfusion of acute myocardial infarction, infarct size reduction may be limited by deleterious events occurring at the time of reperfusion—myocardial reperfusion injury. The present study demonstrates that the intravenous administration of adenosine during the reperfusion period after 90 minutes of regional ischemia resulted in a persistent reduction in infarct size; this was associated with enhanced regional contractile function. Results of the present study corroborate previous research in our laboratory that reperfusion injury contributes significantly to infarct size in the canine model after 90 minutes of ischemia and that it is attenuated by intravenous adenosine. Regardless of whether myocyte salvage was due to abolition of the no-reflow phenomenon, improved postreperfusion blood flow may provide other beneficial effects such as increasing scar thickness, accelerating postinfarct ventricular remodeling and thereby reducing infarct expansion and aneurysm formation, and decreasing the incidence of ventricular arrhythmias.48–50 Further inquiry into the mechanisms of the beneficial effects of adenosine is warranted as well as studies assessing the clinical efficacy in humans in an attempt to limit infarct size and preserve left ventricular function beyond timely reperfusion alone.

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KEY WORDS • adenosine • reperfusion injury • endothelium
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C J Pitarys, 2nd, R Virmani, H D Vildibill, Jr, E K Jackson and M B Forman

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