Intracoronary Adenosine Administered After Reperfusion Limits Vascular Injury After Prolonged Ischemia in the Canine Model

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Myocardial salvage after reperfusion may be limited by deleterious vascular changes in the previously ischemic microcirculatory bed. This could result in a progressive decrease in blood flow in the capillary bed to potentially viable myocytes (no-reflow phenomenon). The effect of intracoronary adenosine on these changes was assessed in 15 closed-chest dogs subjected to 2 hours of proximal left anterior descending artery (LAD) occlusion followed by 3 hours of reperfusion. Animals randomly received adenosine (n=8) 3.75 mg/min into the proximal LAD or an equivalent volume of saline (control) (n=7) for 1 hour after reperfusion. Endothelial-dependent and independent coronary vasodilator reserve was determined using a chronically implanted volume-flowmeter on the mid-LAD at baseline and 1 and 3 hours after reperfusion with acetylcholine and papaverine infusions, respectively, into the proximal vessel. Regional myocardial blood flow was measured serially with radioactive microspheres and regional contractile function with contrast ventriculography. Both agonists produced a significant increase in LAD flow before occlusion. Endothelial-dependent and independent vasodilatory reserve was significantly reduced (p<0.05) at 1 and 3 hours after reperfusion in control animals compared with adenosine treatment. A progressive decrease in mid-LAD flow and increase in coronary vascular resistance after reperfusion was observed in control animals (p<0.05). The treated group manifested improved regional myocardial blood flow in endocardial regions from the central (0.73±0.15 versus 0.24±0.11 ml/g/min; p<0.02) and lateral ischemic zones (0.80±0.15 versus 0.34±0.12 ml/g/min; p<0.05) 3 hours after reperfusion. A significant reduction (p<0.05) in endocardial and midmyocardial flow compared with baseline was seen in control animals at 3 hours. Intravascular and interstitial neutrophil infiltration was reduced in adenosine animals and this was associated with relative ultrastructural preservation of endothelial cells. Regional ventricular function in the ischemic zone was improved in the adenosine group 3 hours after reperfusion (13.4±3.9% versus −5.3±1.6%; p<0.001). This study demonstrates that selective administration of adenosine after reperfusion significantly attenuates functional and structural abnormalities in the microvasculature after prolonged (2 hours) regional ischemia in the canine model. Prevention of microvascular injury and the no-reflow phenomenon by adenosine may preserve reversibly injured myocytes following restoration of blood flow to previously ischemic myocardium. (Circulation 1989;80:1388–1399)

Timely reperfusion either pharmacologically, mechanically, or surgically has been demonstrated to have beneficial effects on infarct size and ventricular function in experimental models and in humans.1–6 However, the restoration of blood flow to the previously ischemic but reversibly injured endothelial and myocardial cells has been postulated to result in irreversible injury to some of these cells (reperfusion injury).7–6 Reperfusion results in both a rapid influx of neutrophils into the previously ischemic bed and a significant increase in oxygen-derived free radicals.9–12 Neutrophils may potentiate cellular damage of viable cells by releasing reactive oxygen species and various potent

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proteolytic enzymes or by mechanically plugging the microvasculature (no-reflow phenomenon). The histologic observation of capillary obstruction by neutrophils and disrupted endothelial cells in the reperfused microvasculature supports the hypothesis that vascular injury may lead to a progressive reduction in regional myocardial blood flow to potentially viable cells.

Adenosine is a potent endogenous coronary arteriolar vasodilator that is found in substantial quantities in endothelial cells. Adenosine appears to be a potentially useful agent in ameliorating reperfusion injury because it acts on many of the postulated mechanisms. These include abolition of microvascular vasoconstriction, inhibition of various neutrophil function such as adherence to endothelial cells and superoxide anion production, reducing platelet aggregation, and replenishment of high-energy stores in endothelial and myocardial cells. We have previously demonstrated that the administration of intracoronary adenosine during the first hour of reperfusion after 90 minutes of regional ischemia resulted in a significant reduction in infarct size in the canine model. The aim of this study was to assess the effect of adenosine on endothelial-dependent and independent vascular reactivity after prolonged regional ischemia. A closed-chest preparation was used to minimize neutrophil activation. Microvascular blood flow was measured serially and correlated with ultrastructural changes. Finally, the effect of treatment on postischemic contractile function was measured using contrast ventriculography.

Methods

Experimental Preparation

Mongrel dogs of both sexes weighing 20–30 kg were quarantined for 2 weeks before study to ensure they were free of canine diseases. One week before the experiment, the animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and ventilated with a Harvard positive-pressure respirator. A left thoracotomy was performed and the proximal and midportions of the left anterior descending artery (LAD) were isolated. An ultrasound transit-time–volume–flow probe (Transonic 2RSF, Transonic Systems, Ithaca, New York) was then placed around the mid-LAD and anchored by suturing a silicone phalange to the myocardium. Care was taken to avoid disruption of septal perforator branches and diagonal arteries in the region of probe placement. A snare (surgical monofilament) enclosed in a polyethylene tubing was implanted on the proximal left anterior descending artery, immediately distal to the first diagonal branch, and secured to the epicardium with two sutures. A small incision was made in the left auricular appendage and a heparinized polyethylene left atrial line was placed using a purse-string suture. The pericardium and chest were subsequently closed and the tubing was buried in a subcutaneous pocket in the subscapular region. Prophylactic antibiotics were administered, and the dogs were allowed 5–7 days to recover before experimental use.

Experimental Protocol

On the day of the experiment, the dogs were randomized to either intracoronary adenosine infusion or control (intracoronary saline infusion) (Figure 1). The animals were reanesthetized with sodium pentobarbital 25 mg/kg i.v., intubated, and placed on a Harvard positive-pressure ventilator to maintain an arterial pH of 7.4±0.5. Adequate anesthesia was maintained throughout the remainder of the experiment with morphine sulfate (mean dose, 15 mg i.v.) and valium (mean dose, 20 mg i.v.). Heart rate and electrocardiographic changes were monitored continuously using leads I, aVF, and aV F. Under sterile conditions, femoral artery and vein cutdowns were performed for subsequent hemodynamic measurements, angiography, and drug infusions. The snare, left atrial line, and volume-flow meter cable were removed from the subcutaneous pocket and flow probe connected to a flowmeter (Transonic Model #T101). A 7F pigtail catheter was used to obtain measurements of phasic and mean arterial blood pressure and left ventricular end-diastolic pressure. A modified 8F right Cordis guiding catheter was used to position a 0.038×145-cm straight, open-ended wire over a 0.014-in. flexible steerable wire (USCI, Billerica, Massachusetts) into the LAD proximal to the snare. The guidewire was then removed and guide catheter disengaged from the left coronary ostium. After baseline hemodynamics had been obtained, a contrast ventriculogram was performed in the right anterior oblique projection using 6–10 ml meglumine diatrizoate (Renografin 76) injected through a power injector. The degree of rotation was noted to ensure all subsequent ventriculograms were obtained in the same view. Regional myocardial blood flow was determined serially with injection of 15-μm microspheres labeled with 125I, 51Cr, 141Ce, 85Sr, and 85Nb (3M Company, St. Paul, Minnesota), respectively, at approximately 2×10⁶ microspheres/5 ml, into the left atrial line followed by 10 ml normal saline. Femoral arterial samples were withdrawn at
a rate of 7.85 ml/min for subsequent calculation of myocardial blood flow. After baseline reactivities were obtained with selective infusions of acetylcholine and papaverine into proximal LAD, each animal was administered 5 mg/kg lidocaine i.v. and the snare was tightened in two stages over 10 minutes. A lidocaine infusion of 0.12 mg/kg was maintained for 1 hour. At 1 hour of occlusion, hemodynamic measurements and LAD flow were repeated and a contrast ventriculogram obtained. Immediately before reperfusion the animals received lidocaine 5 mg/kg i.v., and the snare was gradually released. Streptokinase in a dose of 30,000 units was administered intracoronary, and patency of the LAD was confirmed by volume-flowmeter readings, as well as by angiography. Adenosine (Sigma Chemical, St. Louis, Missouri) was dissolved in normal saline to form a solution of 2.5 mg adenosine/ml NaCl. Animals randomized to the adenosine group received 3.75 mg/min intracoronary infusion through the distal tip of the infusion wire into the proximal LAD. The volume administered was 1.5 ml/min over a 60-minute period for a total volume of 90 ml (225 mg adenosine). The estimated plasma concentration of adenosine in the LAD, assuming a flow of 50 ml/min and hematocrit of 40%, was 0.12 mg/min. Control animals received the same volume of saline into the proximal LAD. Regional myocardial blood flow was subsequently obtained 10–15 minutes after initiation of adenosine therapy, at 1 hour into reperfusion before discontinuation of the adenosine infusion, and at 3 hours after reperfusion. Coronary vasodilatory reserve was repeated 1 hour and 3 hours after reperfusion. After obtaining a ventriculogram, the left thorax was opened and the LAD snare ligated under direct vision. Mammalian blue dye (DuPont) was injected through a pigtail catheter positioned in the ascending aorta in a dose of 1 mg/kg, 2–3 minutes after ligating the snare. After administration of 20–40 meq potassium chloride, the hearts were rapidly excised and washed to prevent counterstaining. The area at risk was measured by computerized planimetry as previously described.28

Coronary Vascular Reactivity

Pilot studies were performed in three animals to determine the dose of each vasodilator that produced maximal vasodilation. Doses of acetylcholine from 5 to 30 μg/min and papaverine 2–8 mg/min were administered by either a 5-minute infusion through a Harvard constant-infusion pump or bolus injection. Infusion rates of 15 μg/min acetylcholine and 4 mg/min papaverine were the lowest doses to produce maximal response in these animals.

Endothelial-dependent and -independent vascular reactivity were evaluated by selective infusion of acetylcholine (15 μg/min) and papaverine 4 mg/min into the proximal LAD artery for 5 minutes at baseline and 1 hour and 3 hours after reperfusion. Bolus injections of each agonist were also performed on all animals at each time interval. The bolus response was used in two animals (one control and one adenosine-treated) because these animals failed to demonstrate maximal vasodilatory response with agonist infusion at baseline. In the remaining animals, there was no difference between bolus and infusion responses. Continuous recordings of heart rate, blood pressure, and phasic and mean coronary arterial flow were obtained. Care was taken to ensure that flow returned to baseline before initiating additional agonist infusion. Acetylcholine was infused first due to its shorter half-life and more rapid metabolism. Coronary vascular resistance was calculated at baseline and at peak vasodilatory response with each agonist by dividing late aortic diastolic pressure by mean LAD flow.

Analysis of Ventricular Function

Regional left ventricular function was measured by digitization of an end-diastolic and end-systolic cineangiogram frame. Regional function was assessed in the ischemic zone (segments that were akinetic or dyskinetic at 1 hour of occlusion) at baseline and 3 hours reperfusion using a radial shortening method with customized software previously validated in our laboratory.28

Calculations of Regional Myocardial Blood Flow

The hearts were cut into 1-cm transverse sections parallel to the atrioventricular groove from apex to base. The third transverse slice was sectioned into three zones: central ischemic (middle of risk region), lateral ischemic (zone immediately adjacent to central region and entirely within risk region), and nonischemic posterior wall. Each zone was further subdivided into epicardial, midmyocardial, and endocardial sections weighing 0.3–1.0 g. Myocardial sections and reference blood samples were counted for 5 minutes in a multichannel analyzer (Model 5986, Packard Instrument, Downers Grove, Illinois) with background correction and the overlapping radioactivity between isotopes corrected using matrix correction method (Compusphere Software, Packard Instrument).28

Light Microscopy

The second slice from all hearts was fixed in 10% buffered formaldehyde. After 3 days of fixation, sections were cut for light microscopy from the ischemic area (region of infarction), the two border zones (lateral), and the posterior wall (nonischemic area), extending from endocardium to epicardium, from eight randomly selected cases (four each from control and adenosine groups). Tissue was dehydrated and embedded in paraffin; sections were cut and stained with hematoxylin and eosin and examined by light microscopy in a blinded manner. The degree of inflammatory infiltrate within vessels and in the surrounding myocardium (interstitium) was assessed in the ischemic, and nonischemic zones. An average of 20 high power fields (×400) per slide.
was evaluated. The degree of neutrophil infiltration was assessed semiquantitatively according to the method of Romson et al29 with a score of 4+ being assigned to the most severe infiltrate and a score of 0 assigned when rare or no changes are seen. The scores from four randomly selected animals in each group were then averaged. The extent of contraction band necrosis was quantified by the method of Tazelaar et al30 with rare presence given a score of 1+ and diffuse presence a score of 4+.

Electron Microscopy

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. The tissue was cut in 1-mm³ pieces and fixed in 3% buffered glutaraldehyde for transmission electron microscopy. Thirty-two specimens were examined from eight (four adenosine-treated and four control) randomly selected animals. Tissue was allowed to fix for 1–6 hours and then transferred to 1% osmium tetraoxide 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 Zeiss 109 IGF electron microscope.

Statistics

Serial results in the groups (i.e., blood pressure, heart rate, myocardial blood flow) were analyzed by repeated measures, two-way analysis of variance. If there was a statistical difference found between some pair of results, then further pairwise analysis was performed by two-tailed t test. Comparisons between the two groups (i.e., vascular reactivity) were analyzed by nonpaired Student’s t test with two-tailed discriminant score.

Results

Twenty-six dogs underwent randomization. Eleven animals (six control and five adenosine-treated) died at occlusion from intractable ventricular fibrillation. The remaining 15 animals (eight adenosine and seven control) form the basis for this report. Two of these animals (one adenosine-treated and one control) were successfully resuscitated from occlusion ventricular tachycardia and completed the experimental protocol.

Hemodynamic Variables

No significant differences were observed in heart rate, systolic or mean blood pressure (data not shown), or rate-pressure product (Figure 2). A small, though statistically significant, reduction in left ventricular end-diastolic pressure was noted in the adenosine treatment group during the first 2 hours of reperfusion.

Figure 2. Plots of hemodynamic changes in adenosine-treated and control animals during experimental protocol. Adenosine was infused at 3.75 mg/min in the proximal left anterior descending artery during first hour of reperfusion. Left ventricular end-diastolic pressure (LVEDP) was significantly increased in control animals 10–15 minutes after reperfusion (REPER). No significant difference in heart rate (HR), systolic blood pressure (SBP), or rate-pressure product (RPP) was observed throughout the study. OCCL, occlusion; REPER, 15 minutes after reperfusion.

Regional Myocardial Blood Flow

Results of regional myocardial blood flow in all animals is illustrated in Figure 3. Baseline flows were significantly higher in the endocardial and midmyocardial sections of the central and lateral ischemic zones in control animals. Both groups of animals demonstrated severe subendocardial ischemia in the central and lateral ischemic zones consistent with comparable poor collateral blood flow to these regions. Endocardial flow in the central ischemic zone was 0.05±0.01 and 0.09±0.04 ml/min/g in control and adenosine animals, respectively (p=NS). This was confirmed by measurement of area at risk, which was similar in adenosine and control animals (38.9±4.7% versus 35.4±5.1%). Selective administration of adenosine at 3.75 mg/min during the first hour of reperfusion resulted in an increase in blood flow in all three zones, which reached statistical significance in the epicardium. An increase in flow in the nonischemic segments (posterior wall) was also noted with adenosine infusion possibly related to recirculation through
the pulmonary vascular bed. Control animals manifested a continual decrease in perfusion to the endocardial and midmyocardial regions at 1 and 3 hours after reperfusion, consistent with the no-reflow phenomenon. Three-hour flow in these regions was significantly reduced compared with baseline value in control animals. This decrease was not observed in the adenosine group, such that endocardial flow was significantly greater 3 hours after reperfusion compared with control animals (0.73±0.15 versus 0.24±0.11 ml/g/min; \( p<0.05 \)). Flow in the mid-LAD also showed a progressive and significant decline during the 3 hours of reperfusion in control animals, and this was associated with an increase in coronary vascular resistance (Figure 4).

**Coronary Vasodilatory Reserve**

At baseline, both acetylcholine and papaverine produced a significant and comparable increase in LAD flow and decrease in coronary vascular resistance in both groups of animals (Figures 5–7). No change in heart rate or systolic blood pressure was noted with either drug. Vasodilatory response was significantly attenuated in control animals at 1 and 3 hours after reperfusion, whereas adenosine dogs manifested a significant increase in flow and decrease in resistance with both agents. Three control animals developed vasoconstriction with acetylcholine after reperfusion. An example of vasodilatory responses from a control and a treated animal is shown in Figure 7. Note relative preservation of vascular reactivity in the adenosine animal.

**Regional Ventricular Function**

Percent radial shortening in the ischemic zone at baseline, 1 hour into occlusion, and 3 hours after reperfusion is illustrated in Figure 8. Baseline contractile function (22.3±2% versus 17.8±2%) and the num-

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**Figure 3.** Plots of serial changes in regional myocardial blood flow in control zone (posterior wall), central, and lateral ischemic zones. A progressive decrease in endocardial and midmyocardial blood flow in ischemic zone (central and lateral) is observed in control animals, which commences within first 15 minutes of reperfusion (R), consistent with “no-reflow” phenomenon. Endocardial flow was significantly higher in adenosine-treated animals 3 hours after reperfusion. 1 HR, 1 hour after reperfusion; 3 HR, 3 hours after reperfusion.

**Figure 4.** Top panel: Mid-left anterior descending artery (LAD) flow measured with a volume flow probe in adenosine-treated and control animals. Control animals demonstrated a continual and significant decline in flow during 3-hour reperfusion period. Bottom panel: Coronary vascular resistance (CVR) was lower in adenosine-treated group after reperfusion reaching significance at 3 hours. OCCL, occlusion; REPER, 15 minutes after reperfusion; 1 HR, 2 HR, 3 HR, 1, 2, and 3 hours after reperfusion, respectively.
**Figure 5.** Vasodilatory responses in mid-left anterior descending artery with selective infusion of acetylcholine and papaverine before occlusion and at 1 and 3 hours after reperfusion. Animals were given either intracoronary adenosine at 3.75 mg/min or equivalent volume of saline for 1 hour after reperfusion. Before occlusion, a significant and comparable increase in LAD flow occurred with each agonist in both groups. At 1 and 3 hours after reperfusion, a significant vasodilatory response was observed only in adenosine-treated animals. Note that baseline flow was also significantly lower in control animals after reperfusion.

**Figure 6.** Bar graphs of coronary vascular resistance (CVR) responses in ischemic bed after infusions of acetylcholine and papaverine in adenosine and control animals. Resistance was significantly higher with both agonists at 1 and 3 hours after reperfusion in control animals.
FIGURE 7. Comparative vasodilatory responses to acetylcholine and papaverine at baseline and 1 hour after reperfusion in animals treated with adenosine and saline. Note that control animal (Panel A) demonstrates minimal increase in left anterior descending artery (LAD) flow with both agonists at 1 hour after reperfusion, whereas vascular is relatively preserved in animal given adenosine (Panel B).
Discussion

Present Study

This study demonstrates that selective administration of the potent coronary arteriolar vasodilator adenosine at 3.75 mg/min for 1 hour after reperfusion significantly preserved endothelial-dependent and independent vasodilatory reserve in a closed-chest canine model subjected to prolonged regional ischemia (2 hours). Control animals manifested a progressive decrease in endocardial and midmyocardial blood flow in the ischemic zone commencing 10–15 minutes after reperfusion suggestive of the no-reflow phenomenon. In contrast, myocardial blood flow was maintained in adenosine-treated animals and was similar to baseline values 3 hours after reperfusion. Ultrastructural analysis showed extensive microvascular injury in the capillaries of the ischemic subendocardium associated with luminal plugging by endothelial cell protrusions, neutrophils, platelets, and red cells in control animals. In contrast, these changes were attenuated after adenosine treatment with relative preservation of endothelial cells and only occasional obstruction of capillaries by cellular elements. Regional myocardial contractile dysfunction remained severely depressed in the control group 3 hours after reperfusion, with all animals manifesting dyskinesia. Adenosine treatment resulted in a marked improvement in regional ventricular function, with the development of positive shortening in the ischemic zone. This study and the previous observation in our laboratory that adenosine significantly reduces infarct size after 90 minutes of ischemia highlights the importance of microvascular injury and the no-reflow phenomenon in attenuating salvage of potentially viable myocytes in areas of prior ischemia (reperfusion injury).\textsuperscript{26} Selective adenosine infusion after prolonged regional ischemia reduced structural and functional injury in the reperfused vascular bed, thereby preventing the progressive fall in regional blood flow and enhanced regional contractile function of viable myocytes.

Mechanisms of Vascular Injury

The exact pathogenesis of vascular injury during ischemia and reperfusion remains speculative. We
FIGURE 9.  A, B, and C: Electron photomicrographs from subendocardial region from control animals. Note: capillary endothelial swelling (most marked in B) with decrease in pinocytotic vesicles and moderate (A) to marked (C) endothelial cell protrusions and membrane bound vesicles within lumina of capillaries that are obstructed. D, E, and F: Photomicrographs from adenosine-treated animals showing moderate endothelial swelling, occasional membrane-bound vesicle (seen in D), and minimal endothelial protrusions within lumina. Note: capillary lumina are nonobstructed, in contrast to obstructed capillaries in control animals.
have previously demonstrated that administration of the perfluorochemical (Fluosol-DA), a potent antineutrophil agent, prevented structural and functional endothelial damage 1 hour after reperfusion.31,32 Neutrophils could produce lethal injury to potentially viable cells by releasing numerous potent proteolytic enzymes, myeloperoxidase, and reactive oxygen species.12–15 Although a burst of free radicals has been shown to occur soon after reperfusion in the intact animal, studies using numerous free radical scavenging agents have failed to show consistent effects on myocardial reperfusion injury.11,33–39 Although one study suggests that superoxide dismutase and catalase reduced ultrastructural changes in the endocardial region after reperfusion, the effects of these agents on vascular responses have not been investigated.40

This study and previous studies from our laboratory demonstrate that reperfusion results in accelerated structural changes in the capillaries of the previously ischemic bed.26,31,32 Endothelial cells exposed to monokines and lymphokinin express an antigen on their surface that enhances neutrophil adhesion.41 We have previously demonstrated that neutrophil adherence is enhanced when cultured endothelial cells are exposed to hypoxia.42 Neutrophils activated by complement fraction C5a produce an increase in coronary vascular resistance during normal coronary perfusion pressure.43 Activated neutrophils introduced into an immunologically primed vasculature at reperfusion may enhance the inflammatory response resulting in accelerated vessel injury, neutrophil plugging, and a continual decrease in microcirculatory flow. The observation by light microscopy in this study of a significant reduction in neutrophil infiltration 3 hours after reperfusion in treated animals associated with relative preservation of endothelial structural supports a role for the neutrophil as a mediator of vascular injury.

**Functional and Histologic Abnormalities in the Vasculature After Ischemia and Reperfusion**

The large surface area occupied by the endothelium of the epicardial and myocardial coronary circulation and the observation that it is metabolically very active suggests that it plays an important role in regulating blood flow to myocardial cells.21,44 Previous studies have shown that endothelial-dependent relaxation is significantly reduced both in vitro and in vivo in experimental models of reperfusion.29,45–47 The present study further highlights the importance of structural and functional vascular abnormalities in the pathogenesis of reperfusion injury. Impaired perfusion of posts ischemic myocardium commences within the first 15 minutes of reperfusion and progresses over, at least, the next 3 hours. Selective administration of adenosine for 1 hour after reperfusion resulted in relative preservation of endothelial-dependent and independent vasodilatory reserve and prevention of the no-reflow phenomenon. Extensive endothelial cell injury in control animals was associated with impaired functional responses, as reported by our laboratory and others.31,44–47 This study also demonstrates abnormalities in endothelial-independent vascular responses in blood reperfused animals. These findings may be due to concomitant injury of smooth muscle cells in muscular arterioles.

The exact mechanism for the striking vascular protection achieved by exogenous adenosine in this study remains to be clarified. Adenosine modulates the effects of various cellular elements that may be involved in the pathogenesis of reperfusion injury. Adenosine could enhance the functional recovery of endothelial cells by restoring the metabolic machinery of the cell either through replenishment of ATP stores or by enhanced oxygen delivery to the microvasculature due to arteriolar dilatation.20,21 Mechanical obstruction of capillary channels by neutrophils may be reduced by vasodilation, inhibition of platelet aggregation and thromboxane release, reduction in neutrophil adherence to endothelial cells, or all these processes.20,23,24 Adenosine may also reduce neutrophil-mediated cellular damage by inhibiting release of reactive oxygen species from activated inflammatory cells.22 Continued functional viability of endothelial cells due to adenosine therapy would allow maintenance of microcirculatory flow with oxygenated hemoglobin. This hypothesis is supported by the observation that pretreatment with 5-amino-4-imidazole carboxamide-riboside (AICA-riboside), a substance that augments adenosine release from energy-deprived cells, significantly improved regional myocardial blood flow after 60 minutes of ischemia in the dog.48

**Effect of Adenosine on Postischemic Ventricular Contractile Function**

The significant improvement in regional ventricular function noted in treated animals suggests that adenosine attenuated the prolonged ventricular dysfunction of viable myocytes after ischemia (stunning).49 Numerous mechanisms have been postulated for this phenomenon including depletion of ATP stores and excess production of oxygen-derived free radicals during early reperfusion.35,39,50,51 The improved contractile function in this study may be related to replenishment of high energy stores by administration of supraphysiological doses of its precursor. Alternatively, adenosine may have reduced reactive oxygen production during reperfusion by reversing intramyocardial production through breakdown of purine compounds or by inhibiting release of superoxide anion from activated neutrophils. Removal of neutrophils with filters has been shown to prevent myocardial stunning after 15 minutes of regional ischemia.52 Finally, augmentation of regional myocardial flow alone may be responsible for improved function, as has been observed with other vasodilators such as papaverine.53
Conclusions

This study demonstrates that coronary vasodilatory reserve is markedly attenuated after prolonged ischemia in the closed-chest canine model of reperfusion. Intracoronary administration of adenosine after reperfusion significantly reduced these functional abnormalities, and this was associated with decreased neutrophil infiltration and relative preservation of endothelial cell ultrastructure in the capillary bed. Adenosine treatment also attenuated postischemic contractile dysfunction of viable myocytes in ischemic region. Prevention of vascular injury in the previously ischemic bed could increase salvage of potentially viable myocytes.

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