Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon

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ABSTRACT We hypothesized that the endogenous coronary vasodilator adenosine may reduce infarct size by progressively increasing reflow in a preparation of coronary occlusion-reperfusion. After 90 min of proximal left anterior descending artery occlusion, 20 dogs were randomized to blood reperfusion with (n = 10) or without (n = 10) adenosine into the proximal left anterior descending vessel at 3.75 mg/min for 60 min after reperfusion. Regional myocardial blood flow was determined serially with microspheres and regional ventricular function was assessed by a computerized radial shortening method. At 24 hr, the area at risk was defined in vivo with monostral blue dye and area of necrosis was determined after incubation of left ventricular slices in triphenyltetrazolium chloride. Hemodynamic variables were similar in the two groups during the experimental protocol. Infarct size was significantly reduced in treated animals, both when expressed as a percentage of the area at risk (9.9 ± 2.8% vs 40.9 ± 6.6%, p < .001) and as a percentage of the left ventricle (4.6 ± 1.3% vs 18.0 ± 3.4%, p = .002). This was associated with significant improvement in radial shortening in the ischemic zone 24 hr after reperfusion (10.1 ± 2.5 vs -2.8 ± 2.2%, p < .01). Regional myocardial blood flow was significantly increased in endocardial and epicardial regions from the lateral ischemic zone 1 hr after reperfusion in adenosine-treated animals. Light microscopy demonstrated decreased neutrophil infiltration in the ischemic zone and electron microscopy showed relative preservation of endothelial structure in the subendocardium with reduced neutrophil and red cell stagnation of capillaries in the treated group. These findings suggest that intracoronary administration of adenosine after reperfusion significantly reduces infarct size and improves regional ventricular function in the ischemic zone in the canine preparation.


EARLY REPERFUSION remains the most effective way of reducing infarct size in both the canine preparation and in man.1, 2 However, the amount of myocardium salvaged may be limited by anatomic and metabolic events occurring at the time of reperfusion, known as “reperfusion injury.”3 Multiple theories have been put forward to explain the mechanisms and pathogenesis of reperfusion injury. These include incomplete return of flow to areas of the microcircu-

lotion (the no-reflow phenomenon), generation of toxic reactive oxygen metabolites, and neutrophil-mediated endothelial cell damage.4-7 Preservation of endothelial cell structure and function may be an important mechanism of enhancement of myocardial salvage after successful reperfusion.8

Adenosine is an endogenous, potent arteriolar vasodilator that may be an important mediator of coronary autoregulation.9 Adenosine has also been shown to inhibit various neutrophil functions and reduce neutrophil-mediated endothelial damage in vitro.10, 11 We postulated that adenosine, by virtue of these properties, may preserve endothelial cell structure in the ischemic zone and thereby prevent the progressive decrease in microcirculatory flow that is known to occur after successful reperfusion.12 The effects of the selective administration of adenosine into the ischemic bed at reperfusion were assessed by measurement of infarct
size, regional myocardial blood flow, and left ventricular contractility in a 90 min preparation of occlusion-reperfusion in the dog. Endothelial ultrastructural changes and the presence of neutrophils and red cells within capillaries in the ischemic myocardium were also determined.

Methods

Experimental preparation. Thirty mongrel dogs of both sexes weighing 23 to 30 kg were used. The dogs were initially anesthetized with intravenous sodium pentobarbital (25 mg/kg), intubated, and ventilated with a Harvard positive-pressure respirator. The heart was exposed by a left thoracotomy, and the left anterior descending artery was isolated distal to the first diagonal branch. A surgical monofilament snare enclosed in a polyethylene pediatric feeding tube was implanted and anchored to the pericardium with two sutures. The pericardium and chest were then closed, the line was buried in a subcutaneous pocket, and prophylactic antibiotics given.

Experimental protocol (figure 1). The dogs were allowed 5 to 7 days to recover and were then randomly assigned to receive either adenosine or to reperfusion with blood alone (control group). Dogs were then re-anesthetized with 25 mg/kg iv pentobarbital, intubated with a cuffed endotracheal tube, and ventilated with a Harvard respirator. In both groups, similar doses of intravenous morphine (mean dose 8 mg/dog) and diazepam (mean dose 10 mg/dog) were used as supplemental anesthesia during the procedure. The animals did not receive heparin during the study, and arterial pH was maintained at 7.40 ± 0.05. Systemic arterial pressure and electrocardiographic leads I, aVF, and aVL were monitored throughout the procedure. Under sterile conditions, a No. 8F Cordis sheath was inserted into the right femoral artery and the right carotid artery. A No. 7F Cordis sheath was introduced into the left femoral artery, and a No. 7F pigtail catheter was used to obtain measurements of phasic and mean arterial blood pressure and left ventricular diastolic pressure throughout the experiment. Regional myocardial blood flow was assessed at baseline and serially with injections of 15 μm microspheres labeled with 199Sc, 185Re, 46Sc, and 125I (3M Company, St. Paul, MN) at 2 × 10^6 microspheres/injection into the apex of the left ventricle. Previous experience in this laboratory has shown no significant differences in baseline transmural blood flow in the anterior wall (0.71 ± 0.05 vs 0.77 ± 0.05 mL/min/g) and in the posterior wall (0.90 ± 0.04 vs 1.03 ± 0.03 mL/min/g) when microspheres are injected into the left atrium (n = 18) and the left ventricle (n = 26), respectively. Patency of the left anterior descending coronary artery was confirmed by selective injection of the left coronary artery. A baseline ventriculogram was obtained in a right anterior oblique projection with 7 ml of meglumine diatrizoate injected through a power injector. The degree of rotation was carefully noted to ensure that all subsequent ventriculograms were obtained in a similar view. The snare was then retrieved from the subcutaneous pocket, and each dog was given 2 mg/kg iv lidocaine before occlusion. After 1 hr of occlusion, hemodynamic measurements were repeated, and a contrast ventriculogram was obtained. Total occlusion of the left anterior descending artery was confirmed with selective angiography. At 90 min of occlusion, a repeat dose of lidocaine (2 mg/kg iv) was given, and the snare was gradually released over 5 min. In adenosine-treated animals, the left coronary ostium was engaged with a No. 8F Cordis guiding catheter, and a 2.5 mm × 2.0 cm USCI angioplasty catheter with a 0.014 inch flexible, steerable guidewire was positioned in the proximal left anterior descending artery, approximately 1 cm proximal to the snare. The left coronary ostium of control animals was engaged with a No. 8F Cordis guiding catheter, but no further instrumentation was done.

Thirty thousand units of intracoronary streptokinase was given. Patency of the artery was confirmed by an injection of contrast material through the guiding catheter, which was then disengaged from the left coronary ostium. Adenosine (Sigma) was dissolved in normal saline to form a solution of 2.5 mg adenosine/ml NaCl. Animals randomly assigned to the treatment group were given a constant infusion of 3.75 mg/min adenosine through a Harvard pump into the proximal left anterior descending artery through the angioplasty catheter. The volume given was 1.5 ml/min over 60 min or a total of 90 ml (225 mg adenosine). Due to the small volume of adenosine solution administered, control animals were not infused with a similar volume of saline. Regional myocardial blood flow was measured 5 min after commencement of adenosine infusion in treated animals and at a similar time in the control group. Regional myocardial blood flow was also determined 1 hr after reperfusion just before termination of the adenosine infusion. After 3 hr of reperfusion, a repeat ventriculogram was performed, and dogs were weaned from the respirator, extubated, given antibiotics, and allowed to recover. After 24 hr the animals were reanesthetized with 25 mg/kg iv pentobarbital and ventriculography was repeated. The heart was exposed through a left thoracotomy, and the snare was ligated. Monastral blue dye (DuPont) was injected through the pigtail catheter positioned in the ascending aorta in a dose of 1 mg/kg 2 to 3 min after ligating the snare. The animals were then rapidly killed by intravenous administration to 20 to 40 meq of potassium chloride, and the hearts were excised and weighed.

Assessment of infarction. The heart of each dog was sliced in five or six slices at 1 cm intervals parallel to the atrioventricular groove. The slices were then photographed with Ektachrome film to define the area at risk (area unstained by monastral blue dye). All slices were placed in a 2.0% solution of triphenyltetrazolium chloride (TTC) for 10 min at 37°C. This stained the viable myocardium in the area at risk bright red, but the infarcted area remained pale yellow (absence of TTC staining). The slices were rephotographed. An enlarged tracing (×5) was made from each slide with the use of a microscopic slide projector. The areas at risk and infarction were then determined by planimetry of the tracings by an observer blinded to the treatment groups using a computerized program. These areas
were multiplied by the thickness of each slice to obtain the volume of the perfusion bed and infarction.

Analysis of ventricular function. Contrast ventriculograms were analyzed with a modified method previously described by our laboratory. A computerized program was used and a longitudinal axis was constructed that connected the middle of the aortic valve plane to the apex of the heart for both the end-diastolic and end-systolic silhouettes. The midpoint of this axis was determined and 36 radii were constructed from this point at 10° intervals. Radial axes that involved the mitral and aortic valves were excluded from analysis. The ischemic zone was determined from the radii that were akinetic or dyskinetic at the occlusion ventriculogram. Percent shortening of each radius was determined with the following formula: percentage shortening = (end-diastolic length-systolic length) / (end-diastolic length) × 100. Global left ventricular ejection fraction was determined by the area-length method according to Simpson’s rule.

Light microscopy. The middle slice from all hearts was fixed in 10% buffered formaldehyde. After three days of fixation, sections were cut for light microscopy from the ischemic area (region of infarction), the two border zones (lateral), and from posterior wall (nonischemic area) extending from endocardium to epicardium. Tissue was dehydrated and embedded in paraffin; sections were cut and stained with hematoxylin and eosin and Mallory’s trichrome stains and were examined by light microscopy in a blinded manner. Variables assessed included degree of inflammatory infiltrate (the number of leukocytes within capillaries and within the interstitium), hemorrhage, and the presence of contraction band necrosis in the border zones (junction of infarcted and noninfarcted myocardium). An average of 20 high-power fields (HPF) per slide was evaluated. The above variables were assessed semiquantitatively according to the method of Romson et al., with a score of 4+ being assigned to the most severe infiltrate and a score of 0 assigned when rare or no changes were 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. Myocardial biopsy samples were taken within 60 sec of death from the central ischemic zone (anterior wall) and the nonischemic zone (posterior wall) and divided into endocardial and epicardial halves. The tissue obtained at biopsy was cut in 1 mm2 pieces and fixed in 3% buffered glutaraldehyde for transmission electron microscopy. Fifty-six specimens were examined from 14 (seven adenosine-treated; seven control) animals. The tissue was placed in 100 ml ice-cold 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 from ultrathin section cutting, stained with uranyl acetate lead citrate, and examined with Zeiss 109 JGF electron microscope.

Calculations of regional myocardial blood flow. A proximal and distal ventricular slice were chosen for analysis of myocardial blood flow. The slices were cut into 20 endocardial and epicardial sections (0.3 to 1.0 g) from the nonischemic area (posterior wall), and the ischemic region. The area at risk was divided into a central zone and two lateral zones. Myocardial sections and arterial reference samples were counted in a multichannel analyzer (Auto Gamma scintillation spectrometer, Model 5986, Packard Instrument Company, Inc., Downers Grove, IL), and myocardial blood flow was determined in milliliters per minute per gram wet weight as previously described in our laboratory.

Statistical analysis. Intergroup comparisons of infarct size were performed with Student’s t test for unpaired data. Data from multiple groups were analyzed by repeated-measures two-way analysis of variance (ANOVA) with the use of BMDP software (BMDP2V, BMDP Software, Los Angeles, CA). When a significant interaction was noted between the variables, Student’s t test was used to compare individual data points. A p value of less than .05 was used to reject the null hypothesis at equality. All data are expressed as the mean ± SEM.

RESULTS

Thirty dogs underwent coronary artery occlusion and 10 were excluded. Six (three adenosine-treated; three control) developed ventricular fibrillation either during occlusion or after reperfusion. One dog in the control group failed to develop significant ischemia (subendocardial blood flow did not fall and no wall motion abnormality was seen on the ventriculogram). One dog in the treatment group was excluded because of malposition of the angioplasty catheter in the first diagonal branch, and two were excluded because of failure of the snare to release at reperfusion. Data from the remaining 20 dogs form the basis for this report: 10 received intracoronary adenosine during reperfusion and 10 were reperfused with blood (control group).

Hemodynamic variables (figure 2). Heart rate, systolic blood pressure, rate-pressure product, and left ventricular end-diastolic pressure are shown in figure 2. Heart rate was significantly higher in the treatment group at 1 hr after reperfusion. However, the double product (systolic blood pressure × heart rate), an indirect assessment of the myocardial oxygen consumption, and the left ventricular end-diastolic pressure were similar in both groups throughout the study protocol.

Effects on regional myocardial blood flow (figure 3). Results of regional myocardial blood flow in nine adenosine-treated and nine control animals are illustrated in figure 3. Baseline flows in the ischemic and nonischemic zones were similar in both groups. Both adenosine-treated and control animals developed a similar and severe reduction in flow in the central and lateral ischemic zones at 1 hr of occlusion suggestive of comparative poor collateral blood flow in the risk region. Both groups exhibited reactive hyperemia at reperfusion, with adenosine-treated dogs having significantly greater flow in epicardial sections from the lateral zones. This increase coincided with commencement of the adenosine infusion before the flow measurements were obtained. Flow declined at 1 hr in control animals while adenosine treatment resulted in a continual rise in transmural flow in the ischemic zone, with a relatively greater increase in subepicardial flow. Flow in the control zone (posterior wall) tended to be greater 1 hr after reperfusion in the treated group, probably secondary to the recirculation of adenosine through the
pulmonary bed or to enhanced collateral blood flow.

**Effects on infarct size (figure 4).** No significant differences were noted in left ventricular volume (63.4 ± 6.6 vs 66.4 ± 3.7 cm³) or the volume at risk (27.5 ± 2.7 vs 28.0 ± 2.2 cm³) in the groups. A highly significant decrease in infarct size, expressed both as a percentage of total left ventricle and as a percentage of volume at risk, was observed. Percentage of myocardium at risk that became infarcted was 40.9 ± 6.6% in the control group and 9.9 ± 2.8% in the adenosine group (p < .001). The percentage of the left ventricular mass that became infarcted was 18.0 ± 3.4% in the control group and 4.6 ± 1.3% in the adenosine group (p = .002). Infarcts in the treated group were usually confined to the subendocardium in both the central and lateral ischemic zones and tended to be more patchy.

**Relation of infarct size, area at risk, and myocardial blood flow (figures 5 and 6).** The relationship between the percentage of area at risk and transmural mean collateral blood flow measured 60 min after occlusion is shown in figure 5. A linear relationship was noted between these variables (r = .62, p < .01). Figure 6 shows a regression plot of infarct size as a percentage of the area at risk vs mean collateral blood flow in treated and control animals. In control animals, a definite inverse relationship between infarct size and collateral blood flow was present (r = −.75, p = .02), whereas in adenosine-treated animals, infarct size was small irrespective of flow (r = −.57, p = .1). Linear regression lines showed a shift in the slope to the left in the treated group, supporting an effect of treatment on infarct size. However, there was no significant difference in the slopes for the two groups. The difference between the two regression lines was greatest at low collateral flow, suggesting a greater benefit of treatment during severe ischemia.

**Effects on ventricular function (figure 7).** The results of regional and global ventricular function are shown in figure 7. No significant differences were noted in baseline radial shortening (23.3 ± 2.6% vs 22.4 ± 2.2%) or the number of radii in the ischemic zone (14 ± 0.4 vs 15 ± 0.3). Both groups developed similar degrees of dyskinesis in the ischemic zone at 1 hr of occlusion. However, after 3 hr of reperfusion, while control animals continued to be dyskinetic, treated animals demonstrated positive wall motion (−0.43 ± 2.1% vs 7.7 ± 3.6%, p ≤ .05). Adenosine-treated dogs continued to improve at 24 hr, whereas regional wall motion in control animals remained unchanged (−2.8 ± 2.2% vs 10.1 ± 2.4%, p ≤ .01). A similar trend was seen in global ejection fraction in the treated group at 3 and 24 hr after reperfusion, but the difference did not reach statistical significance (37 ± 3% vs 45 ± 4%).

**Light and electron microscopy (table 1; figures 8 and 9).** Seven randomly selected animals from the treatment group and seven control dogs were examined by light microscopy. Confluent hemorrhagic infarction was observed in six control dogs and patchy hemorrhage was seen in one. The number of contraction bands present in the border zone resulted in a score of 2+ to 4+ (moderate to severe) in five control animals. The inflammatory infiltrate (interstitial and intravascular) was moderate to severe (2+ to 4+) in all control animals. All animals treated with adenosine had patchy, subendocardial infarcts. Hemorrhage was absent or 1+ in six of the animals, and contraction band
necrosis was not prominent in any animals treated with adenosine. Inflammation was sparse, with six animals showing no inflammation or 1+ inflammation. One animal had moderate inflammatory infiltration (2+) (table 1).

Electron microscopy was performed in seven randomly selected dogs from the adenosine group and in seven control animals. Both showed prominent 1 bands and swollen mitochondria with disrupted cristae and amorphous matrix densities in the subendocardium of the ischemic zones. The subepicardial zone showed similar changes in four control animals; however, no such changes were seen in the treated group. Marked differences were noted in the ultrastructure of the endothelium between the two groups. The capillary lumen in control dogs showed membrane-bound bodies and endothelial protrusions with red cell and white cell plugging (figure 8). Endothelial cell disruption and loss of pinocytotic vesicles were seen in all control animals with and without fibrin deposition. Adenosine-treated animals showed intact swollen endothelial cells with occasional endothelial folds but no obstructions of capillary lumens in the endothelial ischemic regions (figure 9). Pinocytotic vesicles were not reduced, and endothelial basement membranes were intact. Some capillary lumina contained loose membranes and flocculent material. Obstruction of capillaries by neutrophils and red cells were invariably present in control animals but were rarely seen in the treated group.

FIGURE 3. Serial changes in regional myocardial blood flow in the control zone (nonischemic region) and central and lateral third of the ischemic zone in the two groups. A significant increase was observed in the epicardial ischemic zone immediately and 1 hr after reperfusion in the adenosine-treated animals. In contrast, flow in control animals was significantly less immediately after reperfusion, and fell at 1 hr after reperfusion. The increase in flow in the nonischemic zone in the adenosine-treated animals probably represents recirculation of adenosine through the pulmonary circulation. Numbers in parentheses are n values.

FIGURE 4. A highly significant decrease in infarct size (AN) when expressed both as a percentage of area at risk (AR) or the total left ventricle (LV) was present in the adenosine-treated animals. The percentage of the area at risk of the left ventricle (AR/LV) was similar in both groups.
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reperfusion itself produces histologic and functional derangements in myocytes and endothelial cells that may limit the amount of potentially salvageable myocardium (so-called reperfusion injury).\textsuperscript{3, 5} Such derangements include marked cellular swelling and degradation of cellular membranes with an incomplete return of blood flow to the microcirculation (no-reflow phenomenon).\textsuperscript{5, 8, 12} Although the exact mechanisms producing these changes are not known, the introduction of oxygen and blood elements, particularly neutrophils, during reperfusion into the ischemic bed are

**FIGURE 5.** A significant correlation was noted between the percentage of area of the left ventricle at risk and the transmural mean collateral flow in the central ischemic zone, measured at 1 hr into occlusion ($r = - .62, p < .01$).

**FIGURE 6.** The percentage of the risk region that became infarcted (AN/AR) was related to epicardial collateral flow in the central ischemic zone at 60 min into occlusion. Regression lines have been drawn for both control and adenosine-treated animals. Note that control animals had larger infarcts for the same degree of ischemia as compared with the adenosine-treated animals. This is manifest by a leftward and downward shift in the regression line in treated animals, indicating that infarct size reduction was due to the therapeutic intervention.

**FIGURE 7.** Serial changes in regional radial shortening in the ischemic zone (top) and global ejection fraction (bottom) in the two groups. A significant improvement was noted in radial shortening in the adenosine-treated animals at 3 and 24 hr after reperfusion. Ejection fraction was also increased in treated animals, but this difference did not reach significance.
FIGURE 8. A. Electron micrographs from control animal showing endothelial cell swelling and endothelial protrusions (arrow) causing luminal narrowing with red (R) and white cell plugging (W) (A and B). B. Note almost total absence of pinocytotic vesicles and white cell plugging. C. Capillary with endothelial cell disruption (arrow) and luminal obstruction by red and white cells. (A, Original magnification × 7700; B, original magnification × 12150; C, original magnification × 7700.)
currently thought to be important mediators of reperfusion injury.\textsuperscript{20-23} Oxygen free radicals are reactive, cytotoxic compounds that may result in lipid peroxidation of cellular membranes and protein denaturation.\textsuperscript{6} A burst of free-radical activity has been shown to occur within seconds after coronary reperfusion.\textsuperscript{24} Although the exact cellular site of free-radical production at reperfusion is unknown, the neutrophil, myocyte, and endothelium are potential sources.\textsuperscript{21, 25}

Neutrophils may further contribute to reperfusion injury by mechanically plugging the microcirculation and/or by releasing proteolytic enzymes.\textsuperscript{7, 21, 26}

**Present study.** Adenosine is an endogenous substance produced during the metabolism of ATP. Adenosine is known to cause relaxation of coronary vascular smooth muscle and may be an important autoregulator of coronary blood flow.\textsuperscript{9} Adenosine has also been shown to modulate neutrophil function by reducing neutrophil

**FIGURE 9.** A, Subendocardial region in an adenosine-treated animal. Note capillary within ischemic myocardium with intact cytoplasm, mild endothelial swelling, and membrane-bound structures (arrow) within lumen. B, Another capillary with mild endothelial swelling and increase in endothelial cell folds (arrow). The lumen contains membrane-bound structures and proteinaceous material. C, Capillary with prominent pinocytic vesicles, intact basement membrane, and a few membrane-bound structure. (A, Original magnification $\times 6231$; B, original magnification $\times 20330$; C, original magnification $\times 6230$.)
TABLE 1
Qualitative and quantitative changes in infarct morphology between treated and control animals

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<tr>
<th>Histologic variables assessed</th>
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adherence and cytotoxicity (free-radical and/or proteolytic enzyme release) in vitro.\textsuperscript{10, 11} Although adenosine has been shown to increase coronary blood flow after reperfusion in the experimental preparation, no studies have quantitatively assessed its role in reducing infarct size after reperfusion.\textsuperscript{27} We postulated that adenosine, because of its vasodilatory and antineutrophil properties, may be a useful agent in reducing reperfusion injury.

Our study demonstrates that the selective, intracoronary administration of adenosine after reperfusion results in a 75% reduction in infarct size in the canine preparation when expressed as a percentage of both the area at risk and the total left ventricular mass. Infarcts in the adenosine group were confined to the subendocardium and were patchy, whereas most control animals demonstrated confluent infarctions extending to the midmyocardium. Histologic examination revealed absent or minimal neutrophil infiltration in the border zones and preservation of endothelial cell structure in the ischemic subendocardium of treated animals only. Capillary obstruction by endothelial protrusions and neutrophil and red cell stacking were frequent in control animals, but were either absent or only occasionally seen in the treated group.

Since a major objective of reperfusion is to improve ventricular function, we also assessed myocardial contractility by contrast ventriculography. Since global ejection fraction in the peri-infarction period is influenced by the compensatory, hypercontractile state of the nonischemic ventricular segments, regional radial shortening in the ischemic zone was examined in this experiment. Radial shortening in the adenosine-treated animals was significantly improved compared with that in control dogs at both 3 and 24 hr after reperfusion. The reduced infarct size and improved ventricular function in the adenosine-treated group were associated with a significantly greater myocardial blood flow in the epicardial ischemic bed immediately and at 1 hr after reperfusion.

Possible mechanisms of action. Reperfusion of ischemic myocardium in known to be followed by a progressive decline in blood flow to regions of potentially salvageable myocardium.\textsuperscript{12} Although the mechanism of this progressive no-reflow is not well understood, the endothelium is thought to play a key role since reperfusion is associated with a degree of endothelial disruption that is not present in preparations of permanent occlusion after comparable durations of ischemia.\textsuperscript{5, 8, 28, 29} Although the relationship between no-reflow and the endothelium has not been precisely defined, mechanical (capillary obstruction by endothelial protrusions and aggregated blood elements) and/or functional derangements may be involved.\textsuperscript{5, 8, 23, 26} Endothelial cells regulate coronary vascular reactivity normally by the release of endothelial-derived relaxation factor.\textsuperscript{30} It has recently been shown that hypoxic and anoxic endothelial cells are capable of releasing vasoconstricting agents.\textsuperscript{31} No-reflow may result from the imbalance between vasodilator substances and vasoconstrictors after reperfusion.

Although the mechanism by which adenosine limits infarct size remains unknown, this study suggests several possibilities. Selective administration of adenosine into the ischemic bed increased blood flow in both the endocardial and epicardial regions of the lateral ischemic zone. This increased flow is at least in part attributable to the delivery of a potent, endothelial-independent vasodilator into a vasoconstricted coronary bed, thereby enhancing oxygen delivery to ischemic myocardium.\textsuperscript{9} In addition, histologic analysis by electron microscopy demonstrated better preservation of the endothelium in the ischemic subendocardium of adenosine-treated animals when compared with control. Preservation of the structural and functional integrity of the endothelium would both permit the physiologic release of endothelial-derived relaxation factor and reduce the degree of capillary obstruction by endothelial cell protrusions. Adenosine may therefore be limiting no-reflow by endothelial-independent and endothelial-dependent mechanisms.

The preservation of the endothelium in this study may also be due to the effect of adenosine on neutrophils. Histologic studies demonstrated a reduced neutrophil infiltration in the ischemic zone without neutrophil capillary plugging in adenosine-treated ani-
imals. Activated neutrophils have been shown to disrupt endothelial basement membranes in vitro by the release of proteolytic enzymes or free radicals. By interfering with the neutrophil-endothelial cell interaction, adenosine may be protecting reversibly damaged endothelium. In support of this hypothesis are studies in vitro demonstrating reduced adherence and cytotoxicity of stimulated neutrophils on endothelial cells exposed to adenosine. Adenosine has also been shown to decrease oxygen free-radical production by neutrophils.

The improved ventricular function in adenosine-treated animals as compared with control dogs may be directly related to decreased infarct size and/or to a reduction in “myocardial stunning,” the delayed postischemic recovery of ventricular function. Both ATP depletion and oxygen free-radical formation have been implicated as potential mechanisms of postischemic ventricular dysfunction. As a result of improved flow to the ischemic region with adenosine administration, ATP repletion may be accelerated either by synthesis de novo secondary to decreased ischemia or via increased formation from its precursor adenosine. In the isolated, isovolumetrically contracting rat heart, adenosine has been shown to decrease degradation of ATP during ischemia and facilitate repletion of ATP during reperfusion. Inhibition of adenosine catabolism augments postischemic repletion of total adenine nucleotide content and enhances postischemic recovery of myocardial function in the isolated perfused rat heart. Purine treatment during global myocardial ischemia in the canine preparation has also been shown to improve postischemic myocardial function. ATP catabolism, which is known to occur during ischemia, may result in free-radical generation after the introduction of molecular oxygen at reperfusion, since the superoxide (·O2−) free radical is a byproduct of conversion of the ATP catabolite hypoxanthine to xanthine by xanthine oxidase. By reducing ATP degradation, adenosine may decrease production of oxygen free radicals after reperfusion, thereby limiting the degree of myocardial stunning. Augmentation of coronary blood flow by papaverine and dipyridamole, an adenosine uptake blocker, has also been shown to improve postischemic ventricular dysfunction. The improved contractile function seen in this study may therefore simply be the result of increased regional myocardial blood flow.

Implications. The development of fibrin-specific thrombolytic agents and balloon angioplasty has resulted in reperfusion as a logical maneuver in limiting infarct size in man. The addition of an agent at the time of reperfusion that could enhance myocardial salvage would therefore have important therapeutic implications. This study demonstrates that adenosine given after reperfusion prevents the progressive fall in myocardial blood flow that occurs after reperfusion, resulting in marked reduction in infarct size and improvement in regional ventricular function in the canine preparation. Since the mechanisms for adenosine degradation vary in different animal species and in man, extrapolating these data to patient care must be done with caution. However, these data suggest that the selective, intracoronary administration of adenosine may have a beneficial role in enhancing salvage and improving left ventricular function in man after successful reperfusion.

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LABORATORY INVESTIGATION—REPERFUSION
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