Oxypurinol limits myocardial stunning but does not reduce infarct size after reperfusion

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ABSTRACT  To explore the role of oxygen free radicals produced by the xanthine oxidase pathway on infarct size and left ventricular function, the effect of oxypurinol, an active metabolite of allopurinol and a potent noncompetitive inhibitor of xanthine oxidase, was assessed in a 90 min, closed-chest, canine preparation of occlusion-reperfusion. Animals were randomized to receive 25 mg/kg iv oxypurinol (n = 13) or saline (n = 13) 60 min after occlusion. Regional myocardial blood flow was measured with radioactive microspheres and regional ventricular function with contrast ventriculography. Hemodynamic variables, regional myocardial blood flow, and size of the occluded bed were similar in the two groups. Oxypurinol failed to reduce infarct size 24 hr after reperfusion when expressed as a percentage of the area at risk (36.3 ± 4.9% vs 36.0 ± 5.6%; p = NS). Both groups exhibited comparative radial shortening at baseline and similar degrees of dyskinesia 1 hr into occlusion (−6.6 ± 1.2% vs −4.9 ± 1.0%). However, oxypurinol-treated animals demonstrated an improved regional ventricular function at 3 hr after reperfusion (0.7 ± 2.6% vs −2.8 ± 2.0%) and a significant improvement at 24 hr (5.4 ± 2.5% vs −3.2 ± 1.7%; p < .05). A reduced neutrophil infiltrate was observed in the border zone in treated animals. These findings suggest that oxygen free radicals derived from the xanthine oxidase pathway contribute to stunning of reversibly damaged myocardium but do not determine the final extent of myocardial necrosis in a canine preparation of reperfusion.


FREE RADICALS are reactive compounds that are toxic to cellular membranes and organelles and that are thought to be involved in the pathogenesis of ischemic-reperfusion injury.1,2 Oxygen free radicals have been implicated in the death of potentially salvageable myocardium when blood flow is restored to ischemic areas (“reperfusion injury”) and more recently have been shown to contribute to the prolonged left ventricular dysfunction (“myocardial stunning”) that occurs in reversibly injured myocytes.3–8

The cellular origin of and metabolic pathways involved in free radical generation in the heart remain speculative. One potential source of the superoxide free radical (O2−) in reperfused myocardium is the enzyme xanthine oxidase, which catalyzes the first irreversible step in the degradation of the adenine-based nucleotides.9,10 The effect of allopurinol, a competitive inhibitor of xanthine oxidase, on infarct size after reperfusion has been variable.9,11,12 However, allopurinol has been shown to improve ventricular function in the ischemic-reperfused canine preparation.13,14 Oxypurinol is the active metabolite of allopurinol and may be a more potent inhibitor of xanthine oxidase in the setting of ischemia.11,15,16

The aim of the present study was to assess the effect of oxypurinol administered 60 min after occlusion on infarct size and serial ventricular function in a 90 min canine preparation of occlusion-reperfusion. This duration of ischemia was chosen because it not only results in myocardial necrosis but also salvages greater than 50% of the ischemic bed.17 Since allopurinol has been reported to inhibit neutrophil function and to cause vasodilatation, myocardial neutrophil accumulation was semiquantified with light microscopy and collateral blood flow was measured with microspheres.18–20

Methods

Surgical preparation. Mongrel dogs of either sex weighing 20 to 25 kg were quarantined for 2 weeks to ensure that they were
free of canine diseases. Dogs were prepared approximately 1 week before the experiment under general anesthesia (30 mg/kg sodium pentobarbital) via a left thoracotomy. The left anterior descending coronary artery was isolated distal to the first diagonal, and a surgical monofilament snare enclosed in a polyethylene tubing was implanted and anchored to the myocardium with a small suture. The pericardium and chest were closed, and the snare was buried in a subcutaneous pocket in the subscapular region.

Experimental protocol (figure 1). On the day of the experiment, dogs were randomly assigned to receive either oxypurinol or saline, reanesthetized with 30 mg/kg iv pentobarbital, intubated, and ventilated with a Harvard positive-pressure respirator to maintain an arterial pH of 7.4 ± 0.05. In both groups, similar intravenous doses of morphine (mean dose = 8 mg/dog) and diazepam (mean dose = 10 mg/dog) were used as supplemental anesthesia during the experimental protocol. Electrocardiographic leads I, aVL, and aVF were monitored throughout the study. Cutdowns were performed on both groins, and No. 7F Cordis sheaths were placed into both femoral arteries and the right femoral vein. The snare was retrieved from its subcutaneous pocket. Phasic and mean arterial pressure were measured with either a No. 7F pigtail catheter or a No. 7F modified right Judkins catheter. A Swan-Ganz catheter was inserted via the right femoral vein to the level of the right atrium for the intravenous injection of oxypurinol or saline. The sheath in the left femoral artery was connected to a constant withdrawal pump to collect blood after the injection of microspheres. The dogs were allowed to stabilize for 30 min before manipulation.

Baseline hemodynamic measurements were then made. Regional myocardial blood flow was determined in 15 dogs (seven oxypurinol and eight controls) at baseline, 1 hr into occlusion, and immediately after administration of streptokinase (reperfusion) with 5 ml injections of 15 μm microspheres labeled with 141Ce, 85Sr, and 46Sc, respectively (3M Co., St. Paul), at ~2 × 10^6 microspheres/injection into the apex of the left ventricle through a pigtail catheter. Femoral arterial samples were collected after injection to allow calculation of myocardial blood flow in milliliters per minute per gram. A baseline ventriculogram was obtained in the right anterior oblique projection with 5 to 7 ml of meglumine diatrizoate (Renografin-76) injected through a power injector. Confirmation of patency of the left anterior descending coronary artery was determined by selective coronary angiography.

Each dog was then given 1 mg/kg lidocaine and the snare was tightened. After 1 hr of occlusion, 25 mg/kg oxypurinol or saline in an equivalent volume was infused via the Swan-Ganz catheter over 5 to 10 min. Since oxypurinol requires an alkaline pH (pH = 10) to dissolve, the saline control solution was adjusted to a similar pH with sodium hydroxide before injection. Hemodynamic measurements were then repeated, myocardial blood flow was determined, and a contrast ventriculogram was obtained. After 90 min, occlusion of the artery was documented by contrast arteriography, and the snare was gradually released over 2 to 3 min. To simulate reperfusion with thrombolytic therapy, 30,000 U of streptokinase was administered by the intracoronary route. Patency of the left anterior descending coronary artery was confirmed by selective coronary angiography. No residual thrombi or arterial kinking was noted in any of the animals at the site of occlusion on review of arteriograms. Hemodynamic measurements were repeated immediately. 1 hr, and 3 hr after reperfusion. Blood samples were obtained at reperfusion and 3 hr after reperfusion for determination of plasma oxypurinol levels. Oxypurinol concentrations were measured by a competitive-protein binding assay as previously described. After a 1 mg/kg bolus of lidocaine, a repeat contrast ventriculogram was made at 3 hr after reperfusion and the dogs were weaned from the ventilator, given prophylactic antibiotics (600,000 U Vetricil-AS), and allowed to recover.

After 24 hr, dogs were reanesthetized with 30 mg/kg pentobarbital and given a 1 mg/kg iv bolus of lidocaine. A contrast ventriculogram was repeated with the dogs in a position similar to that at baseline. After the plasma oxypurinol level was determined, the chest was then opened by thoracotomy, and the snare was retrieved and tightened. A dose of 1 ml/kg monastral blue (DuPont) was injected into the ascending aorta 2 to 3 min after ligation of the artery. The animals were then killed within 30 to 60 sec after dye injection with an overdose of potassium chloride, and the heart was rapidly excised.

Analysis of infarction. The excised heart of each dog was weighed, and the left ventricle was sliced in six or seven slices at 1 cm intervals parallel to the posterior atrioventricular groove and photographed to define the area at risk (unstained by monastral blue dye). The slices were immersed in 2% triphenyltetrazolium chloride (TTC) at 37°C for 5 to 10 min and photographed. The region of infarcted myocardium in the area of risk was demarcated by the absence of TTC staining, and the viable myocardium in the area of risk stained bright red. An

**PROTOCOL**

![Graphical representation of the protocol](image)

1. Hemodynamic Measurements (B, 1 hr O, R, I, 3, 24 hrs)
2. RMBF (B, 1 hr O, R)
3. CVG (B, 1 hr O, 3 hrs, 24 hrs)
4. Oxi Levels (R, 3, 24 hrs)

**FIGURE 1.** Summary of experimental protocol. CVG = contrast ventriculogram; ICSK = intracoronary streptokinase; 1 hr O = 1 hr after occlusion; Oxi = oxypurinol; RMBF = regional myocardial blood flow.
enlarged tracing was made from each slide with the aid of a microscopic slide projector. The areas of the perfusion bed and infarction were then determined by computerized planimetry of the tracings by an observer blinded to the treatment groups. These areas were multiplied by the thickness of each cross section. Summation of the volume of each tissue sample yielded the volume of the perfusion bed and the total volume of infarction. The ratio of the infarct volume to the volume at risk was calculated. Since TTC staining may underestimate patchy infarcts because of proximity of reversibly and irreversibly damaged myocytes, accuracy of the area of necrosis was confirmed by planimetry of a midventricular slice stained by Mallory's trichrome stain in four randomly selected animals.

Analysis of contrast ventriculograms. Since most animals manifested frequent premature ventricular contractions at 3 and 24 hr after reperfusion, a 1 mg/kg bolus of lidocaine was given before ventriculography. Most dogs responded, and the ventriculograms were taken only during periods of normal sinus rhythm. The contrast ventriculograms were analyzed by a modified method described by Behar and Stack and their colleagues. Briefly, a longitudinal axis was constructed connecting the middle of the aortic valve plane and apex of the heart for both the end-diastolic and end-systolic silhouettes. Radii were then constructed from the midpoint of the axis to the edge of the ventricular silhouette at 10 degree intervals (36 segments). Percent shortening of each radius was calculated according to the formula: percentage shortening = (end-diastolic length - systolic length/end-diastolic length × 100). 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 hr of occlusion. Changes in all radii in the ischemic zone were averaged to determine the regional shortening at baseline, 3 hr, and 24 hr after reperfusion.

Calculation of myocardial blood flow. Ventricular samples for determination of regional myocardial blood flow were obtained from endocardial and epicardial sections (0.3 to 1.0 g) in a nonischemic area (posterior wall) and in the central area of ischemia (anterior wall). Samples were obtained from a proximal and distal left ventricular slice. Myocardial sections and arterial reference samples were counted in a multichannel analyzer (Auto Gamma scintillation spectrometer, Packard Instrument Co., Inc.). The myocardial blood flow (ml/min/g) as calculated as previously described.

Light microscopy. Myocardial sections not used for microsphere flow determinations were fixed in 10% buffered formaldehyde for 1 week. Transmural myocardial sections were obtained from the ischemic and nonischemic areas, dehydrated, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. Neutrophil accumulation within vessels and the surrounding myocardium was quantified (0 to 4 +) in the ischemic (anterior) and nonischemic (posterior) zones by a pathologist blinded to the treatment groups as previously described.

Statistical analysis. All data are presented as the mean ± SEM. Data from successive time points in the two groups were tested with analysis of variance followed by Duncan’s multiple range test. Intergroup comparisons of infarct size were performed by Student’s t test for unpaired data. Linear regression analysis was performed to examine the relationship between the degree of neutrophil infiltration and the radial shortening in the ischemic zone at 24 hr. Since infarct size in this preparation is dependent on the area at risk, epicardial collateral blood flow, and myocardial oxygen consumption (determined by rate-pressure product), these independent variables were analyzed by linear regression to determine their contribution to infarct size as a percentage of the area at risk in the two treatment groups.

Probability values of .05 or less were required for assumption of statistical significance.

Results

Thirty-one dogs were entered into the study. Three died of ventricular fibrillation during occlusion (two oxypurinol, one control), and two were excluded when the risk volume was determined to be less than 20% of the left ventricle (two control). Twenty-six dogs were included in the final analysis, 13 in the oxypurinol group and 13 controls.

Laboratory and hemodynamic measurements (figure 2). The pH and P O₂ were comparable in both groups at baseline and 3 hr after reperfusion. Heart rate, systolic and diastolic pressures, mean arterial pressure, left ventricular end-diastolic pressure, and rate-pressure product were similar in both groups throughout the study (figure 2). There were no hemodynamic changes during the intravenous administration of oxypurinol or saline. The average oxypurinol concentrations at reperfusion, 3 hr, and 24 hr after reperfusion were 163 ± 64, 70 ± 42, and 3 ± 3 μM, respectively.

Effect on infarct size (table 1). The average heart weight and left ventricular volume were comparable in both groups. The region at risk was similar in the oxypurinol-treated and control groups when expressed as a percentage of the left ventricular mass (38.4 ± 2.9% vs 42.1 ± 2.9%). Although the risk regions varied from 23.7% to 55.1%, reflecting the variability in the distribution of the left anterior descending coronary artery and epicardial collateral blood flow, 95% of the
TABLE 1
Effect of oxypurinol on infarct size 24 hr after reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 13)</th>
<th>Oxypurinol (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>204 ± 12</td>
<td>175 ± 9</td>
</tr>
<tr>
<td>(150–240)</td>
<td>(105–220)</td>
<td></td>
</tr>
<tr>
<td>LV volume (cm³)</td>
<td>72.8 ± 5.0</td>
<td>71.1 ± 4.3</td>
</tr>
<tr>
<td>(50.4–95.7)</td>
<td>(55.1–107.2)</td>
<td></td>
</tr>
<tr>
<td>Infarct volume (cm³)</td>
<td>11.5 ± 2.6</td>
<td>9.2 ± 1.7</td>
</tr>
<tr>
<td>(2.3–31.5)</td>
<td>(2.4–20.2)</td>
<td></td>
</tr>
<tr>
<td>Volume of risk region (cm³)</td>
<td>29.4 ± 2.4</td>
<td>25.9 ± 1.8</td>
</tr>
<tr>
<td>(18.6–38.0)</td>
<td>(15.2–33.7)</td>
<td></td>
</tr>
<tr>
<td>Risk region/LV (%)</td>
<td>42.1 ± 2.9</td>
<td>38.4 ± 2.9</td>
</tr>
<tr>
<td>(26.6–55.1)</td>
<td>(23.7–53.0)</td>
<td></td>
</tr>
<tr>
<td>Infarct/LV (%)</td>
<td>16.8 ± 3.2</td>
<td>14.8 ± 2.6</td>
</tr>
<tr>
<td>(3.0–36.2)</td>
<td>(3.7–29.6)</td>
<td></td>
</tr>
<tr>
<td>Infarct/risk region (%)</td>
<td>36.0 ± 5.6</td>
<td>36.3 ± 4.9</td>
</tr>
<tr>
<td>(17.1–67.7)</td>
<td>(14.4–60.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM, with ranges in parentheses.
LV = left ventricle.

values were clustered within ± 12% of the mean (SD = 5.8%). No significant differences were noted in infarct size between oxypurinol and control animals when expressed either as a percentage of the left ventricular volume (14.8 ± 2.6% vs 16.8 ± 3.2%) or the volume of the region at risk (36.3 ± 4.9% vs 36.0 ± 5.6%).

Relation of infarct size, collateral blood flow, area at risk, and rate-pressure product (figure 3). The contribution of the baseline predictors of infarct size in this preparation (epicardial collateral blood flow, area at risk, and myocardial oxygen consumption as assessed by the rate pressure product) were determined by linear regression analysis. Epicardial collateral blood flow in the central ischemic region was found to be the most accurate predictor of infarct size (r = .65, p = .009), with a loose but significant correlation existing between the area at risk and infarct size expressed as a percentage of the area at risk (r = .48, p = .01). The rate-pressure product was found to be the least accurate predictor of infarct size (r = .36, p = .09). The slopes of the regression lines for all three variables were similar in the oxypurinol-treated and control animals, demonstrating the similarity of the flow baseline predictors. The similar slopes relating infarct size to collateral blood flow also suggest comparable degrees of ischemia in both groups.

Regional ventricular function (figure 4). The number of radii in the ischemic zone (13.3 ± 0.5 vs 14.3 ± 0.4), the global ejection fraction (59 ± 5% vs 58 ± 5%), and the average radial shortening (24.0 ± 2.8% vs 24.3 ± 2.3%) within this zone at baseline were similar in the oxypurinol-treated and control groups. Both groups exhibited similar degrees of dyskinesis in the ischemic zone.
zone 1 hr after occlusion ($-6.6 \pm 1.2\%$ vs $-4.9 \pm 1.0\%$). Oxypurinol-treated animals demonstrated an improved regional radial shortening by 3 hr after reperfusion ($0.7 \pm 2.6\%$ vs $-2.8 \pm 2.0\%$) and a significant improvement at 24 hr after reperfusion ($5.4 \pm 2.5\%$ vs $-3.2 \pm 1.7\%; p < .05$). Global ejection fraction also tended to be greater in the treated group at 24 hr ($40 \pm 3\%$ vs $33 \pm 5\%; p = .07$).

Regional myocardial blood flow (Figure 5). Data on regional blood flow in the central ischemic and nonischemic zones were available in 15 dogs (seven oxypurinol, eight controls) and are shown in Figure 5. No significant differences were noted in subendocardial or subepicardial blood flow in both groups at baseline, 1 hr into occlusion, or immediately after reperfusion. Endocardial flow in the central ischemic zone 1 hr into occlusion was reduced markedly to less than 15% of baseline flow in both oxypurinol-treated and control animals suggestive of comparably severe ischemia ($0.12 \pm 0.07$ vs $0.15 \pm 0.10$ ml/min/g, respectively).

Light microscopy. Light microscopy revealed extensive ($3^+ \text{ to } 4^+$) polymorphonuclear leukocyte infiltrates in the surrounding area of myocardial necrosis in 10 of 13 control animals, whereas only three of 13 treated animals had a similar extent of inflammation. The remaining animals had no or only minimal infiltrates ($0 \text{ to } 2^+$). A loose correlation was noted between the extent of neutrophil infiltration and radial shortening at 24 hr in the ischemic zone ($r = -0.57, p = .01$)

Discussion

Free radicals in ischemic-reperfused myocardium. The acute onset of myocardial ischemia results in the rapid catabolism of the high-energy purine nucleotide adenosine triphosphate (ATP) to adenosine, inosine, and finally hypoxanthine. Ischemia has also shown to result in conversion of xanthine dehydrogenase (which uses NAD+ as an electron acceptor) to xanthine oxidase. Both the dehydrogenase and oxidase enzymes catalyze the irreversible degradation of hypoxanthine to xanthine and xanthine to uric acid; however, the oxidase form utilizes molecular oxygen as an electron acceptor with the production of one superoxide anion ($O_2^-$) for each molecule of hypoxanthine oxidized. The high intracellular concentration of hypoxanthine and the increased xanthine oxidase activity present in ischemic myocardium may therefore result in the production of large amounts of the superoxide free radical with the introduction of oxygen at reperfusion. A burst of free radical production has previously been demonstrated in ischemic myocardial tissue at the time of reperfusion. Free radicals are reactive metabolites (possessing an unpaired electron) that are known to degrade lipids and nucleic acids. Although endogenous enzyme systems, such as superoxide dismutase, catalase, and glutathione peroxidase, protect cells against injury by scavenging free radicals,
Ischemia depletes these enzymes and may therefore allow greater damage of the myocardium during reperfusion and reoxygenation.\textsuperscript{1, 28, 29}

Inhibition of xanthine oxidase by oxypurinol (figure 6). In vitro studies have examined the molecular interactions among allopurinol, oxypurinol, and xanthine oxidase.\textsuperscript{15, 16} Xanthine oxidase is a complex enzyme with a self-contained electron transport chain. The oxidized enzyme interacts with its substrate to produce an oxidized product and a reduced form of the enzyme. To regenerate the active oxidized enzymatic state, electrons are shuttled down the transport chain and eventually transferred to molecular oxygen with the resulting production of the superoxide free radical. Allopurinol is structurally similar to the physiologic purine substrates and therefore acts as a competitive inhibitor of xanthine oxidase. Allopurinol is converted by xanthine oxidase, and possibly by aldehyde oxidase, to oxypurinol. Oxypurinol has also been shown to inhibit xanthine oxidase, but it acts as a “pseudoirreversible” (inactivation without covalent bonding) inhibitor, binding tightly to the reduced state of the enzyme.\textsuperscript{16}

The nature of the interactions among oxypurinol, allopurinol, and xanthine oxidase suggests that oxypurinol is a more potent enzyme inhibitor in ischemic reperfused tissue. Since ischemia is known to elevate the hypoxanthine-xanthine pool as a result of ATP catabolism, allopurinol may be unable to compete with the high concentration of physiologic substrate for the active site of the enzyme when introduced via collaterals or at reperfusion.\textsuperscript{26} Also, during the hypoxic conditions of the ischemic period, the reduced state of xanthine oxidase may predominate and, as such, the enzyme is more susceptible to inhibition by oxypurinol.\textsuperscript{16}

**Xanthine oxidase inhibitors and infarct size.** Previous occlusion-reperfusion studies with allopurinol have demonstrated conflicting results with regard to reduction of infarct size. Chambers et al.\textsuperscript{9} and Werns et al.\textsuperscript{11} have reported a diminished infarct size with occlusion periods of 60 and 90 min, respectively, when animals were killed at 4 and 6 hr after reperfusion. However, Reimer and Jennings\textsuperscript{12} failed to demonstrate reduction of infarct size with allopurinol in a 40 min canine preparation of occlusion-reperfusion when animals were killed after 4 days. A 90 min occlusion time was used in this study because it predictably results in myocardial infarction yet spares 50% to 60% of the ischemic bed.\textsuperscript{17} This damaged but viable epicardial tissue is potentially stunned after reperfusion.\textsuperscript{30}

In this study, administration of the xanthine oxidase inhibitor, oxypurinol, 60 min into occlusion failed to reduce infarct size when measured 24 hr after reperfusion. These results are in agreement with other studies that showed no reduction of infarct size with allopurinol and the free radical scavenging enzymes superoxide dismutase and catalase.\textsuperscript{12, 31, 32} It may be that the inhibition of xanthine oxidase activity only delays myocardial necrosis, since early studies reported positive results when animals were killed within 6 hr of reperfusion.\textsuperscript{9, 11} However, the discrepancy may reflect the differing administration schedules, drug doses, and animal preparations, as suggested by Werns et al.\textsuperscript{11}

**Functional recovery after ischemia: stunned myocardium.** Recent studies have suggested that functional recovery of the myocardium after coronary occlusion
and reperfusion may require several days. Such dysfunction has been termed "stunning" and is known to occur even in the histologic absence of necrosis. Stunning has been recorded after brief periods of ischemia (5 to 15 min) and is therefore a phenomenon that occurs in reversibly injured cells. However, longer periods of ischemia (40 to 120 min) may also cause stunning as a result of nonlethal injury to the epicardial border zones surrounding infarcted myocardium.

The physiologic mechanisms responsible for stunning have not yet been determined. Although initial studies reported that stunning was a manifestation of a delay in the restoration of normal cellular ATP levels, additional work has shown that abnormal contractility may persist in the presence of normal high-energy phosphate concentrations. Recently, there has been increasing evidence suggesting that free radicals may be important mediators of myocardial stunning. Several studies using the free radical scavengers superoxide dismutase and catalase have shown improved postischemic left ventricular function in regionally ischemic preparations. In addition, two studies have examined the effect of free radical production by the xanthine oxidase pathway on left ventricular function. Stewart et al. found that dogs pretreated with allopurinol 72 hr before undergoing 1 hr of global ischemia has significantly improved myocardial contractility compared with control, and Charlat et al. demonstrated that intravenous allopurinol enhanced regional myocardial function in a canine preparation of reversible ischemia.

In our 90 min occlusion-reperfusion protocol, oxypurinol-treated animals had significantly improved ventricular function at 24 hr after reperfusion when compared with controls. Since the dysfunctional area remained the same throughout the experimental protocol, and since infarct size was similar in treated and control animals, this finding implies that the contractility of the ischemic yet surviving epicardial myocardium was improved by oxypurinol. Although this study does not pinpoint the time when oxypurinol exerted a protective effect, it seems probable that oxypurinol was most beneficial during reperfusion, since this is the time when a marked increase in free radical production is known to occur. However, since ischemia is known to deplete cells of protective free radical scavenging enzymes, it is conceivable that some protection was afforded before reperfusion in our study.

Since the location and quantities of xanthine oxidase in myocardial tissue are not known, the exact cellular sites whereby free radicals mediate stunning remain speculative. Potential sources of the free radicals in myocardial tissue include the myocyte, the endothelial cell, and neutrophils. The role of neutrophils in the pathogenesis of postischemic myocardial dysfunction remains controversial, as does the effect of xanthine oxidase inhibition on neutrophil function. However, our results suggest that neutrophils may contribute to postischemic dysfunction, since reduced numbers of neutrophils were observed in the lateral border zones of the treated group.

The improved ventricular function in this study may also be due to other mechanisms. As an inhibitor of xanthine oxidase, oxypurinol may block the catabolism of the purine bases, potentially sparing the intracellular adenine pool. It has been hypothesized that a spared adenine pool may increase the circulating adenosine level. Adenosine is a potent arteriolar vasodilator in the coronary vasculature and could therefore improve myocardial blood flow during reperfusion. However, oxypurinol failed to increase regional blood flow in this study as assessed by the microsphere technique. Nevertheless, adenosine has also been shown to have a significant antileukocyte action. In vitro studies have demonstrated reduced neutrophil adherence and cytotoxicity to endothelial cells when exposed to adenosine. Adenosine has also been shown to reduce free radical production by the neutrophil. The decreased neutrophil infiltration in the border zones of treated animals in this study may therefore be related to an increased circulating adenosine level secondary to xanthine oxidase inhibition.

Critique of methods. Because infarct size may be extremely variable in the canine preparation, it is essential to determine important predictors of infarct size when assessing the efficacy of any therapeutic intervention. These variables include myocardial oxygen consumption, the collateral blood flow within the ischemic region, and the area of the occluded bed at risk of infarction. When these variables were analyzed by linear regression, no significant differences were noted in any with reference to the final infarct size as a percentage of the area of risk. Therefore both treatment groups were comparable regarding the major predictors of infarct size.

The area at risk was measured in vivo in this study because it may approximate the physiologic situation more reliably than techniques in vitro. Whereas in vitro dye methods inject colored dyes of variable viscosity at a fixed pressure, methods in vivo allow for the administration of a dye of known volume and viscosity. The dye is injected 2 to 3 min after ligation of the artery,
and the animal is then killed immediately. Infarct size in this study was measured by TTC quantification. Although it is possible that TTC may underestimate small patchy infarcts due to proximity of irreversibly and reversibly damaged myocytes, confirmation of the reliability of this histochemical method was confirmed by planimetry of a midventricular slice stained with Mallory’s trichrome stain in four randomly selected animals. Infarct size expressed as a percentage of the area at risk was slightly larger by histologic assessment (27.8 ± 2.8%) compared with TTC staining (24.9 ± 0.5%).

Regional ventricular function was assessed by a computerized radial shortening method, which is a modification of the centerline analysis developed by Behar and Stack and their colleagues. Whereas ultrasonic crystals measure thickening over a small discrete region of the ischemic zone, radial shortening on contrast ventriculography measures regional function over a larger area without the theoretical disadvantage of intramyocardial trauma associated with insertion of crystals.

Implications. The widespread use of potent thrombolytic therapy in evolving myocardial infarction has increased the number of patients undergoing successful reperfusion. However, reperfusion of reversibly damaged myocytes may result in prolonged postischemic dysfunction. Although the mechanisms of this dysfunction remain unknown, free radicals have been implicated as a cause. The potent inhibition of xanthine oxidase by oxypurinol in this study resulted in significantly improved regional ventricular function 24 hr after reperfusion. These findings suggest that xanthine oxidase activity (free radical production and/or purine catabolism) contributes to the stunning that occurs in reversibly damaged myocardium in the canine preparation.

We thank Randy Stinson, Andrew Manlove, and James Phillips for expert technical assistance, William K. Vaughn, Ph.D., for statistical analysis, and Linda Grayson for manuscript preparation. We are grateful to Dr. Steven Lindberg (Burroughs-Wellcome Co.) for providing oxypurinol and advice on dosage.

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Oxypurinol limits myocardial stunning but does not reduce infarct size after reperfusion.
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Circulation. 1987;76:678-686
doi: 10.1161/01.CIR.76.3.678

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