Xanthine Oxidase Inhibition Does Not Limit Canine Infarct Size

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Background. Evidence supporting the role of xanthine oxidase in myocardial reperfusion injury is based on studies with pharmacological interventions used to inhibit enzyme function. Controversy exists, however, regarding the true role of xanthine oxidase in reperfusion injury. This study was performed to determine whether xanthine oxidase inhibition limits myocardial injury due to coronary artery occlusion and reperfusion.

Methods and Results. Anesthetized dogs underwent coronary artery occlusion (90 minutes) and reperfusion (6 hours). Oxypurinol (28 mg/kg) or amflutizole (30 mg/kg), chemically unrelated inhibitors of xanthine oxidase, or vehicle was infused intravenously 15 minutes before and 3 hours after reperfusion. Regional myocardial blood flow was determined with radiolabeled microspheres. Infarct size was determined with the tetrazolium method. Myocardial infarct size (percent of risk region) was less in oxypurinol-treated dogs, 32±16%, compared with that of the control group, 46±15%. Infarct size for the amflutizole-treated dogs, 40±21%, was not significantly different from that of the control group. There were no differences in rate-pressure product or collateral blood flow to account for differences in infarct size. Uric acid concentration in the coronary venous plasma increased after reperfusion in the dogs treated with vehicle but not in the drug-treated dogs. Xanthine oxidase inhibition was demonstrated in each of the drug treatment groups, but only oxypurinol limited the extent of myocardial injury.

Conclusions. Previously reported cardioprotective effects of allopurinol, noted to occur only when the drug was administered chronically, may be related to a property of oxypurinol, a major metabolite of allopurinol. The beneficial effect of oxypurinol is unrelated to inhibition of superoxide formation during xanthine oxidase–catalyzed oxidation of xanthine and hypoxanthine. (Circulation 1991;83:995–1005)

Activated species of oxygen generated by the enzyme xanthine oxidase have been postulated to cause cellular injury during reperfusion of ischemic tissue.1 Treatment with either oxygen radical scavengers or allopurinol, an inhibitor of xanthine oxidase, has attenuated the tissue damage caused by transient ischemia in the kidney,2 intestine,3,4 and skeletal muscle.5 The role of xanthine oxidase in the pathogenesis of ischemic myocardial injury is controversial because the effects of xanthine oxidase inhibitors on canine myocardial infarction have been variable.6–10 This study was performed to compare the effects of two chemically unrelated inhibitors of xanthine oxidase, oxypurinol and amflutizole, in a canine preparation of regional myocardial ischemia and reperfusion.

Methods

Protocol 1

Surgical preparation. Detailed methods have been published previously.11 Male mongrel dogs (12–17 kg) were anesthetized with Dial-urethane (0.6 ml/kg i.v.) and were ventilated with room air. A left thoracotomy was performed, and the proximal left circumflex coronary artery was isolated and instrumented...
with an electromagnetic flow probe. After measurement of basal coronary blood flow, a stenosis was affixed to the artery by a silk ligature tied around the vessel and an 18- or 19-gauge needle. The degree of partial constriction was adjusted to reduce by at least 70% the hyperemic response to a 10-second occlusion without altering basal flow. The left circumflex coronary artery blood flow; carotid artery pressure; and leads II, III, and aVF of the electrocardiogram were monitored.

Drug administration. One group of dogs (n=18) received oxypurinol (Burroughs Wellcome Co., Research Triangle Park, N.C.) 28 mg/kg 15 minutes before reperfusion and 28 mg/kg 3 hours after reperfusion. A second group of dogs (n=17) received amfutizole (4-amino-3-[3-(trifluoromethyl)phenyl]-5-isothiazole carboxylic acid; Eli Lilly Research Laboratories, Indianapolis) 30 mg/kg 15 minutes before reperfusion and 30 mg/kg 3 hours after reperfusion. Dogs serving as controls (n=29) were randomized throughout the study with the other two groups and received a drug diluent solution administered at the same time points in the experimental protocol. Each injection of oxypurinol (dissolved in 50 ml 0.9% NaCl, pH 10.3–10.6), amfutizole (dissolved in 45 ml 8% NaHCO₃), or the control solution (50 ml 0.9% NaCl, pH 10.3–10.6, or 45 ml 8% NaHCO₃) was infused continuously during 15 minutes through a peripheral vein.

Evaluation of ischemic injury. All dogs underwent 90 minutes of regional myocardial ischemia produced by occlusion of the left circumflex coronary artery with a snare occluder made of silicone surgical tape (Retract-O-Tape, Quest Medical, Inc., Carrollton, Tex.) inserted in a short length of polyethylene tubing. The mechanical occlusion was gradually released during 30 minutes to prevent reperfusion-induced ventricular fibrillation. Predetermined exclusion criteria were heart worms, a failure to manifest electrocardiographic evidence of regional myocardial ischemia (no ST segment elevation after coronary occlusion), and intractable ventricular fibrillation (four attempts at defibrillation were permitted). After the 6-hour reperfusion period, the heart was fibrillated electrically and was excised immediately.

Histochemical determination of the anatomic area at risk and the zone of infarction was accomplished with a dual-perfusion technique previously described. The aorta was perfused retrogradely with 0.25% Evans blue dye, and the left circumflex coronary artery was perfused with 1.5% triphenyltetrazolium chloride in 20 mM potassium phosphate buffer (pH 7.4, 37°C). The solutions were infused simultaneously for 5 minutes at 100 mm Hg with the heart suspended in a water bath (37°C). The heart was cut into 1-cm-thick transverse sections, which were fixed in 10% formalin. Both surfaces of each section were traced onto clear plastic overlays for subsequent calculation of the area at risk (denoted by the absence of Evans blue dye) and the infarct zone (denoted by the absence of red formazan pigment within the area at risk) by planimetry using a Graphic tablet and Apple IIe computer (Apple Computer, Inc., Cupertino, Calif.). A custom-made software program was used to calculate the masses of the infarct zone and the area at risk from the planimetered areas and the weights of each transverse ventricular section. Previous studies demonstrated that there is an excellent correlation between infarct size derived by the planimetric method and the direct gravimetric measurement.

Histological examination. The measurement of infarct size using the triphenyltetrazolium chloride technique was correlated with infarct size measured by histological examination. Transmural tissue blocks from the area at risk were obtained from three transverse sections of the hearts. The blocks were embedded in paraffin, were cut to a thickness of 6 μm, and were stained with hematoxylin and eosin. Light microscopy was used to calculate the extent of irreversible injury. The histological criteria for necrosis were contraction bands, cytoplasmic eosinophilia, and nuclear pyknosis.

Evaluation of regional myocardial blood flow. With standard techniques, regional myocardial blood flow was determined with radiolabeled microspheres (15-μm diameter, New England Nuclear, Boston, Mass.), labeled with cesium-141 or ruthenium-103. The microspheres were prepared for administration by sonication in an ultrasonic bath and agitation with a vortex mixer. An aliquot of 1–2 million microspheres was diluted in 10 ml saline (37°C) and infused into the left atrial appendage during 30 seconds, followed by two flushes with 10 ml saline. Dual reference blood samples were withdrawn simultaneously from one femoral and one carotid artery at 3.50 ml/min. Microspheres were injected 70 minutes after coronary artery occlusion. After ex vivo staining, fixation in 10% formalin, and tracing of the heart sections as described above, the central ischemic zones and the nonischemic zones of the three basal sections were dissected into epicardial, midmyocardial, and endocardial thirds. A gamma spectrometer (model 1185, Tracer, Inc., Austin, Tex.) was used to measure the radioactivity of the tissue and blood samples. An Apple II+ computer was used to perform overlap corrections and myocardial blood flow calculations.

Protocol 2

Surgical preparation. During the course of the study, additional experiments were performed to determine the effects of oxypurinol or amfutizole on the formation and appearance of uric acid in coronary venous blood during reperfusion of the myocardium after a 90-minute period of regional ischemia. As described above, dogs were anesthetized with Dial-urethane (0.6 ml/kg i.v.), and a left thoracotomy was performed. Dogs were treated with control solution (n=6), oxypurinol (n=4), or amfutizole (n=6) as described in protocol 1. The dogs treated with control solution or oxypurinol underwent occlusion...
of the left anterior descending coronary artery, and coronary venous blood was collected with a 7F Courand catheter inserted in the right atrial appendage and advanced to the junction of the anterior interventricular vein and the great cardiac vein. The dogs treated with amflutizole underwent occlusion of the left circumflex coronary artery, and a vein draining the circumflex coronary artery bed was cannulated with an 18-gauge catheter for collection of coronary venous blood. Coronary artery occlusion was maintained for 90 minutes followed by reperfusion for 6 hours as in protocol 1.

Plasma uric acid. The blood samples for analysis of uric acid concentration were collected in heparinized tubes, were immediately placed on ice, and were then centrifuged at 0°C. The plasma was frozen at −70°C until subsequent analysis. The concentration of uric acid was determined by high-pressure liquid chromatography as described previously.16

Plasma amflutizole. Blood samples were obtained from seven dogs to measure the concentration of amflutizole. Nine milliliters of blood was mixed with 1 ml 3.8% sodium citrate. After centrifugation at 0°C, the plasma was stored at −70°C until subsequent analysis. The thawed samples were extracted 1:1 (vol/vol) with ethyl acetate containing 2% glacial acetic acid, mixed on a shaker (Eppendorf, Inc., Fremont, Calif.) for 5 minutes and centrifuged in a microfuge (Eppendorf) for 4 minutes. Twenty microliters of the organic extract was injected onto an octadecyl reversed-phase column (4.5×150 mm) by an automatic sampler (Du Pont Co., Wilmington, Del.). Samples were eluted for 20 minutes by a mobile phase consisting of 55% methyl alcohol in water and 1% glacial acetic acid. The flow rate was maintained by an Altec pump (model 110, Beckman Instruments, Inc., Carlsbad, Calif.) at 1 ml/min.

Amflutizole standards were prepared in methyl alcohol and diluted 100-fold into dog plasma. After mixing, the samples containing various amounts of amflutizole were subjected to the same extraction procedure and high-pressure liquid chromatography separation. Amflutizole was detected by measuring absorbance at 343 nm in a Kratos Spectroflow 773 (Advanced Instruments, Inc., Needham, Mass.). The mean retention time for amflutizole was 12.5±0.17 minutes. The serum amflutizole concentration was calculated from the standard curve obtained with the standards.

Xanthine oxidase assay. The enzyme assay was performed at room temperature according to the procedure of Kalckar.17 Xanthine oxidase activity was measured by the rate of uric acid formation from xanthine. In a total volume of 1.0 ml, the incubation mixture contained 50 μmol potassium phosphate buffer, pH 7.4, 0.05 μmol xanthine, and 0.01 unit of the enzyme preparation (chromatographically purified milk xanthine oxidase, grade III, Sigma Chemical Co., St. Louis). The absorbance change at 292 nm was recorded by a recording spectrophotometer (Gilford), and the uric acid that was formed was calculated with an extinction coefficient of 12 mM/cm at 292 nm.

Statistics

All data are expressed as mean±SD. Differences were considered significant at a probability level less than 0.05. The preocclusion and postreperfusion uric acid concentrations were compared by a paired t test. Analysis of variance and Scheffé's multiple comparison confidence intervals were used to detect group differences in plasma uric acid, collateral blood flow, heart rate, mean arterial pressure, rate–pressure product (heart rate multiplied by mean arterial pressure), and area at risk in the left ventricle. Analysis of covariance, with the transmural regional myocardial blood flow as the covariate, was used to determine group differences in extent of infarct per area at risk. The incidence of fatal and nonfatal ventricular fibrillation among the three treatment groups was analyzed by the χ2 test, but the sample sizes were insufficient to draw definitive conclusions.

Results

Survival

Sixteen animals did not satisfy the criteria for myocardial ischemia after coronary artery occlusion and were excluded from the designated protocol. Ventricular fibrillation occurred in 15 of the remaining 72 dogs: nine of 34 control dogs, three of 21 dogs that were treated with oxyipurinol, and three of 17 dogs that were treated with amflutizole. Defibrillation was unsuccessful in four control dogs, one of which was treated with oxyipurinol and three of which were treated with amflutizole. The incidence of fatal and nonfatal ventricular fibrillation was not significantly different among the three groups of dogs.

Extent of Myocardial Injury

Figure 1 and Table 1 summarize the extent of myocardial injury in the 55 dogs that survived occlusion and reperfusion of the left circumflex coronary artery. The percentage of the left ventricle at risk of infarction (area at risk) for the entire population was 42±6%. This value is consistent with that of previous studies that assessed the size of the left circumflex coronary artery bed by either the dual-perfusion method with colored dyes19,13,14,18 or radiographic analysis of postmortem angiograms.19 The mean size of the risk regions of the three study groups did not differ from each other.

The extent of myocardial injury expressed as a percentage of the area at risk was 40±13% for the entire group of control dogs (n=25) and 46±15% for the subset of control dogs with collateral blood flow data (n=11). The extent of infarction expressed as a percentage of the left ventricle was 17±6% for the entire group of control dogs and 18±7% for the subset of control dogs with collateral blood flow data. The infarct sizes of the control group did not differ from our previously reported values in other control
groups subjected to occlusion of the left circumflex coronary artery for 90 minutes followed by reperfusion for 6 hours.9,13,14,18

Expressed as a percentage of the left ventricle, the extent of myocardial injury for the amflutizole-treated dogs was 18±8% for the entire group (n=14) and 16±8% for the subset with collateral blood flow data (n=10). The extent of myocardial injury expressed as a percentage of the area at risk was 43±20% for the whole group of amflutizole-treated dogs and 40±21% for the subset with collateral blood flow data.

Expressed as a percentage of the left ventricle, the extent of myocardial injury was 12±6% for the whole group of oxypurinol-treated dogs (n=16) and 13±6% for the subset with collateral blood flow data (n=10). The extent of myocardial injury expressed as a percentage of the area at risk was 28±15% for the entire group of oxypurinol-treated dogs and 32±16% for the subset with collateral blood flow data.

**Histological Examination**

After 90 minutes of ischemia followed by 6 hours of reperfusion, the area at risk contained regions of injury characterized by cytoplasmic eosinophilia, contraction bands, and early nuclear pyknosis. A polymorphonuclear leukocyte infiltrate was present in areas bordering the infarcted tissue. Quantitative analysis of one control heart revealed an excellent correlation between the extent of infarction measured by the triphenyltetrazolium chloride technique, 50.4% of the area at risk, and histological examination, 56.9%. Quantitative analysis of the heart from a dog treated with oxypurinol also demonstrated a close correlation between the histochemical measurement of infarct size, 37.1% of the area at risk, and the histological assessment of infarct size, 40.4%.

**Regional Myocardial Blood Flow**

Previous studies performed in this laboratory consistently demonstrated a mean infarct size of approximately 40% of the area at risk among control animals that satisfy our electrocardiographic criteria for myocardial ischemia, that is, ST segment elevation. Nevertheless, collateral blood flow was measured in 36 dogs to confirm that unrecognized differences in collateral blood flow did not account for the smaller infarct size in the group of dogs treated with oxypurinol.20 Measurement of regional myocardial blood flow was attempted in 14 control dogs, 11 dogs treated with oxypurinol, and 11 dogs treated with amflutizole. Five experiments were unsuccessful (two, fatal arrhythmia; two, failure of the withdrawal pump; and one, failure of the infarct-staining technique). Thus, the results of 31 successful experiments are presented in Table 1 and Figures 2 and 3. There were no significant differences between the control group and either drug group with respect to heart rate, mean arterial pressure, rate–pressure product, or area at risk expressed as a percentage of the left ventricle (Table 1). There were no significant differences among the groups with respect to endocardial, midmyocardial, epicardial, or transmural myocardial blood flow within the ischemic region during coronary artery occlusion (Figure 2). The infarct size of each group was compared by analysis of covariance with the transmural mean collateral blood flow to the ischemic region as the covariate. The infarct size of the oxypurinol group, but not the amflutizole group,
TABLE 1. Infarct Size, Collateral Blood Flow, and Rate–Pressure Product in Dogs With Regional Myocardial Blood Flow Data

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<th>IS/LV (%)</th>
<th>IS/AR (%)</th>
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AR, area at risk; IS, infarct size; LV, left ventricle; RPP, rate–pressure product (heart rate multiplied by mean arterial pressure divided by 100); ANOVA, analysis of variance; ANCOVA, analysis of covariance.

was significantly smaller than that of the control group ($p<0.01$, Figure 3).

**Coronary Venous Uric Acid**

Aortic and coronary venous plasma samples were obtained from 15 animals that underwent occlusion of either the left anterior descending coronary artery (five control dogs and four oxypurinol-treated dogs) or left circumflex coronary artery (six amflutizole-treated dogs). The uric acid concentration of coronary venous plasma increased from $81\pm17 \mu M$ before coronary artery occlusion to $127\pm28 \mu M$
(p=0.01) 15 minutes after reperfusion in the control group. An increase in the uric acid concentration of coronary venous plasma was not observed in the dogs treated with oxypurinol or amflutizole. Expressed as a percentage of the preocclusion concentration, the differences between the preocclusion and postreperfusion uric acid concentrations were 58±33% for the control group, which is significantly different from -22±22% for the oxypurinol group and -54±14% for the amflutizole group.

The transcardiac gradients of uric acid, that is, coronary venous concentration minus aortic concentration, are illustrated in Figure 4 and summarized in Table 2. Transcardiac gradients were not significantly different among the groups before coronary occlusion, whereas the gradients were significantly different after reperfusion (p<0.05). The transcardiac gradient of uric acid increased from a negative value to a positive value in four of the five control dogs; the mean gradient for the control group was -6.8±6.2 μM before occlusion and 17.4±26.6 μM after reperfusion. Eight of the nine drug-treated dogs had a negative transcardiac gradient of uric acid after reperfusion. Before occlusion, the mean gradient was 3.1±12.7 μM for the oxypurinol group and 1.8±5.9 μM for the amflutizole group. After reperfusion, the mean gradient was -10.5±5.8 μM for the oxypurinol group and -0.5±1.3 μM for the amflutizole group. Thus, the transcardiac gradients suggest that uric acid was released from the hearts of control dogs but not from the hearts of dogs that were treated with amflutizole or oxypurinol.

Inhibition of Xanthine Oxidase In Vitro

Amflutizole, compared with allopurinol, is a potent inhibitor of xanthine oxidase, with an IC50 of 3.8×10-8
and $3 \times 10^{-6}$M for amflutizole and allopurinol, respectively (Figure 5). Kinetic studies indicated that inhibition by amflutizole was apparently noncompetitive with a $K_i$ value of $1.24 \times 10^{-8}$M (Figure 6). Allopurinol, however, is a structural analogue of hypoxanthine and behaves as both a substrate and an inhibitor of xanthine oxidase.\textsuperscript{21} Thus, allopurinol demonstrated an apparent competitive inhibition with a $K_i$ value of $5.5 \times 10^{-7}$M, a value in close agreement with published data.\textsuperscript{22}

**Serum Amflutizole Concentrations**

The plasma concentrations of amflutizole were determined for seven dogs, two of which had fatal arrhythmias 30 minutes after reperfusion. The plasma concentration of amflutizole was $189 \pm 17$ \(\mu\)g/ml ($n=7$) at the time of reperfusion, $32 \pm 12$ \(\mu\)g/ml ($n=5$) 3 hours after reperfusion (immediately before the second dose of amflutizole), and $103 \pm 12$ \(\mu\)g/ml ($n=3$) 6 hours after reperfusion. Thus, throughout reperfusion, the plasma concentration of amflutizole exceeded the $I_{50}$ of 0.011 \(\mu\)g/ml.

**Discussion**

This study demonstrates that treatment with amflutizole, an inhibitor of xanthine oxidase, beginning 15 minutes before coronary reflow did not alter the extent of canine myocardial injury after regional ischemia for 90 minutes followed by reperfusion for 6 hours. Treatment with oxypurinol initiated at the same time point, however, did limit the extent of injury. There were no differences between the control and drug-treated groups with respect to heart rate, mean arterial pressure, or the rate-pressure product, suggesting that the smaller infarct size in the oxypurinol-treated group was not due to a lower myocardial oxygen demand. There were no significant differences in collateral blood flow among the dogs with regional myocardial blood flow data, indicating that the smaller infarct size in the oxypurinol-treated dogs was not due to a less-severe degree of regional ischemia.

Renewed interest in the effects of xanthine oxidase inhibitors on ischemic myocardium was stimulated by the recognition that xanthine oxidase generates oxygen radicals and by the observation that free radical scavengers attenuated myocardial injury due to global and regional myocardial ischemia following reperfusion.\textsuperscript{1} The xanthine oxidase activity of canine myocardium has been reported to increase during coronary artery occlusion,\textsuperscript{6} but there have been variable effects of xanthine oxidase inhibitors on canine myocardial injury due to regional ischemia (Table 3). Treatment with allopurinol beginning at least 1 day before coronary artery occlusion has been shown to limit the extent of canine myocardial injury after coronary occlusion for 60 or 90 minutes followed by reperfusion for 4 or 6 hours, respectively. Dogs treated with allopurinol for 48 hours before coronary artery occlusion have exhibited decreased myocardial stunning after regional ischemia for 15 minutes followed by reperfusion.\textsuperscript{23} The beneficial effects of allopurinol have been attributed to alleviation of tissue injury during reperfusion, but further studies have found that pretreatment with allopurinol 24 hours before the onset of ischemia limited the extent of myocardial injury in dogs subjected to coronary artery occlusion for 24 hours without reperfusion.\textsuperscript{7,24}

The cardioprotective effects of allopurinol appear to depend on a period of long-term pretreatment
before coronary artery occlusion. The short-term administration of allopurinol beginning 30 minutes before coronary artery occlusion has not reduced ultimate infarct size in dogs undergoing 40 minutes of coronary occlusion followed by reperfusion. Treatment with allopurinol beginning 1 or 30 minutes after coronary artery occlusion has not limited the extent of injury after 24 hours of ischemia. The ineffectiveness of short-term treatment regimens suggested that the amelioration of xanthine oxidase–mediated myocardial injury by allopurinol, a competitive inhibitor of xanthine oxidase, requires a more prolonged treatment schedule that would allow the metabolic conversion of the parent compound to its active metabolite, oxypurinol, a noncompetitive inhibitor with a longer half-life. Thus, the elevated concentrations of xanthine and hypoxanthine, which occur within ischemic tissue, may attenuate the inhibitory effect of allopurinol but not that of oxypurinol. The results of other recent studies, however, indicate that short-term therapy with allopurinol may be sufficient to inhibit xanthine oxidase. Short-term administration of allopurinol has inhibited the formation of uric acid by rat hearts subjected to global ischemia and reperfusion.

Despite the evidence that both oxypurinol and amiflitzole inhibited xanthine oxidase activity, only oxypurinol reduced the extent of myocardial injury. Thus, an unrecognized action of oxypurinol other than the inhibition of xanthine oxidase may account for oxypurinol’s effect in this study and allopurinol’s effect in previous studies. Based on in vitro data, previous studies suggest that oxypurinol acts as a scavenger of hydroxyl radicals or of hypochlorous acid, an oxidant produced by activated neutrophils by the myeloperoxidase-catalyzed reaction involving hydrogen peroxide and chloride anion. Hypochlorous acid and its derivatives may potentiate tissue injury by inactivating α1-antiprotease, a protein that protects tissue elastin from hydrolysis by elastase. Hypochlorous acid also mediates the neutrophil-induced depletion of ATP in target cells. Oxypurinol, but not allopurinol, has prevented the inactivation of α1-antiprotease by hypochlorous acid. The metabolism of allopurinol to oxypurinol is relatively slow; in dogs treated with allopurinol, the plasma concentration of oxypurinol has been shown to peak at 13 hours after injection of allopurinol. Thus, the lack of efficacy of the short-term dosing schedules of allopurinol used in previous studies may indicate that the plasma concentrations of oxypurinol achieved during the early phase of reperfusion were inadequate to counteract the cytotoxic actions of hypochlorous acid formed by neutrophils. The treatment of cats with allopurinol, however, did not enhance the capacity of plasma or lymph to scavenge hypochlorous acid or inhibit the peroxidation of lipids by hydrogen peroxide and myoglobin. Thus,
the apparent cardioprotective effect of oxypurinol remains unexplained.

The cardioprotective effect of the oxypurinol regimen used in this study probably would not be sustained beyond reperfusion for 6 hours. Short-term administration of a drug may merely delay tissue injury because progressive myocardial damage occurs after reperfusion. For example, the infusion of iloprost, a stable analogue of prostacyclin, during coronary occlusion for 90 minutes and the first 2 hours of reperfusion was found to reduce infarct size after 6 hours but not 72 hours of reperfusion. The continuation of iloprost therapy until 48 hours after reperfusion did limit the extent of injury assessed 72 hours after reperfusion. Thus, it is not surprising that administration of oxypurinol before reperfusion failed to limit the ultimate extent of myocardial injury in dogs subjected to 40 or 90 minutes of coronary artery occlusion followed by reperfusion for 1 or 4 days.

Histochemical reagents such as triphenyltetrazolium chloride and nitro blue tetrazolium impart pigmentation to myocardium that retains the activities of dehydrogenase enzymes and their coenzymes, which may not be synonymous with cell viability. Nevertheless, the calculation of the extent of myocardial infarction using triphenyltetrazolium chloride or nitro blue tetrazolium correlates with the assessment of infarct size by electron microscopy, histopathology, and the depletion of creatine kinase activity, the uptake of infarct-avid radionuclides, or antimony antibodies. According to Kloner et al., electron microscopic analysis of myocardium confirmed that triphenyltetrazolium chloride... can reliably identify those areas of myocardium that demonstrate severe ultrastructural damage during the early phases of myocardial infarction, prior to the development of frank necrosis on histologic examination.” Triphenyltetrazolium chloride and nitro blue tetrazolium may underestimate the size of small infarcts because of admixture of reversibly and irreversibly damaged tissue at the infarct border. Thus, the degree of myocardial salvage by pharmacological agents may be overestimated.

Collateral blood flow is an important determinant of the extent of canine myocardial injury during coronary artery occlusion. With collateral blood flow as a covariate, analysis of covariance demonstrated that the infarct size of the oxypurinol-treated dogs was significantly less than that of the control dogs. Although measurement of regional myocardial blood flow was not performed in every dog in this study, analysis of the infarct sizes of the dogs with collateral blood flow data yielded the same results as the analysis of the entire population of dogs. Therefore, the data support the conclusion that treatment with oxypurinol limited the extent of myocardial injury under the experimental conditions used in this study.

Studies reported by this and other laboratories imply that the primary phase of ischemic myocardial injury that is terminated by reperfusion may be followed by a secondary phase of tissue damage that can be alleviated by the administration at the time of reperfusion of agents that protect tissue from oxidants produced by neutrophils (extracellular) or other sources (intracellular). Currently, the beneficial effects of allopurinol and oxypurinol are interpreted as evidence supporting the hypothesis that reperfusion of ischemic tissue elicits injury caused by the xanthine oxidase reaction, which generates superoxide anions and hydrogen peroxide from molecular oxygen concomitant with the oxidation of xanthine and hypoxanthine. The present study suggests that the ability of oxypurinol and, perhaps, allopurinol to attenuate ischemic myocardial injury is unrelated to their inhibition of xanthine oxidase. Other laboratories also have obtained results that support the hypothesis that the addition of allopurinol or oxypurinol to the coronary perfusate has improved the contractility, compliance, and ATP concentration of pig hearts that were subjected to global ischemia followed by reperfusion. Treatment with allopurinol for 1 week has preserved the left ventricular function, the cellular and mitochondrial ATP concentrations, the ATPase activity of the sarcolemma and sarcoplasmic reticulum, and the myocardial ultrastructure of rabbit hearts that underwent coronary occlusion followed by reperfusion. The administration of allopurinol to rabbits beginning 1 day before coronary artery occlusion has not altered the extent of myocardial injury as determined by the triphenyltetrazolium chloride method. The discordant effects of allopurinol on myocardial injury in the rabbit may relate to the different durations of treatment, the different experimental end points, or other unknown factors.

Extrapolation from the experimental data on xanthine oxidase to clinical therapeutics requires consideration of the existing knowledge about xanthine oxidase activity in human organs. Analysis of human liver and small intestine has revealed significant xanthine oxidase activity, although the activities were lower than those measured in other mammals. Minimal xanthine oxidase activity was present in the human kidney. Although biochemical assays have not detected xanthine oxidase activity in homogenates of human myocardium, Jarasch et al. observed immunohistochemical evidence of xanthine oxidase in the capillary endothelium of the human heart. Measurement of xanthine oxidase activity by chemiluminescence has indicated the presence of substantial xanthine oxidase activity in human umbilical vein endothelial cells. Human umbilical vein endothelial cells subjected to anoxia followed by reoxygenation have released superoxide anions. Treatment with superoxide dismutase plus catalase has attenuated the microvascular injury observed after transient occlusion of a canine coronary artery,
suggesting that oxygen radicals are responsible for the endothelial injury associated with myocardial ischemia and reperfusion.\textsuperscript{50} Recently, Huizer et al\textsuperscript{61} studied the arteriogenous urea difference across the hearts of patients undergoing coronary angioplasty. Balloon inflation resulted in production of uric acid by the heart, suggesting that the human heart does contain active xanthine oxidoreductase activity. Thus, additional studies are needed to ascertain the mechanism of action of allopurinol and oxypurinol, and closer examination of the xanthine oxidase activity of cardiac endothelial cells is warranted to determine the importance of xanthine oxidase and other potential sources of oxygen radicals in the pathogenesis of postischemic myocardial injury.

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