ALLOPURINOL IMPROVES MYOCARDIAL REPERFUSION INJURY IN A XANTHINE OXIDASE-FREE MODEL

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The ability of allopurinol to protect against reperfusion injury in the heart has usually been attributed to its xanthine oxidase (XO)-inhibiting properties. Human myocardium however, has exhibited low levels of XO activity. To investigate the effects of allopurinol in an XO-free model and determine whether pretreatment is necessary, 12 domestic pigs (15 kg to 20 kg) underwent occlusion of the left circumflex for 8 minutes followed by reperfusion for 4 hours. One group received allopurinol infusion (5 mg/kg IV) at occlusion over 45 minutes and a control group (n = 6) received a saline infusion (same volume). Left ventricular and aortic pressure, electrocardiograms, and regional wall motion (sonomicrometry) were monitored throughout the process. Regional blood flow (microspheres) were obtained before, during, and 5, 10, and 30 minutes after ischemia. Occlusion decreased transmural flow at the midpapillary level by 75% (0.28 versus 1.10 mL/minute/g). The allopurinol-treated group exhibited a mild, generalized hyperemia at 5 minutes (ischemic zone: 1.44 versus 1.10 mL/min/g, which returned to control levels at 10 and 30 minutes. In contrast, the control group was associated with only 80% restoration of resting blood flow at 5 minutes (0.84 versus 1.10 mL/min/g), which stabilized at 63% of control levels at 10 and 30 minutes. When evaluated for the propensity of arrhythmias using an arbitrary arrhythmia score, the allopurinol group demonstrated no myocardial ectopy when compared with the focal ectopy routinely encountered in the control group at all time intervals. Since pigs have no detectable levels of XO activity, allopurinol must exert its protectant effect during myocardial reperfusion by an alternative mechanism. Because protection was evident without pretreatment, beneficial effects may not necessarily be the result of allopurinol degradation products; therefore, pretreatment with allopurinol may not be necessary. These results are clinically important when considering the use of allopurinol in an emergent coronary angioplasty or coronary artery bypass grafting. (*J Natl Med Assoc.* 1995;87:480-484.)

Key words • pretreatment • oxypurinol • swine • myocardial shortening • microspheres

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Restoration of myocardial function following global ischemia associated with cardioplegic arrest and coronary artery bypass surgery is a major concern to

cardiothoracic surgeons. Poorly contracting myocardium caused by reperfusion of previously ischemic tissue produces an injury, or "stun," that is believed to be a factor in the induction of fatal arrhythmias and heart failure, which ultimately may lead to death.[1]

In an attempt to reduce this injury, the effectiveness of several pharmacological interventions have been investigated, including glucose, insulin, potassium, hyaluronidase, β-blockers, and calcium channel antagonists.[1,2] More recently, therapies directed at reducing the impact of reactive oxygen species, oxygen-derived free radicals (ODFRs), have generated much scrutiny. One such therapeutic agent is allopurinol.

Beneficial effects observed with allopurinol in the setting of ischemia and reperfusion injury in the myocardium have been attributed to its ability to reduce the formation of ODFRs by inhibiting the enzyme XO.[3-6] However, these data largely have been generated from animal models (dogs and rats), which have demonstrated substantial XO enzyme activity. However, allopurinol also has been reported as therapeutic in animal systems with no detectable XO activity.[7,8] Human myocardial biopsies have demonstrated very little XO enzymatic activity.[6] Therefore, the exact role of allopurinol in limiting the effects of ODFRs and myocardial reperfusion injury remains uncertain. Clearly, the data suggest that allopurinol reduces myocardial reperfusion injury by mechanisms other than inhibition of XO-generated ODFRs. At least one suggestion is that pretreatment with allopurinol leads to accumulation of the degradation product oxypurinol, which has ODFR-scavenging effects.[8] To further evaluate the efficacy of allopurinol and/or pretreatment, a "stun" paradigm was investigated in a pig model which has been shown to exhibit no XO activity.[6,8]

METHODS

Twelve domestic pigs (15 kg to 20 kg) were anesthetized using sodium pentobarbital (10 mg/kg bolus; 7 mg/kg/hour infusion), intubated, and ventilated with positive pressure and room air. A median sternotomy was performed. Left atrial, left ventricular, arterial, and venous cannulas were placed. Pairs of sonomicrometry crystals were placed in the distribution of the circumflex (ischemic, posterior) and the anterior (control) descending coronary arteries. Segment length was measured according to electrocardiogram (ECG)-gated times for end-systole and end-diastole, and relative shortening was calculated as an index of myocardial function in each region.

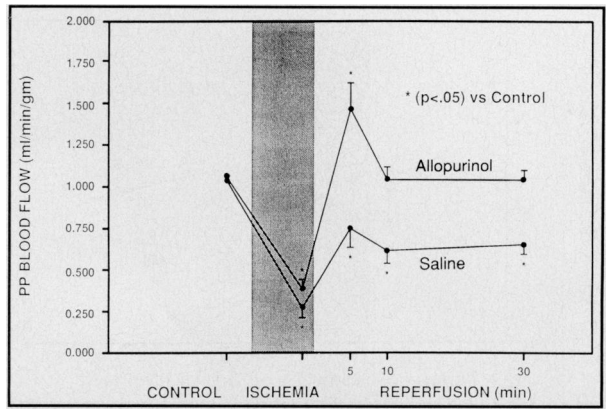

Figure 1. Mean transmural blood flow in the posterior papillary region. Note that both groups showed significant decreases during occlusion, as expected, while only the allopurinol-treated group demonstrated remarkable hyperemia and restoration of baseline flows during reperfusion.

The left circumflex artery was gently dissected free of interstitial connections, and a snare was placed. The animal was then allowed to stabilize. Following stabilization, local occlusion of the left circumflex artery was accomplished and maintained for 8 minutes. After the 8-minute ischemic period, the left circumflex artery was reopened and allowed to reperfuse the ischemic myocardium for a period of 4 hours. One group (n = 6) received an intravenous allopurinol infusion (5 mg/kg) beginning at the time of occlusion and continuing for 45 minutes (37th minute of reperfusion). A second group (n = 6) received a comparable saline infusion (vehicle control) over the same period. Left ventricular and aortic pressure, ECG (lead II), and regional wall motion (sonomicrometry) were monitored and recorded throughout the experiment.

Regional myocardial blood flow, using the reference withdrawal method as established in our laboratory,[9] was determined using microspheres before, during, and 5, 10, and 30 minutes after ischemia was produced. At the end of the experiment, the animal was killed by lethal overdose, and the heart was removed. After formalin fixation for 5 days, the myocardium was sectioned, and tissue was analyzed for radioactivity, which was then converted to flow by computer analysis and normalized for sample weight. This produced final blood flow measurements expressed in mL/minutes/g.

An arrhythmia score was developed: 0 = no ectopy,

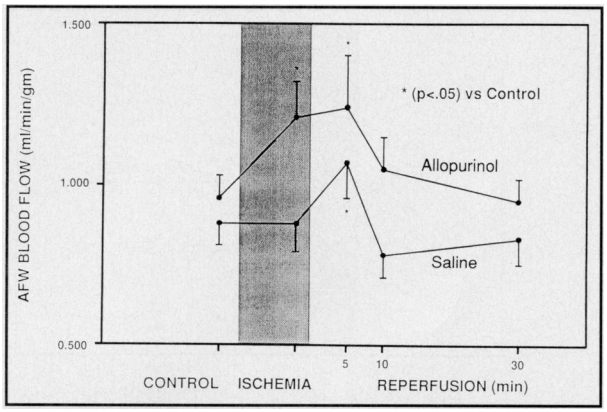

Figure 2. Mean transmural flow in the anterior papillary region. Note that the allopurinol-treated group displayed a significant hyperemia during the occlusive episode in the adjacent region, which was sustained early during reperfusion. Blood flow patterns later during reperfusion were not significantly different.

no tachycardia; 1 = isolated ventricular ectopy or periods of sinus tachycardia; 2 = interrupted runs of ventricular tachycardia (V-tach); 3 = sustained V-tach (continuously for 10 seconds); and 4 = ventricular fibrillation. Arrhythmogenicity during ischemic and reperfusion periods was evaluated.

All experiments were conducted under protocols approved by the institutional animal care and use committee, and methods of anesthesia and euthanasia were consistent with National Institutes of Health guidelines. All data were analyzed using multivariate analysis of variance for repeated measures, using Dunnett's multiple range test as appropriate. Significance was determined when $P<.05$.

RESULTS

Blood Flow

Occlusion of the left circumflex artery decreased transmural blood flow 75% in both groups (0.28 versus 1.10 mL/minutes/g; $P<.01$). During reperfusion, the allopurinol-treated group demonstrated a mild, generalized hyperemia at 5 minutes that was significantly greater than baseline (1.44 versus 1.10 mL/minutes/g; $P<.01$). In addition, the allopurinol-treated group sustained baseline blood flow levels at 10 and 30 minutes of reperfusion. In contrast, the saline group exhibited a defective reperfusion. At 5 minutes of reperfusion, flow remained 20% below baseline (0.84 versus 1.10 mL/minutes/g; $P<.05$). At 10 and 30 minutes, the untreated group showed a further decrease in adequate reperfusion. Blood flow stabilized at only 63% of baseline levels. In summary, allopurinol was associated with preservation of physiologic reactive hyperemia and preservation of normal flow distribution during reperfusion. Both were defective in comparable controls (Figure 1). In addition, allopurinol treatment was associated with improved perfusion to the nonischemia anterior segments during occlusion (Figure 2). The improved hyperemia in the nonischemic segments may have important ramifications in supporting compensatory recruitable wall motion adjacent to the ischemic tissue.

Arrhythmogenicity

Using the defined arrhythmia score, the propensity of the reperfused myocardium to develop arrhythmias was evaluated. The allopurinol-treated group demonstrated no myocardial ectopy, while dramatic, focal, and myocardial ectopy was routinely encountered in the untreated group at all time intervals ($P<.05$).

Wall Motion

In general, allopurinol treatment did not have a significant effect on wall motion in the nonischemic myocardium, except during the immediate occlusion, when there was a remarkable retention of shortening compared with saline-treated groups (Figure 3). Thus, in the nonoccluded region, the effect of allopurinol is manifest during ischemia in the adjacent region, but little effect was observed during reperfusion. In contrast, wall motion in the occluded region was not preserved in the allopurinol-treated group; however, with reperfusion, allopurinol treatment was associated with a much stronger, sustained recovery of myocardial shortening (Figure 4). Together, both wall motion data and perfusion data suggest a bimodal efficacy for allopurinol treatment, with a beneficial effect during occlusion manifest in the nonischemic tissue beds, and a beneficial effect during reperfusion in the previously ischemic tissue but not the nonischemic tissue.

DISCUSSION

Allopurinol has been suggested to improve myocardial reperfusion injury by reducing the number of ODFRs secondary to competitive inhibition of XO.[3-5] Xanthine oxidase uses molecular oxygen as an electron acceptor.[5] These ODFRs are able to produce myocardial alterations characteristic of ischemic-reperfusion injury, including disruption of plasma membranes and lysosomes, damage to mitochondria and sarcoplasmic

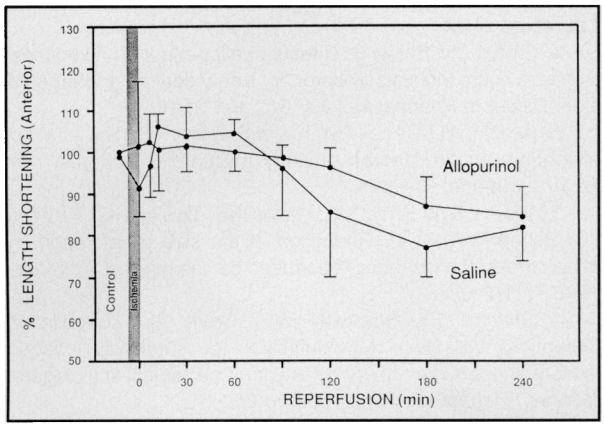

Figure 3. Change from baseline in shortening fraction in the anterior region. Note that while shortening was generally comparable throughout the experiment, during the occlusion of the adjacent region, allopurinol was associated with wall motion preservation that was 10% better than the control group. While this may not be a factor in otherwise-normal myocardium, as in these experiments, this difference may be important in diseased myocardium.

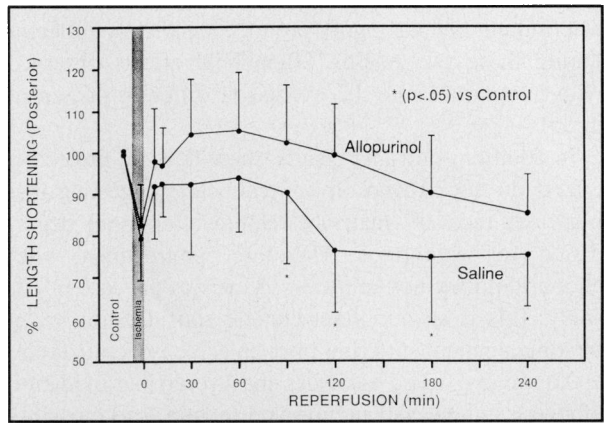

Figure 4. Change from baseline in shortening fraction in the posterior region. Note that, in contrast to the anterior region, no effect of allopurinol treatment could be demonstrated during ischemic occlusion. However, on reperfusion, a sustained recovery in shortening was observed. Allopurinol was associated with a full recovery of wall motion in the previously ischemic tissue within 30 minutes, while saline control had recovered only 75% of baseline function as late as 4 hours after reperfusion had been established.

reticulum, increases in vascular permeability, and the initiation of ventricular arrhythmias.[1]

Despite this observation, the involvement of XO and allopurinol in myocardial preservation during ischemic-reperfusion remains unclear. The activity of XO has varied from one study to another, as well as from species to species, which is also confusing. No XO activity has been reported in pig myocardium. Similarly, rabbit and human myocardial biopsies have exhibited little XO enzymatic activity. However, moderate to high levels of XO have been detected in both dogs and guinea pigs.[6]

Using the pig model, our results demonstrate that allopurinol does have beneficial and protective effects during myocardial reperfusion after an acute, non-necrosing ischemic period. Since no XO enzyme activity was present in this model, the beneficial effects observed with allopurinol administration must be attributed to a mechanism other than inhibition of XO.

The allopurinol-treated group exhibited superior restoration of blood flow during the reperfusion period when compared to the untreated group. Myocardial ectopy also was reduced dramatically in the allopurinol-treated group. Our data are consistent with other investigations which have shown allopurinol to exert beneficial effects in rabbit hearts, the rabbit being an animal that also is reported to have little XO activity.[1]

Many factors are believed to contribute to the generation of reperfusion injury. In general, these factors may be categorized into three groups: injuries related to ultrastructural damage and the inability to regulate calcium flux, injuries related to the production of ODFRs, and injuries that result from acute inflammatory responses in the previously ischemic region.[7] Das et al[8] used biochemical analysis to demonstrate that both oxypurinol and allopurinol may salvage myocardial function during ischemia and reperfusion by scavenging ODFRs rather than by inhibiting XO. Together, these data suggest that xanthine oxidase probably is not the principal source of ODFRs and that allopurinol has a direct scavenging effect on ODFRs.

Certain protective actions of allopurinol, such as the preservation of cellular adenosine triphosphate (ATP) levels and of mitochondrial ATP-generating capacity, have been shown to be exerted predominantly during the ischemic phase of injury. Other beneficial actions, such as preventing a decrease in left ventricular pressure, decreasing sodium and calcium accumulation, and preserving sarcolemmal and sarcoplasmic reticular ATPases, are greater during the reperfusion phase of injury. This would imply that the molecular processes

determining cellular injury are mechanistically different during these two phases.[1] Beneficial effects observed with allopurinol may be exerted at different phases of injury.

In addition, our data clearly show that the protective effects do not depend on pretreatment, suggesting that improved recovery may not require allopurinol degradation to oxypurinol. Therefore, pretreatment with allopurinol may not be necessary, as was the case in our study. This is an important clinical consideration when treating a patient suffering from an acute ischemic event.

Xanthine oxidase catalyzes the irreversible oxidation of hypoxanthine to xanthine and to uric acid, possibly accelerating the ischemic breakdown of purine nucleosides and bases that are needed for the resynthesis of ATP during reperfusion.[6,10] DeWall et al[2,11] have suggested that XO inhibition might be protective by facilitating purine salvage, thereby enabling ischemic tissues to maintain their ATP scores. However, others have suggested that this mechanism is unlikely because the washout of nucleotide degradation products is too rapid.[12]

SUMMARY

Despite controversy, there is a growing body of evidence to support the theory that allopurinol exhibits its beneficial effects by more than one mechanism and pathway. The results of this study demonstrate clearly that allopurinol exerts a significant protective effect during myocardial reperfusion injury by mechanisms or pathways other than the inhibition of XO, and pretreatment with allopurinol is not required. Future studies to specifically identify these mechanisms and pathways, as well as dosage strategies, are needed.

Acknowledgments

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