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REACTIVE OXYGEN SPECIES MAY CAUSE MYOCARDIAL REPERFUSION INJURY

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SUMMARY. The pathogenic mechanisms responsible for heart damage following temporary coronary artery occlusion are unknown. Some damage may be mediated by a normal cellular enzyme, xanthine dehydrogenase, which converts to xanthine oxidase during myocardial ischemia. Reperfusion, with restoration of oxygen supply, may then lead to formation of superoxide by xanthine oxidase, possibly initiating a cascade of oxidative events. In support of this, reperfusion of transiently ischemic canine myocardium leads to a rapid loss of cellular glutathione and a decrease in catalase activity, both indicative of enhanced generation of activated oxygen. Allopurinol—an inhibitor of xanthine oxidase — ameliorates both biochemical damage and functional deficits ordinarily triggered by ischemia and reperfusion, suggesting one possible mode of pharmacologic intervention following acute myocardial infarction.

Reperfusion of transiently ischemic myocardium occurs spontaneously following ∿50% of acute myocardial infarctions (1) and is the desired result of thrombolytic intervention. Although brief ischemia may deplete cellular energy stores, morphologic cellular damage is most evident after reperfusion when oxygen is reintroduced (2). It has, therefore, been proposed that damage occurring after reperfusion may be due, in part, to oxidation of various cellular components (3). Such oxidation might arise from intracellular production of activated oxygen. During ischemia, myocardial xanthine dehydrogenase is converted to xanthine oxidase (4). Both forms of this enzyme are involved in purine catabolism, in the step-wise oxidation of hypoxanthine to uric acid. Although xanthine dehydrogenase employs NAD as an electron acceptor in these reactions (yielding NADH), xanthine oxidase utilizes molecular oxygen (forming superoxide, O_2^-). Furthermore, an accumulation of oxypurines is the expected consequence of hypoxia-induced dephosphorylation and degradation of intracellu-

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lar ATP. Hence, during reperfusion of ischemic myocardium, xanthine oxidase, oxypurines and molecular oxygen are all present and might mediate generation of intracellular O_2^-.

O_2^- and H_2O_2 are normally metabolized by several enzymes, including superoxide dismutase, catalase, glutathione peroxidase, and by reductants such as ascorbic acid and α-tocopherol. Little is known of the status of these defense systems during reperfusion of transiently ischemic myocardium in an *in vivo* model. The object of the present investigations was to determine whether depletion of antioxidant defenses occurs following transient ischemia and if xanthine oxidase might be involved in activated oxygen generation *in vivo*. As indicators of intracellular oxidant stress, we chose to measure both glutathione content and catalase activity. Intracellular glutathione, which is ordinarily in the reduced form, is oxidized upon reaction with H_2O_2 and organic hydroperoxides (reactions usually mediated by glutathione peroxidases). In the event of intense oxidant stress, decreases in total cellular glutathione may occur because oxidized glutathione is actively transported from cells (5) and may also be lost as mixed disulfides with protein thiols. Similarly, increased intracellular H_2O_2 predisposes to the formation of catalase "complex II", an inactive form of the enzyme (6,7). We find that myocardial glutathione and catalase activity decrease following transient ischemia and that these changes are diminished by an inhibitor of xanthine oxidase, allopurinol.

MATERIALS AND METHODS. Our studies were performed in 15-25 kg adult mongrel dogs. Biochemical changes were assessed before and after ischemia by analysis of serial punch biopsies of ischemic and non-ischemic (control) areas. Mongrel dogs under general anesthesia were ventilated with room air and supplemental oxygen to maintain physiologic arterial blood gases. A left thoracotomy was performed and the heart supported in a pericardial sling. The dogs were given loading doses of intravenous lidocaine and maintained on lidocaine for the duration of the procedure. Acute myocardial ischemia was produced by ligature of the mid-left anterior descending coronary artery (biopsy dogs) or the mid-circumflex artery (functional dogs). A control biopsy was obtained from the distal distribution of the left anterior descending coronary artery. The ligature was then tightened to occlude this vessel for one hour and a repeat biopsy taken proximal to the initial biopsy site. The ligature was then released allowing the involved area to be reperfused and serial biopsies were taken. Each biopsy was taken proximal to the previous site and the perforation was closed by a single suture. Biopsies (approximately 2 mm in diameter comprising a tissue weight of ∿25 mg) were frozen immediately in liquid nitrogen and

stored at -70 °C until the biochemical analyses were performed. For studies on function, 2-D directed m-mode echocardiographic data were obtained prior to coronary occlusion and then during ischemia and reperfusion.

Total glutathione levels (i.e., the sum of both reduced and oxidized glutathione) were determined in homogenates of the punch biopsies by the method of Tietze (8). Biopsies were homogenized and a TCA extract was used for the assay. Protein was determined by the method of Lowry (9). For the measurement of catalase activity, tissue samples were homogenized in 50 mM sodium phosphate buffer, pH 7.2, and centrifuged 15 minutes at 20,000 x g. Catalase activity in the supernatant was assayed in triplicate spectrophotometrically.

Myocardial function was assessed echocardiographically. Function was expressed as fraction of baseline systolic thickening of a 2-D directed m-mode echocardiogram. Negative values indicate thinning during systole.

In studies of the effects of allopurinol, the drug was given orally 50 mg/kg for the two days preceding the experiment and 50 mg/kg was infused into the left atrium for 15 minutes beginning 30 minutes prior to coronary occlusion.

RESULTS AND DISCUSSION. In partial confirmation of the idea that reperfusion may exert an oxidative stress, myocardial glutathione levels were not significantly altered during a one-hour period of ischemia but showed significant drop after 30 minutes of reperfusion (Figure 1). The baseline glutathione

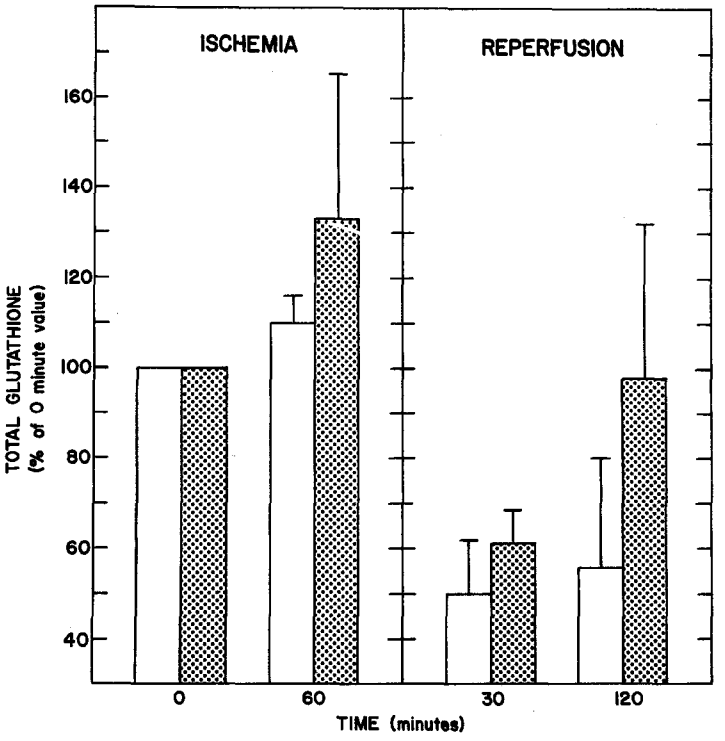

Figure 1. Total glutathione, expressed as percent of baseline biopsies in serial biopsies from seven untreated (open bars) and four allopurinol-treated (stippled-bars) dogs.

levels in control (0') biopsies averaged 1.0 μmol/g protein. Biopsies taken from non-ischemic areas during experimental ischemia and reperfusion failed to show any change in glutathione content.

A second series of dogs was studied in the same manner for determination of catalase activity during ischemia and reperfusion. The results show a moderate (∿ 40%) decrease in catalase activity during the initial 30 minutes of reperfusion, with a return toward normal thereafter (Table I). Thus, the declines in myocardial glutathione and catalase activity early in reperfusion are consistent with the generation of activated oxygen during the period of reperfusion.

Importantly, these decrements in total glutathione and catalase activity of reperfused myocardium parallel changes in myocardial function. Myocardial function measured echocardiographically by systolic wall thickening deteriorates rapidly during ischemia induced by sudden coronary occlusion (Figure 2). Within one minute of ischemia, the involved wall actually thins during systole rather than thickening. After an occlusion of one hour, reperfusion is followed by poor recovery of wall function (Figure 2). It is not known whether this defective myocardial function is due to cellular damage from activated oxygen species. However, it is worth noting that oxygen radicals can inactivate sarcoplasmic reticulum calcium ATPases (10) and that hydroxyl radical generated in an *in vivo* model leads to decreased developed tension in isolated perfused rabbit ventricular septa (11).

Table I. Catalase activity in canine myocardium during experimental ischemia and reperfusion

Sampling time	Catalase activity (% of control) (± 1 S.E.M.)	n
0'	100	5
60' ischemia	84 (± 8)	4
30' reperfusion	55 (± 4)*	5
120' reperfusion	97 (± 11)	5

* Significantly different from 0' values, $p < 0.05$.
Results shown represent serial biopsies from five dogs.

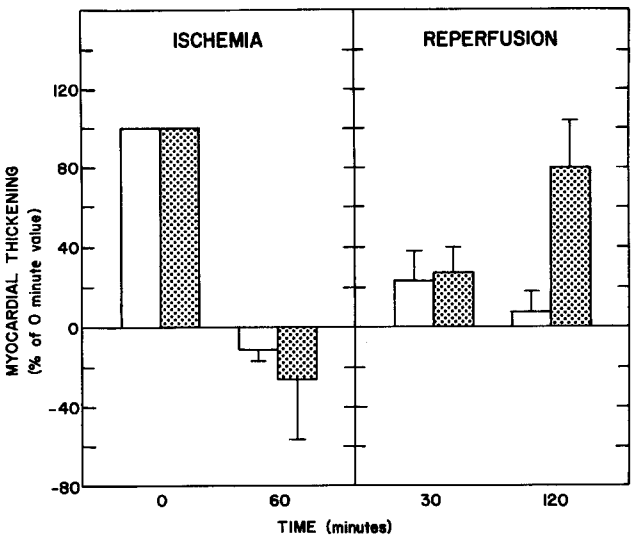

Figure 2. Myocardial function in five untreated (open bars) and five allopurinol-treated (stippled bars) dogs during ischemia and reperfusion.

To determine whether xanthine oxidase might be a source of activated oxygen production following reperfusion, we pretreated nine dogs with allopurinol, an inhibitor of xanthine oxidase. Interestingly, although myocardial glutathione levels fell after 30 minutes of reperfusion, the glutathione content actually returned to normal two hours post-ischemia in the four dogs pretreated with allopurinol (Figure 1). The baseline glutathione levels in control and allopurinol dogs were 1.0 and 1.37 μmol/g protein, respectively. In both groups, total glutathione was significantly lower at 30 minutes of reperfusion vs. 0 and 60 minutes ischemia ($p < 0.05$ in all cases). At 120 minutes reperfusion, glutathione in allopurinol-treated dogs is significantly higher than control ($p < 0.05$) and does not differ from pre-ischemia values.

Interestingly, almost complete functional recovery occurred within two hours in a series of five allopurinol-treated animals, whereas all seven untreated dogs still showed profound functional deficits after this time (Figure 2). Changes in myocardial thickening in both control and treated animals during ischemia and 30 minutes reperfusion are highly significant vs. 0 values, as are those at 120 minutes reperfusion for the control dogs ($p < 0.001$ in all cases). However, at 120 minutes reperfusion, myocardial function in the allo-

purinol-treated animals is much better than in controls ($p < 0.01$) and does not differ significantly from the 0 time values for either group ($p < 0.4$).

Because the one clear physiologic effect of allopurinol is inhibition of xanthine oxidase, these results imply that xanthine oxidase is one likely source of activated oxygen production in the ischemic, reperfused canine myocardium. In support of this, allopurinol as well as SOD and catalase have been found to limit infarct size following temporary coronary occlusion and reperfusion (12,13). It is unlikely that allopurinol acts by sustaining ATP levels through blockade of purine catabolism because systolic function during reperfusion does not correlate with ATP levels (14).

In aggregate, the data presented above indicate that, following reperfusion of myocardium subject to prolonged ischemia, two important antioxidants — glutathione and catalase — show decrements and functional recovery is delayed and/or incomplete. This reperfusion damage may be mediated, in part, by xanthine oxidase activity because allopurinol ameliorates the long-term biochemical and functional deficits. Our results provide evidence for the occurrence of oxidants within reperfused myocardium and suggest that such oxidants may be at least partially responsible for the damage which occurs following transient myocardial ischemia. It should be emphasized, however, that our observations by no means indict oxidation as the sole, or even primary, cause of irreversible cardiac damage following circulatory occlusion and reperfusion. Nonetheless, the finding that allopurinol diminishes the biochemical and functional sequellae of transient ischemia suggests the possibility of rational pharmacologic intervention following acute myocardial ischemia in humans.

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