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ALLOPURINOL CAN ACT AS AN ELECTRON TRANSFER AGENT. IS THIS RELEVANT DURING REPERFUSION INJURY?

D.A. Peterson*, B. Kelly, and J.M. Gerrard

Research Service, Veterans Administration Medical Center, Minneapolis, MN 55417; and Manitoba Institute of Cell Biology, Winnipeg, Manitoba

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The xanthine oxidase inhibitor allopurinol markedly enhances myocardial function and decreases ventricular irritability during myocardial reperfusion. In the present report, we have evaluated the molecular mechanism of allopurinol action. First, allopurinol was shown to be a weak radical scavenger. Second, allopurinol was found to act as an electron transfer agent from ferrous iron to ferric cytochrome c. The results suggest that the beneficial effect of allopurinol might partially result from its facilitated electron transport during reperfusion when the lipid components of the chain can be expected to be disordered.

Xanthine dehydrogenase has been shown to be converted to xanthine oxidase during ischemia (1). The oxidase uses molecular oxygen as an electron acceptor. The resulting superoxide anion can form other toxic radical species via well-defined pathways. Evidence has been presented from several laboratories that this sequence of events plays a significant role in reperfusion damage in several organs including myocardium. Much of this evidence is based on studies using the xanthine oxidase inhibitor, allopurinol. Using in vivo models it has been shown to limit infarct size, decrease the incidence of ventricular arrythmias, and to lead to increased return of myocardial function in myocardial reperfusion studies involving various animals (2-4). We have examined the possibility that allpurinol might have other mechanisms of action to explain these observations. First, we tested the ability of allopurinol to act as a direct superoxide scavenger. Second we assessed the ability of allopurinol to act as an electron transfer agent. The reasons for the second study are as follows: 1) Unsaturated fatty acids are

* To whom reprint requests are to be sent.

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released during ischemia from membrane phospholipids (5); 2) we have previously presented evidence that unsaturated fatty acids are essential for electron flow through the mitochondrial electron transport chain (6); 3) we hypothesized that in ischemia disordered lipids may be associated with a disordered electron transfer chain and that the lipophilic allopurinol might facilitate electron flow through this chain. At the same time allopurinol might decrease the leakage of electrons and the formation of incompletely reduced oxygen species.

MATERIALS AND METHODS

Allopurinol was obtained from Burroughs Welcome, and cytochrome c, from Sigma Chemical Co. Spectra were obtained on a Beckman DU.

The effect of allopurinol as a superoxide scavenger was studied by the method of Hyland et al. (7). An alkaline-DMSO superoxide generating system was used. Either the Na salt of allopurinol or an aliquot of an equal amount of blank was added before the 20 min incubation at 0 degrees. Cytochrome c reduction was determined at 550 nm.

The effect of allopurinol as a electron transfer agent was determined in a 50 mm Tris pH 7.4 solution containing 200 μM $FeSO_4$ and 45 μM cytochrome c. Reactions were carried out at 20°C and cytochrome c reduction was followed at 550 nM.

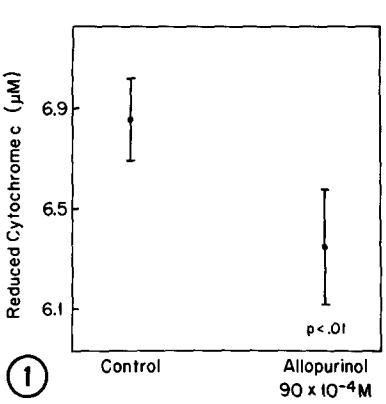
RESULTS

A. Allopurinol as a scavanger of superoxide radicals

An alkaline dimethylsulfoxide generating system was used to generate superoxide (7). The amount of superoxide generated was evaluated by measuring the reduction of cytochrome c. Only at high concentrations was allopurinol able to act as a superoxide scavenger and even under these conditions it was only minimally effective (Figure 1).

B. Allopurinol and electron transfer

A previously developed model (an aqueous solution of ferrous iron and ferric cytochrome c) was used to study the ability of allopurinol to act as a



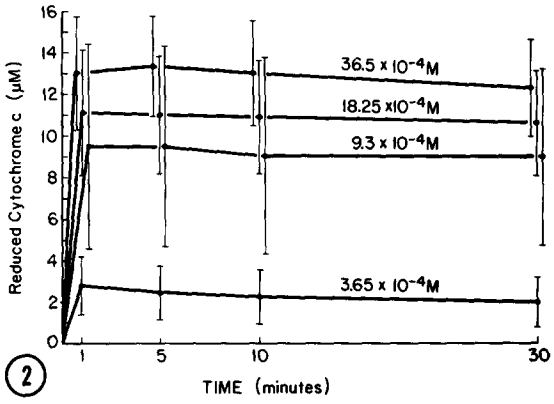


Figure 1: Effect of allopurinol as a superoxide scavenger using an alkaline dimethyl sulfoxide generating system.

Figure 2: The ability of allopurinol to act as an electron transfer agent between ferrous sulfate and ferric cytochrome c compared to an equal volume of blank ($p < 0.05$ compared to blank).

potential electron carrier from the ferrous iron to the ferric cytochrome c. As shown in Figure 2, allopurinol, which by itself did not reduce cytochrome c, was able to enhance the rate of cytochrome c reduction by ferrous iron. This electron transfer was not inhibited by superoxide dismutase (data not shown).

DISCUSSION

The results of the present study show that allopurinol can facilitate the transfer of electrons from ferrous iron to ferric cytochrome c. Thus allopurinol could enhance electron transport during reperfusion when the lipid components of the membrane of the mitochondria may be disordered. In support of this concept is the observation that allopurinol improves function in a rabbit global ischemic/reperfusion model without affecting xanthine oxidase activity as measured by serum xanthine and uric acid levels (8). By facilitating electron transport allopurinol could also enhance the reduction of vital dyes such as triphenyltetrazolium chloride (TTC). This might explain the discrepancy observed in the studies which have been done regarding the effect of allopurinol on infarct size reduction. These studies have shown no effect on long term follow-up of infarct size reduction based on scar

formation (9), but a significant reduction based on dye studies when allopurinol is given (2). Marginally injured tissue which perhaps would recover with or without allopurinol might be able to reduce vital dyes in the presence of allopurinol. The dye used in these studies, TTC, is reduced by succinate dehydrogenase (10), a component of the respiratory chain. By facilitating electron flow in a damaged chain allopurinol could give the impression of reducing infarct size when in fact is is only leading to more rapid recovery (4). In conclusion, by facilitating electron transport, allopurinol may enhance myocardial function during reperfusion as well as lead to an apparent decrease in infarct size in methods using reducible vital dyes while not changing infarct size when the eventual scar formation is assessed.

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