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Prevention of reperfusion injury in ischemic-reperfused hearts by oxypurinol and allopurinol

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Abstract. We investigated the effects of the xanthine oxidase inhibitor allopurinol and its metabolite oxypurinol on isolated rabbit hearts. To assess the potential role of these drugs in preventing reperfusion injury, hearts were perfused using Langendorff techniques, held globally ischemic for 3 h at 15°C, and then reperfused. During perfusion, hearts received Krebs-Henseleit solution maintained at 37°C. Aortic perfusion pressure was held constant at 80 cm H_2O. Prior to ischemia, hearts were arrested with a constant volume of KCl cardioplegia. Using a left ventricular (LV) balloon, developed pressures were measured prior to and following global ischemia. In addition, coronary circulation (CC) was measured before and after ischemia. All hearts were paced at 260 beats/min. We studied four groups: group 1 received 1 m*M* allopurinol, group 2 received 1 m*M* oxypurinol, group 3 received 90 IU/ml superoxide dismutase (SOD) plus 8085 IU/ml catalase (CAT), and group 4 received no treatment and served as a control. Each group consisted of 8 animals. Hearts receiving drug treatment did so during the first 5 min of reperfusion. Displaying all data as a function of LV volume, postischemic values were compared to preischemic values. Multivariate analysis and Tukey tests were used to detect significant differences between groups. When compared to the control group, all drug-treated groups significantly recovered end-diastolic function. Peak systolic pressure decreased significantly in the SOD/CAT group as compared to all other groups. LV isovolumetric work decreased significantly more in the SOD/CAT and control groups than in the oxypurinol group. Coronary circulation decreased significantly in the SOD/CAT and control groups as compared to the allopurinol and oxypurinol groups. Our results demonstrate an enhanced recovery of function when oxypurinol and allopurinol are given at the time of reperfusion. Recent evidence has supported the view that rabbit myocardium, as well as human myocardium, lacks xanthine oxidase. The beneficial effects seen with these drugs may therefore be unrelated to the presence of xanthine oxidase.

Key words: Oxypurinol, in heart preservation - Allopurinol, in heart preservation - Reperfusion injury, prevention of - Heart, prevention of reperfusion injury.

Injury seen during reperfusion of ischemic tissues appears to be caused by toxic oxygen radicals. The superoxide anion ($\cdot O_2^-$) and the highly reactive hydroxyl radical ($OH^\cdot$) have been implicated in the reperfusion event. The enzyme xanthine oxidase catalyzes the conversion of xanthine to urate with $^\cdot O_2$ as a by-product. When present, xanthine oxidase provides a major pathway for radical production under conditions of ischemia and reperfusion. We have previously demonstrated the beneficial effect of allopurinol in preventing reperfusion injury in rat hearts that contain significant amounts of xanthine oxidase [5]. The xanthine oxidase inhibitors allopurinol and oxypurinol have both been shown to exhibit radical scavenging abilities as well [8].

The purpose of this study was to assess the effects of allopurinol, oxypurinol, and the radical scavengers superoxide dismutase (SOD) and catalase

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(CAT) in the isolated, perfused heart in a species whose heart, like that of humans, seems to lack a demonstrable content of xanthine oxidase.

Materials and methods

Female New Zealand white rabbits (2.3-2.8 kg) were heparinized (650 IU/kg IV) and overdosed with pentobarbital (30 mg/kg IV). Hearts were excised and immersed in ice-cold buffer solution. All animals received humane care in compliance with the *Principles of laboratory animal care*, formulated by the National Society for Medical Research, and with the *Guide for the care and use of laboratory animals*, published by the National Institutes of Health (NIH) publication no. 85-23, revised 1985.

Perfusion technique

Using a Langendorff's apparatus with a perfusion pressure set at 80 cm H_2O, hearts were retrogradely perfused through the aorta. The perfusate (37° C) was gassed (95% O_2/5% CO_2), providing an oxygen saturation greater than 99.9%. The hearts received nonrecirculated perfusate only during the first 5 min of reperfusion. Hearts were arrested with cardioplegic solution (15° C) perfused retrogradely through the aorta at a pressure of 80 cm H_2O.

Perfusate content

The standard perfusate was modified Krebs-Henseleit solution (conc/l): NaCl (120 m*M*); KCl (4.7 m*M*); $CaCl_2$ (2.5 m*M*); $MgSO_4$ (1.2 m*M*); KH_2PO_4 (1.2 m*M*); NaHCO3 (25 m*M*); glucose (11.1 m*M*); albumin (2 g). The pH was 7.4. The cardioplegic solution was the same as the standard solution except that it contained 30 m*M*/l KCl and lacked albumin.

Experimental protocol

Following excision and immediate immersion in iced buffer solution, the hearts were retrogradely perfused. After 20 min, baseline functional measurements were taken. Hearts were then arrested with 200 ml of cardioplegic solution. Perfusion to the hearts was shut off and the hearts were immersed and stored for 3 h in a temperature-regulated (15° C) organ bath containing standard perfusate. Hearts were then reperfused again at 37° C. After 20 min, postischemic functional measurements were obtained. Hearts were paced at 260 beats/min during all periods when measurements were taken. Utilizing a separate perfusate reservoir maintained at 37° C, hearts received nonrecirculated standard perfusate with or without drug treatment only during the first 5 min of reperfusion.

Study groups

Four treatment groups were studied, each containing eight hearts. The hearts in group 1 received 1 m*M* allopurinol, those in group 2 1 m*M* oxypurinol, and those in group 3 90 IU/ml SOD plus 8085 IU/ml CAT; hearts in group 4 received no added treatment and served as a control.

Measurement of function

A saline-filled latex balloon was placed in the left ventricle through the mitral valve and secured with a tie. A small stab wound in the apex of the left ventricle allowed for thebesian drainage. The balloon was in direct fluid contact with a pressure transducer and left ventricular end-diastolic pressure (LVEDP) and peak systolic pressure (PSP) were measured. The volume of the balloon was adjusted until an LVEDP of 5 mmHg was achieved. By incrementing the volume of the balloon from this baseline volume (vol. = 0), pressure-volume curves were obtained. Measurements were taken at intervals of 0.2 ml until a volume of 2 ml was reached. The volume of the balloon was returned to baseline prior to cardioplegic arrest. Incrementation of volume postischemically provided correlating pressure-volume curves. LV isovolumetric work was calculated as (PSP-LVEDP) x volume. At volumes of 0, 1, and 2 ml, coronary circulation was measured by collecting all flow exiting the heart. Wet and dry weights of all hearts were measured.

Data analysis

Data were expressed as a function of LV volume. Postischemic and preischemic values were compared and the absolute differences (post-pre) compared between groups. Multivariate analysis was used with the post-pre difference at all volume intervals as a set of dependent measures. Significant group differences ($P < 0.05$) were followed with Tukey tests for multiple comparison in order to identify which groups were significantly different over the range of volumes considered.

Results

Results are shown in Figs. 1-4. The difference between postischemic and preischemic values are displayed as a function of LV volume.

At LV volumes greater than 0.2 ml, all drug-treated groups significantly recovered end-diastolic function as compared to the control group. There were no significant differences between the drug-treated groups, even though LVEDP tended to decrease postischemically in the oxypurinol group. LVEDP in the control group consistently increased postischemically over the volume range, and LVEDP differences were greater at larger volumes. In all drug-treated groups, LVEDP differences generally remained small throughout the LV volume range.

PSP consistently decreased postischemically in all groups, and most noticeably in the SOD/CAT group. Over the full range of volumes (except vol. = 0), PSP decreased significantly in the SOD/CAT group when compared to all other groups. Generally, PSP differences were slightly less in the oxypurinol group than in the control group. However, differences in PSP between the control, allopurinol, and oxypurinol groups were not significant at any LV volume.

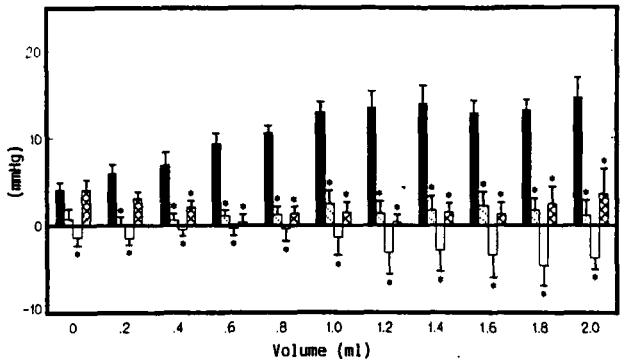

Fig. 1. End-diastolic pressure difference (postischemic minus preischemic) expressed as a function of LV volume. Data displayed as mean ± SEM. *($P<0.05$) relative to control. $n=8$ for each group. ■, Control; □, allopurinol; □, oxypurinol; ▨, SOD+CAT

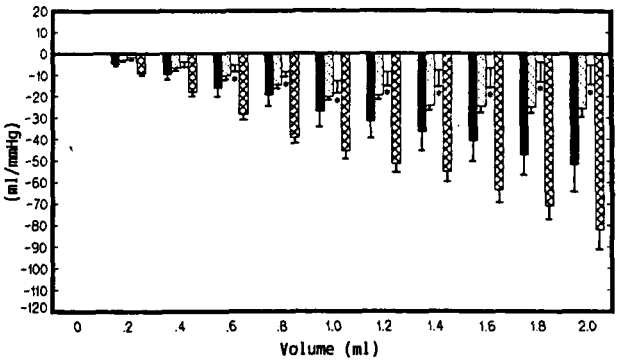

Fig. 3. Isovolumetric work difference (postischemic minus preischemic) expressed as a function of LV volume. Data displayed as mean ± SEM. *($P<0.05$) relative to control and SOD/CAT groups. $n=8$ for each group. Symbols as for Fig. 1

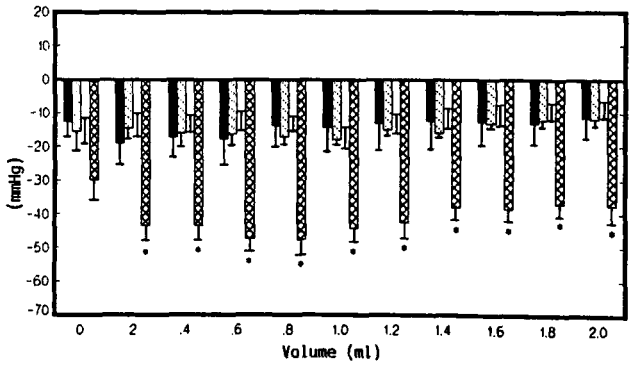

Fig. 2. Peak systolic pressure difference (postischemic minus preischemic) expressed as a function of LV volume. Data displayed as mean ± SEM. *($P<0.05$) relative to control, allopurinol, and oxypurinol groups. $n=8$ for each group. Symbols as for Fig. 1

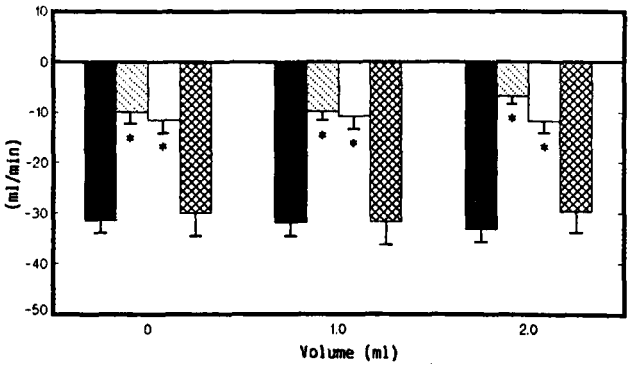

Fig. 4. Coronary circulation difference (postischemic minus preischemic) expressed as a function of LV volume. Data displayed as mean ± SEM. *($P<0.05$) relative to control and SOD/CAT groups. $n=8$ for each group. Symbols as for Fig. 1

LV isovolumetric work consistently decreased postischemically in all groups. Over the entire range of LV volumes, LV isovolumetric work differences in the control and SOD/CAT groups were progressively larger as LV volume increased. At volumes greater than 0.4 ml, LV isovolumetric work showed significantly smaller decreases in the oxypurinol group as compared to the control and SOD/CAT groups. Although LV isovolumetric work differences in the SOD/CAT group tended to be larger when compared to the control group, this was not significant at any volume level. LV isovolumetric work differences in the allopurinol group tended to be less than control group differences, but this was not significant.

Coronary circulation consistently decreased postischemically in all groups. At each volume level measured, differences in coronary circulation in the allopurinol and oxypurinol groups were significantly less than those in both the control and SOD/CAT groups.

Wet to dry weight measurements of hearts showed no statistical differences among groups.

Discussion

The importance of oxygen radicals in reperfusion injury of hearts [21-23] and other organs [2, 13, 17] seems to be well documented. Beneficial effects of allopurinol and various known radical scavengers such as SOD and CAT have been demonstrated [3, 23]. Allopurinol may protect reperfused tissues by preventing oxygen radical production via inhibition of the enzyme xanthine oxidase [6]. Oxypurinol - a metabolite of allopurinol and in itself a xanthine oxidase inhibitor - reduced myocardial injury following ischemic arrest in the rat [7] but failed to reduce infarct size in a closed-chest canine preparation [19].

Although the presence of xanthine oxidase has been well documented in the rat [4, 20] and dog [6] myocardium, xanthine oxidase activity has yet to be detected in the rabbit [10], pig [8], and human heart [11]. One study demonstrated a failure of allopurinol to alter purine washout profiles in the reperfused rabbit heart, suggesting a lack of xan-

thine oxidase [12]. Allopurinol also failed to protect postischemic rabbit myocardial function. Although rabbit myocardium presumably lacks xanthine oxidase activity, free radical generation during reperfusion of rabbit hearts has been detected [24]. In a hypoxic-reoxygenated isolated rabbit heart model, the administration of allopurinol decreased the release of creatine kinase [16]. A recent report demonstrated a reduction of urate release by the human heart when diltiazem was given during coronary angioplasty [9]. These authors presumed that xanthine oxidase therefore is present in human hearts. In another study on human cardiac tissue utilizing a fluorometric assay, xanthine oxidase activity could not be demonstrated [11]. The presence of xanthine oxidase in the human heart therefore remains controversial and certainly not proven.

Since allopurinol and oxypurinol appear to be beneficial to reperfused ischemic myocardium in species lacking xanthine oxidase in the myocardium, other mechanisms must be operative. Although still debated [8], Moorhouse et al. [15] have clearly demonstrated that allopurinol and oxypurinol scavenge the toxic hydroxyl radical. Hydroxyl radicals may be formed in ischemic cells at the time of reperfusion. Stimulated neutrophils are also potent sources of free radicals such as hypochlorous acid (HOCl) [14]. Allopurinol and oxypurinol can inhibit free radical signals from activated neutrophils [8]. Allopurinol may also act by improving ATP production in mitochondria [18].

In our study, allopurinol and oxypurinol protected hemodynamic function when given at the time of reperfusion, the time when free radical production has previously been demonstrated to occur in this model [24, 25]. We assume that the beneficial effect occurred due to scavenging of hydroxyl radicals. The lack of direct demonstration of free radicals in tissue in our studies makes this assumption somewhat speculative. We find it highly unlikely, however, that the effect was due to a direct, positive, inotropic action of these drugs. Allopurinol and oxypurinol were given for only 5 min at reperfusion, but hemodynamic performance remained stable after discontinuation of the treatment. We are not aware of any study that has demonstrated a positive inotropic effect of these drugs.

Possible explanations for the failure of SOD/CAT in this model include inadequate dosage or tissue availability. Since bovine SOD was utilized from a commercial source, contamination may also be possible. We, as other groups, have previously demonstrated a beneficial effect of SOD/CAT on ischemic rat hearts [5]. It should also be mentioned that some of the studies demonstrating beneficial effects of SOD have used higher doses, including a bolus dose of the drug [1, 25].

There appears to be a potentially important role for allopurinol - and especially oxypurinol - in the prevention of reperfusion injury. In a species that, like humans, supposedly lacks myocardial xanthine oxidase, allopurinol and oxypurinol demonstrated a significant beneficial effect. Admittedly, the dose utilized was high, and further experiments with lower doses of drugs should be performed. The fact that the drugs worked when given at the time of reperfusion without pretreatment is especially attractive from a clinical standpoint. The ability of both allopurinol and oxypurinol to easily penetrate cell membranes and to reach intracellular sites of radical production may make them more desirable in this setting than SOD and CAT, both of which have large molecular weights. We believe that further exploration of a potential clinical value of these drugs is needed, especially in in vivo models and, eventually, in clinical studies.

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