Biochemical Pharmacology, Vol. 27, pp. 1397-1400. 0006-2952/78/0501-1397 $02.0C

XANTHINE OXIDASE-INDUCED FOOT-EDEMA IN RATS: INVOLVEMENT OF OXYGEN RADICALS

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It has been reported that leukocytes and macrophages evolve superoxide anion (O_2^-) during phagocytosis (1,2). Superoxide anion was cytotoxic to cultured calf myoblast cells (3). Fong et al. reported that hydroxyl radical (OH·) caused the release of lysosomal enzymes from isolated lysosomes (4). Recently, Kellogg and Fridovich have reported that enzymically generated O_2^- and H_2O_2 caused peroxidatio of liposomes and erythrocyte lysis (5). These active species of oxygen are harmfu to tissues and are predicted to be involved in inflammation (2,6).

The oxidation of hypoxanthine by xanthine oxidase (XOD) yields O_2^- primarily, that is disproportionated into H_2O_2 and O_2 (equation [1]). Hydrogen peroxide will then react with O_2^- to form OH· and singlet oxygen ($^1O_2$) (equation [2]) (4,5,7).

$$2\,O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2 \qquad [1]$$

$$O_2^- + H_2O_2 \longrightarrow OH^- + OH\cdot + {}^1O_2 \qquad [2]$$

Singlet oxygen, however, may be readily scavenged either by hypoxanthine or by xanthine and urate which are the products of this enzymic reaction as reported by Kellogg and Fridovich (5).

We investigated whether these active species of oxygen could be stimuli of inflammation by employing XOD-HPX.* The present communication describes several lines of evidence that active species of oxygen such as O_2^- and OH· are involved directly in the induction of inflammation.

XOD-HPX system consisted of 25 mM potassium phosphate buffer (pH 7.0), 1 mM hypoxanthine and 2 mg/ml of xanthine oxidase (Boehringer Mannheim; 0.4 U/mg). Where indicated, various agents were dissolved in this mixture. Male rats of Sprague-Dawley strain weighing 140-160 g were used throughout this work (n=4-6).

By injecting 0.1 ml of XOD-HPX into the hind foot paw of rats, an acute foot-edema was induced at the injected site. The foot volume was measured by wate displacement method. The edema reached maximum in about 20 min and decreased gradually thereafter, as shown in Fig. 1. This acute paw swelling was dependent or hypoxanthine, in particular, in the initial phase. Omission or bioling of XOD resulted in a marked reduction of the paw swelling. Xanthine or uric acid which a oxidation products of hypoxanthine by XOD were not inflammatory. Other miscellaneous proteins like bovine serum albumin, lactate dehydrogenase or horse radish peroxidase showed slight inflammatory activities compared with XOD. These results suggest that the foot-edema was induced specifically by XOD-HPX and that O_2 or other active oxygen species derived from O_2^- would play a major role in the inflammation.

* XOD-HPX : xanthine oxidase plus hypoxanthine

In order to assure this assumption, effect of allopurinol, superoxide dismutas (SOD) and catalase on XOD-HPX-induced foot-edema was investigated as illustrated in Fig. 2. These agents were injected directly into the foot paw by mixing with XOD-HPX. Allopurinol, an inhibitor of XOD (8) considerably suppressed the paw swelling, thus indicating that turnover of XOD is essential for the inflammation. That a low but significant paw swelling was still observed in the presence of

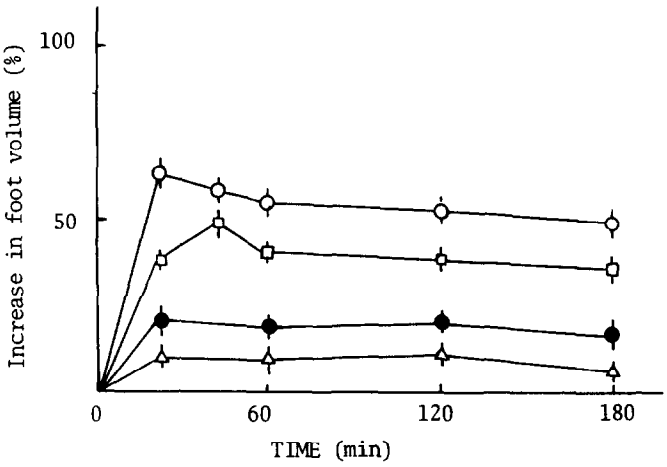

Fig. 1. Time-course of XOD-HPX-induced foot-edema in rats.
(—○—) complete system (XOD-HPX), (—□—) minus hypoxanthine, (—●—) minus XOD
(—△—) complete system (XOD was boiled at 100°C for 5 min.)

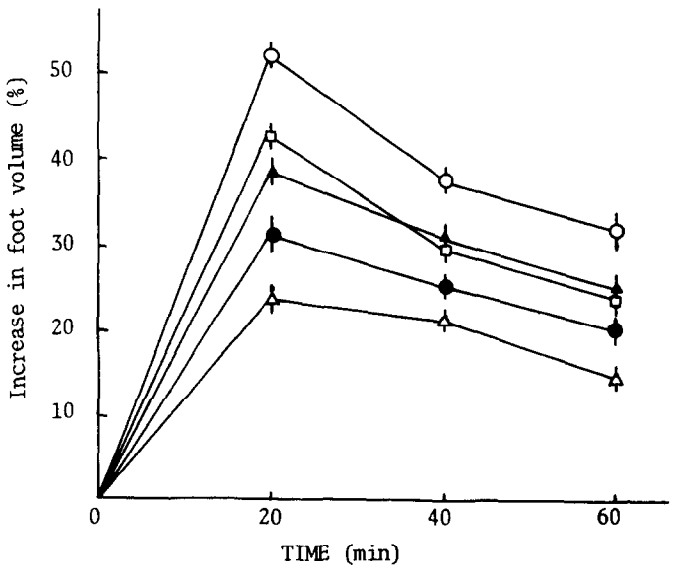

Fig. 2. Effect of allopurinol, SOD and catalase on XOD-HPX-induced foot-edema in rats.
All agents were injected into the foot paw by mixing with XOD-HPX.
(—○—) control (XOD-HPX), (—△—) + allopurinol (1 mM), (—▲—) + SOD (200 units),
(—□—) + catalase (250 units), (—●—) + SOD (200 units) and catalase (250 units).

allopurinol suggests that XOD itself is somewhat inflammatory. On the other hand, the foot-edema induced by XOD alone was similarly inhibited by allopurinol (Data were not shown). Therefore, it sppears that the foot-edema in the absence of hypoxanthine would be due to the utilization of endogenous substrates by XOD.

In order to determine which of these active species of oxygen are really responsible for the foot-edema, the effect of scavengers of active oxygens was examined. These agents were injected directly into the foot paw by mixing with XOD-HPX. As shown in Fig. 2, both SOD and catalase slightly inhibited foot-edema. The inhibition, however, was enhanced when both enzymes were used in combination. The extent of inhibition did not exceed 50% since these enzyme proteins, themselves were somewhat inflammatory. Superoxide dismutase catalyzes the disproportionation of O_2^- to form H_2O_2 and O_2. The reaction is faster by 10^4-fold than the corresponding nonenzymic reaction (equation [1]) (9). Therefore, it is likely that SOD promotes the formation of OH$^\cdot$ according to equation [2] through supplying H_2O_2 as proposed by Fong et al. (4). On the other hand, p-benzoquinone that reduces H_2O formation by oxidizing O_2^- to O_2 and would, therefore, decrease the production of OH$^\cdot$, inhibited the paw swelling markedly as indicated in Table 1. Furthermore, hydroxyl radical scavengers like benzoic acid and D-mannitol prevented the foot-edema markedly (Table 1). These results suggest that OH$^\cdot$ plays a major role i XOD-HPX-induced foot-edema in rats. This would explain why SOD could not suppress the paw swelling to a considerable extent. The involvement of OH$^\cdot$ in inflammation has been predicted by some authors (4,10).

Table 1. Effect of oxygen radical scavengers on XOD-HPX-induced foot-edema in rats.

Oxygen radical scavengers		% swelling	% inhibition
control		48.5 ± 1.6	-
+p-benzoquinone	1 mM	25.9 ± 1.5	46.6
+benzoic acid	1 mM	21.1 ± 2.2	56.5
+D-mannitol	1 mM	29.2 ± 4.5	39.8

All agents were injected into foot paw by mixing with XOD-HPX. Paw swelling was measured at 20 min after injecting the stimulus.

β-Carotene which is a typical scavenger of singlet oxygen did not inhibit the foot-edema significantly (Data not shown). Singlet oxygen may not be involved in this inflammation because it is readily scavenged in XOD-HPX system as reported by Kellogg and Fridovich (5).

In the presence of catalase, H_2O_2 derived from O_2^- will be decomposed into H_2O and O_2 immediately, thus leading to the decrease in the formation of OH$^\cdot$. Therefore, superoxide anion would be a major species of active oxygen under this condition. The observation that the foot-edema was suppressed only partially in th presence of catalase suggests that superoxide anion itself is also involved in the foot-edema. The combination of SOD and catalase that leads to the decrease in the levels of both O_2^- and OH$^\cdot$ would result in an enhanced suppression of the paw swelling induced by XOD-HPX. And this was the case as illustrated in Fig. 2.

Injection of 2 mM H_2O_2 into the foot paw did not cause a significant paw swelling. This does not, however, rule out the possibility that H_2O_2 may participate in the stimulation of the foot-edema induced by O_2^- and $OH^\cdot$.

The results described above showed that active species of oxygen like O_2^- and $OH^\cdot$ could be stimuli of inflammation. Of anti-inflammatory agents tested, diphenhydramine was the most effective in suppressing XOD-HPX-induced foot-edema, thus indicating that histamine is one of the most important mediators. Preliminary experiments showed that XOD-HPX caused histamine release from isolated rat peritoneal mast cells. The results will be published in the forthcoming paper.

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