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LIVER XANTHINE OXIDASE INCREASE IN MICE IN THREE PATHOLOGICAL MODELS

A POSSIBLE DEFENCE MECHANISM

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Abstract—Liver xanthine oxidase (XO) levels were determined in mice during bacterial or protozoal infection or with Ehrlich ascitic carcinoma. A long-lasting, but not permanent, increase in XO activity was observed in all three pathological models. Direct administration of liver XO or cow milk XO to mice with bacterial infection resulted in a significant decrease in mortality rate. Administration of superoxide dismutase (SOD) to infected animals significantly increased the mortality rate. A non-specific defence mechanism is indicated, probably involving enhanced oxidative processes.

In previous studies we have found a significant increase in liver xanthine oxidase (XO) activity in animals during the 24–48 hr following bacterial infection [1]. This increase, due to exo-endotoxins, is prevented by cortisone [2] and appears to be unrelated to bacterial pathogenicity. Other investigations have shown increased liver XO activity in various pathological conditions, including mice infected with *Listeria* [3], *Schistosoma mansoni* [4] or certain viruses [5]; in normal compared with germ-free newborn mice [6]; in starved re-fed rats [7] and in chickens kept in a low-temperature environment [8].

Our previous results suggest that increased liver XO activity may be a defence mechanism. To test this hypothesis further, we have investigated (i) the duration of the liver XO increase by defining time–effect relationships, and (ii) its specificity using models of bacterial and protozoal infection and of carcinoma.

MATERIALS AND METHODS

Charles River Swiss CD_1 albino mice, weighing 23 ± 2 g, were injected with a culture of *Staphylococcus aureus* CN6538 (2.5×10^8/10 g) or *Plasmodium berghei* I.S.S. (1×10^5/10 g). Male albino mice, weighing 23 ± 1 g, were injected i.p. with Ehrlich hyperdiploid carcinoma cells (4×10^6/animal). Median lethal time, determined on groups of 10 mice each, was 17.5 ± 0.9 days.

Groups of 6–10 animals per day were decapitated. The livers were removed, weighed and homogenized with a glass Teflon homogenizer in 5 vol. of 0.1 M phosphate buffer (pH 7.4) containing 0.005% EDTA w/v. The homogenate was centrifuged at 1000 r.p.m. for 20 min at 4°. In preliminary experiments, supernatants were dialysed for 3 hr at 4° against three changes of 100 vol. each of 0.1 M phosphate buffer (pH 7.4) containing 0.005% EDTA w/v. There were no differences between dialysed and non-dialysed samples and this procedure was omitted in later experiments.

XO levels were determined in air using Fried's

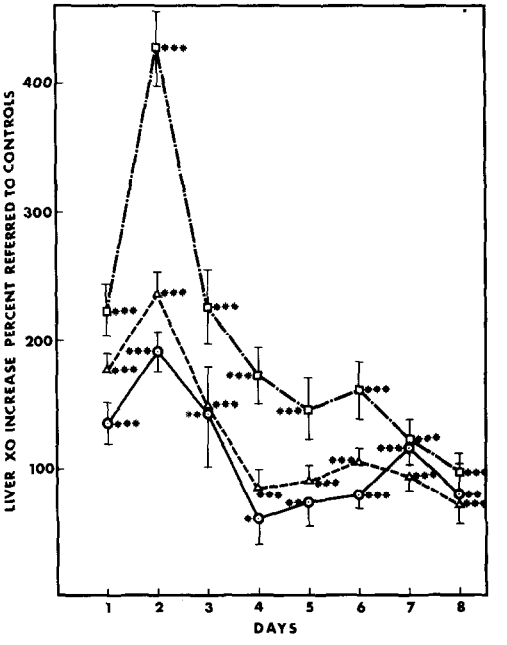

Fig. 1. Liver xanthine oxidase in 22 ± 1 g female albino mice infected i.v. with *S. aureus* CN6538 (2.5×10^8 microorganisms/10 g). Six infected and six control animals were killed daily by cervical dislocation, their liver excised, weighed, immediately homogenized and the XO levels determined. Values are reported as per cent increase of enzymic activity in infected animals (controls = 0); ○——○ refers to total XO per liver, △---△ to XO per mg fresh tissue, and □—·—□ to XO per mg protein. After the fourth day, infection was always ascertained by the presence of kidney abscesses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[9] modification of the spectrophotometric method of Nachlas *et al.* [10].

To assess how much of the XO activity determined by this aerobic method may be attributed to the oxidase, rather than dehydrogenase, activity of the enzyme, determinations were carried out in the presence of varying amounts of superoxide dismutase (SOD). A comparison of the xanthine oxidase/xanthine dehydrogenase ratio in infected and control animals was carried out using parallel experiments. The effect of the presence or absence of a fixed amount of SOD (768 U/ml) on liver XO activity was determined in groups of 5 mice infected 2 days previously i.p. with *S. aureus* CN6538 (2.5×10^8 microorganisms/0.2 ml/10 g) and in groups of 5 control animals.

The effect of direct administration of XO to infected animals was investigated. Partially purified liver XO liver from Charles River Swiss CD_1 albino mice was administered s.c. to mice 72, 48, 24 and 0.5 hr before infection with *S. aureus* Smith ATCC13709. The liver XO was extracted according to the method of Murray [11] and suspended in 0.01 M phosphate buffer (pH 7.8) containing 50 mcg/ml EDTA Na_2. Cow milk XO (Boehringer, Mannheim GmbH, F.R.G.) was administered s.c. to mice at various time intervals before and after i.p. infection with *S. aureus* Smith ATCC13709 and *E. coli* I.S.S.406 and CN6455. The milk XO was previously dialysed for 24 hr at 4° in 0.01 M phosphate buffer (pH 7.8) containing 50 mcg/ml EDTA Na_2 (ratio of XO:suspension buffer 1:1000 v/v), and then suspended in the same buffer. All controls received the same volume of buffer as the treated animals. In all experiments, mortality was evaluated 8 days after infection and the results were statistically treated with the Fisher exact test.

To assess the effect of SOD on experimental infection, the survival rate of mice infected i.v. with *S. aureus* Smith ATCC13709 and injected s.c. with 7125 units/kg SOD, was evaluated. Diethyldithiocarbamate (15 mg/kg), an *in vivo* inhibitor of SOD [12], was administered i.p. to mice infected i.v. with *S. aureus* NCTC8719.

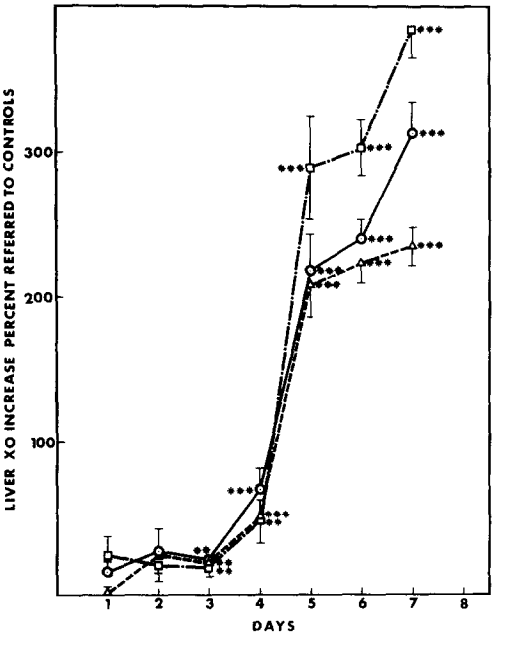

Fig. 2. Liver xanthine oxidase increase in 24 ± 2 g male albino mice infected i.p. with *Plasmodium berghei* I.S.S. (1×10^5/10 g). Ten infected and ten control animals were killed daily by cervical dislocation, their liver excised, weighed, immediately homogenized and the XO levels determined. Values are reported as per cent increase of enzymic activity in infected animals (controls = 0); O——O refers to total XO per liver, △---△ to XO per mg fresh tissue, and □·—·□ to XO per mg protein. The experiment was interrupted when animals started to die. ** $P < 0.01$, *** $P < 0.001$, without asterisk: not significant.

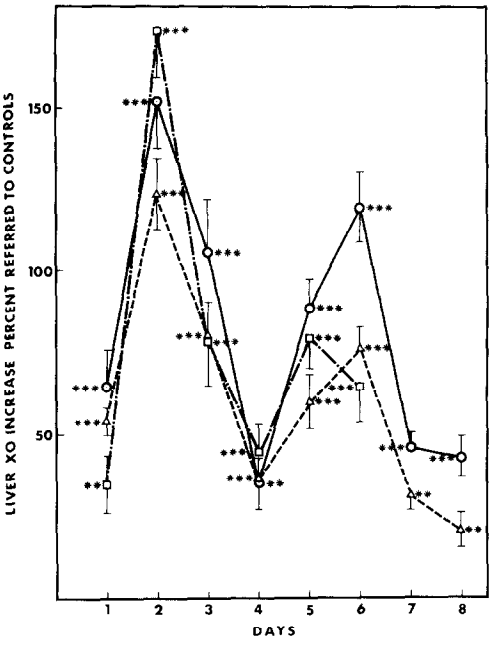

Fig. 3. Liver xanthine oxidase in 23 ± 1 g male albino mice injected i.p. with Ehrlich hyperdiploid carcinoma cells (4×10^6/animal). Ten injected and ten control animals were killed daily by cervical dislocation, their liver excised, weighed, immediately homogenized and the XO levels determined. Values are reported as per cent increase of enzymic activity in injected animals (controls = 0); O——O refers to total XO per liver, △---△ to XO per mg fresh tissue, and □·—·□ to XO per mg protein. ** $P < 0.01$, *** $P < 0.001$.

RESULTS

In Fig. 1, maximum liver XO activation occurs on the second day after bacterial infection and progressively decreases during the next 6 days. The high values of XO/mg protein are probably a consequence of liver steatosis during *S. aureus* infection.

During *Plasmodium berghei* infection (Fig. 2), liver XO activity is characterized by a 4-day induction phase followed by a sharp response on day 5, which continues to increase during subsequent days. Figure 3 illustrates liver XO increase in Ehrlich ascitic carcinoma. The curve shows two maxima, the first on day 2 and the second, which is less pronounced, on

Table 1. The effect of SOD on liver XO activity in controls and in animals infected with *S. aureus* CN6538*

	XO activity (mcg of iodoformazan/ mg protein/min/37°)		
	No external SOD	+SOD	Inhibition (%)
Controls	16.7 ± 1.00	5.9 ± 0.7	64.7
Infected animals	28.9 ± 2.1	6.5 ± 1.1	77.5

* Liver XO activity determined according to Fried [9]. Each value is the mean from 5 animals.

day 6. The two peaks are separated by a distinct minimum on day 4.

Addition of SOD during the determination of liver XO results in a decrease in apparent XO activity; the relationship of inhibition of XO activity to SOD concentration (expressed as units/mg liver protein) is linear in a semi-log plot. In Table 1, the effect of SOD on control and infected animals is compared. Inhibition of XO activity by SOD is evident for both groups, although a slightly higher value is observed with the infected animals.

Administration of partially purified liver XO or cow milk (a heterologous enzyme) to infected animals affords significant protection against bacterial infection (Table 2). The effect is greater when the enzyme is administered before, rather than after, infection. Table 3 gives the results of administration to infected mice of allopurinol and adenine, two well-known inhibitors of XO. Both substances lead to an increase in mortality rate, showing a linear dose–effect relationship.

Administration of SOD to infected animals sig-

Table 2. The effect of administration of mouse liver XO and cow milk XO on bacterial infection in mice

Group	Infecting micro-organism (i.p.)	No. of micro-organisms/ 0.25 ml/10 g	XO dose (U/10 g)*	Administration	Dead/ Total	P
Controls	*S. aureus* Smith ATCC13709				14/16	
Mouse liver XO-treated	*S. aureus* Smith ATCC13709	1.1×10^9	0.0203	72, 48, 24, 0.5 hr b.i.†	4/16	≤0.01
Controls	*S. aureus* Smith ATCC13709	1.75×10^9 in 5% hog gastric mucin			18/18	
Cow milk XO-treated	*S. aureus* Smith ATCC13709	1.75×10^9 in 5% hog gastric mucin	0.018	3 hr b.i.	0/18	<0.001
Controls	*E. coli* I.S.S.406	4.7×10^9			19/22	
Cow milk XO-treated	*E. coli* I.S.S.406	4.7×10^9	0.018	3 hr b.i. 20, 40 hr a.i.‡	11/22	<0.01
Controls	*E. coli* CN6455	2.75×10^6			16/18	
Cow milk XO-treated	*E. coli* CN6455	2.75×10^6	0.020	24, 5, 1 hr b.i.	6/18	<0.01
Cow milk XO-treated	*E. coli* CN6455	2.75×10^6	0.020	1, 5, 24 hr a.i.	14/18	n.s.§

* U = Kalckar units [13].
† b.i. = before infection.
‡ a.i. = after infection.
§ n.s. = not significant.

Table 3. Effect of some XO inhibitors on infections in mice

Exp. No.	Drug	Dose (mg/kg)	Route of adminis-tration	Administration	Infecting micro-organisms	No. of i.m./ ml/10 g	Route of infection	Times of evaluation of deaths	Dead/ total	Mortality (%)	ED_{50} and C.L. (mg/kg)
	Controls								0/15	0.0	
	Allopurinol	5	s.c.	5 and 1 hr	*E. coli* CN6538	1.4×10^7	i.p.	66 hr after	1/15	6.67	23.8
1	Allopurinol	15	s.c.	before,		/0.25 ml		infection	5/15	33.33	(15.4–37.7)
	Allopurinol	45	s.c.	18 hr after infection					11/15	73.33	
	Controls								0/10	0.0	
	Allopurinol	5	or.	5 and 1 hr	*E. coli* CN6538	1.4×10^7	i.p.	66 hr after	0/10	0.0	18.7
2	Allopurinol	15	or.	before,		/0.25 ml		infection	4/10	40.0	(18.4–19.0)
	Allopurinol	45	or.	18 hr after infection					9/10	90.0	
	Controls								4/16	25.0	
	Allopurinol	5	s.c.	On days 0, 2	*P. berghei*	5.54×10^6	i.p.	16 days after	6/15	40.0	9.25
3	Allopurinol	15	s.c.	3, 4 and 5		/0.20 ml		infection	8/16	50.0	(8.1–10.6)
	Allopurinol	45	s.c.	divided into 2 daily doses					13/16	81.2	
	Controls								4/16	25.0	
	Allopurinol	5	or.	On days 0, 2	*P. berghei*	5.54×10^6	i.p.	16 days after	9/16	56.2	2.83
4	Allopurinol	15	or.	3, 4 and 5		/0.20 ml		infection	12/16	75.0	(1.6–5.1)
	Allopurinol	45	or.	divided into 2 daily doses					13/16	81.2	
	Controls								6/20	30.0	
	Adenine	25	s.c.	5 and 1 hr	*K. pneumoniae*	2×10^6	i.v.	48 hr after	9/20	45.0	33.3
5	Adenine	75	s.c.	before,		/0.25 ml		infection	13/20	65.0	(31.5–35.2)
	Adenine	225	s.c.	18 hr after infection					18/20	90.0	

Using the same administration schedule as in experiments No. 1 and 2, LD_{50} of allopurinol administered s.c. was 192 (147.7–249.6) mg/kg. LD_{50} of allopurinol orally administered was 430 (344.0–537.5) mg/kg.

nificantly reduces the survival rate. If diethyldithiocarbamate, an *in vivo* inhibitor of SOD [12] is administered, the survival rate of the infected animals is significantly improved.

DISCUSSION

Enhancement of liver XO levels has not previously been considered of importance as a possible defence mechanism, with the single exception of Buengener [14], who postulated a role for this enzyme in malarial infection. We have found that liver XO activity is significantly increased in bacterial infection, plasmodium infection and in Ehrlich ascitic carcinoma. The magnitude and duration of the observed increase and the similarity of response in such different pathological models lends support to our hypothesis that the phenomenon represents a non-specific defence mechanism involving increased oxidative processes. We have found that most of the XO activity is expressed as an oxidase, especially in infected animals (Table 1). Our results also seem to rule out the possibility that the dehydrogenase is converted into the oxidase form of the enzyme as a response to infection.

The protection against bacterial infection observed when XO is administered directly to the animals, particularly before infection (Table 2), is further evidence of a non-specific mechanism. In agreement, administration of XO inhibitors to infected animals worsens the infection (Table 3). The results of allopurinol administration (Table 3) suggest that allopurinol-treated patients may become more susceptible to infection. This possibility is ruled out by clinical observations on patients normally prone to infections, such as cancer patients. Moreover, in our experiments with mice, the deleterious effect of allopurinol on the infection is observed with doses 9–27 times greater than the therapeutic dose for human patients. It remains to be clarified whether the need for this high dosage is due to different species specificity or is associated with varying drug penetration at particular sites.

It has been stated that XO, localized in liver cells in the same cellular compartment as SOD, is the main producer of superoxide anions in the body [15]. Superoxide anions play a definite role in leucocyte bactericidal activity during phagocytosis [16, 17], and an investigation into the effect of SOD on experimental infection is relevant. We found that SOD significantly reduced the survival rate of mice infected with *S. aureus*, whilst administration of an *in vivo* inhibitor of SOD significantly improved the infection. With these results in mind, we believe that the physiological role of XO should be reconsidered in the light of its possible impact on the defence systems through the production of superoxide anions.

All results reported in this paper refer to liver XO; as an obvious development, levels of this enzyme in other areas will be studied, commencing with polymorphonuclear leucocytes and macrophages. These might, in fact, represent the final sites of the liver enzyme.

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