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ENHANCED XANTHINE OXIDASE ACTIVITY IN MICE TREATED WITH INTERFERON AND INTERFERON INDUCERS

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Administration to mice of either interferon (IFN) or IFN inducers resulted in a marked increase of xanthine oxidase (XO) activity in different organs. Dose response studies revealed that serum XO was increased by administration of polyinosylic-polycyticylic acid (poly I-C) at doses as low as 0.1 mg/kg. In view of the well known ability of XO to generate superoxide radicals it is suggested that its induction might play a role in several biological effects of IFN.

Investigation on the biochemical mechanisms by which IFN acts has led to the discovery of some IFN-activated metabolic pathways involving oligo-A-synthetase, protein kinase, phosphodiesterase (1), indoleamine dioxygenase (2) a and guanylate cyclase (3). It has recently been suggested that superoxide radical might play a role in the mechanism of action of IFN (4). Pottathil et al. (5) have shown that the establishment of IFN-mediated antiviral state depends on the presence of superoxide dismutase (SOD) or a related copper-containing enzyme while superoxide is a substrate for an IFN-induced enzyme, namely indoleamine dioxygenase (6). Furthermore, genetic studies have shown that the genes coding for cytoplasmic SOD and IFN receptor are synthenic in both mouse and man and map to chromosomes 16 and 21, respectively (7). Here we report an induction of XO, a well known superoxide generating enzyme (8), in mice given IFN or IFN-inducers.

MATERIALS AND METHODS

Materials. Poly I-C was from Sigma, bacterial lipopolysaccharide (LPS, from E. Coli 055-B5) was fromDifco 8-^{14}C-hypoxanthine (specific activity 53mCi/mmol) was from Amersham. Cellulose thin layer chromatographic plates were from Merck (20x20 cm, thickness 0.1 mm). Tilorone, an orally active IFN-inducer, was obtained from Merrel Dow Pharmaceutical Inc., Cincinnati, Ohio, through the courtesy of Dr. W.J. Hudak. Partially purified mouse α, β IFN from Litton Bionetics, Lot. No. 231-75-14, was a kind gift of Dr. Chirigos, Frederick, Md. Purified recombinant leukocyte IFN (IFL or A/D) was from Hoffman-La Roche; Nutley, N.J.

Animals and tissue preparation. Male Swiss albino mice (CD1, Charles River, Italy) weighing 20-25 g were used. Animals were killed 24 hours after the treatment (except for time course experiments), organs were removed, washed in cold isotonic saline and homogenized in ice-cold 0.1 M phosphate buffer (pH 7.8) at a 1:4 (w:v) ratio (Potter homogenizer). Homogenates were cen-

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trifuged at 20,000 g for 30 min at 0-4°C. Supernatants were dialyzed overnight against 1,000 volumes of the same buffer.

Enzyme assay. Liver XO was determined, unless otherwise indicated, at 25°C by the UV method by Bergmeyer et al. (9). Enzyme levels are expressed as nmoles of uric acid formed/minute x g of liver, taking a molar extinction coefficient at 292 nm for uric acid = 7.6×10^{-3}.
Serum XO, due to the low activity, was measured with a radiometric method using 8-^{14}C-hypoxanthine (HX) as a substrate (10). Briefly, 10 μl of serum diluted 1:10 were incubated 10 min at 37°C with 5 μl of 0.2 M Tris-Cl (pH 7.8) and 5 μl of HX (9 μCi/ml). Reaction was stopped by the addition of 10 μl of perchloric acid 1M and centrifuged. Five μl of supernatant were spotted on TLC-cellulose along with 2.5 μl of a 10^{-2} solution of HX, Xanthine and uric acid. TLC was developed with butanol:methanol:water:NH_4OH 25% (60 :20:20:1), purines were located under UV light and the radioactivity of the spots was measured by liquid scintillation in 10 ml of Filter-Count (Packard) after scraping the portions of the chromatogram with HX alone and both xanthine and uric acid. XO activity was espressed as nmoles of HX oxidezed to xanthine + uric acid/minute x ml serum. When XO was measured in organs by this method, the dialyzate was appropriately diluted (tipically liver supernatant was diluted 20-50 fold).

RESULTS AND DISCUSSION

Table 1 shows that all the IFN-inducers tested caused a marked increase of liver XO activity in CD1 mice 24 hours after treatment. Also the administration of the two IFN preparation tested, increased liver XO, suggesting that IFN mediates the effect of the three IFN-inducers. In six different experiments after a single administration of poly I-C (10 mg/kg, i.p.) to CD1 mice, liver XO was increased from 3 to 10 fold. Similar results were ob-

Table 1: Liver XO levels after administration of IFN or IFN inducers

Treatment	XO Activity (nmoles/min x g liver)
Control	20.2 ± 1.5
LPS	92.1 ± 12.4**
poly-IC	95.1 ± 12.0**
Control	12.7 ± 3.4
Tilorone	43.5 ± 3.1*
Control	13.7 ± 3.9
α, β-IFN	69.6 ± 13.7**
IFLrA/D	35.3 ± 3.9*

Poly I-C (10 mg/kg) and LPS (10 mg/kg) were given i.p. in 0.2 ml of pyrogen-free sterile saline; controls received saline alone. Tilorone (75 mg/kg) was given orally in 0.2 ml of water; controls received water alone. IFN preparations in MEM with 2% fetal calf serum were diluted with saline to a concentration of 50,000 U/0.2 ml. IFN was given at the dose of 50,000 U/mouse; controls received 0.2 ml of MEM with serum diluted in the same way.

All values represent the mean ± S.E.
* $p<0.05$ ** $p<0.01$ by Student's t-test.

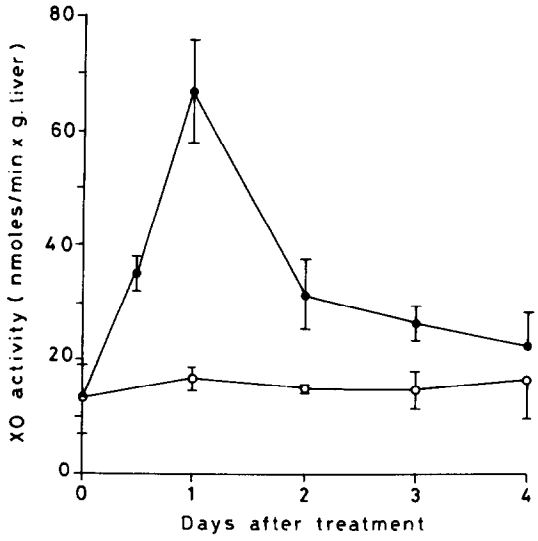

Fig. 1. Time course of liver XO after a single i.p. injection of 10 mg/kg of poly I-C (●—●) or saline (o—o).

tained with inbred strains of mice such as the C57Bl/6 and the C3H/HeN (data not shown). The time course of liver XO activity in CD1 mice after a single i.p. administration of poly I-C (10 mg/kg, i.p.) showed that the most prominent effect was observed 24 hours after treatment (Figure 1).

As shown in Table 2, the induction of XO by poly I-C is not limited to the liver but occurs in various organs. This fact rules out the possibility that

Table 2: Effect of poly I-C on XO levels in different tissues

Tissue	XO (nmoles/min x g wet tissue	
	saline	poly I-C
Liver	80.7±12.5	324.7±28.7**
Brain	0.8±0.3	1.9±0.5
Heart	10.2±2.5	43.0±3.2**
Intestine	206.5±34.8	241±18.9
Kidney	13.7±3.8	173.3±20.8**
Lung	79.2±6.8	182.2±9.9**
Serum	4.5±0.4	15.0±0.7**
Spleen	13.0±0.6	43.5±9.2*

Poly I-C (10 mg/kg i.p.) was given 24 hrs before sacrifice.
XO was measured by the radiometric method. Activity is expressed as nmoles/min x g tissue or nmoles/min x ml of serum.
All values represent the mean ± S.E.
* $p<0.05$; ** $p<0.01$ by Student't-test.

Table 3: Liver Xanthine Oxidase and Dehydrogenase activities after 10 mg/kg poly I-C

	saline	poly I-C
XO	116.7±5.7	245.9±20.8
XO+XD	265.8±45.6	497.5±56.7
XO/XO+XD	0.44	0.49

XO was determined by the radiometric method.
For XO+XD determinations 1mM NAD^+ was included in the assay. Enzyme activities (nmoles/min x g wet tissue) are given as means ± S.E.

the increase in liver and serum XO could be merely secondary to an hepatotoxic effect of poly I-C. As XO and xanthine dehydrogenase are known to be two different forms of the same enzymic protein and the conversion of XD into XO by proteolitic degradation and sulphidryl group oxidation was reported (11), we have investigated the possibility of an increased conversion of XD into XO in poly I-C treated mice. Table 3 shows that poly I-C also increases liver XD activity and that no significant change was observed in the XO/XD ratio, suggesting that the effect of IFN is possibly the result of an increase in the amount of the enzymic protein.

The protein in serum XO reported here might be of particular interest with respect to a possible study on the effect of IFN treatment on XO activity in humans and to a possible use of XO for monitoring patients treated with IFN. Dose response studies with poly I-C revealed that the minimal effective dose for increasing serum XO was 0.1 mg/kg (Table 4), that is well in the range of the minimal effective dose for antiviral protection and IFN production reported for Swiss albino mice (12). As early as 1947, Bauer found that XO was increased in the brain of mice with yellow fever encephalitis, lymphocytic choriomeningitis and lymphogranuloma inguinale (13) and high XO has also been observed in liver and polymorphonuclear leukocytes of mice during bacterial

Table 4: Serum XO levels 24 hrs after different doses of poly I-C

poly I-C (mg/kg, i.p.)	serum XO (% of control)
0.02	93 ± 14 N.S.
0.1	139 ± 9*
0.5	146 ± 4**
2.0	179 ± 15**
10.0	330 ± 16**

Mean ± S.E. N.S.: not significantly different; *$p<0.05$ and **$p<0.01$ vs. saline controls (Student's t-test).

infection (14, 15). The results reported here suggest that IFN might account for induction of XO in these pathological conditions.

The role of XO in the killing of S. Aureus by polymorphonuclear leukocytes was demonstrated using allopurinol and oxopurinol to selectively inhibit the enzyme (16).

IFN has diverse biological effects and it is unclear whether and how all of them can be accounted for by the induction of the known IFN-induced enzymes. For instance, a recent paper showed that induction of the two major IFN-mediated, double-stranded RNA activated enzymes, oligo A-synthetase and protein kinase is not sufficient for the antiproliferative activity of IFN (17).

Here we report that IFN augments the activity of XO, an enzyme generating reactive oxygen species. Reactive oxygen species are known to be involved in phagocyte mediated microbicidal activity (18), in cellular cytotoxicity (19-20) and in tissue damage, including destruction of cytochrome P-450 (21). Therefore, it is tempting to speculate that the augmentation of XO activity may play a role in the various biological activities of IFN, such as enhanced resistance to bacterial infections in mice treated with IFN inducers (22,23) enhanced activity of natural killer cells and monocytes (24), cell growth inhibition (25), toxicity in neonatal mice (26) and depression of liver drug metabolizing enzymes (27).

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