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# INDUCTION OF XANTHINE OXIDASE AND DEPRESSION OF CYTOCHROME P-450 BY INTERFERON INDUCERS: GENETIC DIFFERENCE IN THE RESPONSES OF MICE

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**SUMMARY:** Interferon and interferon inducing agents depress hepatic cytochrome P-450 systems. They also induce hepatic xanthine oxidase activity. It has been suggested that free radicals produced by xanthine oxidase may cause the loss of P-450. High titers of serum interferon are induced by poly IC (poly riboinosinic acid·polyribocytidylic acid) in both C57Bl/6J and $C_3H$/HeJ mice; Newcastle disease virus (NDV) induces a high titer of interferon in C57Bl/6J mice but not in $C_3H$/HeJ mice. The induction of xanthine oxidase activity by NDV in $C_3H$/HeJ mice was less than half that seen in C57Bl/6J mice, thus demonstrating a relationship between the induction of xanthine oxidase, the depression of P-450 and a genetically determined difference in responsiveness of mice to interferon inducers. © 1985 Academic Press, Inc.

**INTRODUCTION:** The administration of interferon inducing agents or interferon *per se* depresses hepatic P-450 systems in laboratory animals (1-4). Recently, Ghezzi and associates (5,6) showed that these agents produce an increase in hepatic xanthine oxidase activity. They have suggested that the increased formation of free oxygen radicals that results from the induction of xanthine oxidase may account for the loss of P-450. DeMaeyer and DeMaeyer-Guignard (7) have described four genetic loci in inbred strains of mice which influence the induction of interferon by specific viruses. One autosomal locus (IF-1) determines a 10-fold difference in serum interferon levels among strains of mice. Singh and Renton (8) exploited this strain difference to demonstrate an inverse relationship between interferon induction and the depression of P-450. C57Bl/6J mice, which carry the high production allele ($IF\text{-}1^h$) at the IF-1 locus, exhibited both a high serum titer of interferon and a depression of P-450 in response to NDV. No change in the P-450 level and no measurable titer of serum

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interferon was detected in $C_3H/HeJ$ mice, which carry the low allele ($IF\text{-}1^l$) for the production of interferon at the IF-1 locus. Poly IC, which induces interferon via loci other than IF-1, induced high levels of serum interferon and depressed P-450 systems in both strains of mice. In the current study, these two strains of mice were used to determine if there is a correlation between the induction of hepatic xanthine oxidase and the depression of P-450 and the P-450-dependent N-demethylation of ethylmorphine.

**METHODS**: Male C57Bl/6J and $C_3H/HeJ$ mice (19-26 g), obtained from Jackson Laboratories (Bar Harbor, ME) were injected intraperitoneally with saline, poly IC (10 mg/kg), or NDV ($2.5 \times 10^6$ PFU/mouse). The animals were killed 24 hr after injection and microsomes were prepared as described previously (9) except that the livers were not perfused. Xanthine oxidase exists in two interconvertible forms (10): as an oxidase (type O) and as an $NAD^+$-dependent dehydrogenase (type D). The 100,000g supernatant fraction from the sedimentation of microsomes was dialyzed at 4°C for 21 hr against 1.15% KCl to remove inhibitors and then assayed for type O and type D xanthine oxidase activities by the methods of Della Corte and Stirpe (11). Type O xanthine oxidase was converted to type D xanthine oxidase by using 10 mM dithiothreitol. Microsomes were assayed for their ethylmorphine N-demethylase activity as described previously (9) and for their P-450 content by the method of Matsubara et al. (12). All spectral measurements were made with an Aminco DW2 spectrophotometer. Students t test was used for the statistical analysis of data. NDV was a gift from Dr. John Newman, Department of Veterinary Pathology, College of Veterinary Medicine, University of Minnesota.

**RESULTS**: The results are summarized in Fig. 1. As anticipated, results obtained with poly IC were very similar in both strains of mice. Ethylmorphine N-demethylase activity was depressed 45% and 54% in $C_3H/HeJ$ mice and C57Bl/6J mice, respectively; corresponding depressions of the P-450 level were 31% and 36%. Type O xanthine oxidase activity was induced 6.7- and 4.8-fold by poly IC in $C_3H/HeJ$ and C57Bl/6J mice, respectively. Quite different results were obtained with NDV in the two strains of mice. In C57Bl/6J mice, losses of ethylmorphine N-demethylase activity and P-450 and the induction of type O xanthine oxidase were very similar to those observed with poly IC. However, in $C_3H/HeJ$ mice, NDV produced no significant loss of demethylase activity or P-450 and the increase in type O xanthine oxidase was much less than that seen with poly IC (2.6- vs. 6.2-fold).

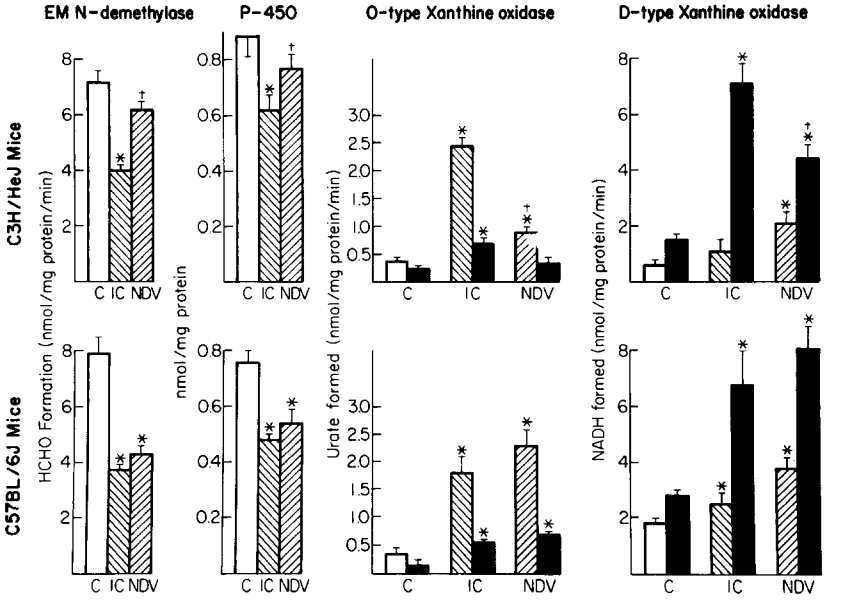


Fig. 1. Effect of poly IC and NDV on the P-450 content, ethylmorphine (EM) N-demethylase activity and oxidase (O-type) and dehydrogenase (D-type) xanthine oxidase (XO) activities of C57Bl/6J and $C_3H$/HeJ mice. Mice received (i.p.) saline (C), poly IC (IC; 10 mg/kg), or Newcastle disease virus (NDV; 2.5 x $10^6$ PFU mouse); their livers were removed 24 hr later. Microsomal fractions were prepared and assayed for their EM N-demethylase activity and P-450 content; the 100,000g fraction separated from the microsomal pellet was assayed for O- and D-types of xanthine oxidase. O- and D-type xanthine oxidase values indicated by solid bars were obtained after the addition of dithiothreitol (10 mM). Values represent the mean ± S.E. *different ($p<0.05$) than corresponding control (C) value. †different ($p<0.05$) than corresponding value obtained with C57Bl/6J mice. N=4.

It is to be noted in Fig. 1 that the induction of xanthine oxidase by poly IC is due almost entirely to an increase in the type O form and that this form is convertible almost completely to the D-form by dithiothreitol.

DISCUSSION: The hepatic monooxygenase systems largely responsible for the biotransformation of drugs and other xenobiotics are comprised of NADPH-cytochrome P-450 reductase and multiple forms of cytochrome P-450. All interferon inducing agents that have been tested as well as highly purified interferon, depress hepatic P-450 levels when administered to laboratory animals (1-4). The mechanism responsible for this effect remains unknown, although it

is a reasonable hypothesis that the interferon-induced antiviral proteins that interfere with the replication of virus might also interfere with the replication of some cellular proteins, including apocytochrome P-450. Recently, Ghezzi and associates (6) proposed another mechanism. They observed that xanthine oxidase is induced 3- to 10-fold in the liver, heart, kidney, lung, spleen and serum by a variety of interferon-inducing agents (poly IC, E. coli lipopolysaccharide, tilorone) and by purified mouse interferon. Since P-450 is known to be destroyed by oxygen radicals (for review see 13), and type-O xanthine oxidase generates superoxide (14), they hypothesized that P-450 may be destroyed by interferon because interferon induces xanthine oxidase. However, because interferon is known to depress levels of many enzymes and increase others in animals and cultured cells (for review see 15), a causal relationship between the increase in xanthine oxidase activity and the destruction of P-450 cannot be assumed. In fact, two observations suggest that direct causality is not the case: a) NADPH-cytochrome P-450 reductase, which is complexed with P-450 can generate oxygen radicals (13) yet it is depressed, not increased by the interferon inducer: tilorone (16); b) Hepatic aldehyde oxidase, which does not exist as a dehydrogenase (13) produces oxygen radicals. Employing the same conditions used in this communication, we have shown in randomly bred Swiss Webster mice that poly IC depressed aldehyde oxidase activity (for method, see 17) from 1.5 $\pm$ 0.08 $\Delta$OD/mg protein/min, observed with control animals, to 1.08 $\pm$ 0.07 $\Delta$OD/mg protein/min (N=12).

One possibility to be considered is that interferon causes the formation of oxygen radicals by an unknown mechanism; these radicals attack both P-450 and the D-form of xanthine oxidase, which is the predominant, if not the only form of the enzyme that exists normally in the liver (10). That the increase in xanthine oxidase is due almost entirely to an increase in the type O form would support this concept. The conversion of the type D form of xanthine oxidase can occur in two ways: a) through oxidation of its thiol groups, which can be reversed with dithiothreitol or b) by proteolysis, an irreversible process (10, 11). Heme or the iron released from P-450 catalyzes the production of oxygen

radicals (18,19) that could convert the type D form of xanthine oxidase to the type O form. El Azhary and Mannering (20) have shown that δ aminolevulinic acid (ALA) synthetase is depressed markedly within 4 hr after the administration of poly IC to rats. In theory, ALA synthetase is depressed when an unassigned heme pool becomes enlarged (21, 22), presumably in this case by heme released from P-450 or by the accumulation of de novo heme that continues to be synthesized at its normal rate while the rate of synthesis of apoP-450 declines. In the current study, the type O xanthine oxidase induced by poly IC was almost entirely reversed by dithiothreitol, thus showing that its conversion from the type D form was oxidative rather than proteolytic. To carry the hypothesis further, it is not inconceivable that the induction of hepatic xanthine oxidase may result from the failure of the organism to recognize type O xanthine oxidase as a viable enzyme. With this in mind, it is of interest that vitamin E deficiency, which would encourage accumulation of oxygen radicals, increases hepatic xanthine oxidase activity 10-fold (23). In accordance with this hypothesis, the relationship of the depression of P-450 and the induction of xanthine oxidase need not be determined by common genetic factors per se, but more simply by the genetic difference that regulates the response to the induction of interferon by NDV in the two strains of mice. Thus, it would seem likely that the induction of xanthine oxidase results from the interferon mediated destruction of P-450 rather than from some more direct action of interferon.

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