Role of Xanthine Oxidase in the Interferon-mediated Depression of the Hepatic Cytochrome P-450 System in Mice

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ABSTRACT

Interferon, interferon inducers, and a variety of other immunomodulators are known to depress the hepatic cytochrome P-450 drug-metabolizing system. Two concepts have been proposed to explain this phenomenon. (a) The steady-state of cytochrome P-450 is altered through decreased synthesis and increased degradation of cytochrome P-450 apoprotein. (b) Interferon induces xanthine oxidase; superoxide generated by interferon-induced xanthine oxidase destroys cytochrome P-450. The current study investigated the second concept. Administered polyribonucleotides [polyribioosinic acid-polyribocytidylic acid (poly IC), polyribioosinic acid-polyctylic acid, polylysine and carboxymethylcellulose, mismatched poly IC], recombinant murine gamma-interferon, and a natural murine alpha/beta-interferon were shown to depress hepatic cytochrome P-450 and selected microsomal cytochrome P-450-dependent monooxygenase reactions and to induce hepatic xanthine oxidase activity. The feeding of tungstate in the drinking water largely depleted xanthine oxidase in mice; cytochrome P-450 levels and monooxygenase activities were not affected by tungstate treatment. Tungstate rendered the level of xanthine oxidase much below that in mice that had not received tungstate regardless of whether or not they had received poly IC or interferon; nevertheless, poly IC and interferons produced losses of cytochrome P-450 and monooxygenase activities in these tungstate-treated mice equivalent to those observed in mice that had not received tungstate. The administration of N-acetylcysteine did not prevent the loss of cytochrome P-450 induced by poly IC, as has been reported, nor did the incubation of microsomal cytochrome P-450 with buttermilk xanthine oxidase and hypoxanthine cause a loss of cytochrome P-450, which has also been reported. It is concluded from these studies that the induction of xanthine oxidase and the loss of cytochrome P-450 generated by interferon are coincidental rather than causally related phenomena.

INTRODUCTION

Although it has been known for more than a decade that interferon, interferon inducers, and many other immunomodulators depress the hepatic cytochrome P-450 drug metabolizing system (see Refs. 1–3 for reviews), the mechanism has not been revealed. Recent studies from our laboratory in which [3H] leucine and [14C]bicarbonate were incorporated into the proteins of individual organelles of hepatocytes led to the conclusion that the interferon inducer, poly IC, decreases the content of proteins of the endoplasmic reticulum, including cytochrome P-450, by decreasing rates of synthesis and increasing rates of degradation (4). In a more recent study (5) we showed that poly IC decreased the in vitro translation of mRNAs for tyrosine aminotransferase, prealbumin, and cytochrome P-450 apoprotein. This suggests that interferon may depress cytochrome P-450 because it regulates gene expression by altering levels of hepatic proteins via increased transcription and decreased translation.

Ghezzi and associates (6, 7) have proposed an alternate mechanism for the depression of cytochrome P-450 by interferon which implicates the induction of hepatic xanthine oxidase. Since cytochrome P-450 is destroyed by oxygen radicals (8) and xanthine oxidase generates superoxide, they proposed that interferon depresses cytochrome P-450 because it induces xanthine oxidase. The studies to be reported here attempt to evaluate this concept.

MATERIALS AND METHODS

Materials. Mouse alpha/beta-interferon (Lee BioMolecular, San Diego, CA), 1.2 x 10^7 units/mg protein) was provided by a National Cancer Society research grant. Mouse rIFN (1.3 x 10^7 units/mg protein) was a gift from Genentech (South San Francisco, CA). Mismatched poly IC (Ampligen) was a gift from HEM Research (Rockville, MD). Poly ICCL (poly IC, 2 mg/ml; poly-L-lysine, 1.5 mg/ml; sodium carboxymethylcellulose, 5 mg/ml) was supplied by the National Cancer Institute. The following materials were purchased from Sigma Chemical Co. (St. Louis, MO): poly IC, 5-azacytidine, NAC, DTT, xanthine, buttermilk xanthine oxidase, mouse serum albumin. Ethylmorphine was obtained from Merck and Co. (Rahway, NJ); aminopyrine from K and K Labs, Inc. (Plainview, NJ); benzylpyrene and 7-ethoxycoumarin from Aldrich Chemical Co. (Milwaukee, WI); and ADT from Herdt and Charton, Inc. (Montreal, Quebec, Canada). AIA was a gift from Hoffmann-LaRoche (Nutley, NJ).

Treatment of Animals. Swiss Webster random-bred male mice (14–22 g), supplied by Lab Supply Co. (Indianapolis, IN) or Taconic Farms (Germantown, NY), were used in all experiments. They were fed a crude, autoclaved mouse chow (Taconic Farms) or purified diet (Normal Protein Test Diet) from United States Biochemical Co. (Cleveland, OH) and spring water from Glenwood Inglewood, (Minneapolis, MN). The mice were maintained in a controlled lighting cycle (12 h on, 12 h off). The mice that were to be fed 330 ppm of sodium tungstate (200 ppm W) in their drinking water were transferred to the purified diet on the day of their arrival (day 0). Tungstate was added to their drinking water on day 2. On day 12 these animals received i.p. saline (0.9% NaCl), poly IC (10 mg/kg), or 2 injections of 50,000 units of alpha/beta-interferon 24 and 18 h before they were killed. Some mice were given NAC in drinking water as a 2.0% solution, neutralized with 1 N NaOH, for 2 days starting 1 day before treatment with poly IC. A fresh solution of 2% NAC was prepared daily. The mice were killed 48 h after their first exposure to NAC. Other mice were injected i.p. with NAC (1000 mg/kg) 24 and 18 h prior to killing. ADT was dissolved in corn oil and injected i.p. (12 mg/kg) 24 h before killing. A 1.0% solution of mouse serum albumin was the vehicle for injections of alpha/beta mouse interferon and mouse rIFN.

Methods. Hepatic microsomes were prepared as described previously (9), or in one experiment, by the procedure used by Ghezzi et al. (6). Assays of the microsomal content of cytochrome P-450 and ethylmorphine N-demethylation, aminopyrine N-demethylation, ethoxyoumarin O-deethylation, and benzylpyrene hydroxylase activities were those used previously (9). The 100,000-g supernatant fraction from the sedimentation of microsomes was assayed for type 0 xanthine oxidase activity by measuring urate formation from xanthine (10) or for type D xanthine oxidase by measuring the NADH formed during the conversion of xanthine to urate (10) as described previously (11). Type O xanthine oxidase was assayed by measuring urate formation from xanthine (10) or for type D xanthine oxidase by measuring the NADH formed during the conversion of xanthine to urate (10) as described previously (11). Type O xanthine oxidase was assayed by measuring urate formation from xanthine (10) or for type D xanthine oxidase by measuring the NADH formed during the conversion of xanthine to urate (10) as described previously (11). Type O xanthine oxidase was assayed by measuring urate formation from xanthine (10) or for type D xanthine oxidase by measuring the NADH formed during the conversion of xanthine to urate (10) as described previously (11).
RESULTS

Effects of Polyrribonucleotides and Interferon on the Cytochrome P-450 System and Xanthine Oxidase. The effects of poly IC on murine cytochrome P-450, ethylmorphine N-demethylase activity, and xanthine oxidase activity over a 24-h time course are shown in Fig. 1. Loss of cytochrome P-450 and ethylmorphine N-demethylase activity were first observed 3 and 12 h, respectively, after the administration of poly IC. After 24 h, losses were near 50%. A 3-fold increase in xanthine oxidase activity was induced by poly IC within 6 h of its administration; a 5-fold increase was observed after 24 h. Thus, in general, there was an inverse relationship between the depression of the cytochrome P-450 system and the induction of xanthine oxidase activity throughout the 24-h period.

Poly ICLI2 (12), a complex of poly IC with poly-L-lysine and carboxymethylcellulose, and poly (I)-poly (C12U), a “mismatched” analogue of poly IC (13), are potent interferon inducers. Fig. 2 shows that these polynucleotides compare favorably with poly IC as depressors of the cytochrome P-450 system and inducers of xanthine oxidase activity. While it may appear that the induction of xanthine oxidase activity by ICLC is disproportionately high relative to its depressant effect on cytochrome P-450, it is to be borne in mind that the maximal depression of cytochrome P-450 achievable by any of these agents is about 50%; conceivably, the maximal induction of xanthine oxidase and the depression of cytochrome P-450 could have been reached with less than the 5 mg/kg dose of ICLC.

GIF 1. Time course of the effects of poly IC on murine hepatic cytochrome P-450 content (P-450), ethylmorphine N-demethylase activity (EM) and O-form xanthine oxidase activity (XO). Mice were killed at indicated times after the i.p. administration of poly IC. Values, mean ± SE., n = 3. * P < 0.05. ** P < 0.01.

Fig. 2. Effects of poly IC (IC), mismatched double-stranded RNA (ICL), and ICLC on ethylmorphine N-demethylase activity (EM), cytochrome P-450 content (P-450), and O-form xanthine oxidase activity (O-form XO) activity. Mice were killed 23 h after i.p. injection of 5 mg/kg of each of the three polynucleotides. Values, mean ± SE., n = 3. *, P < 0.05.

Fig. 3. Effects of poly IC (10 mg/kg) and murine rTIFN(rT) on ethylmorphine N-demethylase activity (EM), aminopyrine N-demethylase activity (AP), cytochrome P-450 content (P-450), and O-form xanthine oxidase activity (O-form XO). Poly IC was injected (i.p.) 21 h before and interferon (1000 or 5000 units i.p.) 24, 21, and 18 h before the mice were killed. Values, mean ± SE., n = 3. P ≤ 0.05.

Effects of the Administration of Phenobarbital, Azacytidine, and AIA on Hepatic Xanthine Oxidase. Phenobarbital induces (for reviews see Refs. 16 and 17) and azacytidine depresses (18) cytochrome P-450 by mechanisms that are not well understood. AIA depresses cytochrome P-450 through a suicidal reaction involving the alkylation of cytochrome P-450 heme (see Ref. 19 for review). It was of interest to determine whether fluctuations in the content of hepatic cytochrome P-450 per se would affect hepatic xanthine oxidase activity. Fig. 4 shows that these agents produced their anticipated effects on the P-450 system but had no effect on xanthine oxidase activity.

Effects of the Administration of Tungstate and Poly IC on Cytochrome P-450, Xanthine Oxidase, Xanthine Dehydrogenase, and Aldehyde Oxidase. If superoxide generated by interferon-induced xanthine oxidase attacks cytochrome P-450, one might expect that cytochrome P-450 would not be depressed after the administration of interferon or poly IC if hepatic xanthine oxidase could be maintained at less than normal levels. Hepatic xanthine oxidase activity of mice was inactivated in vivo by displacing its molybdenum with tungstate supplied in the drinking water (20, 21). Results obtained from mice fed tungstate in their drinking water are summarized in Fig. 5. The following conclusions are derived from Fig. 5. (a) Tungsten, supplied at a level of 200 ppm in the drinking water for 10 days, lowered xanthine oxidase activity by about 90% without depressing cytochrome P-450 or any of the 4 monooxygenase activities evaluated in this study. (b) Cytochrome P-450 and monooxygenase activities of hepatic microsomes from these animals were depressed by the administration of poly IC to about the same extent as those observed in preceding
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Fig. 4. Effects of phenobarbital (PB), azacytidine (AzC), and allylpropylacetamide (APA) on ethylmorphine N-demethylase activity (EM), cytochrome P-450 content (P-450), and O-form xanthine oxidase (O-form XO) activity. PB was fed in the drinking water (1 mg/ml) for 4 days before the mice were killed. AzC was given i.p. (25 mg/kg) 24 h before the mice were killed. APA (400 mg/kg) was given s.c. 2 h before the mice were killed. 100% saline control values: EM, 11.1 ± 0.12 nmol/mg microsomal protein/min; P-450, 1.19 ± 0.15 nmol/mg microsomal protein; O-form XO, 0.63 ± 0.12 nmol urate formed/mg 100,000 g supernatant protein/min. Values, mean ± S.E., n = 3. *, P ≤ 0.05.

Fig. 5. Effects of tungstate and poly IC on murine hepatic xanthine oxidase activity. O-form XO, xanthine dehydrogenase activity (type D form) (22-23). It is converted to the type O form when its sulfhydryl groups are oxidized or it undergoes partial proteolysis (10). Type O xanthine oxidase formed by oxidation is reconverted to the type D form by DTT whereas that formed by proteolysis is not. It can be seen in Fig. 5 that almost all of the type O form is convertible to the type D form by DTT.

Like xanthine oxidase, aldehyde oxidase is a molybdenum enzyme that generates superoxide (24). In Fig. 5 it can be seen that in contrast to what was observed with xanthine oxidase, aldehyde oxidase activity was depressed by poly IC, not induced.

The effects of murine α/β-interferon on type O and D forms of xanthine oxidase, the reversion of the type O form to the type D form by DTT, the cytochrome P-450 level, and ethylmorphine N-demethylase activity of livers from mice that received tungstate are summarized in Fig. 6. It can be seen that even though tungstate lowered xanthine oxidase activities to levels that were not readily detectable, it did not affect the level of ethylmorphine N-demethylase activity; i.e., α/β-interferon produced a 50–60% loss of demethylase activity regardless of whether or not the mice received tungstate. Neither form of xanthine oxidase was detectable in the liver preparations of mice with or without the addition of DTT. A 40% depression of cytochrome P-450 was produced by α/β-interferon in this study but no significant change was elicited by poly IC. This may be due to a reversal of the depressant effect of poly IC on the cytochrome P-450 system occasionally observed when the dose is raised from 5 to 10 mg/kg. If this is the case, a form(s) of cytochrome P-450 that is not associated with ethylmorphine N-demethylase must be induced because this demethylase is depressed maximally despite the lack of depression of total cytochrome P-450. It is to be noted that a maximal induction of xanthine oxidase activity occurred in these mice despite the experiments (Figs. 1–3), which used mice with normal or elevated levels of hepatic xanthine oxidase activity. (a) Poly IC induced xanthine oxidase activity in mice given tungsten, but this induced level was very much lower than that observed in mice that had not received tungstate or given poly IC without tungstate. (b) In mice that did not receive tungstate, hepatic xanthine oxidase levels were about the same whatever animals were fed the crude diet (Figs. 1–3) or the purified diet (Fig. 5); on the other hand, the cytochrome P-450 system was considerably depressed in mice that received the purified diet relative to that observed in mice that received the crude diet.

Xanthine oxidase is believed to exist primarily, if not exclusively, in the in situ hepatocyte as xanthine dehydrogenase (type D form) (22-23). It is converted to the type O form when its sulfhydryl groups are oxidized or it undergoes partial proteolysis (10). Type O xanthine oxidase formed by oxidation is

Fig. 6. Effects of tungstate and poly IC or murine α/β-interferon on D (O-form XO) and D forms of (D-form XO) hepatic xanthine oxidase activity, ethylmorphine N-demethylase activity (EM), and cytochrome P-450 content (P-450). The diet and administration of poly IC and tungstate were the same as described in Fig. 5. 50,000 units interferon were injected i.p. 24 and 21 h before the mice were killed. DTT, 10 mM. Values, mean ± S.E., n = 3. ∩, saline; ‡, Poly IC; ‡‡, α/β-interferon. *, P ≤ 0.05 versus saline. N.D., not detectable.
failure of poly IC to produce a significant loss of cytochrome P-450.

Effects of NAC and ADT on the Depression of Cytochrome P-450 and Ethylmorphine N-Demethylase Activity and the Induction of Xanthine Oxidase by Poly IC. Ghezzi and associates (6, 7) published data that showed that NAC (2.0 or 2.5% in the drinking water for 2 days) reversed the depressant effect of poly IC (2 mg/kg i.p. given after the first day of feeding of NAC) on cytochrome P-450 and the O-deethylation of ethoxycoumarin. Fig. 7 illustrates experiments of similar design which used either injected or fed NAC or injected ADT, a dithiolthione known to prevent CCl4-induced lipid peroxidation (25). None of these treatments prevented the loss of cytochrome P-450 or ethylmorphine N-demethylation activity produced by poly IC, nor did they prevent the induction of xanthine oxidase activity.

Effect of Bovine Buttermilk Xanthine Oxidase and Hypoxanthine on Microsomal Cytochrome P-450. Ghezzi and associates (6) reported that the incubation of hepatic mouse microsomes with bovine buttermilk xanthine oxidase and hypoxanthine produced a loss of cytochrome P-450. NAC prevented most of this loss. This experiment was repeated without the addition of NAC by using microsomes as prepared by Ghezzi et al. (6) or as prepared in our laboratory (9). Bovine buttermilk xanthine oxidase did not produce a loss of cytochrome P-450 in either microsomal preparation (Table 1).

**DISCUSSION**

Two concepts have been proposed for the depression of the hepatic cytochrome P-450 system by interferon and interferon inducers. (a) The steady-state of cytochrome P-450 is altered through depressed synthesis of cytochrome P-450 apoprotein, through increased degradation of cytochrome P-450 apoprotein, or a combination of both processes (4). (b) Interferon induces xanthine oxidase; superoxide generated by induced xanthine oxidase destroys cytochrome P-450 (6, 7). The discussion that follows addresses the evidence that supports or argues against each of these concepts.

Cytochrome P-450 is only one of several enzymes and other proteins that are known to be depressed by interferon (3). Many of these proteins are not hemoproteins and therefore not readily subject to destruction by oxygen radicals. By analogy to other agents that alter protein synthesis (e.g., actinomycin D), it is tempting to suggest that a single mechanism may be responsible for the alteration of the steady state of all of the proteins known to be affected by interferon. It is generally accepted that interferon produces its antiviral effect by inactivating the RNA responsible for virus replication through the induction of (2′5′)oligo(A)synthetase and protein kinase. If the activities of the interferon-induced synthetase and kinase are not restricted to their effects on RNA involved in virus replication but, as many believe, they may affect both viral and some host RNA, it is understandable that proteins such as cytochrome P-450 might be depressed by the same mechanisms which prevent replication of virus. In other cases, when one or more RNAs may compete with each other for rate-limiting components required for their expression, superinduction (for review see Ref. 26) may occur. Xanthine oxidase is only one of several proteins known to be induced by interferon (3). In short, depression and induction of proteins by interferon may involve the same antiviral synthetase or kinase.

Recent studies from this laboratory (4) support the concept of a depressant effect of interferon on the steady state of cytochrome P-450 apoprotein. The effects of poly IC on the in vivo incorporation of [3H]leucine and [14C]bicarbonate into the proteins of individual organelles of the hepatocyte led to the conclusion that this interferon inducer decreased the content of proteins, including cytochrome P-450, by decreasing rates of synthesis and increasing rates of degradation. In another study (5), it was shown that poly IC increased the transcription of total RNA but decreased the translation of mRNAs for tyrosine aminotransferase, prealbumin, and cytochrome P-450 apoprotein. This suggests that interferon may depress cytochrome P-450 because it regulates gene expression by altering levels of hepatic proteins via increased transcription and decreased translation.

The concept that cytochrome P-450 is destroyed by superoxide generated by interferon-induced xanthine oxidase was
developed by Ghezzi and associates (6, 7) from their observations that the depressive effect of interferon on cytochrome P-450 was reversed by allopurinol, a potent inhibitor of xanthine oxidase, and by the oxygen radical scavengers, NAC and superoxide dismutase. Allopurinol inhibits xanthine oxidase by reacting with its molybdenum site (27). In the current study cytochrome P-450 was depressed by poly IC even though xanthine oxidase activity was nearly eliminated through tungstate administration. Since both allopurinol and tungstate inhibit xanthine oxidase by reacting at the molybdenum site, and allopurinol reverses the depression of cytochrome P-450, whereas tungstate does not, it follows that allopurinol must be producing its effect by some mechanism other than the inhibition of xanthine oxidase activity.

Ghezzi and associates (6) observed that the administration of NAC reversed the depressive effect of poly IC without reversing its inductive effect on xanthine oxidase activity. This was interpreted to mean that superoxide generated by induced xanthine oxidase was neutralized by NAC. We were unable to repeat this experiment. We have no explanation for this discrepancy of results. Ghezzi and associates also observed that in the presence of hypoxanthine, buttermilk xanthine oxidase (0 form) caused a loss of cytochrome P-450 when incubated with hepatic microsomes. The addition of NAC or superoxide dismutase to the incubation medium prevented this loss. We were not able to demonstrate a loss of cytochrome P-450 in microsomes incubated with milk xanthine oxidase and hypoxanthine (Table 1). Again we are unable to explain this discrepancy of results.

Ali et al. and Koizumi et al. (28, 29) reported that poly IC induces in vitro lipid peroxidation in hepatic microsomes of mice; they implied that this could account for the depression of cytochrome P-450 by poly IC. We have not been able to show that poly IC induces lipid peroxidation in hepatic microsomes (data not shown). Moreover, ADT, which prevents lipid peroxidation (25), did not alleviate poly IC-induced loss of cytochrome P-450 (Fig. 7).

The oxygen radical concept of the destruction of cytochrome P-450 depends on the speculation that the 0 form of xanthine oxidase exists or can be made to exist in vivo. There is no clear evidence to date that the 0 form of hepatic xanthine oxidase does in fact exist in vivo under normal conditions; the preponderance of evidence favors the view that the 0 form exists only as an artifact derived during isolation of the enzyme (22, 23). This is not to say that the 0 form cannot be formed in vivo under certain conditions. One might conceive of a condition where an accumulation of heme may lead to the formation of oxygen radicals which in turn convert the D form of xanthine oxidase to the 0 form. 0-form xanthine oxidase generated in this manner would then produce superoxide which would attack cytochrome P-450 heme and thereby complete a cycle of destruction. An interferon-induced lowering of the steady state of cytochrome P-450 apoprotein could result in a temporary accumulation of heme because heme and cytochrome P-450 syntheses proceed independently. An interferon-induced lowering of the steady state of cytochrome P-450 apoprotein could lead to an temporary accumulation of heme while heme synthesis is accommodating to the lesser need for heme mandated by the loss of apoprotein. The effects of poly IC on ALA synthetase and tryptophan 2,3-dioxynegase suggest that interferon produces a transient accumulation of heme (30). ALA synthetase, the rate-limiting enzyme in the synthesis of heme, is regulated by a negative heme feedback mechanism (31); poly IC produced a rapid depression of ALA synthetase activity thus indicating that the heme pool had been enlarged. A rapid addition of heme to apotryptophan 2,3-dioxynegase follows the administration of poly IC to rats (30). In view of these considerations, it is conceivable that once the loss of cytochrome P-450 had been initiated by interferon, further loss might be perpetuated by oxygen radicals generated by the 0 form of xanthine oxidase formed through heme degradation; xanthine oxidase induced independently by interferon would facilitate this second phase of the destruction of cytochrome P-450. However, in the absence of evidence for these speculations, it seems more likely that the induction of 0 and D forms of xanthine oxidase and the loss of cytochrome P-450 generated by interferon are coincidental rather than causally related phenomena.

REFERENCES

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