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DIFFERENTIAL EFFECTS OF RECOMBINANT INTERFERON α ON CONSTITUTIVE AND INDUCIBLE CYTOCHROME P450 ISOZYMES IN MOUSE LIVER

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Abstract—The hepatic cytochrome P450 (P450)-dependent monooxygenase system is subject to regulation by a variety of xenobiotics and endogenous factors. During infection and inflammation the P450 system is usually suppressed, but the factors responsible for this phenomenon and the P450 isozymes involved have not been identified conclusively. We have studied the effects of a specific inflammatory mediator, recombinant interferon α, on the constitutive and inducible expression of P450 isozymes(from the CYP1A,CYP2B and CYP2C) gene families using isozyme preferred substrates and Western blot analysis. Both increases and decreases in P450 levels occurred in response to interferon α. Suppression of constitutive P450 isozyme expression occurred and was shown to involve a decrease in steady-state protein expression. The induction of 7-ethoxyresorufin *O*-deethylase activity by 3-MC was potentiated whereas induction of 7-pentoxyresorufin- and 7-benzyloxyresorufin *O*-dealkylases by PB was suppressed by interferon α. These data demonstrate that the effects of interferon α on the P450-dependent monooxygenase system are complex, involving differential regulation of several isozymes. Both direct and indirect mechanisms may participate in these phenomena.

Among the most important routes by which cells metabolize xenobiotics is that involving the cytochrome P450-dependent monooxygenase system. Cytochrome P450s (P450s¶) comprise a polymorphic multigene family of haem-containing enzymes which are essential to the Phase I metabolism of drugs and carcinogens [1]. The P450s may participate in either activation or detoxification of carcinogens and anticancer drugs [2]; thus an understanding of these enzymes is essential to any programme aimed at the prevention or treatment of cancer. Induction of P450s by drugs and carcinogens has been extensively studied ever since the identification of these cytochromes some 30 years ago; endogenous regulation of P450s also occurs during normal development and in various disease states [3]. Parasites, bacteria and viruses also cause inflammation leading to changes in P450-dependent drug metabolism [4, 5]. Many of the inflammatory stimuli which affect P450-dependent drug metabolism also induce interferon synthesis. Inducers of interferons α and β include viruses, double-stranded RNA, intracellular microorganisms, protozoans, pyran copolymers and polyvinyl sulphate as well as certain small molecules whilst molecules which activated T-cells, such as antigens and mitogens, are inducers of interferon γ and endotoxin causes heterogeneous induction of both type I (α and β) and type II (γ) interferons [6–8]. A variety of interferon inducers have been used to study the effects of activating host defence mechanisms on P450-associated activities. Initially it was observed that tilorone (2,7-bis[2-diethyl aminoethoxy]fluoren-9-one) suppressed P450 and its dependent activities [9]; this led to studies on a variety of other interferon inducers, all of which were found to have similar effects [10]. Circumstantial evidence indicated that the effects of interferon inducers on P450 expression were indeed mediated by interferon [11, 12], but proof of its involvement in the depression of hepatic P450 awaited the availability of a pure interferon that was active in a laboratory animal. This came in the form of hybrid HuIFNr-AD, which possessed antiviral activity in the mouse. HuIFNr-AD depressed the P450 system of the mouse; HuIFNr-A and huIFNr-D, which have no antiviral activity in the mouse, had little or no effect. Pure recombinant mouse interferon γ also depressed the P450 activities [13].

Previous studies on the effects of inflammation on the monooxygenase system have concentrated on constitutive P450-dependent activities; few studies have examined the effects of concomitant treatment with a P450 inducer and an inflammatory agent. Those studies which have done so suggested that

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¶ Abbreviations: BROD, 7-benzyloxyresorufin O-debenzylation; ECOD, 7-ethoxycoumarin O-deethylation; EROD, 7-ethyoxyresorufin O-deethylation; i.p., intraperitoneally; 3-MC, 3-methylcholanthrene; P450, cytochrome P450; PB, phenobarbital; PROD, 7-pentoxyresorufin O-depentylation; SD, standard deviation; SEM, standard error of the mean; TBST: 50 mM Tris–HCl pH 7.9 containing 0.15 M NaCl and 0.05% Tween 20.

Table 1. Effects of interferon α on the murine hepatic P450-dependent monooxygenase system

	Cytochrome P450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)
Control	0.942 ± 0.093 (100%)	0.687 ± 0.024 (100%)
Control + interferon	0.736 ± 0.022 (78%)	0.641 ± 0.012 (93%)
PB	1.439 ± 0.076 (153%)	0.904 ± 0.030 (132%)
PB + interferon	0.954 ± 0.050* (101%)	0.688 ± 0.010* (100%)
3-MC	0.840 ± 0.108 (89%)	0.800 ± 0.023 (116%)
3-MC + interferon	0.892 ± 0.036 (95%)	0.735 ± 0.018 (107%)

Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. The table shows mean level ± standard error of the mean (SEM) in nanomoles per milligram microsomal protein for individual hepatic microsomes from three mice.

* Significantly different from corresponding non-interferon-treated sample, $P < 0.01$.

inducing agents, including phenobarbital (PB), 3-methylcholanthrene (3-MC), SKF 525A and Aroclor 1254 offered some protection against interferon-mediated P450 suppression. In each of these studies only one, or at most two parameters of P450 expression were measured and the specific isozymes involved were not identified [14–17]. Previous work in this laboratory suggested that low doses of endotoxin could in fact potentiate P450 induction by PB and 3-MC [18]. If it is the case that inflammatory agents can potentiate P450 induction, susceptibility to carcinogens could be increased during inflammation.

In previous studies the criteria used to assess effects on the P450 system were extremely general; activities such as 7-ethoxycoumarin O-deethylation (ECOD) are mediated by several P450 isozymes, total P450 encompasses all P450 isozymes present and drug clearance depends not only on a number of P450s but also on rates of blood flow, binding of drugs to serum albumin and activities of conjugating enzymes. More specific studies indicated that all P450 isozymes are not equally suppressed by interferon or interferon inducers. Comparison of the effects of tilorone, poly IC, and adjuvant treatment indicated that in rat liver several proteins in the molecular weight range 43,000 to 54,000 were significantly suppressed. Different treatments led to the suppression of different proteins, although the proteins affected were not shown to be P450s [19]. In the studies described below, the effects of treatment of mice with PB or 3-MC together with interferon α were considered and more clearly defined enzyme activities (dealkylation or resorufin ethers) and immunological techniques (Western blot analysis) were used to elucidate the effects of interferon α on specific P450 isozymes.

MATERIALS AND METHODS

Sources of reagents. Reagents for this study were obtained from BDH (Macfarlane Robson Ltd, Thornliebank, Glasgow) or Sigma Chemical Co. (Poole, Dorset) except for the following: ammonium persulphate, bromophenol blue, 4-chloro-1-naphthol and *N,N,N',N'*-tetramethyl ethylene diamine (TEMED) were from Biorad Laboratories Ltd (Watford, Herts), horseradish peroxidase linked anti-rabbit IgG was from the Scottish Antibody Production Unit (Glasgow and West Scotland Blood Transfusion Service, Lanarkshire) and 7-benzyloxy, 7-ethoxy- and 7-pentoxyresorufin and resorufin were from Molecular Probes Inc (Eugene, OR, U.S.A.). Nitrocellulose filters were obtained from Anderman and Co. Ltd (Kingston-upon-Thames, Surrey).

Animal treatment and sample preparation. Male CBA mice (25 g) were fed on standard laboratory chow and allowed to acclimatise for 7 days in a controlled environment with a 12 hr light/dark cycle, then treated intraperitoneally (i.p.) with sodium phenobarbitone (80 mg/kg body wt in 0.9% NaCl) or 3-methylcholanthrene (40 mg/kg body wt in corn oil). Immediately following the administration of P450 inducers, recombinant interferon α (25,000 units/mouse/day in 0.9% NaCl) was injected i.p. Treatment was carried out on 3 consecutive days. The animals were starved overnight on day three of the experiment and killed by cervical dislocation on day four. The livers were removed and washed with 10 mM phosphate buffer pH 7.4 containing 1.15% KCl. Microsomal fractions were prepared as described previously [20]. The protein content of samples was estimated according to the method of Lowry *et al.* [21] using bovine serum albumin as a standard.

Enzyme and P450 assays. The level of cytochrome P450 in hepatic microsomes was estimated by carbon monoxide difference spectroscopy according to the method of Omura and Sato [22]. Cytochrome P450 reductase activity was estimated using cytochrome *c* as a substrate according to the method of Phillips and Langdon [23]. 7-Ethoxycoumarin *O*-deethylase (ECOD) activity was assayed by the method of Ullrich and Weber [24] and benzphetamine *N*-demethylase by the method of Hewick and Fouts

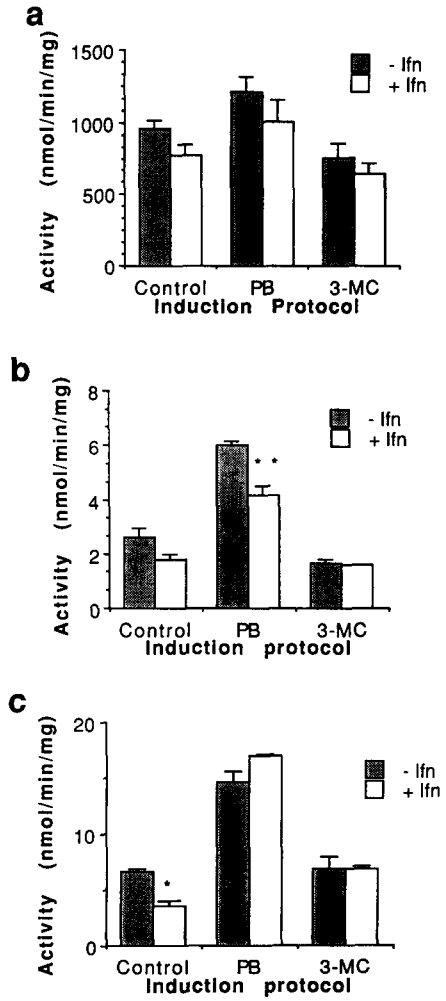


Fig. 1. Effects of recombinant interferon α on hepatic NADPH-cytochrome P450 reductase and monooxygenase activities in male CBA mice. Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. The figure shows mean activity ± SEM for individual hepatic microsomal samples from three mice. (a) NADPH-cytochrome P450 reductase activity (nmol cytochrome *c* reduced/min/mg protein). (b) Benzphetamine N-demethylation (nmol product produced/min/mg protein). (c) 7-Ethoxycoumarin O-deethylation (nmol product produced/min/mg protein). *Significantly different from non-interferon-treated sample ($P < 0.05$). **Significantly different from non-interferon-treated sample ($P < 0.05$).

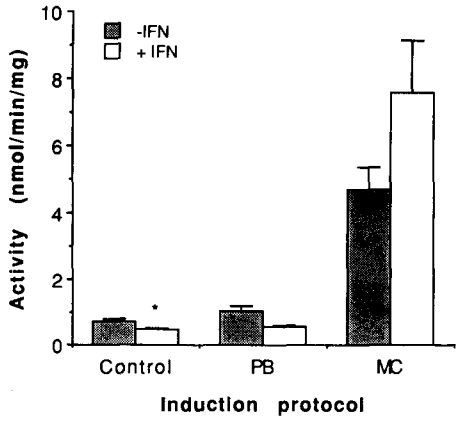


Fig. 2. Effect of recombinant interferon α on hepatic microsomal ethoxyresorufin metabolism in male CBA mice. Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. The figure shows mean EROD activity ± SEM in nmol/min/mg microsomal protein for individual hepatic microsomal samples from three mice. *Significantly different from non-interferon-treated sample ($P < 0.05$).

[25], measuring formaldehyde production by the Hantsch reaction [26]. The alkoxyresorufin activities 7-ethoxyresorufin *O*-deethylase (EROD), 7-pentoxyresorufin *O*-depentylase (PROD) and 7-benzyloxyresorufin *O*-debenzylase (BROD) in rodent liver microsomes were assayed by a modification of the method of Burke and Mayer [27]. The method used was similar for each of the alkoxyresorufin substrates used. The assay was carried out in a Perkin–Elmer LS3 Fluorescence Spectrometer using a built-in temperature regulator set at 37°. The reaction mixture consisted of substrate (1 mL of 1 μM in 100 mM Tris–HCl pH 7.4, equilibrated to 37° before use) plus sample. The reaction was started by adding 10 μL of NADPH (10 mM in 100 mM Tris–HCl pH 7.4). The fluorescence of the reaction mixture (excitation 530 nm, emission 585 nm) was monitored continuously using a Perkin–Elmer 561 Chart Recorder. After about 3 min, 10 μL of resorufin (10 μM in ethanol) was added as a standard and the activity of the sample calculated directly from the observed gradient.

Immunochemical analysis. Western blot analysis was performed by a modification of the method of Towbin *et al.* [28] using polyclonal antisera raised against purified rat hepatic P450 isozymes which have been used before to study murine P450 expression [29]. For this study the antisera were characterized by Ouchterlony double immunodiffusion and Western blot analysis (L. A. Stanley, unpublished results). In each case the antiserum labelled its corresponding antigen most strongly, although the antisera raised against isozymes CYP1A1 and CYP1A2 did cross-react weakly with the alternate member of family 1 and the antiserum raised against CYP2C6 cross-reacted with several members of family CYP2C. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis was performed as described by Laemmli [30], loading 7.5 μg of microsomal protein per track. Following electrophoresis the gel was transferred electrophoretically to a 0.45 μm nitrocellulose filter. The

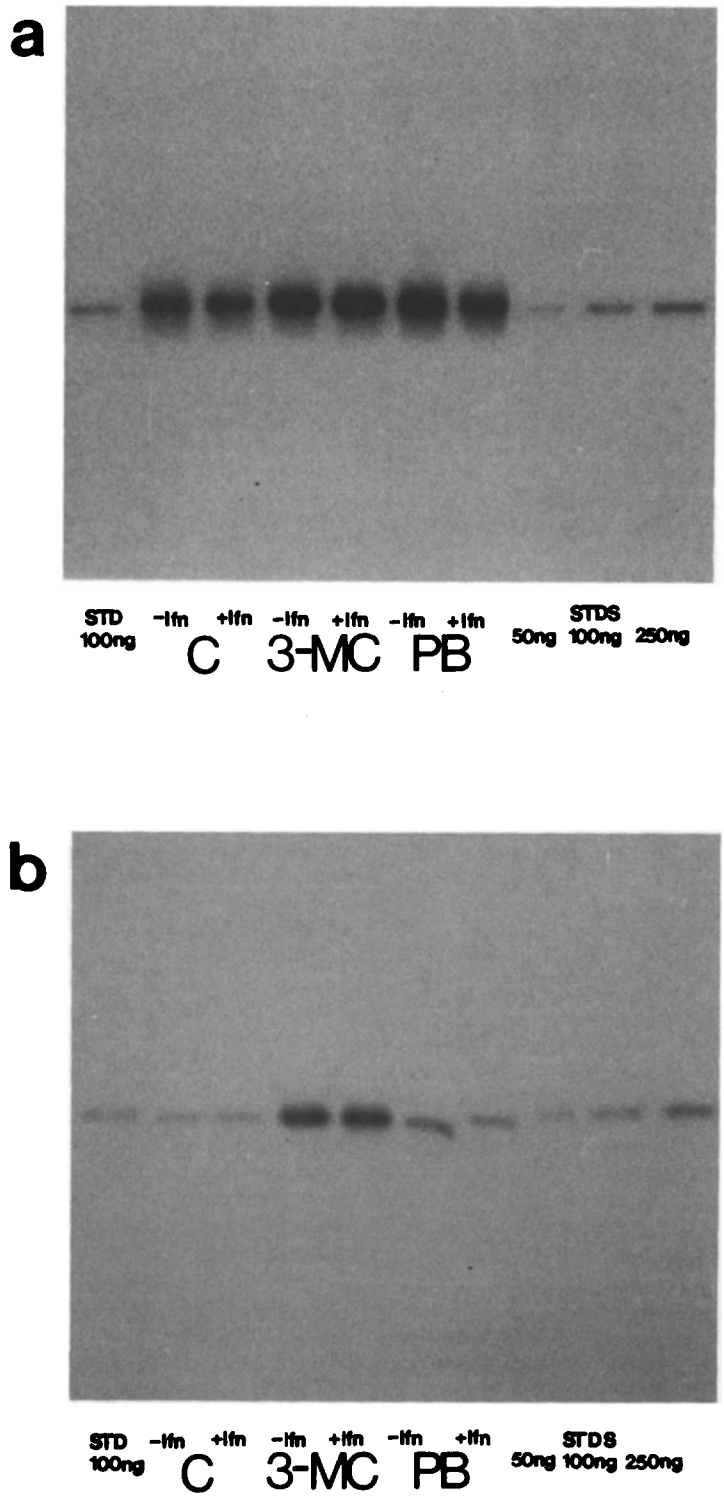


Fig. 3. Effect of recombinant interferon α on the hepatic expression of isozymes CYP1A2 and CYP1A1 in male CBA mice. Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions from three mice were pooled together and analysed by Western blot analysis loading 7.5 μg of protein per track. (a) Expression of isozyme CYP1A2. (b) Expression of isozyme CYP1A1.

nitrocellulose filter was washed twice (10 min each) in 50 mM Tris–HCl pH 7.9 containing 0.15 M NaCl and 0.05% Tween 20 (TBST) at room temperature, then blocked in 3% low-fat milk powder in TBST for 1 hr. After blocking, the filter was washed twice, incubated with anti-P450 antiserum (diluted 1/500 in TBST) for 1 hr, washed four times, incubated with horseradish peroxidase-conjugated goat antiserum raised against rabbit immunoglobulin G (diluted 1/1000 in TBST) and washed again four times. It was then exposed to the horseradish peroxidase substrate 4-chloro-1-naphthol until bands became visible, incubated with ^{125}I-conjugated protein A (5 μCi in 50 mL TBST) for 45 min and repeatedly washed with TBST until the radioactivity in the washes returned to background. The filter was air-dried overnight and exposed to Kodak XAR-5 film at −70°. In order to obtain an estimate of the relative amounts of a particular P450 in different samples, some Western blots were further analysed by densitometric scanning using a Joyce-Loebl Chromoscan 3 densitometer. For this analysis, slightly underexposed autoradiographs were used in order to ensure that the film had not been saturated. The region of the band was scanned using a green filter with a scan length of 0.5–1 cm and a slit width of 0.3 mm. The relative intensity of each band was calculated as a percentage of that of a control band after checking that the intensities of the purified P450 bands were linear with respect to the amount of protein loaded. It should be noted that although this precaution was taken this method is only semi-quantitative; small variations in protein loading, labelling and film background can significantly affect band intensity.

RESULTS

Status of the P450-dependent monooxygenase system in interferon α-treated mice

The constitutive levels of cytochrome P450, cytochrome b_5, P450 reductase, ECOD and benzphetamine *N*-demethylase activity were all reduced in the male CBA mouse liver following treatment with interferon α (Table 1, Fig. 1); however, only in the case of ECOD activity was this suppression significant ($P < 0.01$). Some of the values may have reached significance if a larger group of animals had been studied. Interferon α did not significantly affect reductase or ECOD activity in PB-treated animals, but induction of P450, cytochrome b_5 and benzphetamine *N*-demethylase activity was reduced when mice were treated concomitantly with interferon α ($P < 0.01$). In order to identify the P450 isozymes involved in these contrasting phenomena, the metabolism of a series of phenoxazone (resorufin) ether substrates was next assayed and compared with the levels of P450 isozymes detected by Western blot analysis using specific polyclonal antisera.

Effects of interferon α on members of the 3-MC-inducible class of P450 isozymes (family 1)

Interferon α caused significant suppression of EROD activity in uninduced mice ($P < 0.05$), but in mice treated with both interferon α and 3-MC hepatic EROD activity was approximately 1.6 times that in mice treated with 3-MC alone (Fig. 2). In order to discover whether this phenomenon could also be detected at the level of protein expression the samples were analysed by Western blot analysis using antisera directed against isozyme CYP1A2, the constitutively expressed member of P450 family 1, and isozyme CYP1A1, the highly 3-MC-inducible member of this gene family [31, 32]. Interferon α appeared to suppress CYP1A2 expression in uninduced animals, in agreement with the finding that EROD activity was suppressed in these samples (Fig. 3a). Isozyme CYP1A2 was not induced by 3-MC, but this inducing agent appeared to offer some protection against interferon-mediated suppression of isozyme CYP1A2 (Fig. 3a). Western blot analysis showed that in non-interferon treated animals the extent of induction of isozyme CYP1A1 correlated closely with that of EROD activity (Fig. 3b). However, neither suppression of constitutive expression, nor potentiation of induced expression of this isozyme by interferon α was observed, in contrast to the results of the EROD assay. In order to confirm and extend these findings, Western blots of individual mouse liver microsomes were analysed by densitometry (Table 2). This analysis showed

Table 2. Use of densitometry to analyse the effects of interferon α on CYP1A expression in mouse liver

	Relative band intensity			
	Control		3-MC	
	− Interferon	+ Interferon	− Interferon	+ Interferon
Anti-CYP1A2	105 ± 5	61 ± 2*	121 ± 11	108 ± 25
Anti-CYP1A1	100 ± 6	82 ± 5	586 ± 54	517 ± 41

Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. Relative band intensity was calculated as a percentage of that of the first control sample. The values shown are mean ± standard deviation (SD) for three individual mouse liver microsomal samples run on the same blot.

† Significantly different from non-interferon treated sample $P < 0.005$.

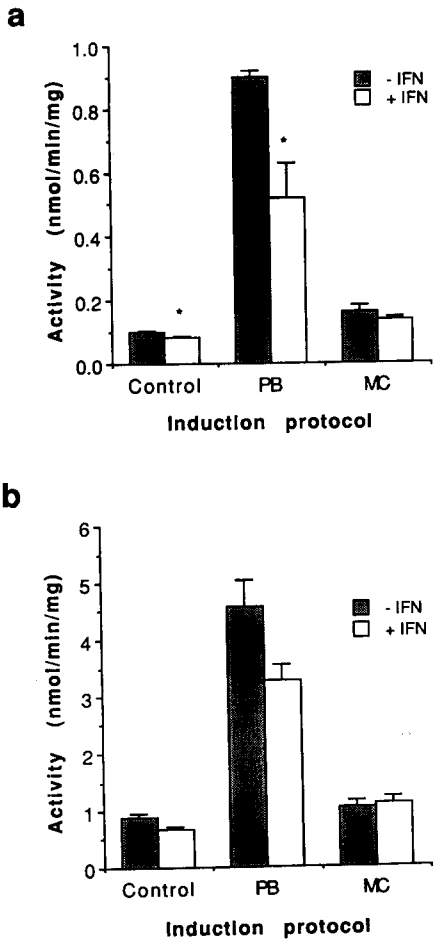


Fig. 4. Effect of recombinant interferon α on hepatic microsomal ethoxyresorufin metabolism in male CBA mice. Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. The figure shows mean activity ± SEM in nmol/min/mg microsomal protein for individual hepatic microsomal samples from three mice. (a) PROD activity (nmol resorufin formed/min/mg microsomal protein). (b) BROD activity (nmol resorufin formed/min/mg microsomal protein). *Significantly different from non-interferon-treated sample ($P < 0.05$).

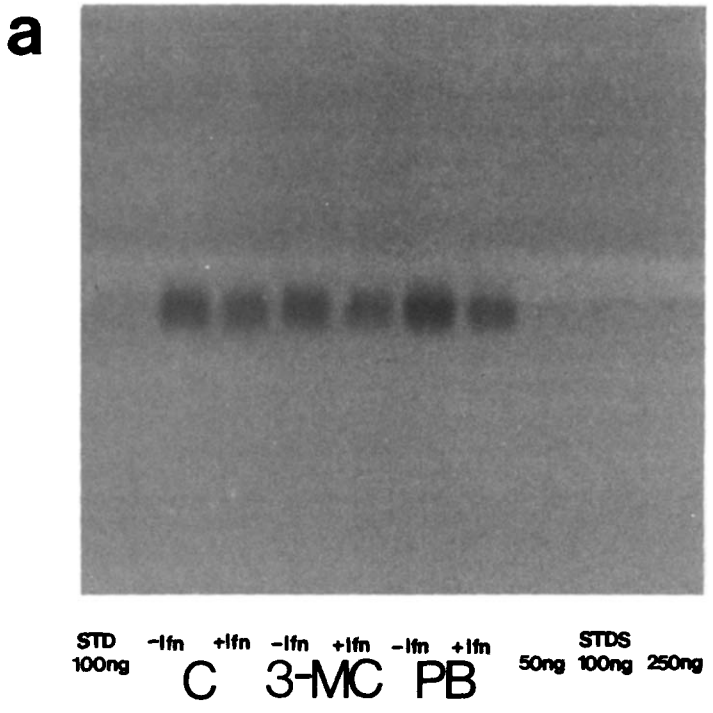
that significant interferon-mediated suppression of CYP1A2 occurred ($P < 0.005$) but that CYP1A1 expression was not significantly affected by interferon α. This finding implicates other mechanisms in the regulation of enzymic activities performed by this isozyme.

Effects of interferon α on members of the PB-inducible P450 isozymes

Two resorufin ethers, 7-pentoxyresorufin and 7-benzyloxyresorufin, were used as markers of the effects of interferon α on members of the CYP2B gene subfamily. These substrates are metabolized by PB-inducible P450 isozymes, chiefly isozyme CYP2B1, the major hepatic PB-inducible P450 isozyme [33]. Interferon α caused significant suppression of both constitutive and PB-induced PROD activity ($P < 0.05$), and its effects on BROD activity were similar, though non-significant (Fig. 4). Western blot analysis was used to compare the effects of interferon on PB-inducible isozyme expression with its effects on these enzyme activities. Antisera directed against isozyme CYP2C6, a marginally inducible member of the P450 CYP2C family which is expressed constitutively in the liver, and isozyme CYP2B1, the major hepatic PB-inducible P450 isozyme [34, 35], were used for this analysis. Western blot analysis of pooled microsomal samples suggested that expression of a CYP2C isozyme was suppressed by interferon α in all three experimental groups, but that interferon α had little effect on the expression of isozyme CYP2B; the constitutive level of this isozyme was too low for suppression to be discerned by this method (Fig. 5). Densitometric analysis of a fresh set of blots made using individual mouse liver microsomes confirmed that interferon α caused significant suppression of CYP2C protein in individual livers ($P < 0.005$) in agreement with the suppression in EROD and BROD activities (Table 3). However, in this second experiment the effects of interferon α on CYP2C expression in the livers of PB- or 3-MC-treated mice were found to be less marked and not statistically significant. We are inclined to place more credence upon the data shown in Table 3, since this experiment was carried out on individual samples and significance tests on the densitometric data could be applied.

DISCUSSION

The initial results obtained in this study indicated that three parameters related to the hepatic P450-dependent monooxygenase system of the mouse, spectrophotometrically determined total P450 level, ethoxycoumarin *O*-deethylase activity and benzphetamine *N*-demethylase activity, were suppressed by treatment with interferon α. Slight suppression of P450 reductase activity was observed in response to interferon α, but this was insufficient to account for the suppression of P450 activities. This finding agrees with a number of recent reports which state that recombinant interferons suppress constitutive P450-dependent activities including the metabolism of benzo(*a*)pyrene, benzphetamine, zoxazolamine, hexobarbital, 7-ethoxycoumarin and acetaminophen [36–39]. The results of the present study also suggested that interferon α might potentiate the induction of certain activities by xenobiotics, in agreement with previous studies on the effects of *Escherichia coli* endotoxin on P450 expression [18]. It was decided to pursue this phenomenon in more detail by examining the effects of interferon α on specific P450 isozymes in order to determine which isozymes were affected by interferon and at what level regulation occurred. This approach was made possible by the availability of more specific enzyme assays using a series of resorufin ethers which are metabolized by specific P450 isozymes [40], and by the technique of Western blotting in which isozyme



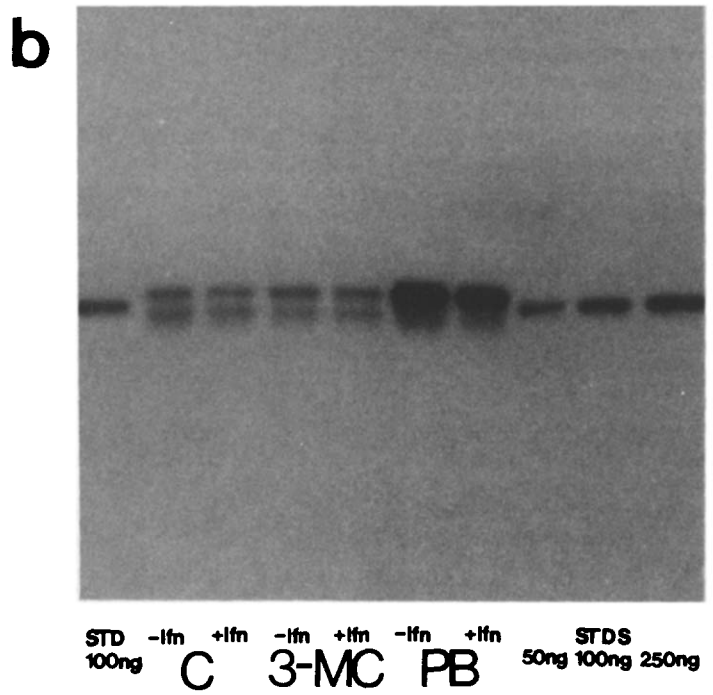


Fig. 5. Effect of recombinant interferon α on the hepatic expression of isozymes CYP2C6 and CYP2B1 proteins in male CBA mice. Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions from three mice were pooled together and analysed by Western blot analysis loading 7.5 μg of protein per track. (a) Expression of isozyme CYP2C6 protein. (b) Expression of isozyme CYP2B1.

Table 3. Use of densitometry to analyse the effects of interferon α on P450 CYP2B and CYP2C isozyme expression in mouse liver

	Relative band intensity			
	Control		PB	
	− Interferon	+ Interferon	− Interferon	+ Interferon
Anti-CYP2C6	101 ± 4	71 ± 1*	97 ± 4	94 ± 4
Anti-CYP2B1	124 ± 12	132 ± 14	970 ± 98	886 ± 88

Male CBA mice were treated three times at 24 hr intervals with 3-MC (40 mg/kg body wt in corn oil, i.p.) or PB (80 mg/kg body wt in 0.9% NaCl, i.p.) with or without interferon α (25,000 units/mouse in 0.9% NaCl, i.p.). On day four the mice were killed and the livers frozen. Microsomal fractions were prepared and assays carried out as described. Relative band intensity was calculated as a percentage of that of the first control sample. Values are mean ± SD for three individual mouse liver microsomal samples run on the same blot.

* Significantly different from non-interferon treated sample $P < 0.005$.

specific polyclonal antisera are used to assess the expression of specific P450 isozymes at the protein level [28].

The two constitutively expressed isozymes studied, CYP1A2 and a member of the CYP2C gene family, were significantly suppressed at the protein level by interferon α according to Western blot analysis, and corresponding decreases in enzyme activities were also observed. The observed reduction in EROD activity in animals treated with interferon α alone was probably chiefly due to changes in the expression of the CYP2C6 protein which mediates constitutive hepatic aryl hydrocarbon hydroxylase activity [41]. The data suggest that these isozymes are subject to transcriptional or translational regulation by interferon α, since there was a close correlation between changes in enzyme activities and protein levels in uninduced animals. Indirect effects such as diversion of the hepatic protein synthetic machinery to production of interferon-induced proteins may have been involved. Interferon-induced decreases in the stability of these proteins would also be consistent with the above data, since Western blot analysis measures steady-state protein expression rather than the rate of protein synthesis. Assessment of the expression of these isozymes at the mRNA level using the techniques of Northern blot analysis and nuclear run-off assays, together with metabolic labelling studies of the proteins, would make it possible to identify the level at which these isozymes are regulated by interferon α, and might help to elucidate the means by which xenobiotics protect isozymes P4501A2 and CYP2C6 against interferon-mediated suppression. Such studies may have implications for the use of concurrent therapy with drugs and interferon in the treatment of cancer.

The effects of interferon α on the inducible isozymes CYP1A1 and CYP2B1 were complex and difficult to interpret. In animals treated with xenobiotics but not interferon α there was close association between the enzyme activities measured using resofurin ether substrates and isozyme expression assessed by Western blot analysis. However, when the levels of activities and isozyme expression in induced animals were compared with those in animals treated with both inducing agent and interferon α the results appeared to come into conflict as changes in enzyme activities did not mirror changes in protein expression. It should, however, be borne in mind that small changes in protein expression are very difficult to detect by Western blot analysis, this could help to explain our observation. However, further experiments are needed to clearly establish the mechanism by which EROD activity is altered by interferon. Some other possibilities include alterations in post-translational protein processing, changes in the cell membrane leading to altered interactions between P450s and the reductase enzyme, or changes in the efficiency of binding of haem to P450 apoproteins. Altered phosphorylation of P450s may also occur in response to interferon α: the activities of many enzymes are regulated by interferon-induced phosphorylation [42]. Some P450s are kinase substrates; phosphorylation of isozyme CYP2B1 has been shown to cause a decrease in its enzymic activity, although the activity of isozyme CYP1A1 does not appear to be regulated by phosphorylation [43–45].

The results discussed above contribute to the present understanding of interferon-mediated P450 regulation by elucidating the effects of recombinant interferon α on both constitutive and induced expression of specific isozymes. Few published reports have examined the effects of interferons on P450 induction. In one study treatment with poly IC or tilorone was shown to inhibit hepatic induction in the mouse; no potentiation of 3-MC-induction occurred, although CYP1A1-dependent activities were relatively resistant to the suppressive effects of interferon [46]. In a more recent report the effects of interferon on P450 induction were found to be time-dependent; suppression of P450 induction by interferons was short-lived and 3-MC-induced, but not PB-induced, activities returned to their induced level a few days after interferon treatment [47]. Such a pattern is consistent with the observations made during the present work; 3-MC-induced activities might have recovered from the initial effects of interferon in the 3 days of interferon treatment which preceded sacrifice.

It is clear that P450 regulation by interferons is extremely complex. Using a "consensus interferon", IFN α CON1, it was shown that 3 hr after interferon administration to hamsters, P450, aminopyrine *N*-

demethylase and benzo(*a*)pyrene hydroxylase were elevated relative to controls, but that they declined to a significantly suppressed level after 24 hr [48]. A gradual recovery then began, lasting for several days. Similar results were obtained in the lung, spleen and adrenal but not in the kidney where suppression started 3 hr after interferon treatment. Induced P450s were also studied, but only after 24 hr treatment with 10^6 units of interferon. This dose suppressed induction of all P450-dependent activities examined, though 3-MC-inducible activities were again the most resistant to suppression by IFN α CON1. It is not clear whether these findings are applicable to interferon-mediated P450 regulation in other species, and much more detailed work is needed before it becomes possible to derive a coherent model of the effects of interferon on different P450 isozymes.

The results discussed above serve to emphasize the complexity of P450 regulation during inflammation. Considerable data now exists to indicate that interferons suppress P450 levels in the mouse liver, but the significance of these observations is still debatable since studies exist in which, although the capacity of the mouse liver to metabolize model substrates *in vitro* was suppressed by human IFNαAD (BgI) and murine interferon γ, the effect of the agents on drug clearance *in vivo* was marginal; this may be due to effects on other drug metabolizing enzymes or to a compensatory increase in the amount of hepatic endoplasmic reticulum [49, 50]. A great deal of work is still required to clarify this aspect of P450 regulation: it is still not clear whether interferon acts by modifying the inductive process itself, or whether interferon simply depresses P450 expression to a new steady state, which can then be increased by inducers in the same proportion as in control animals, thus yielding a lower induced P450 level. One major problem in previous studies has been the fact that each investigator used a different model, with widely varying doses of inflammatory agents and time courses. More detailed experiments using a single system, with homologous inflammatory mediators and careful attention to dose and time course will help to clarify the situation. Another way to simplify the study of P450 regulation by inflammatory agents is to use a cell culture model, a possibility which we have also investigated (manuscript in preparation).

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