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Interferon suppresses the isoform-specific activities of hepatic cytochrome P450 in female rats

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There are important sex differences in hepatic drug metabolism in rats. Adult females have 10–30% lower levels of hepatic cytochrome P450 (P450*) than males and the activities of several P450-dependent mixed function oxidases differ several-fold [1, 2]. These differences are accounted for by the presence of sex-specific P450 isoforms [3, 4].

Interferon (IFN) down-regulates hepatic P450 in male rats [5]. The principal isoform suppressed by IFN was P4503A2 [5], a male-specific protein that is not expressed in adult female rat liver [4, 6]. A variety of IFN inducers including poly IC, tilorone, endotoxin, Bacillus Calmette-Guérin and *Corynebacterium parvum* have been reported to decrease the level and catalytic activity of P450 in female rats [7–11]. However, it is controversial whether IFN inducers produce a greater impairment of drug metabolism in females than males [7–9, 11]. The purpose of the present study therefore, was to determine whether IFN alters drug metabolism in female rats. We reasoned that, if it did so, this must involve changes in the activity of different isoforms to those suppressed in male rats.

Materials and Methods

Ethylmorphine was from McFarlan Smith Ltd (Edinburgh, U.K). [4-^{14}C]Androstenedione (59 mCi/mmol) and [4-^{14}C]progesterone (sp. act. 56 mCi/mmol) were purchased from Amersham Australia. Testosterone, unlabeled androstenedione and progesterone, 6β-hydroxy- and 16α-hydroxyandrostenedione, 5α-androstanedione, 16α-hydroxy- and 21-hydroxyprogesterone, as well as all other biochemicals were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 7α-Hydroxyandrostenedione and 6β-hydroxyprogesterone were obtained from the MRC Steroid Reference Collection, Queen Mary's College (London, U.K.). Recombinant rat IFN-γ was kindly supplied by Dr P. H. van der Meide, TNO Primate Center, Rijswijk, The Netherlands.

Adult female Wistar rats (200–250 g) were raised at the Westmead Hospital animal facility. Animals were administered either 7.5×10^5 U of rat IFN-γ or vehicle (H_2O) as a single intramuscular injection 24 hr before being killed. The liver was removed from rats anaesthetized with ether and hepatic microsomal fractions were prepared as described previously [12]. Microsomal protein concentration [13], P450 [14] and ethylmorphine *N*-demethylase activity [12] were determined by standard methods. Androstenedione hydroxylase activity was assayed as described previously [12]. Progesterone hydroxylase activity was assayed using the method of Halpert *et al.* [15] with minor modifications. Separation of the hydroxylated metabolites of androstenedione and progesterone was by TLC [12, 15]. Serum oestradiol and testosterone were determined with direct radioimmunoassay kits (CIS, Gif Sur Mur, France). All results were expressed as mean ± SD. Comparisons were performed using Student's *t*-test (unpaired, two-tailed). A P value < 0.05 was considered to be statistically significant.

* Abbreviations: IFN, interferon; P450, cytochrome P450; rat IFN-γ, recombinant rat interferon-γ.

Results and Discussion

Hepatic light microscopic morphology and hepatic microsomal protein content did not differ between IFN-treated and control animals (data not shown). There was no significant alteration in serum oestradiol levels compared with control (162 ± 68 vs 98 ± 41 pg/mL, N = 5, NS); serum testosterone levels were below the lowest standard (0.1 ng/mL) in all animals.

The data presented in Table 1 indicate that rat IFN-γ decreased hepatic microsomal P450 levels to 81% of control ($P < 0.005$) and ethylmorphine N-demethylation to 82% of control ($P < 0.05$). Thus, rat IFN-γ suppressed the level and catalytic activity of hepatic P450 to a similar extent as previously observed in male rats [5]. This finding is in keeping with some (but not all) earlier studies which suggested that gender was unimportant in determining the magnitude of the fall in P450 produced by treatment with IFN inducers [7–9, 11]. In female rats, ethylmorphine N-demethylation is predominantly catalysed by two quantitatively important isoforms, P4502C6 and P4502C12 [4, 16]. P4502C6 is the major sexually undifferentiated, hormonally independent isozyme present in female rat liver [3]. P4502C12, which is absent from normal male rat liver, is the principal female-specific P450 [4, 17]; its expression is regulated by sex steroids [3, 18].

Progesterone hydroxylation was used to assess further the suppression of female P450 isoforms by IFN. The activity of the progesterone 21-hydroxylase (P4502C6) was decreased by 14% ($P < 0.05$) following IFN administration

Table 1. Cytochrome P450, ethylmorphine *N*-demethylase activity and progesterone hydroxylase activities in hepatic microsomes from female rats treated with rat IFN-γ or vehicle

Treatment*	Cytochrome P450 (nmol/mg protein)	Ethylmorphine *N*-demethylase (nmol formaldehyde/mg protein/min)	Progesterone hydroxylation pathway (nmol/mg protein/min)		
			16α	6β	21
Vehicle	0.98 ± 0.07	1.92 ± 0.13	0.51 ± 0.06	0.23 ± 0.05	0.35 ± 0.03
Rat IFN-γ	0.79 ± 0.06	1.57 ± 0.22	0.43 ± 0.06	0.23 ± 0.05	0.30 ± 0.03
P	<0.005	<0.05	NS	NS	<0.05

Values are mean ± SD of results from five individual microsomal fractions.
* Rats were injected with either rat IFN-γ (7.5×10^5 U) or vehicle (H_2O) 24 hr before being killed.
NS, not significant.

Table 2. Androstenedione metabolism in hepatic microsomes from female rats treated with rat IFN-γ or vehicle

Treatment*	Androstenedione hydroxylation pathway				5α-Androstanedione	Testosterone
	16α	16β	6β	7α	(nmol/mg protein/min)	
Vehicle	0.12 ± 0.02	0.22 ± 0.04	0.23 ± 0.04	0.67 ± 0.07	0.15 ± 0.08	1.22 ± 0.60
Rat IFN-γ	0.10 ± 0.01	0.19 ± 0.02	0.19 ± 0.04	0.53 ± 0.09	0.15 ± 0.05	1.36 ± 0.66
P	<0.05	NS	NS	<0.05	NS	NS

Values are mean ± SD of results from five individual microsomal fractions.
* Adult female rats were injected intramuscularly with either rat IFN-γ (7.5×10^5 U) or vehicle (H_2O) 24 hr before being killed.
NS, not significant.

(Table 1). This minor decrease in P4502C6 activity would not, therefore, be sufficient to explain either the observed decrease in ethylmorphine N-demethylation or in total P450, because P4502C6 comprises only about one-third of total P450 in female rat liver [3]. It seems possible, therefore, that the female-specific P4502C12 may also be down-regulated by IFN. However, this enzyme has minimal catalytic activity towards unconjugated steroid substrates. Thus, measurements of steroid hydroxylation on female hepatic microsomes provide no information regarding P4502C12. Rates of formation of the 16α- and 6β-hydroxyprogesterone metabolites were not different from control microsomes (Table 1). In male rat liver, it appears that P4503A2 is the predominant isozyme involved in progesterone 6β-hydroxylation [19]. Since P4503A2 is not expressed in the liver of untreated adult female rats [6], it seems likely that P4501A2, which also displays progesterone 6β-hydroxylase activity [19], and which is present in untreated female rat liver microsomes [3], is not suppressed by IFN.

The activity of the relatively minor androstenedione 16α-hydroxylation pathway was decreased in IFN-treated microsomes to 83% of control levels ($P < 0.05$) (Table 2). In females, androstenedione 16α-hydroxylase activity is probably catalysed by the closely related forms, P4502B1 and 2 [19]. However, an inconsistent finding in the present study was the failure of IFN to decrease the activity of the P4502B1 and 2-dependent androstenedione 16β-hydroxylation pathway (Table 2). Although androstenedione 16β-hydroxylase activity appeared to be reduced in female microsomes (to 86% of control) by IFN this change was not significant ($P = 0.09$). Interestingly, a similar (but significant) reduction in the activity of the P4502B1 and 2-dependent androstenedione 16β-hydroxylation pathway (to 83% of control) was previously noted in male hepatic microsomes after IFN treatment [5]. Thus, it remains unresolved from the present study whether IFN down-regulates the minor P4502B1 and 2 forms in female rat liver. IFN produced no alteration in the activities of the two P450-independent steroid metabolising enzymes 5α-reductase and 17β-hydroxysteroid oxidoreductase in female rats (Table 2).

Another finding of the present study was that IFN decreased androstenedione 7α-hydroxylation to 79% of control ($P < 0.05$) (Table 2). This pathway of steroid hydroxylation is catalysed in female rats by P4502A1 exclusively [3, 20], which is present in higher levels in female hepatic microsomes than males [3, 18]. Expression of P4502A1 is regulated by sex steroids and growth hormone [18, 21]. In male rats, androstenedione 7α-hydroxylation was unchanged by rat IFN-γ administration [5]. Thus, an effect of IFN on down-regulation of P4502A1 but not P4502A2 would explain the decreased androstenedione 7α-hydroxylase activity observed in female but not male rat hepatic microsomes. Since changes in serum sex steroids were not observed, the reason for the IFN-mediated reduction in P4502A1 catalytic activity is not clear. Serum growth hormone levels are elevated in certain patients receiving IFN [22]. Although an altered growth hormone secretion profile could account for suppression of P4502A1 and P4502C12 [17, 21], there is no evidence that growth hormone is important in the regulation of P4502C6 [23]. Thus, it seems likely that in female rats, non-hormonal factors (possibly IFN itself) are important in the IFN-mediated suppression of hepatic P450. Administration of endotoxin has been shown to down-regulate P4502C12 in female rats [24]. The present findings with IFN may therefore relate to a selective suppression of certain P450 genes by inflammatory mediators.

In summary, the results from the present study indicate that rat IFN-γ decreases hepatic P450 levels and catalytic activities in female rats. The magnitude of the down-regulation of P450 is similar to that found in male rats. Furthermore, the activities of specific P450 isoforms (P4502A1 and P4502C6 and possibly P4502C12, and P4502B1 and 2) are decreased by IFN. Hence, the effect of IFN in female rats appears to involve several, but not all, hepatic P450 isoforms. Both hormone-dependent and hormone-independent isoforms are suppressed by IFN. The mechanism for this effect does not, however, appear to be mediated by changes in serum levels of sex steroids.

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