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INDUCTION AND DEPRESSION OF CYTOCHROME P-450-DEPENDENT MIXED-FUNCTION OXIDASE BY A CLONED CONSENSUS α-INTERFERON (IFN-αCON$_1$) IN THE HAMSTER*

SHABBIR M. MOOCHHALA† and KENNETH W. RENTON‡
Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7
and
NOWELL STEBBING§
Amgen Inc., Thousand Oaks, CA, U.S.A.

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Abstract—A novel analogue of human α-interferon (IFN-αCON$_1$) was tested for its ability to modify the hepatic cytochrome-P-450-dependent mixed-function oxidase system in the hamster. This cloned interferon was derived by selecting the most frequently observed amino acid sequences at each position in the known human α-interferon subtypes. IFN-αCON$_1$ had a biphasic effect on cytochrome P-450 and related drug biotransformation in the hamster causing an initial increase followed by a significant depression. IFN-αCON$_1$ also had a biphasic effect on cytochrome P-450 in the lung, adrenal and spleen but only a depressant effect in the kidney. This effect was not due to morphological damage and followed the species specificity for this type of interferon. Both the increase and the decrease in cytochrome P-450 could be prevented by the administration of the protein synthesis inhibitor puromycin. Various isozymes of cytochrome P-450 induced by phenobarbital, β-napthaflavone and clofibrate were also depressed by this interferon. The results presented in this report suggest that IFN-αCON$_1$ interferon will likely depress drug biotransformation in humans because the antiviral effects and the "anti-cytochrome P-450" effect of interferons cannot be separated, and this interferon has antiviral properties in both hamster and human cells. Clinically relevant drug interactions may be common during the concomitant use of this interferon and other drugs that are metabolized by cytochrome P-450.

In 1976, two laboratories demonstrated that interferon inducers decrease the metabolism of drugs in the liver by depressing the content of cytochrome P-450 [1–3]. At that time considerable controversy existed as to whether this depression was due to interferon itself or resulted from other actions common to these agents (e.g. immunomodulation). Direct evidence for the involvement of interferon in depressing mixed-function oxidase was established followed the cloning of interferon and the production of a highly purified product by recombinant DNA technology [4, 5]. The depression of the mixed-function oxidase system by interferon might be a problem during the clinical use of interferon as this will interfere with the concomitant utilization of other drugs and increase the frequency of untoward drug reactions. The loss of drug biotransformation which occurs during viral infections is thought to be due to the production of interferon during the course of the infection [6, 7].

Following the discovery of how to synthesize complete gene sequences, a novel human interferon was conceived called alpha consensus one (IFN-αCON$_1$) which is derived by selecting the most frequently observed amino acid at each position of the known α-interferon subtypes [8]. This interferon (IFNα-CON$_1$) has significant antiviral activity in human and hamster cell lines and is active against viral infections and experimentally induced tumors in hamsters [9]. Because of the potential clinical use of this interferon as an anticancer or antiviral drug, the effect of IFN-αCON$_1$ on hepatic drug metabolism is of concern. In this study, we investigated the effects of IFN-αCON$_1$ interferon on the mixed-function oxidase system in various organs of the hamster.

MATERIALS AND METHODS

Animals and drug treatment. Adult male golden Syrian hamsters (100–200 g) were obtained from Canadian Breeding Farm, Hals Harbour, Nova Scotia, Canada, and Swiss Webster strain mice (20–25 g) were obtained from Jackson Laboratories, Bar Harbor, ME, U.S.A. All animals were allowed to acclimatize in our own facilities for a period of 1 week before use. Animals were housed on clay chip bedding and fed water and Purina Chow. Interferons and poly rI·rC were administered i.p. in saline in the doses and for the times indicated in Results. Various isozymes of cytochrome P-450 were induced in the liver with phenobarbital (80 mg/kg daily for 3 days), β-napthaflavone (40 mg/kg daily for 3 days)

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† Present address: Department of Pharmacology, University of Singapore, Singapore.
‡ Author to whom all correspondence should be addressed.
§ Present address: ICI Pharmaceuticals Division, Alderney Park, Macclesfield, England.

Table 1. Depression of hepatic cytochrome P-450-dependent drug oxidation by IFN-αCON$_1$ interferon preparations in the hamster

Treatment	Cytochrome P-450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	Aminopyrine *N*-demethylase (nmol HCHO/mg protein/hr)	Benzo[*a*]pyrene hydroxylase (nmol 3-OHBP/mg protein/hr)
Control	0.83 ± 0.06	0.46 ± 0.10	424 ± 7	9.44 ± 1.27
IFN-αCON$_1$ (5×10^4 units)	0.75 ± 0.09	0.49 ± 0.03	436 ± 38	12.56 ± 0.24
IFN-αCON$_1$ (1×10^6 units)	0.62 ± 0.02*	0.45 ± 0.04	342 ± 4*	3.10 ± 0.97*
IFN-αCON$_1$ (2×10^7 units)	0.42 ± 0.08*	0.52 ± 0.07	153 ± 14*	6.53 ± 0.53*
IFN-αCON$_1$ (1×10^7 units daily for 3 days	0.41 ± 0.06*	0.38 ± 0.07	282 ± 24*	5.42 ± 0.82*
Buffy coat interferon (1×10^6 units)	0.48 ± 0.02*	0.45 ± 0.01	262 ± 14*	7.38 ± 0.51*
Poly rI·rC (10 mg/kg)	0.56 ± 0.02*	0.50 ± 0.04	234 ± 9*	7.32 ± 0.34*

Animals were treated i.p. with various doses of interferon which are indicated in parentheses. After 24 hr, animals were killed, and hepatic microsomes were prepared. In the group that received three daily doses of interferon, the animals were killed 24 hr after the final dose. Each value is the mean ± SE, N = 4.

* Significantly different from corresponding control, $P < 0.05$.

or clofibrate (250 mg/kg daily for 4 days). In the experiments designed to determine if the action of interferon required the synthesis of an intermediate protein, animals were treated with puromycin (20 mg/kg) for the times indicated in Results. In all experiments, control groups received saline in equivalent volumes.

Interferons. IFN-αCON$_1$ interferon, which was supplied by Amgen Inc., Thousand Oaks, CA, was prepared using recombinant DNA procedures and was expressed in *Escherichia coli* [8]. The specific activities of the various batches of this interferon were between 2×10^8 and 2×10^9 units/mg protein (as calibrated against NIH standard GA-23-902-530) and exceeded 95% in purity as assessed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Buffy coat interferon was derived from human leukocytes (Amgen Inc.). The single batch of buffy coat interferon utilized had a specific activity of 1×10^8 units/mg protein.

Microsomal drug biotransformation. Microsomes from the liver and other organs were prepared from hamsters and mice by ultracentrifugation by the method described by El Defrawy El Masry and Mannering [10]. Microsomal protein was determined by the method of Lowry *et al.* [11]. Cytochrome P-450 and cytochrome b_5 were measured by the method of Omura and Sato [12]. In the case of lung microsomes, cytochrome P-450 was determined by the method of Johannesen and De Pierre [13] to minimize interference from hemoglobin. NADPH–cytochrome *c* reductase activity was measured by the method of Williams and Kamin [14]. Heme content and heme oxygenase activity were measured by the methods of Falk [15] and Schacter [16] respectively. Microsomal drug biotransformation was assessed by measuring aminopyrine N-demethylation [17], benzo[*a*]pyrene hydroxylation [18], and lauric acid hydroxylation [19] using procedures described previously.

Histology. Sections of liver removed from the right and left lobes were fixed in 2% glutaraldehyde in sodium cacodylate buffer (pH 7.2). The sections were fixed in Osmium tetroxide and stained with uranyl acetate and lead citrate and examined on a Phillips 300 electron microscope.

Statistics. An unpaired Student's *t*-test was used to compare two variables. In experiments with more than two variables, groups were compared using analysis of variance and the Student–Newman–Keuls test for multiple comparisons.

RESULTS

Effect of IFN-αCON$_1$ interferon on hepatic microsomal mixed-function oxidase in hamsters. The effect of various doses of IFN-αCON$_1$ interferon on hepatic mixed-function oxidase activity in the hamster is shown in Table 1. IFN-αCON$_1$ treatment (single dose) for 24 hr caused a dose-dependent decrease in microsomal cytochrome P-450, but cytochrome b_5 levels remained unchanged. The loss of cytochrome P-450 at each dose of IFN-αCON$_1$ was accompanied by a loss of aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities. Treatment of animals for 3 days with IFN-αCON$_1$ (injected once daily; 1×10^7 units, i.p.) did not cause a further decrease in mixed-function oxidase. Buffy coat interferon (a naturally occurring interferon derived from human leukocytes) also caused a significant depression in cytochrome P-450 and in aminopyrine *N*-demethylase and benzo[*a*]pyrene activities in hamster hepatic microsomes. In contrast to these results obtained in the hamster, IFN-αCON$_1$ interferon had no effect on hepatic mixed-function oxidase activities in the mouse (Table 2). In both the hamster and the mouse, the interferon inducer poly rI·rC depressed cytochrome P-450 and drug biotransformation (Tables 1 and 2). In all of these experiments, body weight, liver weight and microsomal protein concentration were identical in interferon and control animals. Neither IFN-αCON$_1$ nor poly rI·rC had any effect on hepatic mixed-function oxidase when these agents were incubated directly with hepatic microsomes *in vitro* (Table 3).

Table 2. Effects of IFN-αCON_1 interferon and poly rI·rC on cytochrome P-450 levels in mouse liver

Treatment	Cytochrome P-450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	Aminopyrine *N*-demethylase (nmol HCHO/ mg protein/hr)	Benzo[*a*]pyrene hydroxylase (nmol 3-OHBP/mg protein/hr)
Control	0.758 ± 0.014	0.252 ± 0.015	338 ± 11	7.07 ± 0.11
IFN-αCON_1 (1×10^6 units)	0.788 ± 0.036	0.248 ± 0.15	336 ± 25	6.49 ± 0.32
Poly rI·rC (10 mg/kg)	0.465 ± 0.064*	0.258 ± 0.01	221 ± 8*	6.53 ± 0.24*

Animals were treated with a single dose of interferon or poly rI·rC, i.p., and killed 24 hr later. Control animals received a corresponding volume of phosphate-buffered saline (pH 7.4) at the same time. Each value is the mean ± SE, N = 4.

* Significantly different from corresponding control, $P < 0.05$.

Table 3. Mixed-function oxidase activity in hamster hepatic microsomes incubated *in vitro* with IFN-αCON_1 interferon or poly rI·rC

Treatment	Cytochrome P-450 (nmol/mg protein)	Aminopyrine *N*-demethylase (nmol HCHO/ mg protein/hr)
Control	0.811 ± 0.003	476 ± 3
IFN-αCON_1	0.813 ± 0.002	478 ± 4
Poly rI·rC	0.790 ± 0.001	460 ± 4

Poly rI·rC (1 mg) or IFN-αCON_1 (10^6 units) in 0.1 M phosphate buffer, pH 7.4, was added to a microsomal suspension (1 mg/ml in 0.01 M phosphate buffer, pH 7.4) and incubated for 15 min at 37° in a shaking water bath. Control microsomal suspensions received equivalent volumes of buffer. Each value is the mean ± SE, N = 3.

Temporal changes in hepatic microsomal mixed-function oxidase in hamsters following a single dose of IFN-αCON_1 interferon or poly rI·rC. The time courses for the effects of a single dose of IFN-αCON_1 interferon and poly rI·rC (10 mg/kg, i.p.) on hepatic cytochrome P-450, aminopyrine *N*-demethylase activity and benzo[*a*]pyrene hydroxylase activity in hamsters are shown in Figs. 1 and 2. For both agents a biphasic response was observed with a significant elevation in mixed-function oxidase occurring initially, followed by a significant depression within 24 hr. Except for the effects of interferon on benzo[*a*]pyrene hydroxylase activity, the values for mixed-function oxidase returned to normal values in approximately 1 week. The highest levels of cytochrome P-450 and drug biotransformation occurred at a slightly later time following treatment with poly rI·rC compared to treatment with interferon (6 hr vs 3 hr). This difference was likely caused by the time lag (3–4 hr) which occurs in the appearance of interferon following poly rI·rC treatment [20]. Although not measured throughout the entire time course of IFN-αCON_1 interferon treatment, hepatic ethoxyresorufin deethylase and lauric acid hydroxylase activities were elevated by 87 and 17%, respectively, at 3 hr and were depressed by 40 and 37%, respectively, at 24 hr.

Total microsomal protein was elevated significantly at 3 and 6 hr following treatment with interferon and at 6 hr following treatment with poly rI·rC. At all other times protein content in treated animals was identical to that found in controls. Variables such as body weight, liver weight, microsomal cytochrome b_5 level and NADPH-cytochrome *c* reductase activity remained unchanged throughout these experiments.

The treatment of hamsters with puromycin modified the interferon-induced biphasic changes which occurred in the mixed-function oxidase system. The increase in hepatic microsomal cytochrome P-450 and aminopyrine *N*-demethylase activity that occurred 3 hr after the treatment of hamsters with IFN-αCON_1 interferon could be totally prevented by treating the animals with puromycin (Table 4). This protein synthesis inhibitor itself had no effect on cytochrome P-450. Puromycin treatment also totally prevented the decrease in cytochrome P-450 and aminopyrine *N*-demethylase activity which occurred 24 hr following the administration of interferon (Table 4). Puromycin itself had no effect at this time.

Effect of IFN-αCON_1 interferon on total microsomal heme content and heme oxygenase activity in hepatic microsomes. The effects of a single dose of IFN-αCON_1 (1×10^6 units, i.p.) on total microsomal heme content and heme oxygenase activity are shown in Table 5. No change in total microsomal heme content or heme oxygenase activity was observed 3 hr after the treatment of hamsters with IFN-αCON_1 interferon. After a single dose of IFN-αCON_1 for 24 hr or after three daily doses of IFN-αCON_1, the total heme content of microsomes was depressed significantly and heme oxygenase was elevated significantly.

Effects of IFN-αCON_1 interferon and poly rI·rC on cytochome P-450 and benzo[a]*pyrene hydroxylase activity in extrahepatic tissues.* The effects of a single dose of IFN-αCON_1 (1×10^6 units, i.p.) on microsomal cytochrome P-450 content and benzo[*a*]pyrene hydroxylase activity in extrahepatic tissues are illustrated in Table 6. Because of the low activity of mixed-function oxidase in these tissues, a pool of microsomes prepared from four animals was used for each assay, and the results are expressed as the mean ± SE of four such pools. Cytochrome P-450 in the lung, spleen and adrenals increased 138, 53 and 22% over control, respectively, 3 hr after treatment

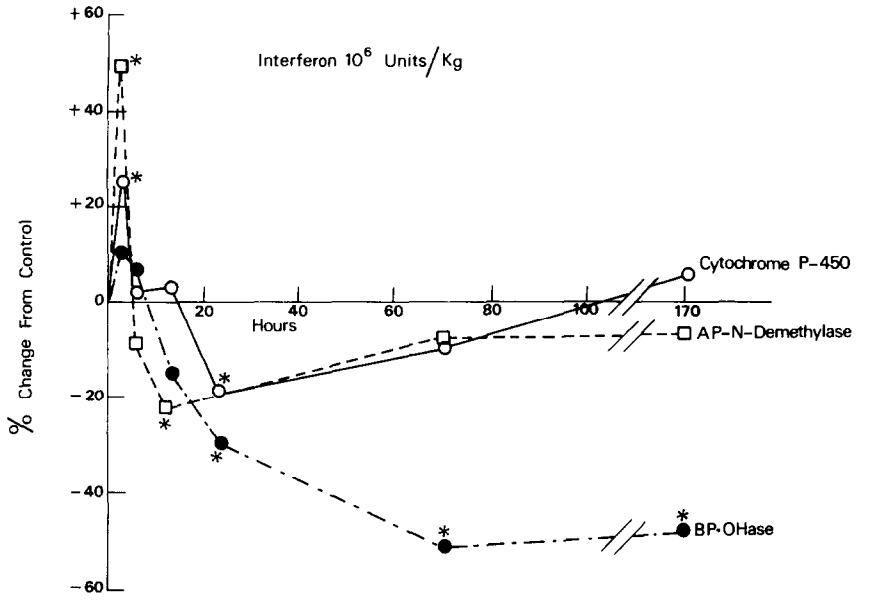

Fig. 1. Hepatic microsomal cytochrome P-450, aminopyrine *N*-demethylase activity and benzo[*a*]pyrene hydroxylase activity in hamsters treated with a single dose of IFN-αCON$_1$ interferon for various times. Data are expressed as percent change from control animals receiving saline for the same time periods. Control values at zero time were: cytochrome P-450, 0.75 ± 0.02 nmol/mg protein; aminopyrine *N*-demethylase activity, 420 ± 13 nmol/mg protein/hr; and benzo[*a*]pyrene hydroxylase activity, 11.7 ± 1.5 nmol 3-OH benzo[*a*]pyrene/mg protein/hr. Each value is the mean of four individual animals at each time period. Key: (*) Significantly different from control, $P < 0.05$.

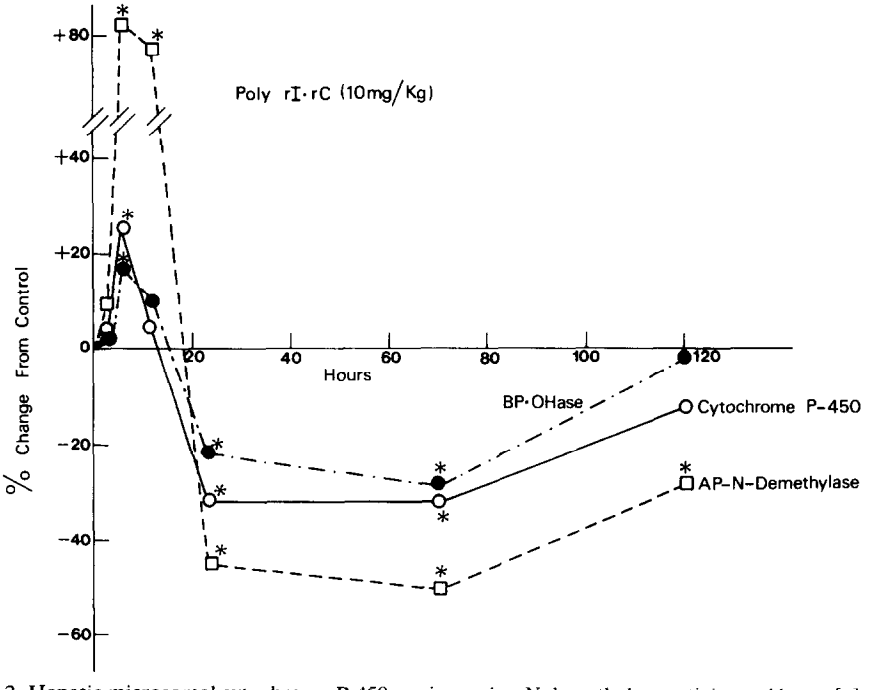

Fig. 2. Hepatic microsomal cytochrome P-450, aminopyrine *N*-demethylase activity and benzo[*a*]pyrene hydroxylase activity in hamsters treated with a single dose of poly rI·rC for various times. Data are expressed as percent change from control animals receiving saline for the same time periods. Control values at zero times were: cytochrome P-450, 0.73 ± 0.01 nmol/mg protein; aminopyrine *N*-demethylase activity, 566 ± 45 nmol HCHO formed/mg protein/hr; and benzo[*a*]pyrene hydroxylase activity, 9.99 ± 0.12 nmol 3-OH-benzo[*a*]pyrene formed/mg protein/hr. Each value is the mean of four individual animals at each time period. Key: (*) Significantly different from control, $P < 0.05$.

Table 4. Effect of puromycin on the interferon-induced stimulation and depression of hepatic mixed-function oxidase in the hamster

Treatment	Cytochrome P-450 (nmol/mg protein)	Aminopyrine *N*-demethylase (nmol HCHO/mg protein/hr)
Control (3 hr)	0.77 ± 0.01	408 ± 10
IFN-αCON$_1$ (3 hr)	1.20 ± 0.08*	645 ± 60*
Puromycin (3 hr)	0.83 ± 0.02	405 ± 32
IFN-αCON$_1$ + puromycin (3 hr)	0.67 ± 0.04	350 ± 31
Control (24 hr)	0.80 ± 0.01	442 ± 14
IFN-αCON$_1$ (24 hr)	0.52 ± 0.01*	332 ± 11*
Puromycin (24 hr)	0.77 ± 0.02	452 ± 6
IFN-αCON$_1$ + puromycin (24 hr)	0.74 ± 0.02	453 ± 12

In the animals treated with interferon for 3 hr (1×10^6 units i.p.), four doses of puromycin (20 mg/kg, i.p.) were administered at −2, 0, 1 and 2 hr. Animals were killed 3 hr after interferon treatment. Control and interferon only treated animals received a corresponding volume of PBS at the same times. In the animals treated with interferon for 24 hr (1×10^6 units, i.p.), six doses of puromycin (20 mg/kg, i.p.) were administered at −2, 0, 1, 3, 4 and 5 hr. Animals were killed 24 hr after interferon treatment. Control and interferon only treated animals received a corresponding volume of phosphate-buffered saline at the same times. Each value is the mean ± SE, N = 4.

* Significantly different from corresponding control, $P < 0.05$.

Table 5. Effect of IFN-αCON$_1$ interferon on heme oxygenase and total heme content in hepatic microsomes in hamsters

Treatment	Total heme (nmol/mg protein)	Heme oxygenase (nmol bilirubin/mg protein/hr)
Control	1.00 ± 0.08	0.06 ± 0.01
IFN-αCON$_1$ (3 hr)	1.30 ± 0.10	0.07 ± 0.02
Control	0.72 ± 0.02	0.05 ± 0.01
IFN-αCON$_1$ (24 hr)	0.53 ± 0.02*	0.10 ± 0.01*
Control	0.71 ± 0.02	0.053 ± 0.01
IFN-αCON$_1$ (three daily doses)	0.54 ± 0.03*	0.111 ± 0.02*

Animals were treated with a single dose of interferon (IFN-αCON$_1$, 1×10^6 units, i.p.) and killed 3 or 24 hr later, or with three daily doses of interferon and killed 24 hr later. Control animals received a corresponding volume of sterile phosphate saline at the same times. Each value is the mean ± SE, N = 4.

* Significantly different from corresponding control, $P < 0.05$.

Table 6. Effect of IFN-αCON$_1$ interferon treatment for 3 and 24 hr on cytochrome P-450 and benzo[*a*]pyrene hydroxylase activity in extrahepatic microsomes

Organ	Treatment	Cytochrome P-450 (nmol/mg protein) 3 hr	Cytochrome P-450 (nmol/mg protein) 24 hr	Benzo[*a*]pyrene hydroxylase (nmol 3-OHBP/mg protein/hr) 3 hr	Benzo[*a*]pyrene hydroxylase (nmol 3-OHBP/mg protein/hr) 24 hr
Lung	Control	0.13 ± 0.02	0.13 ± 0.01	2.55 ± 0.03	2.09 ± 0.11
	IFN-αCON$_1$	0.31 ± 0.01*	0.05 ± 0.00*	4.57 ± 0.30*	1.35 ± 0.01*
Kidney	Control	0.18 ± 0.00	0.16 ± 0.01	5.15 ± 0.10	5.63 ± 0.11
	IFN-αCON$_1$	0.11 ± 0.01*	0.05 ± 0.00*	4.79 ± 0.18	3.44 ± 0.02*
Spleen	Control	0.13 ± 0.01	0.12 ± 0.00	0.65 ± 0.06	0.71 ± 0.05
	IFN-αCON$_1$	0.20 ± 0.01*	0.08 ± 0.00*	0.82 ± 0.11	0.59 ± 0.03
Adrenals	Control	0.22 ± 0.01	0.22 ± 0.01	1.99 ± 0.03	2.76 ± 0.07
	IFN-αCON$_1$	0.27 ± 0.01*	0.13 ± 0.01*	2.33 ± 0.02*	1.95 ± 0.02*

Animals were treated with a single dose of IFN-αCON$_1$ (1×10^6 units, i.p.) and killed 3 or 24 hr later. Control animals received a corresponding volume of sterile phosphate-buffered saline at the same times. Microsomes prepared from four individual animals were pooled for each determination. Each value is the mean ± SE of four sets of microsomes, each containing microsomes pooled from four animals.

* Significantly different from control, $P < 0.05$.

with a single dose of IFN-αCON_1 interferon. In contrast, cytochrome P-450 content in the kidney was depressed to 56% of control. Benzo[*a*]pyrene hydroxylase activity in the lung and adrenal was increased to 76 and 26% over control, respectively, but was unchanged in the kidney and spleen. Twenty four hours following the administration of IFN-αCON_1 cytochrome P-450 was depressed in all four of the extrahepatic tissues studied, and benzo[*a*]pyrene hydroxylase activity was depressed in the lung, adrenals and kidney.

Effect of interferon on xenobiotic-induced cytochrome P-450. The effects of a single dose of IFN-αCON_1 (1×10^6 units, i.p.) for 24 hr on hepatic cytochrome P-450 content and mixed-function oxidase activities were determined in animals induced with phenobarbital, β-napthaflavone and clofibrate (Table 7). Administration of IFN-αCON_1 depressed cytochrome P-450 and aminopyrine *N*-demethylase activity, benzo[*a*]pyrene hydroxylase activity and lauric acid hydroxylase activity to 66, 65, 81 and 63% of control, respectively, in uninduced animals. In phenobarbital-induced animals that received interferon, cytochrome P-450 and aminopyrine *N*-demethylase activity were depressed by 23 and 45%, respectively, compared to the phenobarbital-induced animals that received saline. Benzo[*a*]pyrene hydroxylase activity was unchanged in phenobarbital-treated animals. In β-napthaflavone-induced animals that received interferon, cytochrome P-450, aminopyrine *N*-demethylase activity and benzo[*a*]pyrene hydroxylase activity were depressed by 56, 23 and 18%, respectively, compared to the β-napthaflavone-induced animals that received saline. In clofibrate-induced animals that received interferon, cytochrome P-450, aminopyrine *N*-demethylase activity, and lauric acid hydroxylase activity were depressed by 54, 22 and 31%, respectively, compared to the clofibrate-induced animals that received saline. Benzo[*a*]pyrene hydroxylase activity was unchanged in these animals.

Liver histology. Examination of the liver by electron microscopy was carried out to assess if damage had occurred in the endoplasmic reticulum during IFN-α-CON_1 treatment. No morphological changes could be discerned in the sections taken from hamsters receiving a single dose of IFN-αCON_1 (1×10^6 units, i.p.) for 3 or 24 hr when compared to saline-treated control animals.

DISCUSSION

The results from this study demonstrate that IFN-αCON_1 interferon has a biphasic effect in hamsters causing an initial increase in hepatic cytochrome P-450 and related drug biotransformation followed by a significant depression within 24 hr. This activity of consensus interferon (IFN-αCON_1) in the hamster was quite different from the effects reported in other species for all other interferons tested to date which do not have an inducing effect after short treatment times and cause only a depression of cytochrome P-450 [4, 5, 21, 22].

The increase in cytochrome P-450 content that occurred within the first 3 hr following administration of interferon was associated with an increase in total

Table 7. Effects of IFN-αCON_1 interferon treatment on hepatic mixed-function oxidase in hamsters induced with different xenobiotics

Treatment	Inducer	Cytochrome P-450 (nmol/mg protein)	Aminopyrine *N*-demethylase (nmol HCHO/mg protein/hr)	Benzo[*a*]pyrene hydroxylase (nmol 3-OHBP/mg protein/hr)	Lauric acid hydroxylase (nmol 11- and 12-laurate/mg protein/min)
Saline	None (12)	0.93 ± 0.04	471 ± 20	12.7 ± 0.7	2.20 ± 0.09
IFN-αCON_1	None (12)	0.62 ± 0.03*	306 ± 19*	10.3 ± 0.3*	1.39 ± 0.06*
Saline	PB (4)	1.31 ± 0.01	1709 ± 32	18.2 ± 0.5	ND
IFN-αCON_1	PB (4)	1.01 ± 0.04*	935 ± 42*	16.6 ± 0.5	ND
Saline	B-NF (4)	2.21 ± 0.03	410 ± 16	26.3 ± 1.1	ND
IFN-αCON_1	B-NF (4)	0.98 ± 0.03*	314 ± 9*	21.6 ± 0.8*	ND
Saline	CLF (4)	0.90 ± 0.04	650 ± 27	16.3 ± 0.8	16.71 ± 1.00
IFN-αCON_1	CLF (4)	0.42 ± 0.09*	506 ± 27*	15.8 ± 0.6	11.53 ± 0.29*

Animals, pretreated with phenobarbital (PB), B-napthoflavone (B-NF) and clofibrate (CLF), received a single dose of interferon (IFN-αCON_1, 1×10^6 units, i.p.) and were killed 24 hr later. Control animals received a corresponding volume of vehicles. Numbers in parentheses indicate the number of animals. Each value is the mean ± SE. ND = not determined.

* Significantly different from corresponding inducer treatment, $P < 0.05$.

microsomal protein concentration. This was likely due to *de novo* protein synthesis as the treatment of the animals with the protein synthesis inhibitor puromycin prevented the interferon-mediated increase in cytochrome P-450 and the related drug biotransformation activity. The concentrations of all proteins in the liver, however, were not increased as no increase in NADPH–cytochrome *c* reductase activity or in cytochrome b_5 content was observed. The increase in mixed-function oxidase that was observed in these experiments appears to be a feature of the effects of poly rI·rC and interferon in the hamster as previous reports indicate that only a depressive effect occurs following the administration of these agents to the mouse and the rat [5, 22].

The stimulation of mixed-function oxidase by interferon as opposed to the depression normally reported has also been observed in two specific circumstances. Poly rI·rC or crude mouse interferon preparations increase cytochrome P-450 levels and related enzyme activity in cultured mouse hepatocytes [23], and crude mouse interferon stimulates benzanthracene-induced aryl hydrocarbon hydroxylase activity in fetal mouse cell cultures [24]. Other enzymes that have been enhanced by interferon or interferon inducers include xanthine oxidase [25–27], 2′,5-oligoadenylate synthetase [28], creatine kinase [29], tRNA methylase [30], guanylate cyclase [31], indoleamine 2,3-dioxygenase [32], tryptophan dioxygenase [8], heme oxygenase ([6, 33] and the current study).

The depression in mixed-function oxidase that occurred 24 hr after interferon treatment in hamsters was similar to that previously reported for human IFN-αAD (Bgl) and gamma interferon in mice [4, 5, 21] and poly rI·rC in mice [4] and rats [33]. As previously reported for other interferons, the depressive effect of IFN-αCON$_1$ interferon appears to be selective for the cytochrome P-450 of the mixed-function oxidase system as cytochrome b_5 and NADPH–cytochrome *c* reductase activity were not affected by interferon treatment. Several other investigators have reported that this flavoprotein was depressed by interferon or its inducers [5, 33]. This supports the idea that the interferon inducers poly rI·rC, tilorone or Freunds' adjuvant selectively depress proteins tentatively identified as apocytochrome P-450 [34, 35]. Multiple doses of IFN-αCON$_1$ had no greater effect than a single dose, suggesting that a new steady-state of cytochrome P-450 content was reached within 24 hr of interferon treatment. A similar observation was made previously with the interferon inducers poly rI·rC and tilorone in rats [1, 3] and with human interferon HuIFNr-AD in mice [5].

Throughout these experiments, the depression of cytochrome P-450 by either IFN-αCON$_1$ or poly rI·rC was not accompanied by changes in body weight or decreases in liver weight or microsomal protein concentration, and no ultrastructural abnormalities were observed in sections of tissue examined by electron microscopy. These results demonstrate that the *in vivo* depression of cytochrome P-450 system by interferon is not accompanied by obvious toxic effects to the animal or ultrastructural changes in the tissue.

The idea that the antiviral and "anti-cytochrome P-450" properties of interferon [4, 5] cannot be separated was also supported by the results obtained in this study. IFN-αCON$_1$ interferon, which is antiviral in the hamster and lowered cytochrome P-450 in that species, has no antiviral activity in the mouse and no effect on hepatic cytochrome P-450 or drug metabolism in the mouse. Poly rI·rC, which has antiviral activity in both species, depressed the hepatic cytochrome P-450 system in both species. Human buffy coat interferon (HuIFN-α), derived from leukocytes, has antiviral activity against a hamster cell line (BHK) when challenged with vesicular stomatitis virus and depressed hamster hepatic cytochrome P-450 in the present study. Buffy coat interferon has no antiviral activity in a mouse cell line and no depressive effect on mouse hepatic cytochrome P-450 [4].

IFN-αCON$_1$ interferon was ineffective in lowering the levels of cytochrome P-450 in animals treated with the protein synthesis inhibitor puromycin. This suggests that an intermediate protein which is synthesized in response to interferon is ultimately responsible for the depression of the mixed-function oxidase system. This hypothesis is supported indirectly by data from a number of other studies indicating that cytochrome P-450 is lost at a time after interferon has disappeared. Round and Stebbing [20] have shown that a single dose of poly rI·rC in hamsters induces serum interferon levels that peak at 3–4 hr which is at the time we observed an increase in cytochrome P-450. No detectable level of interferon was observed at 24 hr at which time hepatic mixed-function oxidase activity was depressed. Parkinson *et al.* [5] demonstrated that the cloned interferon, IFN-α AD, disappears rapidly from blood and is absent at the time of the observed decrease in cytochrome P-450 content. These authors suggested that the effects of interferon were not due to its direct action on the mixed-function oxidase system. The nature of the putative protein intermediate is at present unknown, but its existence is consistent with the hypothesis of Ghezzi *et al.* [25, 26] who have suggested that interferon induces xanthine oxidase in the liver and that the free radicals produced by this enzyme destroy cytochrome P-450 in the endoplasmic reticulum. It is interesting to speculate that, in the present experiments, puromycin prevented the synthesis of xanthine oxidase and, therefore, blocked the effect of IFN-αCON$_1$ interferon on drug metabolism. The existence of a protein intermediate might also suggest that the antiviral mechanism of interferon which involves 2′,5′-oligoisoadenylate synthetase [36] might be involved in the loss of cytochrome P-450. Induction of this synthetase ultimately leads to the production of an endonuclease which could degrade the mRNA coding for the isozymes of cytochrome P-450. As the induction of this synthetase requires *de novo* protein synthesis, the antibiotic may act to prevent the initiation of this chain of events. The increase in heme oxygenase which was observed at 24 hr is consistent with either hypothesis. This is likely due to an increase in the regulatory heme pool caused by the dissociation of heme during cytochrome P-450 breakdown or a decrease in apo-protein synthesis leaving an excess

of free heme [33].

The stimulatory and depressant effects of IFN-αCON$_1$ interferon on the mixed-function oxidase system were not restricted to the liver as cytochrome P-450 in the lung, adrenal and spleen were affected in a similar manner. The only exception to this general pattern was in the kidney where cytochrome P-450 content was depressed at both 3 and 24 hr. This depressant effect at 3 hr and the large magnitude of the effect of interferon at 24 hr in the kidney may be related to the high concentrations of interferon reaching this tissue as about one-third of a dose of a human α-interferon labeled with ^{125}I was found in the kidney of mice within 5 min of i.v. administration compared to the liver and stomach which contained only 5.5 and 1.4% of the dose respectively [37].

The effects of IFN-αCON$_1$ interferon on the mixed-function oxidase system in animals treated with different cytochrome P-450 inducers indicate that all isozymes of the hemoprotein are not affected to the same degree. The isozymes of cytochrome P-450 which oxidize benzo[*a*]pyrene appeared to be affected to a lesser degree than constitutive cytochrome P-450 in the control animals or the cytochrome P-450 induced by phenobarbital or clofibrate. It has been shown previously by Renton *et al.* [38] that concurrent administration of poly rI·rC or tilorone suppresses the induction of cytochrome P-450 and mono-oxygenase activity (ethylmorphine *N*-demethylase activity and benzo[*a*]pyrene hydroxylase activity) in phenobarbital- and 3-methylcholanthrene-induced animals. Recently, Crowe *et al.* [39] demonstrated that poly rI·rC depresses the cytochrome P-450 induced with either phenobarbital or 3-methylcholanthrene, but this effect is only temporary in the 3-methylcholanthrene-treated animals since the cytochrome P-450 content returns to the pre-induced level within 72 hr of poly rI·rC treatment. A similar recovery was not obtained in the animals induced with phenobarbital.

In summary, we have shown that in the hamster IFN-αCON$_1$ interferon caused an initial increase and then a decrease in the hepatic mixed-function oxidase system which is responsible for the metabolism of drugs. At the present time only, the depression of drug biotransformation has been observed in humans [7, 22]. The results presented in this report suggest that IFN-αCON$_1$ interferon will likely depress drug biotransformation in humans because the antiviral effects and the anti-cytochrome P-450 effect of interferons apparently cannot be separated, and IFN-αCON$_1$ interferon is antiviral in both hamster and human cells. It will be important to determine if interferon depresses cytochrome P-450 in humans and to assess the potential for interferon to cause clinically significant drug interactions.

REFERENCES

1. K. W. Renton and G. J. Mannering, *Drug. Metab. Dispos.* **4**, 223 (1976).
2. G. A. Leeson, S. A. Biedenback, K. Y. Chan, J. B. Gibson and G. J. Wright, *Drug Metab. Dispos.* **4**, 232 (1976).
3. K. W. Renton and G. J. Mannering, *Biochem. biophys. Res. Commun.* **73**, 343 (1976).
4. G. Singh, K. W. Renton and N. Stebbing, *Biochem. biophys. Res. Commun.* **106**, 1256 (1982).
5. A. Parkinson, J. Lasker, M. J. Kramer, M. T. Huang, P. E. Thomas, D. E. Ryan, L. M. Reik, R. L. Norman, W. Levin and A. H. Conney, *Drug Metab. Dispos.* **10**, 579 (1982).
6. K. W. Renton, *Biochem. Pharmac.* **30**, 2333 (1981).
7. K. W. Renton, in *Biological Basis of Detoxication* (Eds. J. Caldwell and W. B. Jacoby), pp. 307–24. Academic Press, New York (1983).
8. K. Alton, Y. Stabinsky, R. Richards, B. Ferguson, L. Goldstein, B. Altrock, L. Miller and N. Stebbing, in *The Biology of the Interferon System* (Eds. E. Demaeyer and H. Schellkens, pp. 119–28. North Holland, New York (1983).
9. N. Stebbing, M. Downing, K. D. Fagin, B. W. Altrock, E. W. Fish, H. D. Liggitt, S. Moochhala and K. W. Renton, in *The Biology of the Interferon System* (Eds. H. Schellekens and W. E. Stewart), p. 426. Elsevier, Amsterdam (1986).
10. S. El Defrawy El Masry and G. J. Mannering, *Drug Metab. Dispos.* **2**, 267 (1974).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
13. K. A. M. Johannesen and J. W. De Pierre, *Anal. Biochem.* **86**, 725 (1978).
14. C. H. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
15. J. E. Falk, *Biochem. Biophys. Acta Lib.* **2**, 232 (1964).
16. B. Schacter, *Meth. Enzymol.* **52**, 367 (1978).
17. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5**, 186 (1969).
18. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
19. G. L. Parker and T. C. Orton, in *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. J. A. Gustaffson, H. Carlsted, J. Duke, A. Mode and J. Rafter), pp. 373–7. Elsevier, Amsterdam (1980).
20. E. M. Round and N. Stebbing, *J. Interferon Res.* **1**, 451 (1981).
21. M. R. Franklin and B. S. Finkle, *J. Interferon Res.* **5**, 265 (1985).
22. G. J. Mannering and L. B. Deloria, *A. Rev. Pharmac. Toxic.* **26**, 455 (1986).
23. K. W. Renton, L. B. Deloria and G. J. Mannering, *Molec. Pharmac.* **14**, 672 (1978).
24. D. W. Nebert and R. M. Friedman, *J. Virol* **11**, 193 (1973).
25. P. Ghezzi, M. Bianchi, A. Mantovani, F. Spreafico and M. Salmona, *Biochem. biophys. Res. Commun.* **119**, 144 (1984).
26. P. Ghezzi, M. Bianchi, L. Gianera, S. Landolfo and M. Salmona, *Cancer Res.* **45**, 3444 (1985).
27. L. B. Deloria, V. Abbott, N. Gooderham and G. J. Mannering, *Biochem. biophys. Res. Commun.* **131**, 109 (1985).
28. T. T. Krispin, T. M. Parfenova, A. V. Itkes, I. G. Balandin, V. P. Kuznetsov and E. S. Severin, *Biochem. Int.* **8**, 159 (1984).
29. P. B. Fisher, A. F. Miranda, L. Babiss, S. Pestka and I. B. Weinstein, *Proc. natn. Acad. Sci. U.S.A.* **80**, 2961 (1983).
30. K. R. Rozee, L. J. Katz and I. S. McFarlane, *Can. J. Microbiol.* **15**, 969 (1969).
31. D. L. Vesely and K. Cantell, *Biochem. biophys. Res. Commun.* **96**, 574 (1980).
32. R. Yoshida and O. Hayaishi, *Proc. natn. Acad. Sci. U.S.A.* **75**, 3998 (1978).
33. R. El Azhary and G. J. Mannering, *Molec. Pharmac.* **15**, 698 (1979).
34. T. B. Zerkle, A. E. Wade and W. L. Rayland,

Biochem. biophys. Res. Commun. **96**, 121 (1980).
35. F. R. Balkwill, S. Mowshowitz, S. S. Seilman, E. M. Moodie, D. B. Griffin, K. H. Fantes and C. R. Wolf, *Cancer Res.* **44**, 5249 (1984).
36. G. C. Sen, *Pharmac. Ther.* **24**, 235 (1984).
37. A. V. Palleroni and O. Bohoslawer, *J. Interferon Res.* **4**, 493 (1984).
38. K. W. Renton, D. E. Keyler and G. J. Mannering, *Biochem. biophys. Res. Commun.* **88**, 1017 (1979).
39. D. O. Crowe, J. J. Reiners, D. E. Nerland and G. Sonnenfeld, *J. Natn. Cancer Inst.* **76**, 879 (1986).