Effects of interferon- α monotherapy on hepatic drug metabolism in cancer patients

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- 1 The influence of interferon- α (IFN α) on the clearances of theophylline (TH), antipyrine (AP) and hexobarbitone (HB) was studied in seven cancer patients given IFN α as their only treatment. In addition, IFN α effects on drug clearance were correlated with changes in serum inflammatory cytokines and acute phase proteins.
- 2 A 'baseline' study was performed by administering an oral drug 'cocktail' of TH (150 mg), AP (250 mg) and HB (250 mg) with saline injected simultaneously and again 24 h later. One week later, an 'acute' study was performed at the initiation of IFN α therapy, 3×10^6 units injected with the drug cocktail and again 24 h later. After 2 weeks of IFN α treatment three times per week, a 'chronic' study was performed with IFN α injected the day prior to, simultaneously with, as well as 24 h after the drug cocktail.
- 3 Plasma samples were collected over 48 h and the clearances of TH, AP and HB were estimated. Serum samples were collected at various times for the measurement of tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), C-reactive protein (C-RP) and α_1 -acid glycoprotein (AGP).
- 4 IFN α caused a 33% decrease in the oral clearance of TH during the chronic study compared with baseline ($P \leq 0.05$). Although IFN α inhibited TH clearance by 16% during the acute study and AP clearance by 20–21% during both acute and chronic studies, these changes did not reach statistical significance. IFN α caused minimal changes in HB clearance. There were no chronic effects of IFN α on serum cytokines or acute phase proteins.
- 5 The findings confirm that the most commonly used dose of IFN α inhibits the hepatic clearance in humans of some but not all drugs and that this inhibition persists during IFN α therapy. Because inhibition was not associated with increases in serum cytokines or acute phase proteins, the mechanism by which IFN α inhibits cytochrome P450 activities *in vivo* does not appear to involve inflammatory mediators such as TNF, IL-1 or IL-6.

Keywords cytokines interferon- α hepatic metabolism P450 metabolism cancer

Introduction

Biologic response modifiers such as cytokines [1–5] and interferon [6–11] have been shown to modulate hepatic cytochrome P450 enzyme activity in animals. Human data are less extensive but recent studies have shown that the acute administration of interferon- α (IFN α)

causes significant decreases in the hepatic clearances of both TH and AP in patients with stable hepatitis [12, 13] and in healthy subjects [12, 14].

To date, there is limited information on the effects of IFN α on hepatic drug metabolism in non-hepatitis

patients, and correlations with measures of the acute phase response, such as serum cytokines and acute phase proteins have not been reported. Therefore, the present study was conducted to examine the effect of acute and chronic IFN α monotherapy on the *in vivo* metabolism of TH, AP, and HB in stable cancer patients.

Methods

Study design

Seven patients with several types of metastatic cancer who were prescribed a trial of IFN α therapy as a sole agent by their primary physician were entered into this study which was approved by the University of Kentucky Human Subjects Committee and the General Clinical Research Center. Five women and two men (aged 45–70, mean age 59 years) were studied after giving written consent; three had renal cell cancer, two had melanoma, one patient had carcinoid and one patient had adenocarcinoma of an unknown primary site. All patients had been off other cancer chemotherapeutic agents for at least 8 weeks prior to entrance into this study. One patient smoked cigarettes, another chewed tobacco, a third was on glyburide 5 mg per day, and a fourth patient was taking amitriptyline 25 mg per day, nifedipine 30 mg per day, ferrous sulphate 325 mg per day and a multiple vitamin preparation.

To examine the effects of IFN α (3×10^6 units) on hepatic cytochrome P450 mediated metabolism, three studies were performed, a baseline study with no IFN α (saline only), an 'acute' study at initiation of IFN α treatment and a 'chronic' study after 2 weeks of IFN α therapy. In each study TH (150 mg), AP (250 mg) and HB (250 mg) were given as a 'cocktail' after an overnight fast [15]. The patients received no caffeine, theophylline products or non-steroidal anti-inflammatory drugs for 3 days prior to drug administration. During each study the patients received oral paracetamol (650 mg) at 10.00 h, 16.00 h, 22.00 h and 04.00 h (if awake). For those patients on medication and/or using tobacco (smoking or chewing), 'dosing' of medication and tobacco was at specified times according to a log generated during the baseline study which the patients followed for the subsequent acute and chronic studies. The same lots of patient medication and nicotine were used during each study.

Clinical procedure

All studies were conducted in the General Clinical Research Center, University of Kentucky. For each study the subjects received subcutaneous injections of 1 ml saline (baseline study) or IFN α (3×10^6 units Intron-A $^{\circ}$, Schering, Kenilworth, NJ) at 08.00 h and immediately ingested the drug cocktail. Patients received another saline or IFN α injection 24 h later. Blood was drawn before administration of the drug cocktail and at 10, 20, 30, 45 min and 1, 2, 3, 4, 6, 9, 12, 24, 36, and 48 h afterwards. Plasma was collected and stored at -20°C until analysis. Serum was collected at 0 and 2 h for the

measurement of TNF, at 0, 6 and 24 h for the measurement of IL-6 and IL-1, and at 0, 12 and 24 h for the measurement of C-RP and AGP.

One week after the baseline study the acute study was performed at the initiation of IFN α therapy. Following the acute study patients were discharged on IFN α therapy and self-administered 3×10^6 units s.c. three times a week. After 2 weeks of IFN α therapy (total of 7 doses) patients returned for the 'chronic' study. For the 'chronic' study, patients self-administered IFN α 24 h prior to drug ingestion and followed the procedure outlined above.

Analytical methods

Serum C-RP and AGP concentrations were determined using commercial kits (Behring Diagnostic Incorporated, Somerville, New Jersey). Serum IL-1 β was also assayed using a commercial kit (Oncomembrane Incorporated, Seattle, Washington). TNF activities were determined by the L-M cell cytotoxicity bioassay [16] with an intraday assay variability of 20% and normal range <5 units ml^{-1} . Serum IL-6 activity was determined by the B9 cell proliferation bioassay [17]. This assay was capable of measuring 1.6 units IL-6 ml^{-1} , corresponding to 0.3 ng IL-6 ml^{-1} , with an interday and intraday variability of 20% and normal range <5 units ml^{-1} [18]. However, because the IL-6 activities in most of our serum samples were below 1.6 units, we cannot report our IL-6 results as 'standard units' (reciprocal of dilution that gives 1/2 maximal stimulation compared with standard IL-6). Instead we calculated IL-6 activity as 'percent of maximal count', defined as the $d \text{ min}^{-1}$ of the first dilution in the assay minus background ($\approx 400 d \text{ min}^{-1}$) divided by the maximal $d \text{ min}^{-1}$ ($\approx 100,000$) minus background. These low IL-6 activity values were shown to represent true activity since they were inhibited by a specific anti-IL-6 antibody (Clonikine $^{\circ}$, Research and Diagnostic Systems, Minneapolis, MN).

Plasma TH was assayed using the TDX $^{\circ}$ system (Abbott Labs, Irving, TX). The assay had a lower limit of quantification of $0.82 \mu\text{g ml}^{-1}$ and an interday assay coefficient of variation of 3.3%. AP was measured by a modification of the method of Teunissen *et al.* [19] and Chang *et al.* [20]. Briefly, plasma was added to tubes containing phenacetin (internal standard) made alkaline with NaOH and back-extracted into methylene chloride. The organic phase was evaporated under a stream of nitrogen and reconstituted with 100 μl of mobile phase. Twenty μl was injected onto the h.p.l.c. column (Altex ODS 5 μm , 4.6 mm i.d. $\times$ 25 cm) and eluted with a mobile phase of 43/57, MEOH/water and 0.01 M triethylamine. The lower limit of quantification was $0.1 \mu\text{g ml}^{-1}$ and the standard curve was linear from $0.1 \mu\text{g ml}^{-1}$ to $20 \mu\text{g ml}^{-1}$. Inter- and intra-day coefficients of variation were 5.6% and 5.3%, respectively. HB was assayed by h.p.l.c. using a modification of the method by Brouwer *et al.* [21]. Briefly, 200 μl plasma was extracted into a 20/80 mixture of 2-propanol/chloroform after acidification with 0.2 M sulphuric acid. Drug was separated using a C18 column and detected at 225 pm. The lower limit of quantification was $0.1 \mu\text{g ml}^{-1}$ and the interday coefficient of variation of the assay was 5.8%.

Pharmacokinetic analysis

Plasma drug concentration-time data for each individual were analyzed using a non-linear least squares regression program (R-STRIP[®], Micromath Scientific Software, Salt Lake City, Utah). AUC values were estimated using the linear trapezoidal rule with extrapolation to infinity using $C_{\text{last}}/\lambda_z$ where C_{last} was the last measured concentration and λ_z is the terminal elimination rate constant obtained from R-STRIP[®].

Statistical analysis

We assumed that a 30% change in drug clearance would be clinically significant and performed a power analysis using the known intraindividual coefficient of variation (6.8–8.2%) from a previous antipyrine clearance study [22]. This indicated that a study using six subjects should be sufficient to detect such a change. Treatment groups (baseline, acute, chronic) were compared with respect to drug clearances, cytokines, acute phase proteins, liver enzymes, haematocrit, white blood cell count (WBC), total bilirubin and albumin by two way analysis of variance followed by Student's *t*-test. Relationships between change in TH clearance and changes in serum cytokines and acute phase proteins were assessed using regression analysis and the *F*-test. Statistical significance was defined as $P \leq 0.05$ for all tests.

Results

Table 1 shows the characteristics of the seven patients studied. The patients were medically stable throughout the 4 weeks of study. Haematocrits were slightly decreased and liver enzyme levels were slightly increased with no significant effect of IFN α therapy. Most patients had normal liver function tests, except for two whose serum alkaline phosphatase values were slightly above normal, probably due to hepatic metastases. Peripheral white blood cell counts stayed within the normal range during the study. As expected, levels decreased to a statistically significant but not clinically significant degree after 2 weeks of IFN α therapy. Serum albumin levels remained stable, but one patient did have low albumin levels throughout the study.

Serum cytokine and acute phase protein concentrations at various times after saline injection in the baseline study and after both acute and chronic IFN α injection are shown in Table 2. Serum TNF activities were normal in all patients except for one who had elevated activities at all time points. There was also a trend toward lower TNF levels with IFN α therapy throughout the study which reached statistical significance after chronic therapy, although levels remained in the normal range. No IL-1 β was detected in any of the sera examined during the baseline, acute or chronic studies. Serum IL-6 activities were in the low normal range with only minor transient increases 6 h after the initial dose of IFN α in six out of seven patients. The concentrations of C-RP, an indicator of the hepatic acute phase response, were slightly elevated 24 h after the acute dose of IFN α in all

patients, but this increase was not statistically significant. The AGP concentration was very high in one patient and mildly increased in two patients, but no consistent effect of IFN α therapy was noted. These data indicate that IFN α did not cause a systemic 'acute phase response' characterized by cytokine release or increases in acute phase proteins.

Figure 1 shows the plasma concentrations of TH, AP, and HB in a representative patient. The effects of IFN α administration on TH, AP and HB clearances are summarized in Table 3. Significant effects of acute IFN α administration on the oral clearances of TH, AP, and HB were not detected. However, four patients had decreased clearances of TH and six of AP. Chronic exposure to IFN α was associated with a significant lowering of TH clearance (33% compared with baseline; $P \leq 0.05$) with six patients having a decrease. Although AP oral clearances were lower in six patients, the 20% decrease was not statistically significant. IFN α appeared to have no chronic or acute effect on the metabolism of racemic HB. No significant correlations were observed between the changes in TH clearance and changes in serum cytokines or acute phase proteins.

Discussion

We have shown that chronic IFN α therapy administered at the most commonly prescribed dose (3×10^6 units three times each week), impaired the metabolism of TH. This confirms an earlier observation of Jonkman *et al.* [14] who reported a 10% decrease in TH clearance after three doses of IFN α (3×10^6 units) in 11 healthy subjects and of Williams *et al.* [13] who reported a mean 49% decrease (range 33–81%) in five patients with chronic active hepatitis B and four healthy subjects. The related interferon- β has also been shown by Okuno *et al.* [23] to decrease TH clearance by 20% after high dose daily injections. Williams & Farrell [12] reported a 16% decrease in AP clearance in 9 patients with chronic active hepatitis after an acute dose of intramuscular IFN α . We observed a mean 20% decrease in AP clearance after both acute and chronic IFN α therapy, but because of the small number of patients this change did not reach statistical significance.

Our data suggest that there may be differential effects of IFN α on hepatic drug metabolism because of the greater decrease in TH clearance (33%) compared with AP (20%) and HB (no effect). Differential metabolism of TH, AP and HB has also been observed in patients with liver disease [24]. Consequently, specific hepatic cytochrome P450 enzymes may be more sensitive to the effects of IFN α . TH is metabolized principally by the CYP1A2 isozyme [25–30], although other isozymes may also play a role [27, 31]. Studies of selective induction and inhibition of AP metabolic pathways suggest that AP is also metabolized by several isozymes [32, 33], one of which may be common to the metabolism of theophylline [15]. HB appears to be metabolized by members of the 2C family of isozymes [34, 35]. The effect of IFN α on specific P450 isozymes can only be implied with the use of the above substrates. Craig *et al.* [36] recently

Table 1 Haematological and blood chemistry data for the patients studied

	<i>Normal range</i>	<i>Baseline</i>	<i>Study phase</i>	
			<i>Acute</i>	<i>Chronic</i>
HCT (%)	(40–54)	38 ± 6.3 (27–48)	37 ± 5.6 (27–46)	37 ± 5.5 (30–46)
WBC ($\times 10^2/\text{mm}^3$)	(4.8–10.8)	7.5 ± 1.8 (5.5–10.6)	7.1 ± 1.3 (5.4–9.2)	5.3 ± 1.0* (3.8–7.1)
Total bilirubin ($\mu\text{mol l}^{-1}$)	(3–17)	6.8 ± 3.4 (2–12)	6.8 ± 3.4 (2–12)	5.1 ± 3.4 (2–10)
ALT (iu l^{-1})	(10–40)	18 ± 10 (9–37)	26 ± 16 (7–46)	27 ± 12 (7–45)
AST (iu l^{-1})	(13–30)	32 ± 21 (19–78)	38 ± 25 (16–89)	42 ± 34 (15–118)
ALP (iu l^{-1})	(49–120)	138 ± 79 (71–269)	149 ± 87 (75–302)	164 ± 129 (63–412)
ALB (g l^{-1})	(34–48)	34 ± 6 (24–42)	34 ± 5 (26–40)	34 ± 5 (26–40)

Abbreviations: Percent haematocrit (%HCT), white blood cell count (WBC), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), albumin (ALB). Values are mean $\pm$ s.d. for $n = 7$ (range), * $P \leq 0.05$ compared with baseline.

Table 2 Serum cytokine and acute phase protein concentrations

	<i>Normal range</i>	<i>Baseline</i>	<i>Study phase</i>	
			<i>Acute</i>	<i>Chronic</i>
TNF, (u ml^{-1})	(0–5)			
Control		4.4 ± 3.2 (1.3–9.3)	3.2 ± 3.0 (0.5–7.0)	2.6 ± 2.9* (0.3–7.4)
2 h		4.6 ± 3.1 (1.2–8.4)	3.4 ± 3.8 (0.4–11)	3.1 ± 4.0 (0.2–11)
IL-6 (% maximal count) (see text)				
Control		3.8 ± 2.5 (2.3–7.6)	3.6 ± 2.5 (1.7–9.0)	4.1 ± 2.9 (2.2–9.7)
6 h		3.3 ± 2.2 (0.9–6.4)	28 ± 40 (2.4–113)	4.7 ± 3.9 (1.4–11)
24 h		5.5 ± 2.2 (2.8–7.9)	4.0 ± 2.4 (1.7–6.7)	4.3 ± 2.4 (1.1–6.9)
C-RP ($\mu\text{g ml}^{-1}$)	(<5–12)			
Control		25 ± 28 (0–83)	24 ± 24 (0–71)	16 ± 15 (0–40)
12 h		nd	26 ± 28 (0–83)	16 ± 16 (0–34)
24 h		nd	36 ± 32 (2–100)	20 ± 19 (2–48)
AGP (mg dl^{-1})	(55–140)			
Control		125 ± 31 (83–165)	129 ± 44 (82–187)	140 ± 43 (78–195)
12 h		nd	109 ± 29 (69–147)	128 ± 43 (87–204)
24 h		nd	129 ± 37 (88–180)	130 ± 41 (92–204)

Abbreviations: Tumor necrosis factor (TNF), interleukin-6 (IL-6), C-reactive protein (C-RP), α_1 -acid glycoprotein (AGP), not determined (nd). Values are mean $\pm$ s.d. for $n = 7$ (range), * $P \leq 0.05$ compared with baseline.

Figure 1 Plasma drug concentrations in a representative patient after simultaneous oral administration of a) theophylline (150 mg), b) antipyrine (250 mg) and c) hexobarbitone (250 mg) with no interferon (baseline; $\square$), after one dose (acute; $\triangle$) and after 2 weeks of therapy (chronic; $\circ$).

reported a modest but significant ($P < 0.05$) decrease in CYP3A activity using another substrate, erythromycin.

The mechanism(s) by which IFN α inhibits hepatic cytochrome P450 is presently unknown despite many studies in animals [11, 37–41]. It has been suggested that the inhibition of drug metabolism by IFN α requires the production of an intermediate protein within the hepatocyte which may decrease the synthesis of certain apoproteins [42]. Another mechanism that is not mutually exclusive was proposed by Ghezzi *et al.* [43] who suggested that IFN α induces the activity of xanthine oxidase in the liver which, in turn, generates oxygen radicals that may damage or destroy cytochrome P450 apoproteins. More recently, Moomchala & Renton [44] provided additional evidence for this hypothesis by observing that the free radical scavenger α -tocopherol and the xanthine oxidase inhibitor allopurinol prevented IFN α mediated loss of total cytochrome P450.

Because IFN α has been shown to modulate the production of cytokines (e.g. IL-1) in peripheral blood monocytes [45] these components might mediate inhibition of cytochrome P450 metabolism [1–5]. However, we observed no significant correlation between changes in TH clearance and serum cytokines (TNF, IL-1 β or IL-6).

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Table 3 The mean ($\pm$ s.d.) oral clearances ($l\ h^{-1}$) of theophylline, antipyrine and hexobarbitone with and without therapy with interferon- α

	Baseline	Study phase Acute	Chronic
Theophylline	3.31 ± 1.3	2.78 ± 1.0	$2.23 \pm 0.7^*$
Range	(1.1–5.0)	(1.2–4.0)	(1.0–3.1)
Change from baseline		–16%	–33%
CI		[+3% to –26%]	[–18% to –39%]
Antipyrine	2.94 ± 1.3	2.31 ± 1.1	2.35 ± 1.1
Range	(1.1–4.4)	(0.8–3.6)	(0.9–4.0)
Change from baseline		–21%	–20%
CI		[–8% to –42%]	[–8% to –30%]
Hexobarbitone	15.0 ± 4.7	17.3 ± 8.8	14.0 ± 6.0
Range	(7.0–19.5)	(8.1–27.7)	(7.2–24.2)
Change from baseline		+13%	–7%
CI		[+37% to –12%]	[+8% to –22%]

Mean $\pm$ s.d. for $n = 7$.

CI [95% confidence interval (percent change between baseline and respective treatment)].

* $P \leq 0.05$ compared with baseline.

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