Effect of peginterferon alfa-2a (40KD) on cytochrome P450 isoenzyme activity

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Interferons are known to down-regulate the hepatic cytochrome P450 (CYP) system thereby reducing drug metabolism.
- Several studies have evaluated the interactions between interferon and drugs metabolized by various CYP isoenzymes and the data suggest that interferon may reduce the activity of CYP1A2.

WHAT THIS STUDY ADDS

- This study examines the effect of PEG-IFN alfa-2a on drugs metabolized by the CYP450 system, including theophylline (CYP1A2).
- Previous studies have examined the effect of standard IFN or PEG-IFN alfa-2b on the activity of the CYP450 drug metabolizing enzymes. However, PEG-IFN alfa-2a has different pharmacokinetics, dosing and pegylated moiety as compared with standard IFN and PEG-IFN-alfa-2b.
- Any effects on the CYP enzyme system by PEG-IFN alfa-2a (40KD) could have important consequences for patients with hepatitis C virus receiving concomitant drug treatments.

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AIM

Pegylated interferon-based therapy is recommended for treatment of hepatitis C virus (HCV) infection. Because interferons are known to down-regulate hepatic cytochrome P450 (CYP) enzymes, which are involved in drug metabolism and clearance, there is a need to investigate the effect of peginterferon (PEG-IFN) alfa-2a (40KD) on the activity of these enzymes *in vivo*.

METHODS

Fourteen healthy, male volunteers aged 18 to 45 years were recruited into an open label, two period, single centre study in which CYP enzyme activity was measured by administration of the selectively metabolized probe drugs theophylline (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), debrisoquine (CYP2D6) and dapsone (CYP3A4) on day 1 of the study. PEG-IFN alfa-2a (40KD) 180 µg was given subcutaneously each week from day 15 to 36, and probe drugs were re-administered on day 37. Probe drugs and metabolites were quantified in plasma or urine samples and used to derive pharmacokinetic parameters.

RESULTS

PEG-IFN alfa-2a (40KD) significantly increased the area under the serum drug concentration vs. time curve (AUC(0,∞)) for theophylline by 24%, with a reduction in the mean oral clearance of theophylline of 20%. There were no effects on the pharmacokinetics of any of the other probe drugs. The incidence of adverse events was as expected in subjects receiving pegylated interferon.

CONCLUSION

These results suggest there may be an inhibitory effect of PEG-IFN alfa-2a (40KD) on CYP1A2. PEG-IFN alfa-2a (40KD) had no effect on CYP2C9, CYP2C19, CYP2D6 or CYP3A4 in healthy subjects.

Introduction

Hepatitis C virus (HCV) infection is a serious global health problem, with approximately 170 million people currently

infected [1–3]. Three to four million people are newly infected each year, with 50–80% of these individuals developing chronic infection and 2–5% developing hepatocellular carcinoma [4, 5]. Hepatitis C virus is the leading cause

of cirrhosis, hepatocellular carcinoma and liver disease-related morbidity worldwide [6, 7].

Interferon-based therapy is part of the recommended treatment for HCV infection [8, 9]. Initially, subcutaneous interferon-based monotherapy was used as an integral part of treatment of HCV infection, but it had low a sustained virological response (SVR) rate of 29% [10]. The addition of oral ribavirin improved the SVR rate to 52% [11]. Conventional interferon has been superseded by pegylated interferon (PEG-IFN), which has improved pharmacokinetic characteristics. For the past decade, the combination of PEG-IFN plus ribavirin for 24 to 48 weeks has been the standard of care for chronic HCV (CHC) infection [12]. PEG-IFN alfa-2a (40KD) plus ribavirin produces SVR rates of up to 66% in randomized, multinational, clinical trials in patients with CHC [12, 13]. However, PEG-IFN and ribavirin do not work on the HCV virus directly but rather by stimulating the immune system [14]. New direct acting antivirals (DAAs), telaprevir and boceprevir, were recently approved by the US Food and Drug Administration for treatment of genotype 1 CHC in combination with PEG-IFN and ribavirin and work by directly inhibiting the HCV protease [8, 9, 15–17]. The addition of either of these protease inhibitors to PEG-IFN and ribavirin, also known as triple therapy, has substantially improved SVR rates and shortened the duration of treatment in patients compared with PEG-IFN and ribavirin alone. For telaprevir in combination with PEG-IFN and ribavirin, the SVR rate was 75% compared with 44% for PEG-IFN and ribavirin alone after 12 weeks of treatment [17]. Next generation DAAs that inhibit the HCV polymerase are also being developed [18]. Eventually, these DAAs may be combined to achieve a PEG-IFN and ribavirin-free CHC therapy that will maximize potency, be an all oral treatment and lessen the side effects seen with PEG-IFN and ribavirin [19, 20].

Interferons are known to down-regulate drug metabolism by the hepatic cytochrome P450 (CYP) system, and understanding the interactions between PEG-IFN alfa-2a (40KD) and co-administered drugs is of considerable interest. Several studies have evaluated the interactions between conventional (non-pegylated) interferon and drugs metabolized by various CYP isoenzymes [21–23]. Although the results are not consistent, the data suggest that conventional interferon may reduce the clearance of theophylline, a substrate of CYP1A2 [21, 24, 25]. However, little is known about the potential for drug interactions between pegylated interferons and other drugs. One study examined the effect of PEG-IFN alfa-2b on the activity of CYP drug metabolizing enzymes in patients with CHC infection [22]. Weekly administration of PEG-IFN alfa-2b increased the 48-h urinary (carboxytolbutamide+4-hydroxytolbutamide):tolbutamide ratio suggesting a small increase in CYP2C8/9 activity and appeared to increase activity of CYP2D6 in some individuals, as evidenced by increases in the dextrophan:dextromethorphan ratio. In this study, a small reduction of activity was

observed in CYP1A2 with an approximate 8% reduction in the 1,7-dimethylxanthine : caffeine ratio. Metabolic interactions between PEG-IFN alfa-2a, which differs from PEG-IFN alfa-2b with respect to its pharmacokinetics, size and structure of its pegylated moiety and dosing, and the CYP isoenzymes have not been reported. The addition of new DAA therapies has appeared to be safe and well tolerated when combined with PEG-IFN and ribavirin [8, 9, 15–17]. However, most of the DAAs, including boceprevir and telaprevir, are metabolized through the CYP3A4 pathway and some are also potent inhibitors of CYP450-mediated drug metabolism, which could cause additional drug interactions in combination with PEG-IFN beyond those observed with PEG-IFN and ribavirin treatment alone [26, 27].

As the liver is the primary organ for drug metabolism and clearance, understanding changes that may occur to the CYP system, upon treatment with PEG-IFN-based therapy is necessary for proper treatment of CHC patients who are taking additional medications. Hepatitis C virus confers increased risks of other diseases, with the most prevalent comorbidities being diabetes, obesity, end-stage liver disease and depression [28, 29]. In addition, approximately 30% of HIV patients are co-infected with HCV, and 2–10% of HCV patients have hepatitis B virus. All of these diseases need to be managed during HCV treatment. Because CHC eradication requires up to 48 weeks of treatment and many patients will be taking additional drugs to manage these conditions, understanding these drug–drug interactions is critical for optimal patient care [30].

Any effects on the CYP enzyme system by PEG-IFN alfa-2a (40KD) could have important consequences for CHC patients receiving concomitant drug treatments. Thus, the objective of this study was to determine the potential for interactions between PEG-IFN alfa-2a (40KD) and a series of probe drugs metabolized by the CYP isoenzymes, including theophylline (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), debrisoquine (CYP2D6) and dapsone (CYP3A4).

Methods

Participants

Non-smoking healthy male subjects aged 18 to 45 years who did not have HCV were recruited. They had body weights of at least 60 kg, were within 20% of ideal weight and in good health as assessed by medical history, physical examination (including electrocardiogram) and clinical laboratory tests. All subjects were screened during the 28 day period preceding administration of PEG-IFN alfa-2a (40KD) (Pegasys®, Roche, Basel, Switzerland). Routine haematologic analysis included haemoglobin levels and haematocrit, white blood cell counts, absolute neutrophil counts and platelet counts. Thyroid hormone concentrations were assessed at screening and during follow-up. The

Figure 1

Study design. The period marked day -28 to day 1 was the pre-study screening period. Study part I was oral administration of probe drugs with pharmacokinetic sampling and identification of extensive metabolizers, part II was administration of subcutaneous peginterferon alfa-2a (40KD) and part III was oral administration of probe drugs and collection of blood samples with further pharmacokinetic sampling. *P450 isozyme identified by probe

study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Reasons for exclusion from the study included clinically significant abnormal baseline findings, significant history of gastrointestinal, renal, hepatic, pulmonary, cardiac, haematologic, endocrinologic, neurologic (seizure) or clinically significant psychiatric disease, history of drug or alcohol abuse during the 12 months preceding study entry, significant allergy or hypersensitivity to conventional interferon alfa-2a or paracetamol (acetaminophen), history of interferon treatment during the 4 weeks preceding the study, use of prescription, over-the-counter or investigational medicines during the 2 weeks preceding the study or anticipation of need for such medicines, participation in the preceding 4 weeks in any study requiring substantial (≥ 700 ml) withdrawal of blood, or donation of blood during the preceding 8 weeks history of influenza or cold symptoms during the 2 weeks preceding the study and the presence of any self-limiting disease (e.g. gastritis).

Study design

The trial was an open label, two period, single centre, non-randomized study in healthy volunteers, and was carried out in three parts (Figure 1). During parts I (days 1 to 14) and III (days 37 to 42), subjects received single oral doses of the five probe drugs: theophylline 125 mg, tolbutamide 500 mg, mephenytoin 100 mg, debrisoquine 10 mg and dapsone 100 mg (Table 1).

Subjects entered the clinical study unit the evening preceding day 1 to begin part I. After an overnight fast, the five probe drugs were administered orally on the morning of day 1. Blood samples were taken on days 1, 3, 4 and 6.

Table 1

Probe drugs administered with peginterferon alfa-2a (40KD) to assess pharmacokinetic interactions involving the hepatic cytochrome P450 system

Drug	Dose (mg)	Cytochrome P450 isoenzyme tested
Theophylline	125	1A2
Tolbutamide	500	2C9
Mephenytoin	100	2C19
Debrisoquine	10	2D6
Dapsone	100	3A4

Part I of this study was used to identify extensive metabolizers of debrisoquine (CYP2D6) and mephenytoin (CYP2C19). Participants in the study were classified as extensive metabolizers of debrisoquine (CYP2D6) and mephenytoin (CYP2C19) according to previously published criteria [31]. After a 2 week washout, subjects identified by phenotyping as extensive metabolizers (CYP2C19 and CYP2D6) proceeded to part II. In part II, subjects received 180 µg PEG-IFN alfa-2a (40KD) on days 15, 22, 29 and 36 by subcutaneous administration. In part III, on the evening before day 36, subjects entered the unit and received the final dose of 180 µg PEG-IFN alfa-2a (40KD) the next morning. On the morning of day 37, oral administration of the five probe drugs was repeated. The same sampling schedule was used as in part I with collection of additional blood samples on days 39, 40 and 42. A final follow-up visit occurred on day 49.

Urine samples were taken, 0 to 4 h before and 4 to 8 h after the day 1 (part I) and the day 37 (part III) doses of the

probe drugs. No caffeinated drinks were allowed for 48 h before and after the study attendance days, although water was allowed as required. There were no special dietary requirements, but alcohol was not allowed for 48 h before each dose of study medication. Alcohol consumption was not to exceed two units daily throughout the rest of the study. Participants were given meals at 6 h (lunch), 9 to 10 h (dinner) and 13 to 14 h (light snack) after administration of probe drugs. Prophylactic 1 g doses of paracetamol were allowed for anticipated febrile responses and were given 2, 6 and 10 h after administration of PEG-IFN alfa-2a (40KD) and as required thereafter.

Sample collection and analytical methods

Serial blood samples (7 ml) were collected by direct venepuncture or through a cannula in the arm not used for the injection of study drug at pre-dose and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 120 h post-dose starting on days 1 and 37. Pre- and post-dose samples of whole blood were collected into plain glass BD Vacutainer® Blood Collection Tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) at the specified times. Blood samples were allowed to clot (30 min at 4°C) prior to centrifugation (2500 rev min⁻¹, 10 min, 4°C) to isolate serum. Isolated serum was then frozen (–20°C or colder) until analysis. Urine was collected (pre-dose, 0–4 h and 4–8 h) in polyethylene containers and the pH and volume were recorded. For each collection interval, an aliquot (approximately 10 ml) was removed and frozen (–20°C or colder) until analysis.

Quantification of probe drugs in serum

Serum and urine concentrations of probe drugs and the hydroxyl metabolites of dapsone, debrisoquine and mephenytoin were determined by liquid chromatography and mass spectrometry by In Vitro Technologies Inc. (Baltimore, Maryland, USA). The analytical range for the serum assay was 5–600 ng ml⁻¹ for all probe drugs except debrisoquine (0.2–50 ng ml⁻¹). Ranges for urine assays were 62.5–2000 ng ml⁻¹ for mephenytoin, tolbutamide and debrisoquine, 500–16 000 ng ml⁻¹ for theophylline, 375–12 000 ng ml⁻¹ for dapsone, 125–20 000 ng ml⁻¹ for hydroxyl metabolites of dapsone and debrisoquine and 375–60 000 ng ml⁻¹ for hydroxyl metabolites of mephenytoin. Inter-assay coefficients of variation did not exceed 25% for calibration standards and 30% for quality control samples. Serum samples were loaded onto polymer cartridges to remove proteins and other potentially interfering compounds, and analytes were subsequently eluted in methanol. Sulfadiazine was added to act as an internal standard immediately before evaporation and reconstitution. For the urinalysis, a stable isotope of theophylline was used as an internal standard and β-glucuronidase was added to cleave any sulphate or glucuronide conjugates. The samples were subsequently processed via an anion

exchange column to remove potentially interfering anions and were loaded onto polymer cartridges. Analysis followed reconstitution.

Pharmacokinetic and safety analysis

Participants included in the pharmacokinetic analysis were required to have received two doses of the probe drugs, four doses of PEG-IFN alfa-2a (40KD) and at least one derived pharmacokinetic parameter estimated. The safety population included all subjects who had received at least one dose of PEG-IFN alfa-2a (40KD) and who had at least one post-baseline follow-up safety evaluation.

Estimation of pharmacokinetic parameters

The pharmacokinetic characteristics of the five probe drugs were evaluated using WinNonlin® Pro version 1.5 (Pharsight, Sunnyvale, California, USA). After administration of the five probe drugs, the following pharmacokinetic parameters were determined using non-compartmental methods: maximum serum concentration (C_{max}) and time to C_{max} (T_{max}), terminal phase half-life ($t_{1/2}$), derived from the terminal log-linear portion of each profile determined by least squares regression and dividing 0.693 by the slope so obtained, area under the curve (AUC) of serum concentration vs. time from time zero to infinity ($AUC(0, \infty)$), determined by the linear trapezoidal method with extrapolation beyond the last experimental concentration value, partial AUC of serum concentration vs. time from time zero to the time of the last measurable concentration ($AUC(0, t_{last})$), apparent total body clearance (CL/F) = dose/ $AUC(0, \infty)$ and apparent volume of distribution (V_d/F) = dose/(terminal slope $\times AUC(0, \infty)$).

Urinary excretion (8 h) was taken as described earlier, and the ability to hydroxylate dapsone (CYP3A4-mediated) was estimated by using the urinary dapsone recovery index $HDA/(HDA + DP)$, where HDA = dapsone hydroxylamine and DP = dapsone (8 h recoveries in urine). CYP2C19 activity was assessed with the mephenytoin hydroxylation index, expressed as $0.5 \times \text{dose}/8 \text{ h urinary mephenytoin excretion}$. CYP2D6 activity was determined by the debrisoquine recovery index $OH-DQ/(OH-DQ + DQ)$, where OH-DQ and DQ are 8 h urinary recoveries of 4-hydroxydebrisoquine and debrisoquine, respectively.

Safety and tolerability

Adverse events were graded as mild (discomfort with no effect on daily activities), moderate (reduction of or effect on normal daily activities) or severe (inability to work or perform normal daily activities). Influenza-like symptoms were defined as the presence of more than one influenza symptom at any one time (such as headache, fever, malaise and rigor). When these symptoms were seen singly, they were recorded as individual adverse events.

Serious adverse events were those that were fatal or life-threatening, required prolonged hospitalization, were permanently disabling or resulted in congenital abnormal-

ity. Laboratory tests and vital signs were monitored and the Medical Dictionary for Regulatory Activities was used to classify adverse events. Haematology, blood chemistry and urinalysis parameters measured as part of the laboratory assessment were the same as those assessed during screening. Neutrophil and platelet counts were also assessed throughout the study.

Data handling and analysis

Although no formal sample size calculations were performed, on the basis of previous drug interaction studies on the pharmacokinetics of interferons (in 7–17 subjects), we considered that 16 subjects should be recruited to this study [22, 25]. The pharmacokinetic characteristics of the five probe drugs in this study were summarized with descriptive statistics, separated into figures obtained before (in the absence of) and after (in the presence of) PEG-IFN alfa-2a (40KD). Analysis of variance (ANOVA) was performed for the primary pharmacokinetic parameter, $AUC(0, \infty)$, with bioequivalence criteria applied to the probe drugs using the 90% confidence interval (CI). Secondary parameters for bioequivalence testing were $AUC(0, t_{last})$ and C_{max} for the five probe drugs as well as the dapson and debrisoquine recovery indices and the mephenytoin hydroxylation index. A pharmacokinetic interaction was defined as significant if the 90% CI for the ratio of geometric treatment means lies outside the range 0.80–1.25 and did not include 1.0.

Results

Participants

Sixteen healthy white male volunteers with no evidence of HCV entered the study, received at least one dose of PEG-IFN alfa-2a (40KD) and all were included in the safety population. Fifteen subjects completed part I. All subjects were identified as extensive metabolizers as determined by metabolic ratios for debrisoquine (debrisoquine : 4-OH-debrisoquine) of <12.6 and S : R mephenytoin ratios for mephenytoin of <0.8 from the 8 h urine samples. On the basis of this result, all 15 subjects were entered into part II. Fourteen subjects entered part III, completed the study and were therefore deemed evaluable for pharmacokinetic parameters analysis.

Participants were aged 22 to 45 years (mean 33.6 years), with body weights from 63 to 94 kg (mean 77 kg), height of 168 to 184 cm (mean 176 cm) and body mass indexes from 19.6 to 28.3 kg m⁻² (mean 24.8 kg m⁻²). All participants tested negative for interferon antibodies.

Pharmacokinetics

The mean serum drug concentration profiles for theophylline, tolbutamide, mephenytoin, debrisoquine and dapson before and after multiple weekly doses of PEG-IFN alfa-2a (40KD) are shown in Figure 2. There were

no significant differences in the mean serum drug profiles for tolbutamide (CYP2C9), mephenytoin (CYP2C19), debrisoquine (CYP2D6) and dapson (CYP3A4) in the presence of PEG-IFN alfa-2a as compared with when the drugs were administered alone. Compared with baseline, metabolic indices for mephenytoin, debrisoquine and dapson were also similar following PEG-IFN administration. In addition, no statistically significant differences were observed in the pharmacokinetics of these four probe drugs after administration of PEG-IFN alfa-2a (40KD) (Table 2). From this it can be inferred that the drug had no apparent effect on the hepatic isoenzymes CYP2C9, CYP2C19, CYP2D6 or CYP3A4.

The mean serum concentrations for theophylline (CYP1A2) were slightly higher and measurable for longer periods in the presence of PEG-IFN alfa-2a (40KD) than when theophylline was administered alone (Figure 2). In most subjects, the addition of PEG-IFN alfa-2a (40KD) resulted in a reduction of theophylline oral clearance, with a mean reduction by 17%, but this was not statistically significant (Figure 3 and Table 2). Nevertheless, nine of the 14 participants had reductions exceeding 15%. The pharmacokinetic parameters, $AUC(0, t_{last})$ and $AUC(0, \infty)$ of theophylline, were significantly different after multiple once weekly doses of PEG-IFN alfa-2a (40KD), with a mean increase of 22% and 24% and the 90% CIs for the ratio of part III : part I of 1.04, 1.42 and 1.05, 1.47, respectively (Table 3). The reduction in clearance and increase in AUC is consistent with the equation used of: $(CL/F) = \text{dose} / AUC(0, \infty)$. No significant changes were observed for any other pharmacokinetic parameters, including C_{max} , for theophylline after administration of PEG-IFN alfa-2a (40KD).

Adverse events

Adverse events during administration of the probe drugs and PEG-IFN alfa-2a (40KD) were observed. Most adverse events were mild and no severe adverse events or deaths were observed. The incidence of adverse events was higher in part II than in parts I (69%) and III (79%). All 15 subjects had at least one adverse event in part II. Most were generally associated with interferon-based therapy and influenza-like illness was the most common. More adverse events were reported in part III than part I (mean 3.5 vs. 1.8 events per participant). The most common adverse events were headache, dizziness and sore throat in part I. In part III, headache, dizziness, paraesthesia, injection site inflammation, nasal congestion and diarrhoea were observed most frequently and appeared to be carryover effects from part II.

Overall, 11 of 142 events were of moderate intensity (nine in part II and two in part III). One serious adverse event of onset of non-insulin-dependent diabetes mellitus was noted during the study. This was possibly related to drug administration, as hyperglycaemia has been reported, albeit infrequently, in subjects receiving PEG-IFN

Figure 2

Effect of peginterferon alfa-2a (40KD) on serum concentrations of probe drugs. Plots of mean serum concentration ($\pm$ SD) of probe drugs theophylline, tolbutamide, mephenytoin, debrisoquine and dapsone vs. time before and after multiple weekly doses of peginterferon alfa-2a (40KD). —●—, baseline; —○—, after peginterferon alfa-2a (40KD)

Table 2

Pharmacokinetic parameters of probe drugs at baseline and in presence of peginterferon alfa-2a (40KD)

Parameter	Study part	Mean $\pm$ SD for probe drugs (CYP450 isoenzyme affected)				
		Theophylline (1A2)	Tolbutamide (2C9)	Mephenytoin (2C19)	Debrisoquine (2D6)	Dapsone (3A4)
AUC(0, ∞) ($\mu\text{g ml}^{-1}\text{ h}$)	I	45.3 $\pm$ 12.8	803 $\pm$ 249	52.6 $\pm$ 12.6	0.37 $\pm$ 0.35	48.4 $\pm$ 15.6
	III	59.7 $\pm$ 23.9*	827 $\pm$ 411	60.3 $\pm$ 22.4	0.33 $\pm$ 0.24	51.3 $\pm$ 22.1
AUC(0, t_{last}) ($\mu\text{g ml}^{-1}\text{ h}$)	I	40.5 $\pm$ 12.6	760 $\pm$ 254	35.7 $\pm$ 9.1	0.27 $\pm$ 0.24	44.9 $\pm$ 14.8
	III	51.3 $\pm$ 22.5*	782 $\pm$ 400	36.6 $\pm$ 13.7	0.25 $\pm$ 0.23	47.2 $\pm$ 21.6
C_{max} ($\mu\text{g ml}^{-1}$)	I	3.9 $\pm$ 10.7	44.7 $\pm$ 9.8	8.6 $\pm$ 2.8	0.014 $\pm$ 0.008	1.6 $\pm$ 0.32
	III	4.3 $\pm$ 1.6	45.3 $\pm$ 17.7	10.0 $\pm$ 4.4	0.014 $\pm$ 0.006	1.7 $\pm$ 0.38
$t_{1/2}$ (h)	I	9 $\pm$ 4	11 $\pm$ 3	73 $\pm$ 27	20 $\pm$ 15	20 $\pm$ 5
	III	13 $\pm$ 8	9 $\pm$ 2	88 $\pm$ 40	25 $\pm$ 17	21 $\pm$ 4
CL/F (l h^{-1})	I	3.0 $\pm$ 0.9	0.7 $\pm$ 0.3	2.0 $\pm$ 0.5	58 $\pm$ 56	2.2 $\pm$ 0.5
	III	2.5 $\pm$ 1.2	0.7 $\pm$ 0.3	1.8 $\pm$ 0.6	59 $\pm$ 73	2.1 $\pm$ 0.5
V_z/F (l)	I	35 $\pm$ 9	10 $\pm$ 2	206 $\pm$ 77	944 $\pm$ 513	62 $\pm$ 9
	III	39 $\pm$ 10	9 $\pm$ 3	222 $\pm$ 91	1138 $\pm$ 627	62 $\pm$ 13

*Statistically significant interaction based on bioequivalence criteria (90% CI for the ratio of geometric means of part I:part III lies outside the range 0.8–1.25 and does not include 1.0). AUC(0, ∞), area under the curve of serum drug concentration vs. time extrapolated to infinity; AUC(0, t_{last}), area under the curve of serum drug concentration vs. time to last measurable concentration; C_{max} , maximum drug concentration in serum; CL/F, oral clearance; CYP, cytochrome P; SD, standard deviation; $t_{1/2}$, terminal phase half-life; V_z/F , volume of distribution based on terminal phase.

Figure 3

Oral clearance (CL/F) of theophylline at baseline and after administration of peginterferon alfa-2a (40KD). Each point represents a single participant. The line of identity as shown for each drug indicates where CL/F before peginterferon alfa-2a administration is equal to CL/F afterwards. Points below the line of identity illustrate inhibition; points above the line illustrate induction. The nine subjects whose theophylline CL/F was between 15 and 60% lower after peginterferon alfa-2a administration are identified by subject numbers

alfa-2a (40KD). One patient withdrew from treatment due to dizziness, weakness, muscle cramps and pain, loss of coordination, impairment of concentration and breathlessness, all of which may have been related to PEG-IFN alfa-2a (40KD) therapy during part II.

Three participants experienced grade 3 neutropenia during part II that carried over into part III. No changes in vital signs or electrocardiograph readings were observed, with the exception of two transient episodes of clinically

Table 3

Statistical evaluation of theophylline pharmacokinetic parameters at baseline and in presence of peginterferon (PEG-IFN) alfa-2a (40KD)

Parameter	Geometric means		Ratio of geometric means (90% CI)*
	Baseline	After PEG-IFN	
AUC(0, ∞) ($\mu\text{g ml}^{-1}\text{ h}$)	43.6	54.3	1.24 (1.05, 1.47)†
AUC(0, t_{last}) ($\mu\text{g ml}^{-1}\text{ h}$)	38.8	47.1	1.22 (1.04, 1.42)†
C_{max} ($\mu\text{g ml}^{-1}$)	3.8	4.1	1.08 [0.95, 1.22]

*Ratio between after PEG-IFN and baseline. †Statistically significant interaction based on bioequivalence criteria; 90% CI lies outside the interval [0.80, 1.25] and does not include 1.00.

significantly raised diastolic blood pressure. However, both readings normalized within 24 h.

Discussion

In this study, a statistically significant interaction was identified between PEG-IFN alfa-2a (40KD) and an increase in the AUC(0, ∞) of theophylline after administration of four once weekly doses of PEG-IFN alfa-2a (40KD). This suggests an inhibitory effect of PEG-IFN alfa-2a (40KD) on CYP1A2. The pharmacokinetic profiles of the other probe drugs, dapsone, debrisoquine, mephenytoin and tolbutamide, were not significantly altered by administration of PEG-IFN alfa-2a (40KD). These findings imply that the pharmacokinetics of drugs metabolized by the CYP isoenzymes 2C9 (e.g. tolbutamide), 2C19 (e.g. mephenytoin), 2D6 (e.g. debrisoquine) and 3A4 (e.g. dapsone) are unlikely to be altered by co-administration of PEG-IFN alfa-2a (40KD) and may be given concurrently with PEG-IFN alfa-2a (40KD)

without dosage adjustments or additional monitoring. The adverse events and laboratory abnormalities observed following administration of 180 µg of PEG-IFN alfa-2a (40KD) once weekly for 4 weeks to healthy male subjects were as expected following the administration of PEG-IFN. The concomitant administration of an oral 'cocktail' of five probe drugs for the cytochrome P450 isoenzymes was well tolerated.

Due to the large variability of hepatic drug metabolism in patients with liver disease, this study was conducted in healthy volunteers. Healthy volunteers were considered to represent the 'worst case scenario' since the largest effect will most likely be seen in the absence of any baseline liver disease. For patients with compromised livers, who may already have reduced cytochrome P450 activity as baseline, the effect might not be as large but the information from this study can be used to guide clinicians in terms of monitoring co-medications patients may take with PEG-IFN alpha-2a. In fact, lower activity has been observed for CYP1A2 (caffeine) in CHC patients, along with CYP2C19 (mephenytoin), CYP2D6 (debrisoquine), and CYP2E1 (chlorzoxazone) [22, 32]. Also, when administered multiple doses of PEG-IFN alfa-2b, CYP1A2 was inhibited in CHC patients to a limited extent.

The mechanism by which PEG-IFN inhibits P450 activity in the liver is largely unknown. Inhibition of P450 activity can result from changes in expression or activity of specific CYP enzymes, as has been observed during infection and inflammation [33]. Since interferon is immunostimulatory, it can modulate the intrahepatic cytokine network which can then impact CYP450 enzyme activity [25, 34, 35]. This could consequently alter drug responses and increase toxicity. Another theory has suggested that interferon increases generation of reactive intermediates, which can covalently bind to CYP enzymes and decrease their activity [36]. Other potential mechanisms include interferon modulation of mRNA levels of P450 enzymes and protein turnover. Interferon can inhibit CYP3A4 enzyme activity and rapidly down-regulates the mRNA expression of CYP3A4, CYP1A2, CYP2B6 and CYP2E1, independent of *de novo* protein synthesis [21, 23]. Whereas this study did see a decrease in CYP1A2 activity, the activity of other CYP enzymes in subjects was unaffected. There still may have been a decrease in the mRNA expression levels of the other CYP enzymes that did not manifest itself in a decrease of activity physiologically. In addition, these previous studies of mRNA levels were done in cells and would need to be verified in humans. Further studies exploring these theories are needed to identify how regulation of P450 enzymes by interferon occurs.

The observed reduction in clearance of theophylline in the presence of PEG-IFN alfa-2a (40KD) is in accordance with other studies that observed the down-regulation of P450 activity and pharmacokinetics in patients treated with interferon. PEG-IFN alfa-2a (40KD) decreased the oral

clearance of theophylline by 33% ($P < 0.05$) after repeated administration in patients with cancer [37]. No involvement of inflammatory mediators such as tumour necrosis factor or interleukins were observed to play a role in the decrease observed, indicating the decrease in oral clearance was a direct result of PEG-IFN alfa-2a (40KD) administration. Jonkman *et al.* observed 15% changes in mean terminal elimination half-life, AUC and residence time of theophylline after a single infusion of interferon in healthy adults [38]. Interestingly, Williams and colleagues showed in both patients with hepatitis B and healthy volunteers that interferon increased the elimination half-life of theophylline most markedly in rapid metabolizers [39]. In this study, we observed a similar magnitude of findings in healthy subjects compared with the results of conventional interferon studies in HCV patients, suggesting that our results can also be extrapolated to the HCV patient population. The mean oral clearance of theophylline was reduced by PEG-IFN alfa-2a (40KD) 17%, with reductions exceeding 15% in nine of 14 participants. This resulted in an increase in mean AUC(0,∞) of 24%. Since PEG-IFN alfa-2a (40KD) was given in this study as a series of four weekly doses to achieve near steady-state concentrations, maximal inhibition of CYP enzymes would be expected by the time of the second probe drug dose and the pharmacokinetic changes observed at that time would be expected to be the highest achievable.

In conclusion, PEG-IFN alfa-2a (40KD) may have an inhibitory effect on the CYP1A2-mediated metabolism of theophylline. The magnitude of this inhibition is modest and similar to that previously reported with conventional non-pegylated interferon. This effect is likely to be clinically relevant only for narrow therapeutic index drugs, such as theophylline. In these cases, concentrations should be monitored and any necessary dosage adjustments considered in patients receiving concomitant therapy with PEG-IFN alfa-2a (40KD). Importantly, PEG-IFN alfa-2a (40KD) had no significant effect on the pharmacokinetics of drugs metabolized by CYP2C9 (tolbutamide), CYP2C19 (mephenytoin), CYP2D6 (debrisoquine) and CYP3A4 (dapsone). The results suggest that clinically relevant drug-drug interactions between PEG-IFN alfa-2a (40KD) and agents metabolized via the hepatic P450 system are unlikely to occur.

Competing Interests

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