SINGLE-DOSE METHOXSALEN EFFECTS ON HUMAN CYTOCHROME P-450 2A6 ACTIVITY

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ABSTRACT:
Methoxsalen (8-methoxypsoralen) is an effective and selective mechanism-based inhibitor of human hepatic cytochrome P-450 (CYP)2A6 in vitro, and may have utility as a clinical probe for CYP2A6-catalyzed xenobiotic metabolism in humans in vivo. This investigation explored single-dose oral methoxsalen effects on human CYP2A6 activity in vivo, assessed by coumarin 7-hydroxylation. Eleven volunteers received 50 mg of oral coumarin on two occasions in a randomized crossover, 90 min after oral methoxsalen or nothing (controls). Plasma and urine 7-hydroxycoumarin and plasma methoxsalen concentrations were determined by HPLC. Methoxsalen pretreatment diminished area under the curve of plasma 7-hydroxycoumarin versus time by 24% (2.40 ± 0.48 versus 3.20 ± 0.55 μg · h · ml⁻¹; P < .001), and also decreased plasma 7-hydroxycoumarin Cmax (0.80 ± 0.26 versus 1.4 ± 0.5 μg/ml; P < .05); however, 7-hydroxycoumarin concentrations were only diminished 0.75 to 2 h after coumarin administration, but not thereafter. Methoxsalen diminished urine 7-hydroxycoumarin excretion in 0- to 1- and 1- to 2-h samples, but not thereafter, and total excretion was unchanged. Considerable individual variability in methoxsalen plasma concentrations was observed. There were significant correlations between the decrease in plasma 7-hydroxycoumarin Cmax and plasma methoxsalen Cmax, but not between the decrease in plasma 7-hydroxycoumarin area under the curve and methoxsalen disposition. These results show that single-dose oral methoxsalen, in conventional doses, was a moderately effective inhibitor of human CYP2A6 activity in vivo, however, the duration of inhibition was limited. Interindividual variability in the extent of CYP2A6 inhibition appeared attributable to variability in the absorption and first-pass clearance of methoxsalen. Alternative doses, timing, and/or routes of methoxsalen administration are required for greater, longer, and more reproducible CYP2A6 inhibition than that provided by single-dose methoxsalen.

Several strategies are available for identifying predominant cytochrome P-450s (CYPs)¹ responsible for human drug metabolism in vivo. These include investigations with genotypically variant individuals, population analysis correlating rates of candidate drug metabolism with P-450 isoform catalytic activity or protein content, enzyme induction, and isoform-selective competitive or mechanism-based inhibitors. Mechanism-based P-450 inhibitors are particularly useful because they often have considerable isoform specificity, can be administered in single doses that also enhance specificity of isoform inhibition, confer inhibition that is terminated by enzyme resynthesis rather than inhibitor washout, and can be used to ameliorate P-450-mediated toxicities. For example, single-dose furafylline, disulfiram, and troleandomycin are effective and selective mechanism-based inhibitors. P-450 isoforms responsible for biotransformation in vivo (Sesardic et al., 1990; Periti et al., 1992; Kharasch et al., 1993, 1996, 1997).

CYP2A6 is a quantitatively minor component of the human hepatic P-450 pool, comprising 1 to 5% of total P-450. Nevertheless, CYP2A6 participation on the human metabolism of important drugs and xenobiotics has been increasingly recognized. Numerous CYP2E1 substrates also have been shown to undergo metabolism by CYP2A6, such as nitrosamines, butadiene, methoxyflurane, halothane, and acetaminophen (Crespi et al., 1990; Honkakoski and Negishi, 1997; Spracklin et al., 1997; Chen et al., 1998; Oscarson et al., 1998). In addition, CYP2A6 has been shown to be the predominant catalyst of human liver microsomal oxidation of nicotine (Berkman et al., 1995; Messina et al., 1997), cotinine (Nakajima et al., 1996a), hexamethylenephosphoramidethanemethoxyflurane (Thorton-Manning et al., 1997), SM-12502 (Nunoya et al., 1996), and valproic acid (Sadeque et al., 1997), and the reduction of halothane (Spracklin and Kharasch, 1998). Several CYP2A6 substrates are procarcinogens and/or environmental contaminants, but others are therapeutic drugs, such as volatile anesthetics, acetaminophen, disulfiram, valproic acid, and SM-12502 (a platelet-activating receptor antagonist). The prototypic CYP2A6-catalyzed reaction in human liver microsomes is coumarin 7-hydroxylation, which is also the most commonly used in vivo probe for human CYP2A6 activity. CYP2A6 substrates have recently been re-reviewed (Honkakoski and Negishi, 1997; Oscarson et al., 1998).

Methoxsalen was initially identified as a potent, mechanism-based microsomal P-450 inhibitor by Pessayre and coworkers (Tinel et al., 1987). It was subsequently shown that methoxsalen was the most potent, and a rather selective, inhibitor of human liver microsomal CYP2A6 activity (Maenpaa et al., 1994; Ono et al., 1996; Draper et

¹Abbreviations used are: CYP, cytochrome P-450; AUC, area under the curve.

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METHOXSALEN INHIBITION OF HUMAN CYP2A6

al., 1997; Koenigs et al., 1997). Although initial reports suggested that methoxsalen inhibition appeared mechanism based (Tinel et al., 1987), Maenpaa et al. (1994) concluded that methoxsalen was not a substrate for CYP2A6. More recent investigations have clearly demonstrated, however, that methoxsalen is a substrate for CYP2A6 and that inhibition of expressed and microsomal CYP2A6 is mechanism based (Draper et al., 1997; Koenigs et al., 1997; Koenigs and Trager, 1998). Methoxsalen inhibition of liver microsomal CYP2A6 occurs at low concentrations (Maenpaa et al., 1994; Ono et al., 1996; Draper et al., 1997; Koenigs et al., 1997), and is very rapid (Draper et al., 1997; Koenigs et al., 1997).

Mechanism-based rapid inhibition of CYP2A6, together with in vivo concentrations approaching the microsomal Km, suggest that methoxsalen may have utility as an in vivo inhibitor of human CYP2A6 activity and as a clinical probe for CYP2A6-catalyzed drug metabolism. Nevertheless, there is little information regarding methoxsalen effects on in vivo CYP2A6 activity in humans. There is only a single report of methoxsalen effects on CYP2A6 activity, which suggested 47 ± 21% inhibition of urine 7-hydroxycoumarin excretion (Maenpaa et al., 1994). However, we recently administered methoxsalen before a known CYP2A6 substrate (halothane) in humans, and observed only brief inhibition of CYP2A6-mediated halothane oxidation (E.D.K., D.C.H., K. Fenstemaker, and K. Cox, submitted). This unexpected result, along with the limited information on methoxsalen effects in vivo, demonstrates the need for a more thorough characterization of methoxsalen effects on human CYP2A6 activity. This investigation explored the effect of single-dose oral methoxsalen, administered in therapeutic doses, on human CYP2A6 activity in vivo, with coumarin 7-hydroxylation as the CYP2A6 probe.

Materials and Methods

Patient Selection and Clinical Protocol. Eight male and four nonpregnant female nonsmoking volunteers age 28 ± 3 years (range 26–37) and 73 ± 13 kg participated in the investigation after written informed consent was obtained. The investigational protocol was approved by the Institutional Human Subjects Committee. Subjects were in good health, within 20% of ideal body weight, had no history of hepatic or renal disease, and were taking no prescription or over-the-counter medications during the course of the investigation (one subject had a levonorgestrel implant). Exclusion criteria included any of the following: allergy to methoxsalen or coumarin, melanoma, idiosyncratic reactions to psoralen compounds, light-sensitive diseases (lupus erythematosus, porphyria cutanea tarda, erythropoietic protoporphyria, variegate porphyria, xeroderma pigmentosum, albinism), invasive squamous cell carcinoma, or aphakia (congenital or acquired). Subjects abstained from caffeine, grapefruit, grapefruit juice, and ethanol beginning the day before each study day and continuing throughout the period of urine collection.

Coumarin (>99% pure) was synthesized as described previously, purified by silica gel chromatography, recrystallized from ether, and formulated in gel capsules (Kharasch et al., 1998). It was used under an Investigational New Drug exemption from the Food and Drug Administration. The investigation was a crossover design, with subjects randomly assigned first to the control or methoxsalen phase and a washout period of 1 to 2 weeks between phases. Subjects received 50 mg of coumarin with 150 ml of water after an overnight fast, followed 2 h later by breakfast. Pretreated subjects received methoxsalen (ICN Pharmaceuticals, Costa Mesa, CA) orally with 100 to 200 ml of water at the following dose: 50 to 65 kg, 30 mg; 66 to 80 kg, 40 mg; 81 to 90 kg, 50 mg; and 90 kg, 60 mg. Methoxsalen was administered 90 min before coumarin. The timing of methoxsalen administration was based in part on a previous investigation (Maenpaa et al., 1994). Subjects wore UV-absorbing glasses during daylight for the next 8 h, and avoided sun exposure for the remainder of the day. Controls received nothing. Subjects drank at least 150 ml of water per hour throughout the day to permit adequate urine collection.

Venous blood was obtained through an indwelling catheter before and for 8 h after coumarin administration. Plasma was separated and stored at −20°C for later analysis. Urine was collected at specified intervals for 24 h after coumarin administration, the volume was recorded, and an aliquot stored at −20°C until analyzed.

Analytical Methods. Plasma 7-hydroxycoumarin concentrations were measured by HPLC with a modification of a previous method (Kharasch et al., 1998). Plasma (0.5 ml) and 0.5 ml of β-glucuronidase (5000 U/ml in 1.0 M potassium acetate, pH 5.0) were incubated overnight at 37°C, then the internal standard (7-aminouracil, 125 ng) was added, followed by 200 μl of trichloroacetic acid (20% v/v). Tubes were vortexed for 60 s, centrifuged at 3500g for 10 min, and the supernatant transferred into polypropylene tubes. Diethyl ether (5 ml) was added, tubes were capped and vortexed for 5 min, centrifuged for 5 min at 4000g, and the ethereal layer was transferred to glass tubes and evaporated to dryness under nitrogen at 35°C. Samples were reconstituted in 100 μl of methanol, and transferred to autosampler vials. Chromatography was performed with a Microsorb reversed phase C18 column (4.6 × 50 mm, 3 μm) (Rainin Instrument Co., Inc., Woburn, MA) with a Hewlett Packard Series 1050 Series HPLC consisting of an autosampler (sample size 10 μl) and quaternary pump, coupled to a Kratos Spectoflow 980 fluorescence detector (374-nm excitation, 495-nm emission filter), with the ChemStation data system used for system control and data analysis. The mobile phase (1 ml/min) was initially methanol/aqueous acetic acid (0.2%) (30:70). The methanol content was constant for 6 min, then increased linearly from 30 to 47.5% between 6 and 8 min, further increased linearly to 82.5% between 8 and 10 min, held for 1 min, and then returned to 30% over the next 4 min where it remained for 5 min for re-equilibration. Typical elution times for 7-hydroxycoumarin and the internal standard were 4.0 and 5.6 min. Standard curves were constructed by analyzing blank plasma containing 10 to 5000 ng/ml 7-hydroxycoumarin, and were linear (r2 > 0.99). The limit of quantification was the lowest point on the standard curve.

Urine 7-hydroxycoumarin concentrations were similarly determined. Urine (0.5 ml) was hydrolyzed overnight with 0.5 μl of β-glucuronidase (5000 U/ml in 0.1 M potassium acetate, pH 5.0), and the internal standard 7-ethoxycoumarin (2.5 μg) was added. Samples were extracted with 4 ml of diethyl ether, evaporated to dryness, and reconstituted in 100 μl of methanol. Chromatography was performed as described above with a Rainin Microsorb C8 column (4.6 × 250 mm, 5 μm) with an isotropic mobile phase of 40:60 acetonitrile/water (0.2% acetic acid) at 1 ml/min. Retention times for 7-hydroxycoumarin and 7-ethoxycoumarin were 9.9 and 11.3 min, respectively. Urine 7-hydroxycoumarin was quantified with a standard curve of peak area ratios (7-hydroxycoumarin/7-ethoxycoumarin) versus 7-hydroxycoumarin (0.2–50 μg/ml) that was linear (r2 > 0.99).

Plasma methoxsalen concentrations were measured by HPLC with a modification of a previous method (Said et al., 1997). Plasma (0.5 ml) was treated with the internal standard 5-methoxypsoralen (80 ng) and extracted with 1 ml of chloroform by vortexing for 3 min, centrifuging, and removing the aqueous/lipid layer by aspiration. The chloroform layer was transferred to glass tubes and evaporated to dryness under nitrogen at 30°C. Samples were reconstituted in 50 μl of methanol, and transferred to autosampler vials. Chromatography was performed with a Rainin Microsorb C18 column (4.6 × 50 mm, 3 μm) on the system described above, with a Spectoflow 980 fluorescence detector (313-nm excitation, 495-nm emission filter). The isotropic mobile phase was 46:54 methanol/water (0.2% acetic acid) at 1 ml/min. Typical elution times for methoxsalen and the internal standard were 3.0 and 5.6 min, respectively. Standard curves (linear, r2 > 0.99) were constructed by analyzing blank plasma containing 3.9 to 1000 ng/ml methoxsalen.

Data Analysis. Plasma and urine 7-OH-coumarin concentrations in the control and methoxsalen sessions were analyzed by two-way repeated-measures ANOVA followed by post hoc Student Newman-Keuls tests (SigmasStat 2.03; SPSS, Chicago, IL). Pharmacokinetic parameters were determined by noncompartmental methods (WinNonlin I.5; Scientific Consulting, Inc., Cary, NC). Student’s paired t test was used to assess the significance of differences between values for control and treatment groups. Significance was assigned at P < .05. Results are shown as means ± S.D.

Results

Coumarin disposition in untreated subjects resembled that reported previously (Kharasch et al., 1998). Plasma 7-hydroxycoumarin con-
centrations in methoxsalen-treated subjects was significantly reduced compared with controls up to 2 h after coumarin administration (Fig. 1). $C_{\text{max}}$ was significantly decreased (0.8 ± 0.3 versus 1.4 ± 0.5 µg/ml, $P < .05$) by methoxsalen, and $T_{\text{max}}$ was prolonged, although the difference did not reach statistical significance (98 ± 33 versus 70 ± 25 min, $P = .07$). There was a small (24 ± 11%) but statistically significant decrease in the 7-hydroxycoumarin area under the curve (AUC) (134 ± 32 versus 186 ± 34 µg·min·ml$^{-1}$) after methoxsalen pretreatment (Fig. 2). There was considerable variability in the decrease, however, which ranged from 6 to 44%.

Urine 7-hydroxycoumarin excretion is shown in Fig. 3. Excretion was diminished in urine collected 0 to 1 and 1 to 2 h after coumarin, but not thereafter. Neither 0- to 8-h nor total urine 7-hydroxycoumarin excretion were significantly decreased by methoxsalen.

Plasma methoxsalen concentrations also were measured (Fig. 4). The following kinetic parameters were obtained: $C_{\text{max}}$ 224 ± 122 ng/ml, $T_{\text{max}}$ 104 ± 40 min, AUC$_{0\rightarrow\infty}$ 470 ± 203 ng·h·ml$^{-1}$, CL/F 24.9 ± 14.4 ml·min·kg$^{-1}$, $t_{1/2}$ 73 ± 35 min, and $V_d/F$ 2.46 ± 1.46 l/kg. These were similar to those found previously (Herfst and De Wolff, 1983).

There was considerable intersubject variability in plasma methoxsalen disposition ($C_{\text{max}}$ 85 to 507 ng/ml and AUC$_{0\rightarrow\infty}$ 134 to 798 ng·h·ml$^{-1}$).

**Discussion**

Results of this investigation show that single-dose methoxsalen (0.5–0.6 mg/kg p.o.) significantly, although briefly and moderately, diminished coumarin 7-hydroxylation when administered 1.5 h before coumarin. Plasma 7-hydroxycoumarin concentrations 0.5 to 2 h after coumarin, $C_{\text{max}}$, and AUC$_{0\rightarrow\infty}$ were significantly diminished after methoxsalen treatment, as was urinary 7-hydroxycoumarin excretion 0 to 2 h after coumarin. These results suggest that single-dose methoxsalen did inhibit CYP2A6 activity.

Nevertheless, the extent of inhibition was relatively small and less than that reported previously. Maenpaa et al. (1994) gave five volunteers 45 mg of methoxsalen 1 h before 5 mg of coumarin, and found 47 ± 21% inhibition of 7-hydroxycoumarin excretion in 8-h urine. In contrast, we observed no significant decrease in 0- to 8-h urine 7-hydroxycoumarin excretion, which was diminished for only 2 h after coumarin administration. Furthermore, and consistent with the urine data, plasma 7-hydroxycoumarin concentrations were decreased for only 0.5 to 2 h, and there was only a 24% decrease in plasma 7-hydroxycoumarin AUC$_{0\rightarrow\infty}$, whereas $C_{\text{max}}$ was diminished by 44%.

These results are consistent with only brief inhibition of CYP2A6 activity, and minimal changes in the fraction of coumarin metabolized by 7-hydroxylation. One major difference in the conduct of these two investigations is the higher coumarin dose used in the present compared with the previous investigation (50 versus 5 mg).

There was considerable interindividual variability in the methoxsalen-related decrease in 7-hydroxycoumarin $C_{\text{max}}$ (25–71%) and AUC$_{0\rightarrow\infty}$ (6–44%). Methoxsalen is extensively metabolized, has a high systemic clearance (12 ± 3 ml·min·kg$^{-1}$ after i.v. administration) (Billard et al., 1995), and an even higher apparent oral clearance [25 ± 14 ml·min·kg$^{-1}$ in this investigation and 180 ± 178 l/h reported previously (Herfst and De Wolff, 1983)], suggesting extensive first-pass clearance and low bioavailability. These have been evaluated directly, and confirmed (Schmid et al., 1980; Sullivan et al., 1986). More importantly, there is considerable inter- and intrasubject variability in methoxsalen disposition (Herfst and De Wolff, 1983; Walther and Haustein, 1991). Apparent oral clearance varied from 50 to 650 liters/h (Herfst and De Wolff, 1983), and methoxsalen treatment failures appear related to low plasma drug concentrations.

Therefore, we examined the relationship between methoxsalen disposition and effects on coumarin 7-hydroxylation. A significant positive correlation was observed between methoxsalen $C_{\text{max}}$ and the decrease in plasma 7-hydroxycoumarin $C_{\text{max}}$ (Fig. 5A). There was
also a significant and negative correlation between methoxsalen mean residence time and the decrease in 7-hydroxycoumarin $C_{\text{max}}$ (data not shown). An oral drug, apparent mean residence time represents the sum of residence time in the body and absorption time. Thus, the relationship between methoxsalen $T_{\text{max}}$ and the decrease in plasma 7-hydroxycoumarin $C_{\text{max}}$ was examined, and a significant negative correlation was observed (Fig. 5B), suggesting that the negative correlation between methoxsalen mean residence time and CYP2A6 inhibition was due largely to variability in methoxsalen absorption. We administered methoxsalen 90 min before coumarin, even farther in advance than Maenpaa et al. (1994), who gave it 60 min before coumarin (Maenpaa et al., 1994). Nevertheless, peak plasma methoxsalen concentrations occurred after coumarin was administered in several subjects, and these subjects not surprisingly showed lesser CYP2A6 inhibition. Thus, interindividual variability in methoxsalen absorption and first-pass clearance appeared to be a major source of the variability in methoxsalen inhibition of CYP2A6. This might argue for increasing the interval between methoxsalen and probe drug administration; however, rapid methoxsalen elimination may negate this advantage. Indeed, the positive correlation between methoxsalen $C_{\text{max}}$ and CYP2A6 inhibition supports this concern. In contrast, there was no significant relationship between methoxsalen disposition ($C_{\text{max}}$, $T_{\text{max}}$, or AUC) and the change in 7-hydroxycoumarin AUC$_{0\rightarrow\infty}$ (data not shown). Clearly, the timing and frequency of methoxsalen administration relative to the CYP2A6 probe drug requires further investigation.

Single-dose methoxsalen effects on coumarin hydroxylase activity were substantially different from the in vivo effects of other mechanism-based CYP inhibitors. Single-dose oral disulfiram, administered 10 h before the CYP2E1 probe chlorozoxazone, diminished chlorozoxazone elimination clearance and 6-hydroxychlorozoxazone formation clearance to 10 to 15% and 6 to 7% of control values, respectively (Kharasch et al., 1993). Similarly, single-dose oral troleandomycin, administered 2 h before the CYP3A4 probe midazolam, reduced plasma midazolam clearance to 24% of control values (Kharasch et al., 1997). A single 90-mg furafylline dose administered 90 min before caffeine markedly impaired CYP1A2-catalyzed caffeine $N$-demethylation and increased caffeine elimination half-life 10-fold, suggesting 90% inhibition of caffeine clearance (Tarrus et al., 1987).

In vitro-in vivo comparisons are instructive in deciphering the differing effects of these mechanism-based inhibitors. For furafylline, in vitro, the $K_i$ for hepatic CYP1A2 was 0.7 to 3 $\mu$M without (Sesardic et al., 1990; Clarke et al., 1994; Bourrié et al., 1996) or 0.6 $\mu$M with preincubation (Bourrié et al., 1996), although a higher $K_i$ (23 $\mu$M) also was reported (Kunze and Trager, 1993). The $K_i$ for expressed CYP1A2 was 7 $\mu$M (Tassaneeyakul et al., 1994). CYP1A2 inhibition was rapid, with 90% inactivation after 5 min (Kunze and Trager, 1993). The $k_{\text{inact}}$ was 0.87 min$^{-1}$ (Kunze and Trager, 1993) and 0.27 min$^{-1}$ (Clarke et al., 1994) for human microsomes, and 0.2 min$^{-1}$ (Kunze and Trager, 1993) and 0.07 min$^{-1}$ (Tassaneeyakul et al., 1994) for expressed CYP1A2. Diethyldithiocarbamate inactivation of human liver microsomal CYP2E1 is rapid and near complete, with 90% inhibition in a single phase with a 1.5 min half-life and $k_{\text{inact}}$ of 0.4 min$^{-1}$ (Guengerich et al., 1991). The $IC_{50}$ (equivalent to $K_i$ for a noncompetitive inhibitor) in human liver microsomes was 10 to 30 $\mu$M (Guengerich et al., 1991; Newton et al., 1995). The CYP3A inhibitor troleandomycin forms an inactive enzyme-inhibitor complex. The $IC_{50}$ for human liver microsomal CYP3A4 (testosterone 6β-hydroxylation) was <5 $\mu$M without (Newton et al., 1995), and 1 $\mu$M with preincubation (Yamazaki and Shimada, 1998). Inactivation was rapid, with maximal inhibitor complex formation achieved after 5 min (Yamazaki and Shimada, 1998). Thus, the above-described in vivo inhibition of CYP 1A2, 2E1, and 3A4 activities, respectively, by single-dose furafylline, disulfiram, and troleandomycin is highly consistent with the in vitro effects of these inhibitors.

In contrast, the magnitude and duration of single-dose methoxsalen inhibition of CYP2A6 activity in vivo were unanticipated, based on in vitro methoxsalen effects. Methoxsalen is a potent, effective, and rapid mechanism-based inhibitor of human liver microsomal and expressed CYP2A6. The $K_i$ for inhibition of human liver microsomal coumarin hydroxylase activity was reported as 1.5 $\mu$M (Maenpaa et al., 1994), 1.9 $\mu$M (Koenigs et al., 1997), and 0.26 $\mu$M without or 0.04 $\mu$M with a 5-min preincubation (Draper et al., 1997). The $K_i$ for expressed CYP2A6 was 0.8 $\mu$M (Koenigs et al., 1997), and 0.33 $\mu$M without or 0.06 $\mu$M with preincubation (Draper et al., 1997). Inactivation was rapid, with 90 to 95% inhibition of microsomal CYP2A6 activity after a 5-min preincubation (Koenigs et al., 1997). The $k_{\text{inact}}$ was 2.1 min$^{-1}$ in human liver microsomes (Koenigs et al., 1997) and 1.0 min$^{-1}$ (Koenigs et al., 1997) or 0.5 min$^{-1}$ (Draper et al., 1997) with expressed enzyme. Among a series of psoralen analogs, methoxsalen was the most potent mechanism-based inhibitor of expressed CYP2A6. The $K_i$ for inhibition of expressed CYP1A2, 2E1, and 3A4 activities, respectively, by single-dose furafylline, disulfiram, and troleandomycin are unknown. Peak plasma furafylline after a single 90-mg dose was ~8 $\mu$M, and remained elevated for several days, with an elimination half-life of 50 h (Tarrus et al., 1987). Thus, furafylline plasma concentrations in vivo are at or above the $K_i$ for CYP1A2 mechanism-based inactivation. In vitro-in vivo correlations with disulfiram are more difficult because disulfiram is rapidly and extensively metabolized in vivo (Faiman et al., 1984) and in vitro (Madan et al., 1998), and the identity of the metabolite(s) responsible for CYP2E1 inhibition are unknown. Peak diethyldithiocarbamate methyl ester, diethyldithiocarbamate methyl ester, and carbon disulfide concentrations were 0.3, 0.3, and 3 $\mu$M after 400 mg of disulfiram (Johansson and Stankiewicz, 1989; Cardoli et al., 1994); diethyldithiocarbamate methyl ester and carbon disulfide were 1 and 200 $\mu$M after 500 mg (Jensen et al., 1982); and disulfiram, diethyldithiocarbamate, and diethyldithiocarbamate methyl ester concentrations were 1 to 3 $\mu$M, whereas carbon disulfide was 200 $\mu$M (Faiman et al., 1984). IC$_{50}$ values for inhibition of human liver
microsomal CYP2E1 (chlorzoxazone hydroxylation) were 1 to 5 μM for diethylthiocarbamate, diethylthiocarbamate methyl ester, and carbon disulfide (E.D.K., unpublished data). Thus, disulfiram metabolite plasma concentrations in vivo are at or above the IC₅₀ values for CYP2E1 mechanism-based inactivation. Plasma troleandomycin was ~1 μM 2 h after 500 mg and peak concentrations were ~1.6 μM; however, tissue levels are generally severalfold greater (Georgiew et al., 1978). Thus, it appears that troleandomycin concentrations in vivo are at or above the IC₅₀ values for CYP2A6 mechanism-based inactivation.

In contrast, single-dose oral methoxsalen in conventional doses may not afford sufficient hepatic concentrations in vivo for CYP2A6 inhibition. Plasma methoxsalen was 0.4 to 2 μM, which is nominally within the range of Kᵢ values observed for in vitro inhibition of microsomal CYP2A6. Nevertheless, methoxsalen is ~90% protein bound (Busch et al., 1978), thus reducing free plasma (and possibly hepatic) concentrations to 0.04 to 0.2 μM, which is near or below the in vitro Kᵢ.

In addition, methoxsalen is rapidly metabolized in vivo (Busch et al., 1978), presumably in the liver, and this would further reduce parent drug concentrations. Metabolites include 8-hydroxypsoralen (Koenigs and Trager, 1998) and unspecified glucuronides (Busch et al., 1978), presumably indicating methoxsalen hydroxylation. These hydroxyl metabolites are comparatively poor CYP2A6 inhibitors (Koenigs and Trager, 1998), thus, methoxsalen metabolite formation further diminishes the parent drug fraction available to the CYP2A6 inactivation pathway in vivo.

Further highlighting the importance of in vivo inhibitor concentrations is that methoxsalen exhibits some in vivo characteristics of a competitive inhibitor. For example, the dependence of CYP2A6 inhibition on plasma methoxsalen concentration and brief duration of CYP2A6 inhibition, combined with rapid methoxsalen elimination (1-h half-life), is consistent with an early, reversible effect rather than mechanism-based inhibition. In addition, methoxsalen inhibition of CYP2A6 appears dependent on substrate concentration and Kᵢₘ. For example, inhibition of CYP2A6 was greater after 5 mg (47% inhibition of total urine 7-hydroxycoumarin excretion) (Maenpaa et al., 1994) compared with 50 mg of coumarin (Fig. 3, no inhibition). Furthermore, similar doses of methoxsalen inhibited the CYP2A6-dependent clearance of nicotine (Kᵢₘ = 60–90 μM) (Nakajima et al., 1996b; Messina et al., 1997) by 30 to 50% (R. Tyndale, personal communication), but CYP2A6-catalyzed metabolism of coumarin (Kᵢₘ = 0.2–0.7 μM) (Pearse et al., 1992; Draper et al., 1997; Koenigs et al., 1997) by less. This behavior requires further characterization.

These data suggest that single-dose methoxsalen is a partially effective but not optimal clinical CYP2A6 inhibitor probe. CYP2A6 inhibition, however, may be amenable to improvement. We used a conventional, therapeutic dose of methoxsalen, administered orally. Higher or multiple oral doses might achieve greater CYP2A6 inhibition. Alternatively, i.v. administration, which provides sustained plasma methoxsalen concentrations (Billard et al., 1995), and eliminates first-pass clearance, also may confer greater CYP2A6 inhibition. Further investigations are necessary to test these hypotheses.

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