MECHANISM-BASED INHIBITION OF HUMAN LIVER MICROSOMAL CYTOCHROME P450 1A2 BY ZILEUTON, A 5-LIPOXYGENASE INHIBITOR

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ABSTRACT:

Zileuton, a 5-lipoxygenase inhibitor, was evaluated as an inhibitor of cytochrome P450 activity in human liver microsomes. In the absence of preincubation, the racemate was found to be a weak inhibitor (IC₅₀ > 100 μM) of phenacetin O-deethylation (POD) (CYP1A2), p-acetamidoacetophenone 6β-hydroxylation (CYP2C8), diclofenac 4'-hydroxylation (CYP2C9), (S)-mephenytoin 4'-hydroxylation (CYP2C19), bufuralol 1'-hydroxylation (CYP2D6), testosterone 6β-hydroxylation (CYP3A4), chloroxazone 6-hydroxylation (CYP2E1), and bupropion hydroxylation (CYP2B6). When preincubated with NADPH-fortified human liver microsomes in the absence of substrate, zileuton (racemate) was shown to inhibit POD. The effect was NADPH-, time-, and concentration-dependent, and was characterized by a k₈ (maximal rate of enzyme inactivation) and apparent Kᵢ (inhibitor concentration that supports half the maximal rate of inactivation) of 0.035 min⁻¹ and 117 μM, respectively (k₈/Kᵢ ratio of 0.0003 min⁻¹ μM⁻¹). Preincubation-dependent inhibition of POD activity was also observed with the individual (S)-(−) and (R)-(−)-enantiomers of zileuton [S-(−)]zileuton; k₈ = 0.037 min⁻¹, Kᵢ = 98.2 μM, k₈/Kᵢ ratio = 0.0004 min⁻¹ μM⁻¹; (R)-(−)zileuton; k₈ = 0.012 min⁻¹, Kᵢ = 66.6 μM, k₈/Kᵢ ratio = 0.0002 min⁻¹ μM⁻¹). In addition, the inhibition of CYP1A2 was not reversed in the presence of reduced glutathione, catalase, and superoxide dismutase and was refractory to dialysis. Therefore, zileuton was characterized as a mechanism-based inhibitor of human liver microsomal CYP1A2. Mechanism-based inhibition of CYP1A2 may explain why zileuton decreases the oral clearance of antipyrine, propranolol, (R)-warfarin, and theophylline, at doses that have a minimal effect on the pharmacokinetics of (S)-warfarin,phenytoin, and terfenadine.

Zileuton (Zyflo; N-(1-benzoyl-2-thienyl-2-ylthio)-N-hydroxyurea), a substituted hydroxamic acid, is a potent and selective 5-lipoxygenase inhibitor that has been approved for the prevention and chronic treatment of asthma (Isreal et al., 1990; Carter et al., 1991; Bell et al., 1992; Wenzel and Kamada, 1996; Dube et al., 1999). The drug contains a single chiral center (Fig. 1), is administered as a racemic mixture of (R)-(−) and (S)-(−)-enantiomers, and is characterized by a plasma half-life of about 4 h (Wong et al., 1995). As a result of the short half-life, zileuton has to be dosed four times a day (q.i.d.), and a plasma half-life of about 4 h (Wong et al., 1995). Although N-hydroxy glucuronidation has been confirmed with human liver microsomes, zileuton is metabolized in the presence of NADPH (Machinist et al., 1995b; Sweeny and Nellans, 1995). In fact, CYP1A2 and CYP2C9 catalyze ring hydroxylation, whereas sulfoxidation is catalyzed largely by CYP3A4. Both reactions conform to Michaelis-Menten kinetics in human liver microsomes and are described by relatively high Kᵢ values (≥ 0.2 mM).

Zileuton also has been shown to be a weak inhibitor of human liver microsomal CYP3A4, CYP2C9, and CYP2D6 activity in vitro (Machinist et al., 1995b). As anticipated, zileuton has a minimal effect on the pharmacokinetics of drugs that are metabolized by CYP2C9 [e.g., (S)-warfarin, phenytoin, and naproxen] and CYP3A4 (e.g., terfenadine) (Awni et al., 1995b,c, 1997; Samara et al., 1995). In contrast, zileuton decreases the oral clearance of antipyrine, propranolol, (R)-warfarin, and theophylline, which indicates that it is a clinically relevant inhibitor of CYP1A2 (Awni et al., 1995c; Granneman et al., 1995; Machinist et al., 1995b; St. Peter et al., 1995). The effect of zileuton on CYP1A2 has been evaluated using precision-cut liver slices, and the compound was shown to inhibit (≈ 60%) theophylline metabolism at a final concentration of 100 μM (Machinist et al., 1995b). However, the studies were rather limited in scope, and no attempt was made to elucidate the mechanism of inhibition or to conduct in vitro-in vivo correlations. Therefore, the aim of the present study was to further evaluate the interaction of zileuton with the human liver microsomal cytochrome P450 (P450) system. During the

1 Abbreviations used are: P450, cytochrome P450; AUC, area under the plasma concentration versus time curve; AUC₉₅%, AUC (oral dose) in the presence of inhibitor; AUC₉₅% AUC (oral dose) in the absence of inhibitor; C₉₅%, maximum concentration in plasma at steady state; C₉₅%, average concentration in plasma at steady state; LC/MS/MS, liquid chromatography/tandem mass spectrometry; k₉₅%, maximal rate of enzyme inactivation; Kᵢ, inhibitor concentration that supports the maximal rate of inactivation; [I]ₙ, free inhibitor concentration; fₚ, fraction unbound in plasma; fₚ,AUC₉₅%, fraction of dose metabolized by all cytochromes P450; fₚ,CYP1A2, fraction of cytochrome P450-dependent metabolism catalyzed by CYP1A2; k₉₅, observed rate of enzyme inactivation; kᵢ, rate of cytochrome P450 holoenzyme degradation in
analitical or high-performance liquid chromatography grade. Zileuton was isolated from Zyllo filmtab tablets (600 mg) by crushing them with a mortar and pestle, and partitioning the resultant white solid between water and ethyl acetate. The organic layer was filtered through a 0.45-μm membrane, then washed with water, dried over Na2SO4, and re-filtered through a second 0.45-μm membrane. The filtrate was concentrated to give a white solid, which was recrystallized from acetonitrile to give zileuton as clear needles (>99% pure by reverse phase-high performance liquid chromatography with online UV detection at 210 and 254 nm). The identity of the recrystallized material was confirmed by proton NMR and LC/MS-MS. Separation of racemic zileuton using a published procedure (Thomas et al., 1992) yielded the individual (R)(+)- and (S)(−)-enantiomers (>99.8% purity).

**Incubations with Human Liver Microsomes.** CYP1A2 (POD), CYP2B6 (bupropion hydroxylation), CYP2C8 (paclitaxel 6α-hydroxylation), CYP2C9 (diclofenac 4′-hydroxylation), CYP2C19 ([S]-mephenytoin 4′-hydroxylation), CYP2D6 (bufuralol 1′-hydroxylation), CYP2E1 (chlorozoxazone 6-hydroxylation), and CYP3A4 (testosterone 6β-hydroxylation) activity in human liver microsomes was determined using accepted P450 form marker assays (Hesse et al., 2000; Madan et al., 2002).

The incubation mixtures (0.2–0.5 ml) contained microsomal protein (0.2–0.5 mg/ml final concentration) and 100 mM potassium phosphate buffer (pH 7.4). The reaction was initiated with NADPH (1 mM) and was allowed to proceed for 10 to 30 min (37°C in a shaking bath). In all cases, no more than 10% of the substrate was consumed. All chemical inhibitors and substrates were dissolved in 50% (v/v) acetonitrile/water. The final volume of acetonitrile in the incubation was less than 1% (v/v). Activity in the presence of solvent alone was assigned as “control” (100%). For paclitaxel 6α-hydroxylation (CYP2C8) and bupropion hydroxylation (CYP2B6), the reaction was terminated with the addition of methylene chloride (4 volumes). Baccatin and triprolidine were added as internal standards, respectively. Thereafter, the samples were vortexed and centrifuged (3,500 rpm for 10 min), and the supernatant was separated and dried under nitrogen gas. The resulting residue was dissolved in 100 μl of acetonitrile, and aliquots (20 μl) were analyzed by LC/MS-MS. For the other assays, the reactions were terminated with acetonitrile (2 volumes), containing an appropriate amount of internal standard (4′-hydroxybutyranilide, flufenamic acid, phenytoin, propranolol, L-594615, and cortisone for the CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 assays, respectively). The samples were vortexed and centrifuged (3,500 rpm for 10 min), and the supernatant was separated and diluted with an equal volume of 0.05% formic acid in water. Aliquots (20 μl) were subjected to LC/MS-MS analysis.

Mechanism-based inhibition of CYP1A2, CYP2C9, and CYP3A4 was also evaluated. NADPH-activated human liver microsomes (2.0 mg/ml final concentration) were incubated at 37°C (up to 30 min) in the presence of solvent alone, or zileuton (racemic mixture or individual enantiomers), in a shaking water bath (final volume of 0.2 ml). At the appropriate times, aliquots (25 μl) of the incubate were removed and added to separate tubes containing 225 μl of fresh buffer preheated at 37°C. Substrate (phenacetin, diclofenac, and testosterone at a final concentration of 120, 100, and 250 μM, respectively) and NADPH (1.0 mM) were added, and the reaction was allowed to proceed. At the required time, the reaction was terminated as described above. Incubations carried out in the presence of solvent alone (time 0) were designated controls (100%).

**LC/MS-MS Analysis.** Chromatography was conducted on a PerkinElmer high-performance liquid chromatography system, equipped with a series 200 liquid chromatography pump and autosampler, interfaced to a Sciex API 2000 tandem mass spectrometer with an atmospheric pressure chemical ionization ion source operating in the positive or negative (chlorzoxazone assay only) ion mode (PerkinElmerSciex Instruments, Boston, MA). For the POD assay, separations were carried out on an Aquasep column (2.0 × 50 mm, 5 μm; ES Industries, West Berlin, NJ). Mobile phase A consisted of 0.05% formic acid in water and mobile phase B of 0.05% formic acid in acetonitrile (constant flow rate of 1.5 ml/min). The composition of mobile phase B was increased from 5% to 50%. Both product (acetaminophen; mlz; 151.9 and 110.1) and internal standard (4′-hydroxybutyranilide; mlz; 180.2 and 71.0) were resolved.

Analyses of the paclitaxel and mephenytoin incubates were carried out using Zorbax 300 Extend C18 and Zorbax SB-Aq columns (4.6 × 50 mm, 5 μm; MAC-MOD Analytical Inc., Chadds Ford, PA), respectively. Mobile
In the in vitro data describing the mechanism-based inhibition of CYP1A2 by zileuton were used to rationalize previously observed clinical drug interactions. For mechanism-based inhibitors, one can attempt to evaluate the effect on the rate of synthesis of the enzyme. Therefore, with some underlying assumptions (Mayhew et al., 2000; Jones and Hall, 2002), it is possible to use the in vitro data ($k_{\text{inact}}/K_i$ ratio) and simulate changes in substrate AUC over a range of $f_m$ - $f_m,CYP3A4$ values (e.g., 0.2–0.95).

For the effect of zileuton on CYP1A2, the in vitro $K_i$ and $k_{\text{max}}$ data were used in the simulations, in addition to estimates of $K_i$ (Correia, 1991; Ito et al., 1998; Mayhew et al., 2000). The steady-state concentrations of zileuton in plasma ($C_{\text{max}}$ and $C_{\text{ave}}$) were corrected for binding ($f_p = 0.069$) to human plasma proteins in vitro (Awini et al., 1995a; Machinist et al., 1995a). No attempt was made to estimate the concentration of zileuton in the liver or in the portal vein following an oral dose (Ito et al., 1998). The simulated changes in AUC were then compared with the known AUC changes observed with (R)-warfarin, theophylline, propranolol, and antipyrine (Awini et al., 1995c; Granneman et al., 1995; Machinist et al., 1995b; St. Peter et al., 1995). Estimates of $f_p$ for each of these drugs were based on the reported fraction of the dose recovered in human excreta as unchanged parent drug, P450 and non-P450 metabolites (Walle et al., 1985; Troger and Meyer, 1995; Engel et al., 1996; Kaminsky and Zhang, 1996; Yao et al., 2001). Values for $f_p$ were published in vitro P450 reaction phenotyping data (Masubuchi et al., 1994; Ha et al., 1995; Yoshimoto et al., 1995; Zhang and Kaminsky, 1995; Engel et al., 1996; Kaminsky and Zhang, 1996; Shaler and Wrighton, 1996; Tjia et al., 1996; Johnson et al., 2000).

Results

Inhibition of P450 Activities. Zileuton was evaluated as a reversible inhibitor of P450 activity in pooled human liver microsomes (Table 1). Activities selective for different P450 forms (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2B6, CYP2E1, and CYP3A4) were measured, and zileuton was found to be a weak inhibitor (IC$_{50}$ > 100 µM). Negligible inhibition of CYP2D6, CYP2C9, CYP1A2, CYP2E1, and CYP2C8 activity (<20%) was observed at the highest concentration of zileuton tested (100 µM). Approximately 40% inhibition of CYP3A4, CYP2B6, and CYP2C19 activity was observed at this concentration. By comparison, fluvoxamine, quercetin, sulfaphenazole, (R)-N-3-benzylphenobarbital, quinidine, ketoconazole, 4-methylpyrazole, and $N$-(α-methylbenzy)-1-amino benzoic acid inhibited CYP3A4 (IC$_{50}$ < 5.0 µM) of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP2E1, and CYP2B6 activity, respectively (Table 1).

Mechanism-Based Inhibition of CYP1A2. When preincubated with NADPH-fortified human liver microsomes in the absence of substrate, zileuton was shown to inhibit the O-deethylation of phenacetin. The effect was NADPH-, time-, and concentration-dependent (Fig. 2A). Upon further study, it was determined that the kinetics of inhibition were described by a maximal rate ($k_{\text{max}}$) of 0.035 min$^{-1}$ and an apparent $K_i$ of 117 µM (Fig. 2B). In contrast to POD activity, no preincubation-dependent inhibition of diclofenac 4’-hydroxylase and testosterone 6β-hydroxylase activity was observed. Under the same conditions, tiencil acid (10 µM) and L-754394 (1.0 µM) served as potent mechanism-based inhibitors of the two activities (>90% inhibition), respectively (data not shown). Therefore, the data suggested that zileuton did not behave as a mechanism-based inhibitor of CYP2C9 and CYP3A4.

As described in Table 2, a number of attempts were made to reverse the preincubation-dependent inhibitory effect of zileuton on POD activity. The presence of GSH, catalase, and SOD had no effect, which suggested that any reactive intermediate formed could not be scavenged prior to the enzyme inactivation event, and that the inactivation process was likely confined to the enzyme and did not involve peroxidative reactions outside the active site. In addition, dialysis had a minimal effect on the inhibition. Collectively, therefore, the data indicated that zileuton was a mechanism-based inhibitor of human liver microsomal CYP1A2.

The study was extended to encompass the evaluation of the individual enantiomers of zileuton as mechanism-based inhibitors of
CYP1A2. Upon preincubation with human liver microsomes, both enantiomers were shown to inhibit POD activity in a time-dependent manner (Fig. 3). However, the extent of inhibition was greater in the presence of (S)-(-)-zileuton. This was reflected in the $k_{\text{inact}}$, which was higher for the (S)-(-)-enantiomer (0.037 versus 0.012 min$^{-1}$). The $K_e$ values for (S)-(-) and (R)-(+) zileuton were comparable (98.2 and 66.6 $\mu M$, respectively).

**Retrospective in Vitro-in Vivo Correlations.** Having determined that zileuton behaved as a mechanism-based inhibitor of CYP1A2 in human liver microsomes, an attempt was made to relate the in vitro data ($K_e$ and $k_{\text{inact}}$) for the racemate to the pharmacokinetics of zileuton. In turn, the effect of zileuton on the pharmacokinetics (plasma AUC) of CYP1A2 substrates was simulated, assuming that the degradation of CYP1A2 was a first order process ($k_e$ ranging from 0.00026 to 0.0012 min$^{-1}$; half-life of 10–44 h).

Use of $C_{\text{max}}$ (19 $\mu M$) and $C_{\text{ave}}$ (9.0 $\mu M$) values for total zileuton in plasma (Awni et al., 1995a) resulted in overestimates of AUC changes (data not shown). After correction for binding of zileuton ($f_m = 0.069$) to human plasma proteins (Machinist et al., 1995a), more reasonable estimates of AUC ratios were obtained (Fig. 4). For compounds undergoing extensive metabolism by CYP1A2, where the majority of the dose (>70%) is metabolized by the enzyme ($f_m \cdot f_{m,CYP1A2} > 0.7$), zileuton would be expected to increase the AUC 1.4- to 2.2-fold ($k_e 0.00026–0.0005$ min$^{-1}$). When CYP1A2 plays less of a role in clearance ($f_m \cdot f_{m,CYP1A2} < 0.4$), changes in AUC are less marked (<1.3-fold). As shown in Fig. 4, simulations indicated that if the degradation of CYP1A2 in vivo were characterized by a $k_e$ of 0.0012 min$^{-1}$ (half-life of 10 h), then the majority of its substrates would be refractory to the effects of zileuton (<1.3-fold increase in AUC).

**Discussion**

Numerous clinical drug interaction studies have been performed with zileuton. Overall, it appears that the compound is a weak inhibitor of CYP2C9 and CYP3A4, as evidenced by its minimal effect on the pharmacokinetics of orally dosed phenytoin, (S)-warfarin, and terfenadine (Awni et al., 1995c; 1997; Samara et al., 1995). Zileuton also has a minimal effect on the oral clearance (~8% decrease) of naproxen (Awni et al., 1995b). This is expected, because naproxen is largely metabolized by glucuronidation (~70%), and there are no reports that zileuton is an inhibitor of UDP-glucuronosyltransferases in vivo (Rodrigues et al., 1996). At the same time, the O-demethylation of naproxen is catalyzed by CYP2C9 and CYP1A2, but the overall contribution of each enzyme to overall clearance is low ($f_m$, $f_{m,P450} ~< 0.15$).

Weak inhibition of CYP2C9 and CYP3A4 in vivo has been confirmed in vitro. For example, Machinist et al. (1995b) reported zileuton as a weak reversible inhibitor ($IC_{50} > 200$ $\mu M$) of tolbutamide hydroxylation (CYP2C9) and terfenadine oxidation (CYP3A4) in human liver microsomes. These observations have been duplicated in the present study using a larger panel of P450 form-selective substrates (Table 1). In our hands, zileuton was a weak reversible inhibitor of not only CYP2C9 and CYP3A4 (IC$_{50} > 100$ $\mu M$), but also CYP1A2, CYP2C8, CYP2C19, CYP2D6, CYP2E1, and CYP2B6 activity. Most of the drug interaction studies reported to date have used one of two zileuton oral dosing regimens (600 mg q.i.d. or 800 mg b.i.d.), both of which result in a plasma concentration of substrate (paclitaxel, 15 $\mu M$; phenacetin, 100 $\mu M$; diclofenac, 10 $\mu M$; (S)-mephenytoin, 80 $\mu M$; bufuralol, 15 $\mu M$; testosterone, 50 $\mu M$; chlorzoxazone, 200 $\mu M$, and hupropion, 100 $\mu M$) as described under Materials and Methods. The percentage inhibition observed at the highest concentration of zileuton tested (100 $\mu M$) was ~5% (CYP2D6 and CYP2C8), ~15% (CYP1A2, CYP2E1, and CYP2C9), 40% (CYP2B6 and CYP3A4), and 45% (CYP2C19).
et al., 1995c). In addition, CYP1A2 plays a major role in the overall metabolism of theophylline and zileuton increases its AUC almost 2-fold (Granneman et al., 1995; Ha et al., 1995; Troger and Meyer, 1995; Zhang and Kaminsky, 1995; Tjia et al., 1996; Yao et al., 2001).

**Fig. 2.** Concentration- and time-dependent inactivation of POD activity in human liver microsomes by racemic zileuton. A, aliquots were removed from the primary reaction mixture at the indicated time points and were assayed for residual POD activity (see Materials and Methods). Percentage of activity remaining (related to time 0 in the presence of solvent alone) was plotted in the logarithmic scale determined from a single experiment. B, the corresponding double reciprocal plot of the rates of inactivation as a function of zileuton concentration. The $k_{\text{inact}}$ (0.035 min$^{-1}$) and $K_i$ (117 μM) were obtained from the y-intercept and the negative reciprocal of the x-intercept, respectively.
MECHANISM-BASED INHIBITION of CYP1A2 by ZILEUTON

Irreversibility of the inactivation of human liver microsomal POD activity by zileuton racemate

Data represent mean and S.D. of n = 3 determinations and are expressed as the percentage of control (solvent and NADPH only). Human liver microsomes were incubated in the presence and absence of zileuton (200 μM) for 20 min (see Materials and Methods). Some incubations contained GSH, catalase, or SOD. Dialysis was carried out at 4°C for 4 h, which was sufficient time to completely reverse the inhibitory effects of fluvoxamine (not a time-dependent inhibitor of CYP1A2).

<table>
<thead>
<tr>
<th>Assay Component</th>
<th>Percentage of Control</th>
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<tbody>
<tr>
<td>Solvent plus NADPH</td>
<td>100</td>
</tr>
<tr>
<td>Zileuton minus NADPH</td>
<td>91.6 ± 6.17</td>
</tr>
<tr>
<td>Zileuton plus NADPH</td>
<td>49.9 ± 1.32</td>
</tr>
<tr>
<td>Zileuton plus NADPH and GSH (2 mM)</td>
<td>52.4 ± 1.89</td>
</tr>
<tr>
<td>Zileuton plus NADPH and catalase (500 units)</td>
<td>50.8 ± 4.01</td>
</tr>
<tr>
<td>Zileuton plus NADPH and SOD (500 units)</td>
<td>50.7 ± 3.00</td>
</tr>
<tr>
<td>Zileuton plus NADPH and dialysis</td>
<td>61.7 ± 1.17</td>
</tr>
<tr>
<td>Furafylline (10 μM) plus NADPH</td>
<td>22.6 ± 0.94</td>
</tr>
</tbody>
</table>

This increase in AUC is comparable to that reported for more potent reversible inhibitors of CYP1A2 (K_\text{inact} \leq 47 μM) such as fluvoxamine, propafenone, and mexiteline (Lo et al., 1991; Brosen et al.; Spinler et al., 1993; Rasmussen et al., 1995; Rasmussen et al., 1997; Kobayashi et al., 1998; Yao et al., 2001). Overall, zileuton appears to be a clinically relevant inhibitor of CYP1A2 despite being a relatively weak reversible inhibitor of the enzyme in vitro. This inhibition is clinically important, because warfarin and theophylline are both characterized by a relatively narrow therapeutic index, which requires patient monitoring and dose adjustment (Awni et al., 1995c; Graneman et al., 1995).

During the course of the present studies, zileuton was shown to be a mechanism-based inhibitor of CYP1A2 activity in human liver microsomes. The inhibition was concentration-, time-, and NADPH-dependent and was refractory to the effect of dialysis and scavenging agents such as GSH, catalase, and SOD. Moreover, the effect was selective for CYP1A2, despite the fact that three P450s (CYP1A2, CYP2C9, and CYP3A4) are involved in metabolism (Machinist et al., 1995b). Notably, a second hydroxysteroid-containing 5-lipoxygenase inhibitor (ABT-761) is a poor CYP1A2 substrate and has been shown to have a minimal effect on the pharmacokinetics of theophylline (Machinist et al., 1998; Wong et al., 1998). Therefore, the CYP1A2-dependent ring hydroxylation of zileuton and mechanism-based inhibition are linked in some way and may involve the formation of a reactive arene oxide intermediate. Although there is no evidence for CYP1A2-catalyzed sulfation of zileuton (Machinist et al., 1995b), it is also possible that CYP1A2 does catalyze sulfonation to a reactive benzthiophene S-oxide, which gives rise to mechanism-based inhibition (Mansuy et al., 1991; Lopez-Garcia et al., 1994). At least for CYP3A4, the products of sulfonation are stable, and there is no evidence for mechanism-based inhibition of the enzyme.

In our hands, the inhibitory effect of zileuton on POD activity in human liver microsomes was amenable to kinetic analysis, yielding estimates of k_{\text{inact}} (0.035 min^{-1}), K_\text{i} (117 μM), and a k_{\text{inact}}/K_\text{i} ratio of 0.0003 min^{-1} μM^{-1}. The k_{\text{inact}}/K_\text{i} ratio is considerably lower than that reported for furafylline (0.01–0.09 min^{-1} μM^{-1}), which decreases the oral clearance of caffeine 10-fold (Tarrus et al., 1987; Kunze and Trager, 1993; Clarke et al., 1994; Jones and Hall, 2002). Despite a relatively low k_{\text{inact}}/K_\text{i ratio,} we sought to relate the in vitro inhibition parameters for zileuton to its pharmacokinetics and attempted to rationalize the observed clinical drug interactions with CYP1A2 substrates.

Efforts have been made to evaluate the mechanism-based inhibition of P450s using nonphysiology- and physiology-based models (Ito et al., 1998; Mayhew et al., 2000; Jones and Hall, 2002). Overall, it is accepted that the net effect of the inhibitor is dependent upon the k_{\text{inact}}/K_\text{i ratio,} k_\text{e,} and the dose of the inhibitor (concentration of the inhibitor at the enzyme active site). However, the weakest part of the models has been the estimation of k_\text{e for the human P450s. Most of the authors have relied on in vitro human (hepatocyte) data or have made use of in vivo rodent data (Ito et al., 1998; Mayhew et al., 2000; Jones and Hall, 2002; Madan et al., 2002). As a result, in the absence of in vivo human data, the k_\text{e values used in the models have been somewhat arbitrary.}

For the sake of simplicity, the retrospective analysis of the zileuton data were based on a kinetic model (eq. 1), using a range of k_\text{e values for CYP1A2. Correia (1991) has reported that the half-life of CYP1A2 in rat liver is about 10 h (k_\text{e} = 0.0012 min^{-1}), whereas estimates of half-life for a human P450 (e.g., CYP3A4) have ranged from 24 to 44 h (k_\text{e} = 0.0005–0.00026 min^{-1}) (Ito et al., 1998; Mayhew et al., 2000). With such estimates of k_\text{e,} it has been possible to carry out retrospective in vitro-in vivo correlations for mechanism-based inhibitors of CYP3A4 (Mayhew et al., 2000; Jones and Hall, 2002). Therefore, zileuton (racemate) was evaluated in a similar manner using the in vitro k_{\text{inact}} and K_\text{i data (Fig. 2) and the plasma levels (corrected for f_\text{m}) reported in the literature (Awni et al., 1995a). Assuming a k_\text{e of 24 to 44 h, the simulations indicated that AUC changes are marked (up to 2-fold) when a large fraction of the dose (f_\text{m} \cdot f_\text{m,CYP1A2} > 0.6) is metabolized by CYP1A2 (Fig. 4, B and C). When CYP1A2 plays a relatively minor role in the overall clearance (f_\text{m} \cdot f_\text{m,CYP1A2} < 0.4), then the AUC increases elicited by zileuton are less pronounced (<1.3-fold). In light of the reported zileuton clinical data, the simulations employing the reported k_\text{e value for rat liver CYP1A2 (0.0012 min^{-1}}; Correia, 1991) gave relatively poor results (<1.3-fold increase in AUC) over the entire range of f_\text{m} \cdot f_\text{m,CYP1A2} values (Fig. 4A).

Using existing in vitro P450 reaction phenotyping data (f_\text{m,CYP1A2} and clinical data (f_\text{m}), it is possible to obtain estimates of f_\text{m} \cdot f_\text{m,CYP1A2} for both the theophylline (−0.7) and (R)-warfarin (≤ 0.4). The latter is partially metabolized by carboxyl reductase (~40%) and the remainder of the metabolism is more or less equally divided between CYP1A2 and CYP3A4 (Kaminsky and Zhang, 1996; Scordo et al., 2002). In contrast, the majority of theophylline N-demethylation and C_\text{ox} oxidation is catalyzed by CYP1A2 at clinically relevant concentrations (Ha et al., 1995; Troger and Meyer, 1995; Zhang and Kaminisky, 1995; Tjia et al., 1996; Yao et al., 2001). Therefore, zileuton would be expected to increase the AUC of theophylline up to 1.8-fold and have less of an effect (<1.3-fold increase on the AUC of (R)-warfarin (Fig. 4, B and C). In agreement, zileuton increases the AUC of theophylline and (R)-warfarin 1.9- and 1.2-fold, respectively (Awni et al., 1995c; Graneman et al., 1995). CYP1A2 can also play an important role in the overall clearance of antipyrine and propranolol (f_\text{m} \cdot f_\text{m,CYP1A2 as high as 0.56}), and the simulations (Fig. 4C) indicate that AUC increases of up to 1.6-fold are possible (Walle et al., 1985; Masubuchi et al., 1994; Yoshimoto et al., 1995; Engel et al., 1996; Sharer and Wrighton, 1996; Johnson et al., 2000). This is in reasonable agreement with the reported effect of zileuton on the oral clearance of antipyrine (~50% decrease; ~2-fold increase in AUC) and propranolol (~42% decrease; ~1.6-fold increase in AUC) (Machinist et al., 1995b; St. Peter et al., 1995). Therefore, with some assumptions, it has been possible to use the in vitro data and reconcile it with the results of previously reported clinical drug interaction studies.

Interestingly, both enantiomers of zileuton were shown to inhibit CYP1A2 activity in human liver microsomes, although the k_{\text{inact}} (0.037 versus 0.012 min^{-1}) and k_{\text{inact}}/K_\text{i ratio (0.0004 versus 0.0002 min^{-1} μM^{-1})} were higher for the (S)-enantiomer (Fig. 3). Because
the concentrations of both enantiomers in plasma are similar (Wong et al., 1995), the in vitro data suggest that the (S)-enantiomer may play more of a role in vivo. However, $f_{lu}$ is higher (0.122 versus 0.037) for the (R)-enantiomer (Machinist et al., 1995a), which could offset the difference in the $k_{inact}/K_I$ ratio.

Based on the results of this study, it is concluded that zileuton is a...
FIG. 4. Simulating the effect of racemic zileuton on the pharmacokinetics of CYP1A2 substrates.

The effect of zileuton on the pharmacokinetics of CYP1A2 substrates is simulated using eq. 1 (see Materials and Methods). In vitro $k_{inact}(0.035 \text{ min}^{-1})$ and $K_I(117 \mu M)$ data were used in the simulation, along with published values for zileuton plasma $C_{max,ss}(19 \mu M)$ and $C_{ave,ss}(9 \mu M)$ corrected for protein binding ($f_u = 0.069$). The fraction of the dose metabolized by CYP1A2 ($f_m f_{m,CYP1A2}$) is considered for different substrates. The degradation of CYP1A2 is assumed to be a first order process, characterized by a rate constant of $0.0012 \text{ min}^{-1}$ (A), $0.0005 \text{ min}^{-1}$ (B), and $0.00026 \text{ min}^{-1}$ (C).
weak reversible inhibitor of P450 activities in human liver micro-

somes. However, the compound behaves as a mechanism-based in-
hibitor of CYP1A2 (K_{inact}, 0.035 min^{-1}; K_{i}, 117 μM). Despite me-
tabolism by CYP1A2, CYP2C9, and CYP3A4 (Machinist et al., 1995b), the compound is not a mechanism-based inhibitor of CYP2C9 and CYP3A4. This P450 form-selective, mechanism-based inhibition may explain why zileuton elicits clinical drug interactions with CYP1A2 substrates [e.g., theophylline, (R)-warfarin, propranolol, and antipyrine] at doses that have a minimal effect on drugs metabolized by other P450s [e.g., terfenadine, phenytoin, and (S)-warfarin]. More-

over, this effect on CYP1A2 is clinically relevant, despite the enzyme playing a relatively minor role in the overall clearance of zileuton.

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References


