Duration of Cytochrome P-450 2E1 (CYP2E1) Inhibition and Estimation of Functional CYP2E1 Enzyme Half-Life after Single-Dose Disulfiram Administration in Humans

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ABSTRACT

Disulfiram (DSF) is a mechanism-based inhibitor of cytochrome P-450 2E1 (CYP2E1), resulting in loss of CYP2E1 protein and activity, which may be useful in preventing CYP2E1-mediated xenobiotic toxicity. The duration of inhibition after a single DSF dose is, however, unknown. The purpose of this investigation was to determine this duration, and CYP2E1 formation and degradation rates, in humans. Oral chlorzoxazone (CLZ) was used as the selective in vivo probe for CYP2E1. Healthy subjects received CLZ to determine baseline CYP2E1 activity (CLZ plasma clearance and 6-hydroxychlorzoxazone fractional metabolic clearance). One week later, DSF (500 mg orally) was administered at bedtime, and CLZ administered the following morning and 3, 6, 8, 10, and 13 days after DSF. A terminal DSF metabolite, 2-thiothiazolidine-4-carboxylic acid, was also measured in each 24-h urine sample. The mean CLZ clearance and 6-hydroxychlorzoxazone fractional metabolic clearance on the first day declined to 10.2 and 5.5% of baseline values, indicating rapid and profound CYP2E1 inhibition. CYP2E1 activity returned to half that of control on day 3, and to baseline values on day 8. Assuming zero-order synthesis and first-order degradation, the in vivo CYP2E1 synthesis rate and degradation half-life was estimated to be 11 ± 5 nmol/h and 50 ± 19 h, respectively. Significant amounts of 2-thiothiazolidine-4-carboxylic acid were present only on day 1, suggesting that the return of in vivo CYP2E1 activity was not caused by inhibitor washout, but by enzyme resynthesis. Results regarding CYP2E1 disposition may be useful for modeling the effects of CYP2E1 inducers and inhibitors. For prevention of CYP2E1-mediated bioactivation, depending on prototoxicant disposition, a second DSF dose might be necessary to completely prevent toxicity.

Disulfiram (DSF) undergoes oxidation in the body to form a number of mono- and dithiol compounds, some of which are potent mechanism-based inhibitors of cytochrome P-450 2E1 (CYP2E1) (Brady et al., 1991; Guengerich and Shimada, 1991). In animal models, the loss of in vitro CYP2E1 activity after a single DSF dose was paralleled by a loss of hepatic microsomal CYP2E1 protein determined by immunoblot. These data suggested that DSF rapidly caused CYP2E1 degradation. Similarly, in humans, a single DSF dose reduced CYP2E1-mediated chlorzoxazone 6-hydroxylation to less than 10% of baseline values within 24 h (Kharasch et al., 1993). Consequently, DSF has been used to investigate the role of CYP2E1 in human drug metabolism (Kharasch et al., 1994, 1995, 1996, 1999a; Mitra et al., 1995).

CYP2E1 catalyzes the human biotransformation of a number of commonly used xenobiotics, including volatile anesthetics (halothane, enflurane, isoflurane, and sevoflurane), hazardous industrial solvents that are protoxicants and procarcinogens, as well as therapeutic drugs such as acetaminophen. Unfortunately, it is this same pathway that seems to be responsible for bioactivation and the organ toxicity of these compounds (Song, 1996). Because DSF is able to inhibit CYP2E1-mediated oxidation, it may be used to prevent bioactivation, as established for the model compound, halothane. For example, a single DSF dose reduced formation of the putative reactive metabolite, trifluoroacetyl chloride (measured as the stable progeny trifluoracetic acid), to about 15% of control values in humans (Kharasch et al., 1996). However, the optimal DSF dose and duration of inhibition remain unknown. This and several other studies employing single-dose DSF to inhibit volatile anesthetic metab-
olism detected the late appearance, after 3 to 4 days, of CYP2E1-generated metabolites, suggesting the return of CYP2E1 activity (Kharasch et al., 1994, 1995, 1996, 1999a). Nevertheless, the exact duration of single-dose DSF inhibition of CYP2E1 remains unknown, and the kinetics of formation and degradation of CYP2E1 in humans are poorly understood.

Whether additional DSF doses might be necessary to optimally prevent CYP2E1-mediated toxification will depend on the elimination of the substrate and the duration of CYP2E1 inhibition. Therefore, the first objective was to determine the duration of CYP2E1 inhibition after a single oral DSF dose. Because DSF seems to cause a loss of CYP2E1, synthesis of new protein will determine the return of activity. Therefore, the corollary objective of this study was to determine the functional CYP2E1 half-life in vivo.

In this investigation, chlorzoxazone (CLZ) was used as the in vivo probe of CYP2E1 activity to determine the duration of CYP2E1 inhibition after a single DSF dose (Peter et al., 1990). There are two possibilities to explain the time course of return of activity: 1) persistence of the inhibitor or 2) new synthesis of the enzyme. We attempted to identify which of these mechanisms is operant and to characterize the relative rates of synthesis and degradation of CYP2E1 in vivo.

**Materials and Methods**

**Chemicals.** CLZ (Barr Laboratories, Inc., Northvale, NJ) and DSF (Antabuse; Wyeth Ayerst, Philadelphia, PA) were both administered as 500-mg tablets. CLZ, pentoxifylline (PTX), and DSF (Antabuse; Wyeth Ayerst, Philadelphia, PA) were both administered as 500-mg tablets. CLZ, pentoxifylline (PTX), and 2-Thiozolidine-4-carboxylic acid (TTCA) was synthesized by a previously reported method (Peter et al., 1990). 2-Thiozolidine-4-carboxylic acid (TTCA) was synthesized as previously reported (Peter et al., 1990). There are two possibilities to explain the time course of return of activity: 1) persistence of the inhibitor or 2) new synthesis of the enzyme. We attempted to identify which of these mechanisms is operant and to characterize the relative rates of synthesis and degradation of CYP2E1 in vivo.

**In Vivo CYP2E1 Regulation in Humans.** The Institutional Human Subjects Review Committee approved the investigational protocol. Eleven healthy, nonsmoking subjects (six males and five nonpregnant females) within 10% of ideal body weight were recruited, and, after giving written informed consent, instructed to refrain from alcohol, caffeine-containing beverages, and acetaminophen use during the study period. Ten subjects completed the study; one was removed for alcohol consumption during the study period. Each subject received 500 mg of oral CLZ to determine baseline CYP2E1 activity and 1 week later, a single 500-mg dose of DSF was administered at bedtime. Additional 500-mg CLZ doses were administered 10 h later (day 1) and 3, 6, 8, 10, and 13 days after the DSF dose. After each CLZ dose, plasma was collected for 8 to 12 and urine for 24 h for quantification of CLZ and 6-OH CLZ, respectively.

Plasma CLZ concentrations were determined by using an integrated HPLC system (HP 1050 series; Hewlett-Packard) fitted with a C18 reversed-phase column (5 μm, 4.5 × 250 mm; Rainin, Deerfield, IL). An isotonic mobile phase [0.15% ammonium acetate, pH 4.7/acetonitrile (83:17)] at a flow rate of 1.0 ml/min was used. After plasma protein precipitation, a 20-μl sample was injected and the effluent monitored at 280 nm. Under these conditions the CLZ retention time was 8.2 min. Urinary 6-OH CLZ was measured by a previously published method (Kharasch et al., 1993). The standard curve ranged from 10 to 250 mg/l. Briefly, 10 μl of β-glucuronidase (55,000 U/ml) were added to each urine sample and incubated overnight at 37°C. After the addition of PTX as an internal standard, quantification of hydrolyzed samples was performed on the HPLC system described above, except that the mobile phase was ammonium acetate/acetonitrile (82:18), and the effluent was monitored at 290 nm. Under these conditions, 6-OH CLZ and PTX retention times were 9.2 and 11.3 min, respectively.

**Pharmacokinetic Analyses.** CYP2E1 activity was assessed from the plasma CLZ oral clearance, Cl/F CLZ:

\[
\frac{Cl}{F \text{ClZ}} = \frac{\text{Dose CLZ}}{\text{Plasma AUC}}
\]

where AUC is the area under the CLZ plasma concentration-time curve analyzed by a semiparametric computer algorithm (LAGRAN; University of Alberta, Edmonton, Canada). The CYP2E1-mediated oxidation of CLZ to 6-OH-CLZ was quantified by calculating the fractional (formation) clearance, Cl/F 6-OH-CLZ, from:

\[
\frac{Cl}{F \text{6-OH-CLZ}} = \frac{fm \text{6-OH-CLZ} \times Cl/F \text{ CLZ}}{}
\]

where fm 6-OH CLZ is the fraction of the dose excreted as 6-OH-CLZ in a 24-h period. For each subject, the change in the time-averaged fractional metabolic clearance as a percentage from baseline was plotted against the CLZ mean residence time from the DSF dose. The mean residence time was estimated from each dose as 1/k, where k was the terminal CLZ plasma elimination constant. The change in baseline clearance versus time from DSF dose was then fit to a model of time-dependent enzyme induction previously developed (Levy et al., 1979) using nonlinear regression (SPSS for WIN95 v. 7.5; SPSS, Inc., Chicago, IL):

\[
\% \text{ baseline } \frac{Cl}{F \text{ CLZ}} \left( \text{or } \frac{Cl}{F \text{6-OH-CLZ}} \right) = 100\% \left( 1 - e^{-k_{\text{CYP2E1}} \times \text{MRT}} \right)
\]

where kCYP2E1 is the first-order CYP2E1 degradation constant and the other symbols are as previously described.

The CYP2E1 synthesis rate was estimated from the steady-state relationship between synthesis and degradation rates. At steady state, the synthesis rate Ro(CYP2E1) is equal to the degradation rate and can be estimated from:

\[
\text{Ro(CYP2E1)} = k_{\text{CYP2E1}} \times \text{[CYP2E1]}
\]

where [CYP2E1] is the total amount of hepatic CYP2E1. An average value for total hepatic CYP2E1 (720 nmol) was estimated from normal biopsy tissue obtained from patients undergoing abdominal surgery. Wedge biopsies were obtained from 17 patients, without known liver disease, undergoing abdominal surgery. Microsomes were prepared and CYP2E1 content determined by immunoblot (Thummel, 1993). Microsomal CYP2E1 content was 8.5 ± 4.2 pmol/mg protein. CYP2E1 content per gram of liver was calculated using microsomal yield (milligram per gram of liver), multiplied by total liver volume (determined by magnetic resonance imaging), to obtain total liver CYP2E1 content (720 ± 360 nmol) (our unpublished results). These models make the following assumptions: 1) a single DSF dose exists in a rapid and almost complete loss of CYP2E1; 2) CYP2E1 is synthesized by a constant (zero-order) rate and undergoes degradation by a first-order process; 3) DSF and its metabolites are rapidly eliminated relative to the CYP2E1 degradation half-life; and 4) DSF and its metabolites do not influence the synthesis rate of CYP2E1.

**Assessment of DSF Metabolite Residence.** This was determined by measuring urinary excretion of TTCA, a biomarker for the terminal DSF metabolite carbon disulfide (CS2). Urinary TTCA concentration was measured by a sensitive GC/MS assay previously described, with minor modification (Johnson et al., 1996). One milliliter of urine was passed through a syringe fitted with a 0.2-μm filter and transferred to a clean tube containing 800 μl of 1 N HCl. TTCA was extracted into a 2-mi aliquot of ethyl acetate. After centrifug-
tion, the organic layer was transferred to a clean tube. Any remaining water was removed by the addition of magnesium sulfate. After a second centrifugation, the ethyl acetate layer was transferred to a clean tube and evaporated to dryness under a stream of N₂. Methanol (0.5 ml) was then added, followed by excess diazomethane (0.6 ml) to form the dimethyl derivative. The solvent was evaporated once again under N₂ and the residue was dissolved in 100 µl of acetonitrile. A 5-µl aliquot was analyzed by GC/MS and TTCA methyl ester

**Fig. 1.** Mean (± S.D.), plasma CLZ concentrations versus time before (day 0) and after a single 500-mg DSF dose. Each panel represents the study periods as indicated.

**TABLE 1**

<table>
<thead>
<tr>
<th>Study Period</th>
<th>CLZ Clearance</th>
<th>Fraction Metabolized to 6-OH CLZ</th>
<th>6-OH CLZ Fractional Metabolic Clearance</th>
<th>CLZ T&lt;sub&gt;1/2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>liter h⁻¹</td>
<td>%</td>
<td>liter h⁻¹</td>
<td>liter h⁻¹</td>
</tr>
<tr>
<td>Baseline</td>
<td>29.9 ± 20.1</td>
<td>0.56 ± 0.18</td>
<td>16.7 ± 10.7</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.92 ± 1.33*</td>
<td>0.27 ± 0.15*</td>
<td>0.83 ± 0.65*</td>
<td>13.2 ± 6.0*</td>
</tr>
<tr>
<td>Day 3</td>
<td>16.1 ± 10.8*</td>
<td>0.52 ± 0.17</td>
<td>8.4 ± 5.5*</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Day 6</td>
<td>25.6 ± 12.00</td>
<td>0.61 ± 0.15</td>
<td>15.7 ± 7.6</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Day 8</td>
<td>30.3 ± 14.6</td>
<td>0.58 ± 0.19</td>
<td>18.5 ± 11.3</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Day 10</td>
<td>28.9 ± 9.7</td>
<td>0.55 ± 0.16</td>
<td>14.6 ± 6.2</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Day 13</td>
<td>28.5 ± 12.00</td>
<td>0.55 ± 0.23</td>
<td>16.2 ± 12.2</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

*p < .05 versus baseline.
was detected by selected ion monitoring (m/z 132 [M–CO₂CH₃]). Similarly prepared standards (10–2000 ng/ml) were used to quantify TTCA using peak areas. The standard curve was linear over the entire range measured (r² = 0.9998).

Data Analysis. Pharmacokinetic parameters were analyzed by repeated measures ANOVA (SigmaStat 2.03; SPSS Inc., Chicago, IL). Significance was assigned at p < .05.

Results

A single 500-mg dose of DSF resulted in rapid and profound loss of CYP2E1 activity. After the first CLZ dose following DSF (10 h later), the average CLZ plasma clearance and the CYP2E1-mediated 6-OH-CLZ fractional metabolic clearance declined to 10 and 5% of baseline values, respectively. The fraction of the dose recovered as 6-OH-CLZ decreased from 0.58 to 0.27 (Table 1). By day 8, plasma CLZ oral clearance, 6-OH-CLZ fractional metabolic clearance, and the fraction of the CLZ dose metabolized to 6-OH-CLZ had returned to baseline values.

On day 1 following DSF administration, the mean area under the plasma concentration time curve was markedly increased and the terminal elimination phase was prolonged, with the plasma terminal half-life increased from 1.6 h at baseline to 13.2 h (Fig. 1). Although peak concentrations did not change significantly, there was a gradual decline in the half-life with each subsequent study period, declining from 2.6 h on day 3 to approximately the same mean baseline value of 1.7 h by day 6.

Figure 2 depicts the time course of CLZ oral clearance and the 6-OH CLZ fractional metabolic clearance for each individual, expressed as a percentage of the baseline value, following single-dose DSF. There was marked interindividual variability in the return of plasma CLZ oral clearance and 6-OH-CLZ fractional metabolic clearance. In some subjects the clearances exceeded the baseline value. The 6-OH CLZ fractional metabolic clearance exhibited greater interindividual variability than did the CLZ oral clearance.

The largest quantity of TTCA was recovered on day 1 after DSF administration and represented about 0.2% of the administered DSF dose (Fig. 3). The average amount of TTCA recovered on day 3, and all subsequent study periods, was negligible and only represented about 5% of the total average TTCA recovered during the entire study period. A low concentration of TTCA was present in the baseline urine of one of the subjects. This was reported previously in subjects not taking DSF and was attributed to dietary CS₂ sources (Simon et al., 1994).

Table 2 shows the individual estimates for CYP2E1 degradation half-life. As expected from the clearance values, there was considerable variability between subjects (range = 24.9–80.0 h). The mean value for CYP2E1 degradation was 50.5 ± 19.1 h. The estimated CYP2E1 synthesis rate was 11 ± 5 nmol/h.

<table>
<thead>
<tr>
<th>Subject</th>
<th>CYP2E1 Degradation T_{1/2} (h)</th>
<th>CYP2E1 Synthesis (nmol/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>32.5</td>
<td>15</td>
</tr>
<tr>
<td>BB</td>
<td>46.1</td>
<td>11</td>
</tr>
<tr>
<td>DD</td>
<td>24.9</td>
<td>20</td>
</tr>
<tr>
<td>EE</td>
<td>58.3</td>
<td>9</td>
</tr>
<tr>
<td>FF</td>
<td>59.2</td>
<td>8</td>
</tr>
<tr>
<td>GG</td>
<td>32.3</td>
<td>15</td>
</tr>
<tr>
<td>HH</td>
<td>34.5</td>
<td>14</td>
</tr>
<tr>
<td>II</td>
<td>80.0</td>
<td>6</td>
</tr>
<tr>
<td>JJ</td>
<td>63.4</td>
<td>8</td>
</tr>
<tr>
<td>KK</td>
<td>73.5</td>
<td>7</td>
</tr>
<tr>
<td>Mean</td>
<td>50.5</td>
<td>11</td>
</tr>
<tr>
<td>S.D.</td>
<td>19.1</td>
<td>5</td>
</tr>
</tbody>
</table>

Based on analysis of the data in Fig. 2 as described in Materials and Methods.

TABLE 2

CYP2E1 synthesis rate and degradation half-life in individual subjects estimated from return of CLZ clearance following a single DSF dose.
models that identified mechanism-based inhibition and subsequent loss of immunoreactive CYP2E1 protein as the cause for the DSF-induced in vivo reduction of CYP2E1 activity (Brady et al., 1991).

The estimated in vivo CYP2E1 degradation half-life in healthy subjects was $50.5 \pm 19.1$ h after the administration of a rapidly cleared mechanism-based inhibitor. Another estimate of 2.5 days, using CLZ as the metabolic probe, was reported in chronic alcoholics undergoing withdrawal and consequent deinduction of CYP2E1 (Lucas et al., 1995). Despite the contrasting methods to estimate CYP2E1 half-life (after rapid, subtotal protein inactivation or following deinduction), and the obvious differences in study populations, the two results are surprisingly consistent.

The rate of in vivo CYP2E1 synthesis in humans has not been previously reported. The unique design of this study, using CYP2E1 inactivation, now affords such an opportunity. The CYP2E1 synthesis rate, calculated from the observed CYP2E1 degradation rate and an average value for total human liver CYP2E1 content ($720 \pm 360$ nmol) obtained in our laboratory, was $11 \pm 5$ nmol/h. Shimada et al. (1994) also determined hepatic microsomal CYP2E1 content, reporting a higher value ($22 \pm 12$ pmol/mg protein) than found presently. Using an estimate for total hepatic CYP2E1 content based on this value, and the presently observed CYP2E1 degradation rate, the CYP2E1 synthesis rate, thus calculated, is $29 \pm 12$ nmol/h. Hepatic CYP2E1 synthesis in rats in vivo has also been measured using pulse radiolabeling; however, an absolute formation rate was not provided (Song et al., 1989; Tsutsumi et al., 1993). We observed considerable intersubject variability in the CYP2E1 resynthesis rate. The mechanism of this variability is unknown, but may relate to a genetic polymorphism in the regulatory sequence of CYP2E1 that confers higher CYP2E1 metabolic activity in response to induction (McCarver et al., 1998).

Rates of CYP2E1 zero-order synthesis and first-order degradation may be used to define current models of human CYP2E1 regulation. Chien et al. (1997a) presented a model for human CYP2E1 induction by ligand stabilization, which also accounted for enzyme inhibition in the presence of inducer, apparent induction immediately after elimination of the inducer, and normalization of enzyme activity caused by protein degradation. This model was applied to interactions between isoniazid and acetaminophen or CLZ in humans (Zand et al., 1993; Chien et al., 1997b; O'Shea et al., 1997). The model assumed biphasic CYP2E1 degradation (half-lives of 7 and 37 h), based on CYP2E1 turnover in rats determined by radioactive pulse labeling (Song et al., 1989; Roberts et al., 1995). Because the corresponding half-lives in humans were unknown, Chien et al. (1997a) used the rat half-lives, but also evaluated longer values (15 and 79 h), based on an expectation of longer half-lives in humans. That the longer half-lives best fit the observed data is consistent with the present (50 h) and previous (2.5 days; Lucas et al., 1995) estimates for CYP2E1 degradation half-life in humans. In addition, human CYP2E1 models may be further refined by the present estimate for the rate of zero-order CYP2E1 synthesis, which was not previously evaluated (Chien et al., 1997a). The present experimental design did not allow incorporation of two CYP2E1 degradation rates for biphasic degradation.

Because of the pivotal role of CYP2E1 in the bioactivation
and toxification of numerous industrial solvents that are protocinsects and procarcinogens, as well as certain therapeutic drugs such as acetaminophen and halothane, there is growing interest in the development of a specific in vivo inhibitor of CYP2E1 suitable for use in humans, for use both as a probe to identify CYP2E1 participation in bioactivation and as a therapeutic or prophylactic modality for treatment or prevention of CYP2E1-mediated toxification. The ideal inhibitor would produce rapid, complete, and highly selective CYP2E1 inhibition of predictable duration.

To date, DSF, watercress, and chlormethiazole have been explored as potential inhibitor probes for human CYP2E1. A single ingestion of watercress (50 g) 10 h before CLZ, used as a dietary source of the CYP2E1 inhibitor phenethyl isothiocyanate, decreased CLZ oral clearance by 44% (Leecher et al., 1998). Similarly, watercress also caused moderate inhibition of acetaminophen bioactivation to the toxic quinone imine, based on 25 to 43% decreases in urinary excretion of cysteine and mercapturic acid conjugates (Chen et al., 1996). At present, the specificity of watercress toward CYP2E1, effects on other P-450 isoforms, and duration of inhibition are unknown. Chlormethiazole inhibited hepatic CYP2E1 induction in rats, primarily by inhibition of gene transcription, although constitutive CYP2E1 expression was minimally and insignificantly affected (Hu et al., 1994). In humans, chlormethiazole given 10 h before CLZ significantly reduced plasma 6-OH-CLZ/CLZ ratios (used as a marker for CYP2E1 activity) by 81%, with 36 to 60 h duration of inhibition (Gebhardt et al., 1997). Similar inhibition was observed in ethanol-induced patients (94% inhibition of plasma 6-OH-CLZ/CLZ ratios and 69% inhibition of CLZ oral clearance; Gebhardt et al., 1997, Eap et al., 1998). Some information on chlormethiazole specificity in humans is available. Tolbutamide elimination was unaffected in volunteers, suggesting no inhibition of CYP2C9 (Mönig et al., 1993). Antipyrine elimination was also unaffected by chlormethiazole (Mönig et al., 1993), suggesting no effect on CYP1A2 activity; however, conclusions about other P-450 isoforms are precluded because of the multiplicity of P-450s catalyzing antipyrine metabolism and the lack of isoform-selective inhibitor effects on antipyrine clearance (Sharer and Wrighton, 1996; Engel et al., 1996). The mechanism of chlormethiazole inhibition of CYP2E1 is presently uncertain. In rats, gene transcription (mRNA and CYP2E1 protein levels) was inhibited in vivo, and chlormethiazole had no effect on liver microsomal CYP2E1 activity in vitro, suggesting purely transcriptional inhibition (Hu et al., 1994). In contrast, in human liver microsomes, chlormethiazole was a noncompetitive CYP2E1 inhibitor (Kᵢ 12 μM), had no effect on CYP1A2 or -3A activity, and elicited a type II difference spectrum, suggesting direct enzyme inhibition (Gebhardt et al., 1997). Nevertheless, inhibition at the enzyme level alone was discounted, because chlormethiazole inhibition of CYP2E1 activity in vivo occurred when plasma inhibitor concentrations were undetectable (Gebhardt et al., 1997).

The most completely characterized human CYP2E1 inhibitor is DSF. The present and previous (Kharasch et al., 1993) results demonstrated profound (85–95%) single-dose inhibition of CYP2E1 activity, based on CLZ clearance and 6-OH-CLZ fractional metabolic clearance. Single-dose DSF also was an effective inhibitor of CYP2E1-mediated metabolism and drug toxification, evidenced by 82 to 90% inhibition of volatile anesthetic metabolism (Kharasch et al., 1994, 1995, 1996, 1999a), 84 to 90% inhibition of halothane toxification (Kharasch et al., 1996), and 77% inhibition of acetaminophen bioactivation to the toxic quinone imine (J. Slattery, University of Washington, personal communication). Thus, DSF is the most effective in vivo human CYP2E1 inhibitor presently available. DSF (specifically single-dose treatment) also exhibits considerable in vivo selectivity toward CYP2E1, with minimal effects on human CYP 2A6, 2C9, 2C19, 2D6, and 3A4 activity (Kharasch et al., 1998, 1999b), thus minimizing potential adverse reactions caused by inhibition of nonCYP2E1 isoforms. Finally, the mechanism of DSF inhibition has been established, because DSF and its primary metabolite, diethylidithiocarbamate, are known to mechanism-based inhibitors of CYP2E1 (Brady et al., 1991; Guengerich and Shimada, 1991). DSF therefore appears to be an excellent in vivo probe and inhibitor for human CYP2E1.

The present results provide a mechanistic explanation for prior clinical observations in which DSF was used to probe the metabolism of CYP2E1 substrates. Previously, after single-dose DSF, subjects receiving volatile anesthetics (known substrates for CYP2E1) showed slight increases in the formation of CYP2E1-catalyzed oxidative metabolites 3 to 4 days after anesthetic administration (3.5–4.5 days after DSF; Kharasch et al., 1994, 1995, 1996, 1999a). This late metabolic formation can now be attributed to resynthesis of CYP2E1, coupled with prolonged residence of these lipid soluble substrates. These data suggest that if DSF is to be used as an inhibitor of CYP2E1 to prevent bioactivation of xenobiotics, it may be necessary to administer additional doses in some individuals, especially if the compound has a residence time greater than 24 to 48 h.

References


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