IN VITRO BIOTRANSFORMATION AND IDENTIFICATION OF HUMAN CYTOCHROME P450 ISOZYME-DEPENDENT METABOLISM OF TAZOFELONE

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ABSTRACT:

Tazofelone is a new inflammatory bowel disease agent. The biotransformation of tazofelone in human livers and the cytochrome P450 responsible for the biotransformation has been studied. Two metabolites of tazofelone were formed in vitro. A sulfoxide metabolite was identified by cochromatography with authentic standards, and a quinol metabolite of tazofelone was identified by mass spectrometry and proton NMR. Sulfoxidation was catalyzed by a single enzyme system while formation of the quinol metabolite was catalyzed by a two enzyme system. The \( K_m \) and \( V_{max} \) values for sulfoxidation were 12.4 \( \mu \)M and 0.27 nmol/min/mg protein, respectively. The high affinity \( K_m \) and \( V_{max} \) values for the formation of the quinol metabolite were 7.5 \( \mu \)M and 0.17 nmol/min/mg protein, respectively. Tazofelone was incubated at 20 \( \mu \)M concentration with human microsomes to determine which of the cytochrome P450 isozyme(s) is involved in the oxidation of tazofelone. A strong correlation was found between the immunoquantified concentrations of CYP3A and the rates of formation of the sulfoxide and quinol metabolites of tazofelone. Similarly, significant correlations were observed between the formation of midazolam 1'-hydroxylation and the rates of formation of both metabolites of tazofelone. Inhibition studies have indicated that triacetyloleandomycin, a CYP3A specific inhibitor, almost completely inhibited the formation of both of these tazofelone metabolites. Incubations with specific cDNA expressed microsomes indicated that the formation of both the sulfoxide and quinol metabolites was highest with CYP3A4A containing microsomes. The correlation data was confirmed by inhibition studies and cDNA expressed cytochrome P450 systems, demonstrating that the biotransformation of tazofelone to its metabolites is primarily mediated by CYP3A.

Tazofelone\(^1\) has been shown to be highly effective in rat and rabbit models of inflammatory bowel disease and colitis. Previous in vivo disposition studies have indicated that the absorption of tazofelone in rats and dogs is approximately 20%. However, appreciable in vivo biotransformation to primarily a stereoisomeric mixture of sulfoxides results in a tazofelone bioavailability of only 2–3% in both species. Tazofelone is rapidly cleared from the plasma of rats and dogs with half-lives of 2 to 4 hr, respectively. Subsequent biotransformation studies with rat and dog hepatic microsomes have demonstrated that tazofelone is rapidly oxidized to the same stereoisomeric mixture of sulfoxides as observed in vivo (unpublished results).

Cytochrome P450 is a superfamily of heme containing enzymes that play a major role in oxidative metabolism of drugs, endogenous compounds, and xenobiotics (1). These enzymes exhibit broad and often overlapping substrate specificities (2). Moreover, they also show interspecies variation in their expression and catalytic activity (3). Therefore, identification of the cytochrome P450 isozyme responsible for the metabolism of a particular compound has many useful implications in the drug development process, namely, a) to predict drug-drug interactions (4), b) to predict variation in drug metabolism caused by genetic polymorphism of specific enzymes (3, 5), and c) to design new drugs with reduced metabolism and improved bioavail-

1 Abbreviations used are: Tazofelone, (R,S)-5-[(3,5-bis[1,1-dimethylethyl]-4-hydroxyphenyl)-methyl]-4-thiazolidinone; BHT, butylated hydroxytoluene; HPLC, high pressure liquid chromatography; NMR, nuclear magnetic resonance; HMQC, heteronuclear multiquantum correlations.

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grams. Two of these peaks corresponded to two sulfoxide diastereoisomeric mixtures that were previously identified in vivo (unpublished results). To identify the third peak, incubations were carried out with 1 mg/ml human hepatic microsomal protein in pH 7.4 phosphate buffer, 50 μM tazofelone, 1 mM NADPH, and 0.5 U glucose-6-phosphate dehydrogenase, 1 mM glucose-6-phosphate, and 5 mM magnesium chloride in 35 ml for 1 hr. Incubations were stopped by the addition of an equal volume of acetonitrile and the precipitated protein was removed after centrifugation. The supernatant was evaporated to dryness under nitrogen and the residue was dissolved in acetonitrile:water (40:60) and subjected to HPLC analysis.

Rates of formation of sulfoxide and the quinol metabolites were determined in 250 μl microsomal incubations. The incubation mixture contained 0.25 mg human hepatic microsomal protein in 100 mM phosphate buffer, pH 7.4, 1 mM NADPH, and was preincubated for approximately 3 min at 37°C. The reaction was initiated by the addition of 14C-tazofelone dissolved in methanol to give a final concentration of 20 μM tazofelone. The amount of methanol added to the incubation mixture was less than 2% in all incubations. The reaction was terminated at 8 min by the addition of 1 ml of cold acetonitrile, and the supernatant was removed after centrifugation at 13000 rpm for 3 min (Micro-max, IEC). The supernatant was evaporated to dryness under nitrogen and reconstituted in 100 μl of acetonitrile:water (40:60), and 60 μl was subjected to HPLC analysis.

Incubations with cDNA expressed P450 isozymes similar to the procedure described above were performed with hepatic microsomes except that the incubations were carried out in a final volume of 500 μl for 60 min and the incubation mix also contained 0.5 units glucose-6-phosphate dehydrogenase, 1 mM glucose-6-phosphate, and 5 mM magnesium chloride.

The following selective cytochrome P450 inhibitors were examined for their effect on tazofelone metabolism by human liver microsomes: coumarin (CYP2A6) (10), furafylline (CYP1A2) (11), sulfaphenazole (CYP2C9/10) (12), S-mephénytoïn (CYP2C19) (13), diethyliodiocarbarnate (CYP2A6 and CYP2E1) (14), and triacetyloleandomycin (CYP3A) (15). The incubation volume was 250 μl and the concentration of tazofelone was 20 μM. The mechanism based inhibitors, triacetyloleandomycin (TAO), diethyliodiocarbarnate (DDC), and furafylline, were preincubated with human hepatic microsomes and an NADPH-generating system for 30 min, and the reaction was started by the addition of tazofelone. The incubation procedure for the rest of the inhibitors was carried out as described previously.

To examine the role of flavin monoxygenase 3 (FM03) in the sulfoxidation of tazofelone, microsomal incubations were conducted at both pH 7.4 and pH 8.5 (16). Heat inactivated microsomes were heated for 1 min at 55°C in the presence or absence of NADPH, placed on ice for 2 min, and then the incubations were carried out as described previously to determine the formation of sulfoxides.

### HPLC Analysis of Tazofelone Metabolites
Tazofelone, tazofelone sulfoxides, and the quinol metabolite were resolved on a Prodigy C18 250 × 4.6 mm column (Phenomenex). The HPLC system consisted of two Shimadzu LC-10AD pumps, a Kratos Spectroflow 783 UV variable detector, and a Waters Model 710B WISP autoinjector. An isocratic mobile phase composed of acetonitrile:water (60:40) was used at a flow rate of 1 ml/min. The column temperature was 40°C, and the eluate was monitored at 214 nm by UV detection. The fractions corresponding to the metabolite peaks were collected into scintillation vials with a Foxy fraction collector (Isco, Lincoln, NE), and UltimaGold scintillation liquid was added to the fractions and assayed for radioactivity by liquid scintillation counting (Beckman LS7500).

### Identification of Quinol Metabolite
Purification of the quinol metabolite was achieved by a two-step procedure. The first involved the isocratic HPLC method described above. The eluate corresponding to the peak was collected and evaporated to dryness under nitrogen at 40°C and the residue dissolved in acetonitrile:water (20:80). The second step consisted of a linear gradient HPLC from 20:80 to 80:20 acetonitrile:water over 40 min. The peak corresponding to the quinol metabolite was collected and evaporated to dryness and subjected to spectral analysis.

### Spectral Analysis
Proton nuclear magnetic resonance spectroscopy (1H-NMR) at 500 MHz, and homonuclear decoupling were performed on a Bruker 500 MHz FT-NMR using deuterated acetonitrile as solvent. A field desorption mass spectrometry (FD-MS) was generated using a VG Model 70SE magnetic sector mass spectrometer and fast atom bombardment mass spectra (FAB-MS) and high resolution FAB mass spectra (HR-MS) were obtained on a VG ZAB-SE mass spectrometer.

### Kinetic and Statistical Analysis
Kinetic parameters for the formation of tazofelone metabolites were determined using kcat enzyme kinetic software (BioMetallcics, Inc., Princeton, NJ) and Eadie-Hofstee plots were visually inspected to assess whether one or two enzymes were involved. Estimates of the kinetic parameters from the kcat program were used as initial estimates for nonlinear regression analysis using NONLIN (Statistical Consultants, Inc., Lexington, KY) software. For the formation of the total sulfoxides, the following one enzyme model for a saturated enzyme system (S > Km) was used.

\[
v = \frac{(V_{\text{max}} + S)}{(K_m + S)}
\]

where \(C_{\text{int}} = V_{\text{max}}/K_m\). To characterize the kinetics of the quinol metabolite formation, a two enzyme model was used. Under the experimental conditions, eq. 2 described the kinetics best with a high affinity site that was saturated and a low affinity site that was not saturated.

\[
v = \frac{(V_{\text{max}} + S)}{(K_m + S)} + \frac{[Cl_{\text{int}} + S]}{}
\]

Statistical analysis was performed using JMP software (SAS Institute, Inc., Cary, NC).

### Results
**Identification of Metabolites**
Incubation of tazofelone with human hepatic microsomes in the presence of NADPH and oxygen resulted in three major peaks (fig. 1). Two of these peaks were observed in plasma of rats and dogs and have been identified as a pair of diastereomeric sulfoxides which co-elute with authentic sulfoxide standards (unpublished results). Because of the epimerization of the tazofelone sulfoxide stereoisomers at pH values greater than 4, tazofelone sulfoxide was quantitated as the sum of the two HPLC peaks for all analysis. To elucidate the structure of the quinol metabolite, mass spectral and proton NMR studies were conducted on the material isolated and purified by HPLC from human hepatic microsomal incubations. The FD-MS contained a molecular ion peak \((m/z = 337)\), indicating the addition of oxygen to tazofelone (MW = 321) (fig. 2).

This was confirmed by FAB-MS which produced a molecular ion at \(m/z = 338 (\text{MH}^+)\) (fig. 2). The FAB-MS also produced ions at \(m/z = 360, m/z = 320, m/z = 264,\) and \(m/z = 219\) corresponding to the fragments \([\text{M}+\text{Na}]^+, [\text{M}+\text{H}_2\text{O}]^+, [\text{M}+\text{butyl}]^+,\) and [BHT tropolion cation], respectively (fig. 2). Also, the high resolution mass spectrum (HR-MS) indicated a molecular formula of \(C_{24}H_{25}NO_3S\) consistent with the proposed structure. The \(^1H\)-NMR, HMQC, and homonuclear decoupling experiments indicated that the difference between tazofelone and the quinol metabolite was in the chemical shifts of the 5.6, 8/12, and phenol protons (table 1). The spin system corresponding to

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Fig. 1. HPLC chromatogram of tazofelone incubated with human hepatic microsomes.
the five membered ring was present in both tazofelone and the metabolite which suggests that addition of oxygen was in the aromatic system. The six methyls of the t-butyl groups all have the same shift, and there was no new -CH\textsubscript{2}-OH in the NMR spectrum indicating that the incorporation of oxygen was not on the t-butyl substituents. The mass spectral peaks M-57 and M-114 also confirmed the lack of substitution on t-butyl groups. From the variable temperature NMR data, decoupling experiments, and comparison of data with the parent molecule, the proposed structure of the quinol metabolite is shown in fig. 3.

Kinetics of Formation. Kinetic analysis of total sulfoxide and quinol metabolite formation was conducted in human liver microsomes at concentrations ranging from 3.9 \( \mu \text{M} \) to 1500 \( \mu \text{M} \). Nonlinear regression modeling was done on the data to fit Michaelis-Menten kinetics. Eadie-Hofstee plots (not shown) indicated that sulfoxidation was catalyzed by a single enzyme while formation of the quinol metabolite was catalyzed by a two enzyme system. Kinetic parameters for sulfoxide and quinol metabolite formation are presented in table 2. As shown in the table, the apparent \( K_{m} \) and \( V_{\text{max}} \) values for sulfoxide formation were 12.4 \( \mu \text{M} \) and 0.27 nmol/min/mg protein, respectively, which resulted in an intrinsic clearance of 0.022 nmol/min/mg protein. The kinetics of quinol metabolite formation followed a two enzyme model. The apparent \( K_{m} \) and \( V_{\text{max}} \) values for the high affinity enzyme were 7.5 \( \mu \text{M} \) and 0.17 nmol/min/mg protein, respectively. The intrinsic clearance by the high affinity enzyme was 0.023 ml/min/mg protein and by the low affinity enzyme was 0.002 ml/min/mg protein.

Identification of Cytochrome P450 Isozyme. The rates of formation of total sulfoxide and quinol metabolites were determined using microsomes from a bank of 18 human livers. These 18 human livers have been previously characterized for their isozyme-selective catalytic activities, and the immunoquantifiable levels of CYP1A2, CYP2A6, CYP2C8/2C9, CYP2D6, CYP2E1, and CYP3A were characterized for 14 livers (3, 8, 9, 17, 18). The medical history indicates exposure to phenobarbital in liver samples HL-E, HL-I, and HL-O. The immunoquantified CYP3A concentrations and the CYP3A isozyme-specific midazolam 1\textsuperscript{\text{st}}-hydroxylation were the highest in these livers, and correspondingly high formation rates were also noticed for sulfoxide and quinol metabolites (table 3). Of the human liver samples tested, no correlation was observed with sex, age, tobacco, or alcohol consumption. The rates of formation of total sulfoxide and quinol metabolite were correlated with isozyme-selec-
catalytic activities and immunoquantitated concentrations of cytochrome P450 (table 3). As shown in table 4, the rate of formation of total sulfoxide correlated significantly with the rate of midazolam 1'-hydroxylation ($r^2 = 0.80$) and with the immunoquantifiable concentration of CYP3A (r_2 = 0.85). Furthermore, the rate of formation of the quinol metabolite was also significantly correlated with the immunoquantifiable concentrations of CYP3A ($r^2 = 0.93$) and with the rate of midazolam 1'-hydroxylation ($r^2 = 0.95$).

Isozyme selective inhibitors were used to investigate further the involvement of specific isozyme(s) of cytochrome P450 involved in the biotransformation of tazofelone. These experiments were carried out at two concentrations of inhibitors. The effects of inhibitors on the formation of total sulfoxide and the quinol metabolite are shown in fig. 4. The addition of TAO at concentrations of 10 and 50 $\mu$M almost completely inhibited the formation of total sulfoxide and the quinol metabolite. Furafylline, a known CYP1A inhibitor, at concentrations of 1 and 10 $\mu$M exhibited about 40% inhibition of sulfoxide formation while the other isozyme-selective inhibitors showed less than 20% inhibition for both sulfoxide and quinol metabolite formation. The results from these inhibition studies suggest that both sulfoxidation and quinol metabolite formation are CYP3A mediated activities. These results are in agreement with the microsomal correlation analysis.

The catalytic activities for the formation of sulfoxide and the quinol metabolite were investigated in microsomes prepared from the B-lymphoblastoid cell line AHH-1 expressing specific P450s. Fig. 5 shows the rates of formation of the two metabolites from these microsomes. The results show that total sulfoxide and the quinol metabolite are formed to some extent by all the P450 isozymes, but the greatest rate was associated with CYP3A4, which is consistent with the results from the isozyme specific correlation and inhibition studies.

Many sulfur-containing xenobiotics are metabolized to their sulfoxide form by flavine-containing monooxygenases (FMO) (19). To investigate the involvement of FMO mediated sulfoxidation, experiments were conducted at both pH 7.4 and pH 8.5 with human hepatic microsomal preparations heated for 1 min at 55°C with or without NADPH. The heating of microsomes without NADPH at 55°C for 1 min selectively inactivates the FMO system while having little effect on the cytochrome P450 system (16). The addition of NADPH to microsomes prior to heating preserves FMO activity and the differences in the rate of formation of the sulfoxide between the microsomes in which the FMO was inactivated and the microsomes in which the FMO activity was preserved would be large if it were metabolized by FMO. The optimum pH for maximal activity of FMO

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**Table 2**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>$K_{in}$ (µM)</th>
<th>$V_{max}$ (nmol/min/mg protein)</th>
<th>CL_{int} (site 1) (ml/min/mg protein)</th>
<th>CL_{int} (site 2) (ml/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfoxide</td>
<td>12.4 ± 1.4</td>
<td>0.27 ± 0.12</td>
<td>0.022</td>
<td>#</td>
</tr>
<tr>
<td>Quinol</td>
<td>7.5 ± 1.6</td>
<td>0.17 ± 0.02</td>
<td>0.023</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Formation rates of metabolites were measured as described in Material and Methods. $K_{in}$ and $V_{max}$ values were determined by nonlinear regression as described in Material and Methods. Incubations were performed in duplicate using tazofelone concentrations ranging from 3.9 $\mu$M to 1500 $\mu$M.

**Table 3**

<table>
<thead>
<tr>
<th>Human Liver Sample</th>
<th>Sulfoxide (nmol/min/mg protein)</th>
<th>Quinol (nmol/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>HL-A</td>
<td>0.42</td>
<td>0.47</td>
</tr>
<tr>
<td>HL-B</td>
<td>0.33</td>
<td>0.43</td>
</tr>
<tr>
<td>HL-C</td>
<td>0.21</td>
<td>0.43</td>
</tr>
<tr>
<td>HL-D</td>
<td>0.16</td>
<td>0.35</td>
</tr>
<tr>
<td>HL-E</td>
<td>0.75</td>
<td>2.20</td>
</tr>
<tr>
<td>HL-F</td>
<td>0.51</td>
<td>0.72</td>
</tr>
<tr>
<td>HL-G</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td>HL-H</td>
<td>0.33</td>
<td>0.52</td>
</tr>
<tr>
<td>HL-I</td>
<td>0.67</td>
<td>3.45</td>
</tr>
<tr>
<td>HL-J</td>
<td>0.46</td>
<td>0.68</td>
</tr>
<tr>
<td>HL-K</td>
<td>0.27</td>
<td>0.42</td>
</tr>
<tr>
<td>HL-L</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>HL-M</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>HL-N</td>
<td>0.44</td>
<td>0.37</td>
</tr>
<tr>
<td>HL-O</td>
<td>0.65</td>
<td>2.97</td>
</tr>
<tr>
<td>HL-P</td>
<td>0.67</td>
<td>1.06</td>
</tr>
<tr>
<td>HL-Q</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>HL-R</td>
<td>0.23</td>
<td>0.26</td>
</tr>
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</table>

**Table 4**

<table>
<thead>
<tr>
<th>Cytochrome P450 isozyme</th>
<th>Sulfoxide</th>
<th>Quinol</th>
<th>Correlation coefficients ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>0.02</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>CYP2A6</td>
<td>0.33</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>CYP2C8</td>
<td>0.49</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.20</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.21</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.51</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>CYP2E1</td>
<td>0.51</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td>-0.14</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td>0.14</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td>0.85*</td>
<td>0.93*</td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td>0.80*</td>
<td>0.95*</td>
<td></td>
</tr>
<tr>
<td>FMO3</td>
<td>0.15</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Denotes $p < 0.01$.
mediated oxidation is pH 8.5, and an increased formation of metab-
olite would be noticed at pH 8.5 if it were mediated by the FMO
relative to cytochrome P450. The results shown in table 5 indicate that
heat did not appreciably change the rate of formation of total sulfox-
dide. Sulfoxide formation at pH 8.5 was only 50% of the rate of
formation at pH 7.4. Incubation at pH 8.5 is not optimum for cyto-
chrome P450 mediated processes and hence the reduced rate of
formation of the sulfoxides. These data together with the aforemen-
tioned correlation analysis strongly suggest that the sulfoxidation of
tazofelone is mediated by the P450 system and in particular the
cytochrome P450 3A isozyme.

Discussion

In vivo biotransformation studies have demonstrated that tazofelone
undergoes oxidation forming a stereoisomeric mixture of sulfoxides
(unpublished results). The present in vitro study with human liver
microsomes has demonstrated that tazofelone undergoes biotransfor-
mation to a mixture of stereoisomeric sulfoxides and a hydroxy
metabolite. The hydroxy metabolite of tazofelone has been isolated
and shown by mass spectral and proton NMR analysis to be hydroxyl-
ated at the para-position of the t-butyl phenol moiety to the quinol
form of tazofelone. The biotransformation of the butylated hydroxy-
toluen (BHT) moiety of tazofelone to the quinol form is consistent
with existing literature. Thompson et al. have shown that BHT un-
dergoes cytochrome P450 mediated oxidation forming a quinol along
with nine other metabolites in rat liver microsomes (20). Recently,
Ohe et al. have shown that when p-cresol was incubated with rat liver
microsomes, a p-toluquiinol metabolite was formed (21). As with
the quinol metabolite of BHT, the quinol metabolite of tazofelone has not
been identified in vivo.

Table 5

<table>
<thead>
<tr>
<th>Incubation Conditions</th>
<th>Sulfoxide Formation (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.4</td>
</tr>
<tr>
<td>Control</td>
<td>140.2</td>
</tr>
<tr>
<td>No NADPH</td>
<td>0</td>
</tr>
<tr>
<td>Microsomes heated with NADPH*</td>
<td>117.6</td>
</tr>
<tr>
<td>Microsomes heated without NADPH*</td>
<td>105.5</td>
</tr>
</tbody>
</table>

* Microsomal protein was heated for 1 min at 55°C in the presence or absence of NADPH, cooled on ice for 2 min and followed by the normal incubation procedure described in Materials and Methods.

Fig. 4. Effect of isozyme-specific inhibitors on the formation of the total sulfoxide (A) and the quinol (B) metabolites of tazofelone.

Values represent the mean of duplicate measurements determined as described in Materials and Methods using a 20 μM tazofelone concentration and expressed as a percentage of duplicate control measurements. The values in parenthesis indicate the μM concentrations of inhibitors used in the experiment.

Kinetic analysis of the formation of the metabolites in human liver microsomes indicates that tazofelone sulfoxidation is catalyzed by a single enzyme system while tazofelone hydroxylation to the quinol metabolite is catalyzed by a two-enzyme system. The mean peak plasma concentrations of tazofelone achieved in rats after oral administration of a single dose of 30 mg/kg was about 1 μM (unpublished results). It is reasonable to assume that only the high affinity enzyme plays a major role in the metabolism of tazofelone. Hence, a 20 μM concentration of tazofelone was used to identify the isozyme(s) responsible for the metabolism of tazofelone. Identification of the specific isozyme involved in the formation of the sulfoxide and quinol
metabolites was achieved by using correlation analysis, isozyme specific inhibitors, and cDNA expressed P450 isozymes. The correlation analysis was accomplished by comparing the rates of sulfoxidation and hydroxylation of tazofelone with the isozyme-specific catalytic activity and immunoquantified isozyme-specific P450 concentrations. A significant correlation (p<0.01) was seen between the formation of both the sulfide and quinol metabolites of tazofelone and immunoquantified concentrations of CYP3A. A similar correlation (p<0.01) was also noted between the formation of both these metabolites and midazolam 1'-hydroxylation, a CYP3A mediated activity. The correlation analysis strongly suggests the involvement of CYP3A in the metabolism of tazofelone in vitro, and this evidence is supported by the inhibition studies that have been carried out using selective isozyme inhibitors. Triacetyleandomycin almost completely inhibited the formation of the sulfide and quinol metabolites at both 10 and 50 µM concentrations. Since TAO is a known mechanism-based inhibitor of CYP3A mediated metabolism, and TAO significantly inhibited the formation of the sulfide and quinol metabolites, this corroborates the earlier evidence obtained in the correlation analysis. Further evidence was obtained using commercially available cDNA expressed cytochrome P450 isozymes. All the cDNA expressed enzymes formed sulfide and quinol to some extent, but CYP3A4 formed the largest amount of both metabolites. This clearly supports the earlier evidence that CYP3A is involved in the metabolism of tazofelone.

Flavin monooxygenases are known to mediate the sulfoxidation of drugs and other xenobiotics (19). The human liver flavin-containing monooxygenase (FMO3) is known to mediate the S-oxidation of drugs such as cimetidine to their S-oxide metabolites (22, 23). Data presented herein strongly indicate that sulfoxidation is a cytochrome P450 mediated process and that FMO3 may not have any significant role. Moreover, the lack of correlation between the rate of formation of total sulfides and immunoquantified concentrations of FMO3 supports the hypothesis that tazofelone metabolism is a cytochrome P450 mediated process.

In summary, a monohydroxylated quinol metabolite of tazofelone has been identified and characterized in vitro. It was demonstrated that tazofelone undergoes oxidative metabolism forming stereoisomeric sulfoxides and a quinol metabolite. The correlation analysis, inhibition studies, and cDNA expressed isozyme specific microsomal studies implicate CYP3A4 as the isozyme responsible for the formation of both tazofelone sulfide and quinol metabolites.

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References