IN VITRO METABOLISM OF CLINDAMYCIN IN HUMAN LIVER AND INTESTINAL MICROSONES

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ABSTRACT:

Incubations with human liver and gut microsomes revealed that the antibiotic, clindamycin, is primarily oxidized to form clindamycin sulfoxide. In this report, evidence is presented that the S-oxidation of clindamycin is primarily mediated by CYP3A. This conclusion is based on several lines of in vitro evidence, including the following. 1) Incubations with clindamycin in hepatic microsomes from a panel of human donors showed that clindamycin sulfoxide formation correlated with CYP3A-catalyzed testosterone 6β-hydroxylase activity; 2) coincubation with ketoconazole, a CYP3A4-specific inhibitor, markedly inhibited clindamycin S-oxidase activity; and 3) when clindamycin was incubated across a battery of recombinant heterologously expressed human cytochrome P450 enzymes, CYP3A4 possessed the highest clindamycin S-oxidase activity. A potential role for flavin-containing monooxygenases (FMOs) in clindamycin S-oxidation in human liver was also evaluated. Formation of clindamycin sulfoxide in human liver microsomes was unaffected either by heat pretreatment or by chemical inhibition (e.g., methimazole). Furthermore, incubations with recombinant FMO isoforms revealed no detectable activity toward the formation of clindamycin sulfoxide. Beyond identifying the drug-metabolizing enzyme responsible for clindamycin S-oxidation, the ability of clindamycin to inhibit six human P450 enzymes was also evaluated. Of the P450 enzymes examined, only the activity of CYP3A4 was inhibited (~26%) by coincubation with clindamycin (100 µM). Thus, it is concluded that CYP3A4 appears to account for the largest proportion of the observed P450 catalytic clindamycin S-oxidase activity in vitro, and this activity may be extrapolated to the in vivo condition.

Metabolism and Disposition

Chemicals. Radiolabeled clindamycin, synthesized with a uniform carbon-14 radiolabel (specific activity 19.97 µCi/mg), was obtained from Pharmacia Corporation (Kalamazoo, MI). The radiochemical purity of [14C]clindamycin was 99.8% as determined by high performance liquid chromatography (HPLC) with radiochemical detection. Other chemicals, including nonlabeled clindamycin (PHA21251F), clindamycin sulfoxide (PHA25026A), and N-desmethyldclindamycin (PHA26285A), [14C]delavirdine, and broprinimine, were obtained from Pharmacia Corporation. [14C](S)-Mephenytoin, [14C]diclofenac, and [14C]chloroxazone were purchased from Amersham Biosciences Inc. (Piscataway, NJ); [14C]testosterone was obtained from PerkinElmer Life Sciences (Boston, MA); [14C]para-nitrophenol, ketoc-zaonole, quinidine, sulfaphenazole, coumarin, cyclosporin A, methimazole, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). (S)-Me-

Abbreviations used are: P450, cytochrome P450; HPLC, high performance liquid chromatography; FMO, flavin-containing monooxygenase; ACN, acetonitrile; LC/MS, liquid chromatography/mass spectrometry; APCI, atmospheric pressure chemical ionization.

Clindamycin (methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidin-2-carboxamido]-1-thio-1-threo-D-galacto-octopyranoside monohydrochloride) is an antibiotic of the “lincosamide” class (Brodasky et al., 1968). The lincosamide antibiotics seem to be most useful against the bacteria classified as Gram-positive cocci. In addition, clindamycin is helpful against protozoans such as Toxoplasma and Mycoplasma as well as many anaerobic bacteria (Luft and Remington, 1988; Dannemann et al., 1991; Mazur et al., 1999).

In humans, absorption of clindamycin is rapid and virtually complete (90%) following oral administration (DeHaan et al., 1972; Metzler et al., 1973). Concentrations of clindamycin in the serum increase linearly with increased dose, and levels exceed the minimum inhibitory concentration for most indicated organisms for at least 6 h following administration of the recommended dose. Clindamycin is widely distributed throughout the body and has an average biological half-life of 2.4 h. The major bioactive metabolites excreted in urine and feces are clindamycin sulfoxide and N-desmethylclindamycin (Seaberg et al., 1984; Flaherty et al., 1988; Gatti et al., 1998).

To date, there are no published reports that comprehensively describe the metabolic pathways associated with clindamycin clearance in humans. Identification of the drug-metabolizing enzymes responsible for the biotransformation of clindamycin and the interindividual differences in the expression and catalytic activities of those enzymes may help explain and/or predict population variability in the metabolic clearance of clindamycin. In vitro methodologies using human liver tissue have been developed to aid in the prediction of possible variation in metabolic clearance in vivo and drug-drug interactions for a variety of drugs. The specific objectives of the current study were to: 1) duplicate the major clindamycin metabolites observed in vivo using appropriate in vitro systems, 2) characterize the drug hepatic enzyme(s) responsible for metabolism formation, and 3) assess the drug-drug interaction potential of clindamycin toward select human P450 enzyme activities.

Material and Methods

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Phenytoin was a gift from Dr. W. F. Trager, Department of Medicinal Chemistry, University of Washington (Seattle, WA). Ultima-Flo-M liquid scintillant was purchased from PerkinElmer (Meriden, CT). All other reagents and solvents were of analytical grade.

**Microsomes.** Human livers were acquired from the International Institute for the Advancement of Medicine (Exton, PA). Liver microsomal protein isolation and the specific catalytic activity of individual enzymes of P450 were determined as previously described (Wienkers et al., 1996). For FMO heat liability experiments, incubations were performed as described above, except that microsomal preparations were preincubated with or without NADPH at 50°C for 2 min. Microsomes from a baculovirus-insect cell line expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9(*1), CYP2C9(*3), CYP2C19, CYP2C18, CYP2D6(*1), CYP2E1, CYP3A4, CYP3A5, CYP4A11, FMO1, FMO3, and FMO5 were purchased from BD Gentest (Woburn, MA). AntiCYP3A4 inhibitory antibodies and control sera were purchased from BD Gentest. Microsomes from human ileum and jejunum were purchased from Tissue Transformation Technology (Edison, NJ).

**Incubation Conditions.** A typical incubation (final volume 0.1 ml) consisted of 0.1 to 0.3 mg/ml microsomal protein in 100 mM potassium phosphate buffer (pH 7.4). Stock solutions of clindamycin were prepared in sterile water. The drug was added to each incubation well, followed by the assay buffer containing microsomes. Reactions were started by the addition of NADPH (1 mM final concentration) and continued for 30 min at 37°C. For control incubations, NADPH was omitted. Reactions were terminated upon addition of 50 μl of acetonitrile (ACN), after which samples were vortex mixed and centrifuged for 10 min at 1750g. The subsequent supernatants were injected directly from the 96-well plate, in a refrigerated plate holder using radio-HPLC analysis.

**Radio-HPLC.** Analytical separation of clindamycin and its metabolites was achieved using a HPLC system equipped with a PerkinElmer Series 200 pump and autosampler (PerkinElmer Instruments, Norwalk, CT) equipped with a chilled sample tray maintained at 4°C. The analytical column was a reverse-phase Zorbax C-18 (250 × 4.6 mm, 5-μm particle size) (Agilent Technologies Inc., Wilmington, DE). The mobile phase consisted of A (water/methanol/acetic acid, 90:10:0.02%) and B (methanol/water/acetic acid 90:10:0.02%). Chromatographic separation was achieved using a gradient run with a flow rate of 1 ml/min 100% A to 100% B over 15 min. Quantitation of clindamycin and its metabolites was performed using a FLO-ONE/Beta Series A500 flow-through radioactivity detector (PerkinElmer Life Sciences), and peak areas were integrated with Windows-based Radio-HPLC Workstation software (FLO-ONE/Ddata for Windows). Ultima-Flo-M liquid scintillant was introduced postcolumn at a rate of 2.5 ml/min. Rates of formation of the clindamycin sulfoxide metabolite were determined from the fractional conversions of [14C]clindamycin apparent from the radiochromatogram. Peaks were identified using retention time comparison with authentic clindamycin and clindamycin sulfoxide standards. In addition, all standards were chromatographed and structure was confirmed using LC/MS.

**LC/APCI/MS and Metabolite Confirmation.** The identities of the primary in vitro clindamycin metabolites were confirmed using an LCQ ion-trap (Thermo Finnigan, San Jose, CA) operated in positive-ion APCI mode. The APCI vaporizer temperature was 450°C, and the discharge current and spray voltage were set at 5 μA and 4.5 kV, respectively. Nitrogen (99.9% pure;
AGA, Maumee, OH) was employed as a drying gas at a sheath pressure of 80 psi and auxiliary flow rate of 20 ml/min, and the heated capillary was set at 250°C. Analytical separation of clindamycin and its metabolite was accomplished using the HPLC conditions described above. Under these conditions, authentic standards of clindamycin and the clindamycin sulfoxide derivative were characterized by retention time, molecular ion, and fragmentation pattern. The collision energy used was 1.3 and 1.6 V for clindamycin and clindamycin sulfoxide, respectively.

In Vitro Enzyme Kinetics. For kinetic studies in human liver microsomes, clindamycin concentrations ranged from 10 to 500 μM, with 0.2 mg/ml microsomal protein in each incubation. All other conditions were as already described. Analysis of clindamycin S-oxidation was performed using a PerkinElmerSciex API 150 single quadrupole mass spectrometer connected to PerkinElmer Series 200 micropumps and autosampler. Ions generated by electrospray ionization were detected using selected-ion monitoring in positive-ion mode. Clindamycin sulfoxide formation was detected by analysis of [M + H]⁺ molecular ion at m/z 441, with carbamazepine as internal standard (m/z 237). Analytical separation was accomplished using a Waters symmetry C8 2.1 mm x 150 mm column with mobile phase A (20 mM ammonium acetate, pH 4.5) and B (100% acetonitrile) delivered at 0.3 ml/min at an initial ratio of 80%:A:20%:B with a linear gradient to 90%:B over the first 2 min, held for another 1.5 min, then back to initial conditions for re-equilibration over the next 4.5 min. Data were analyzed using Sciex Analyst version 1.2 software, and kinetic parameters for clindamycin S-oxidation by CYP3A4 were estimated by nonlinear regression analysis (Michaelis-Menten equation) with GraphPad PRISM 3.0 software (GraphPad Software, Inc., San Diego, CA).

Incubations with Recombinant Human P450s. The metabolism of clindamycin was examined in microsomes prepared from a baculovirus-insect cell line expressing for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9(*1), CYP2C19, CYP2C19, CYP2C18, CYP2D6 (*1), CYP2E1, CYP3A4, CYP3A5, and CYP4A11. The incubations were conducted in a manner essentially as described above, with 100 μM [14C]clindamycin and equivalent P450 concentrations (10 pmol) of each P450 enzyme in 100 mM potassium phosphate buffer, pH 7.4. Incubations identical to those described above, containing clindamycin (1–1000 μM), were conducted using recombinant expressed CYP3A4 to determine the $K_{m}$ and $V_{max}$ for clindamycin metabolism.

Correlation Analysis. The rates of formation of clindamycin sulfoxide were determined across a panel of microsomes prepared from 12 human livers, 7 human jejunum, and 6 human ileum donors and compared with the catalytic activities previously characterized for specific P450 substrates (Wienkers et al., 1996). For this study the concentration of clindamycin was 50 μM, incubation conditions and sample work-up were carried out as described under Incubation Conditions. Coefficient of determination ($r^2$) for enzyme activities was determined by linear regression analysis using the graphical/statistical program Prism 3.0 (GraphPad).

Chemical Inhibition Experiments. [14C]Clindamycin (100 μM; approximately the apparent $K_{m}$ for clindamycin toward CYP3A4) was incubated at a single concentration in pooled human liver microsomes in the presence of a panel of compounds, which interacted selectively with various cytochrome P450 enzymes. The following P450 enzyme substrates/inhibitors were examined for their ability to inhibit the microsomal metabolism of clindamycin: bropirimine/CYP1A2 (200 μM), α-naphthoflavone/CYP1A2 (10 μM), coumarin/CYP2A6 (20 μM), sulfaphenazole/CYP2C9 (5 μM), (S)-mephentoin/CYP2C19 (200 μM), quinidine/ CYP2D6 (5 μM), ketoconazole/CYP3A (5 μM), cyclosporin A/CYP3A (50 μM), methimazole/FMO3 (200 μM). The inhibitors were dissolved in ACN and were added to the incubations such that the final amount of solvent was 1%. Control incubations (minus inhibitor) also contained 1% ACN.

Incubations with Anti-CYP3A4. To further study metabolism by CYP3A4, clindamycin (100 μM) was incubated with pooled human liver microsomes (0.3 mg protein/ml). Each sample was incubated with and without anti-CYP3A4 antibody (0.1 mg protein). The anti-CYP3A4 antibody was added to microsomes and placed on ice for 15 min. The incubations were then carried out in a manner identical to that of the human liver microsomal incubations.

P450 Inhibition Screen. The ability of clindamycin to selectively inhibit enzyme activity was investigated against six different recombinant human cytochrome P450 enzyme systems (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). Incubations were conducted in triplicate, and each incubation contained recombinant P450 microsomal protein (0.1–0.3 mg/ml), NADPH (1 mM final concentration), P450 marker substrate ([S] = $K_{m}$), and clindamycin at concentrations of 0 (minus inhibitor control), 1, 10, and 100 μM, in a final volume of 0.1 ml of 100 mM, pH 7.4, potassium phosphate buffer. Incubation reactions, sample work-up, and quantitation of P450 marker metabolite formation were conducted as previously described (Wynalda and Wienkers, 1997).

Results

Metabolism of Clindamycin. Human liver, ileum, and jejunum microsomes and recombinant CYP3A4 and CYP3A5 microsomes...
were incubated with \([^{14}\text{C}]\text{clindamycin}\) (100 \(\mu\text{M}\)), and the metabolite profiles were defined by radiochromatographic analysis (Fig. 1). For all incubations examined, clindamycin sulfoxide was the principal metabolite formed and accounted for greater than 90% of the total clindamycin consumed. In addition, a minor metabolite was also identified as the \(N\)-desmethyliclindamycin in human liver microsomes (Fig. 2). Both metabolites were characterized by LC/MS (Fig. 3) as well as cochromatography with that of the authentic metabolite standards (data not shown). Preliminary in vitro studies revealed that clindamycin sulfoxide formation increased linearly with time for up to 60 min (data not shown). Unless specified, an incubation time of 30 min was used to ensure initial rate conditions for the formation of clindamycin sulfoxide.

**In Vitro Kinetic Analysis.** The effects of substrate concentration on the rate of \(S\)-oxidation for clindamycin were determined in three human liver microsomal preparations and are listed in Table 1. For clindamycin, formation of clindamycin sulfoxide appeared to undergo saturable kinetics. Moreover, analysis of the kinetic data using Eadie-Hofstee graphical analysis (data not shown) revealed a linear relationship between \(V\) (the rate of clindamycin sulfoxide formation) and

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**Fig. 3.** Positive ion electrospray mass spectrum of clindamycin (A), \(N\)-desmethyliclindamycin (B), and clindamycin sulfoxide (C) obtained from human liver microsomal incubations.

Incubation conditions as well as LC/MS analysis were as described under Materials and Methods.
V/[S] (the velocity divided by the substrate concentration) that suggests that a single enzyme or two enzymes with similar $K_m$ values were responsible for clindamycin $S$-oxidation. This observation was then substantiated through a comparison of goodness-of-fit values generated for clindamycin sulfoxide velocity data modeled to single $K_m$ and multiple $K_m$ equations using sum-of-squares nonlinear regression analysis.

**Incubations with Recombinant Human P450s.** Of the 10 human baculovirus-insect cell-expressed P450 enzymes investigated, only CYP3A4 and, to a minor extent, CYP3A5 were able to catalyze the formation of clindamycin sulfoxide (Fig. 4). Enzyme kinetic experiments revealed that the apparent $K_m$ value for clindamycin sulfoxide metabolite formation by the recombinant CYP3A4 was consistent with the apparent $K_m$ values determined in human liver microsomes (Table 1).

**Cytochrome P450 Correlation Studies.** The rates of clindamycin sulfoxide formation were determined in 12 different human liver microsomal preparations. As shown in Fig. 5A, at a clindamycin concentration of 50 $\mu$M, the rate of clindamycin sulfoxide formation correlated with CYP3A-catalyzed testosterone activity ($r^2 = 0.97$). Moreover, the formation of this metabolite did not correlate with any other measured P450 activity in the individual livers (Table 2). Additionally, correlation studies conducted in individual intestinal microsomal preparations from human ileum ($n = 6$) and jejunum ($n = 7$) exhibited marked correlation with the measured CYP3A activity ($r^2 = 0.93$ and $r^2 = 0.95$, respectively) (Fig. 5, B and C).

**Cytochrome P450 Chemical Inhibition.** In addition to the correlation data, clindamycin was coincubated with the following P450 enzyme-specific substrate/inhibitors: bropirimine (CYP1A2), α-naphthoflavone (CYP1A2), coumarin (CYP2A6), sulfaphenazole (CYP2C9), mephenytoin (CYP2C19), quinidine (CYP2D6), ketoconazole (CYP3A), cyclosporin A (CYP3A4), and the FMO inhibitor methimazole. The agents were examined for their ability to inhibit clindamycin sulfoxide formation at a substrate concentration of 100 $\mu$M, as described in Fig. 6. The data in Fig. 6 reveal that only inhibitors of CYP3A were able to attenuate the rate of formation of clindamycin sulfoxide.

**Immunoinhibition Studies with Pooled Human Liver Microsomes.** To confirm the P450 reaction phenotyping results obtained with chemical inhibitors and cDNA-expressed P450s, [14C]clindamycin (100 $\mu$M) was incubated with pooled human liver microsomes in the absence and presence of immunoinhibitory anti-CYP3A4 peptide antibodies. The results with clindamycin indicated that the majority (~90%) of the clindamycin $S$-oxidase activity in human liver microsomes was attributable to CYP3A4 (Fig. 7).

**Contribution of FMO in Clindamycin S-Oxidation.** Flavin-containing monoxygenases are also known to oxidize thiethers to sulfoxides. Therefore, with incubations in human liver microsomes,

### TABLE 1

<table>
<thead>
<tr>
<th>Microsomes</th>
<th>$K_m$ ($\mu$M)</th>
<th>$V_{max}$ (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-18</td>
<td>130.2</td>
<td>5.1</td>
</tr>
<tr>
<td>HL-20</td>
<td>86.7</td>
<td>5.1</td>
</tr>
<tr>
<td>HL-23</td>
<td>1140.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Mean$^a$</td>
<td>110.3</td>
<td>5.5</td>
</tr>
<tr>
<td>S.D.$^a$</td>
<td>21.9</td>
<td>0.6</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>99</td>
<td>106$^c$</td>
</tr>
</tbody>
</table>

$^a$ HL, human liver.

$^b$ Obtained from three samples of human liver microsomes.

$^c$ $V_{max}$ presented as pmol/min/pmol P450.

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**Fig. 4.** Relative clindamycin $S$-oxidase activity in microsomes from human baculovirus-insect cell line expressing various drug-metabolizing enzymes.

A substrate (200 $\mu$M clindamycin) was incubated at 37°C for 45 min with microsomes (0.2 pmol) from human baculovirus-insect cells expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9(*1), CYP2C9(*3), CYP2C19, CYP2C18, CYP2D6(*1), CYP2E1, CYP3A4, CYP3A5, and CYP4A11, as well as (0.3 mg/ml) recombinant FMO1, FMO3, and FMO5. Each column represents the mean of triplicate experiments. ND, not detectable.
the potential involvement of FMO in clindamycin sulfoxide formation was examined. When clindamycin (100 μM) was incubated with recombinant FMO1, FMO3, and FMO5, no measurable clindamycin sulfoxide was formed (Fig. 4). In addition, there was no effect upon clindamycin sulfoxide formation following incubations with heat-inactivated microsomes compared with control microsomes (Fig. 6), as well as with coincubation with the FMO inhibitor, methimazole (Rawden et al., 2000) (Fig. 6). Lastly, clindamycin sulfoxide formation in human liver microsomes as a function of pH in a range of 6 to 10 revealed a single maximum at 7.5, which further suggests sole P450 involvement (Hoskins et al., 2001).

Effect of Clindamycin and Clindamycin Sulfoxide on Select P450 Activities. The selectivity of clindamycin to inhibit six human P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2E1, CYP2D6, and CYP3A4) was evaluated using a simple in vitro inhibition screen (Wynalda and Wienkers, 1997). When the P450 enzymes were tested against clindamycin at various concentrations (0, 1, 10, and 100 μM), only CYP3A4 testosterone hydroxylase catalytic activity was inhibited (~26% inhibition), at 100 μM clindamycin (Table 3).

Discussion

In the present study, it has been established that the in vitro biotransformation of clindamycin primarily involves an S-oxidation reaction which, based upon in vitro kinetic data, is mediated by a human P450. The involvement of CYP3A, in particular, CYP3A4, in the in vitro metabolism of clindamycin is supported by several lines of evidence. 1) A good correlation was found between the rate of clindamycin sulfoxide formation and measured CYP3A4 activity across a panel of human liver microsomes as a function of pH in a range of 6 to 10 revealed a single maximum at 7.5, which further suggests sole P450 involvement (Hoskins et al., 2001).

Table 2

<table>
<thead>
<tr>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP2E1</th>
<th>CYP3A4</th>
<th>Sulfoxide</th>
</tr>
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<td>1.00</td>
<td>0.05</td>
<td>1.00</td>
<td>0.00</td>
<td>1.00</td>
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<td>0.97</td>
</tr>
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<td>0.06</td>
<td>0.08</td>
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</tr>
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<td>0.19</td>
<td>0.07</td>
<td>0.00</td>
<td>1.00</td>
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<td>1.00</td>
</tr>
<tr>
<td>0.00</td>
<td>0.31</td>
<td>0.11</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
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</table>
clindamycin sulfoxide; and 4) only microsomes prepared from the insect cell line expressing CYP3A4 and, to a lesser extent, CYP3A5 were capable of oxidizing clindamycin to clindamycin sulfoxide. Moreover, the metabolite profile for clindamycin oxidation in recombinant CYP3A4 was qualitatively similar to that of pooled human liver, ileum, and jejunum microsomal preparations, formation of clindamycin sulfoxide correlated with measured testosterone 6-hydroxylase activity, a marker reaction for CYP3A4 (Waxman et al., 1991; Wang et al., 1997). In addition, the y-intercepts for each of these correlations are near the origin, which suggests a single enzyme being primarily responsible for the formation of metabolites (Fig. 5). In the experiment using microsomes expressing recombinant human P450 enzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5 and 4A11), only recombinant CYP3A4 and, to a lesser degree, CYP3A5 possessed measurable clindamycin S-oxidase activity (Fig. 4). Subsequent kinetic analysis of the interaction between clindamycin and CYP3A4 revealed an apparent \( K_m \) value of 99 \( \mu \)M for the reaction (Table 1).

When clindamycin S-oxidase activity was examined across human liver, ileum, and jejunum microsomal preparations, formation of clindamycin sulfoxide correlated with measured testosterone 6-hydroxylase activity, a marker reaction for CYP3A4 (Waxman et al., 1991; Wang et al., 1997). In addition, the y-intercepts for each of these correlations are near the origin, which suggests a single enzyme being primarily responsible for the formation of metabolites (Fig. 5). In the experiment using microsomes expressing recombinant human P450 enzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5 and 4A11), only recombinant CYP3A4 and, to a lesser degree, CYP3A5 possessed measurable clindamycin S-oxidase activity (Fig. 4). Subsequent kinetic analysis of the interaction between clindamycin and CYP3A4 revealed an apparent \( K_m \) value of 99 \( \mu \)M for the reaction (Table 1).

The CYP enzyme-selective inhibitors bropirimine (CYP1A2; Wynalda and Wienkers, 1997), coumarin (CYP2A6; Pearce et al., 1992), sulfaphenazole (CYP2C9; Baldwin et al., 1995; Newton et al., 1995), (S)-mephenytoin (CYP2C19; Wrighton et al., 1993), and quinidine (CYP2D6; Guengerich et al., 1986; Sai et al., 2000) all failed to

### Table 3

<table>
<thead>
<tr>
<th>P450 Enzyme</th>
<th>Percentage Control of P450 Marker Substrate Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ( \mu )M Clindamycin</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>97 ± 16</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>102 ± 2</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>101 ± 9</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>90 ± 6</td>
</tr>
</tbody>
</table>

Clindamycin sulfoxide; and 4) only microsomes prepared from the insect cell line expressing CYP3A4 and, to a lesser extent, CYP3A5 were capable of oxidizing clindamycin to clindamycin sulfoxide. Moreover, the metabolite profile for clindamycin oxidation in recombinant CYP3A4 was qualitatively similar to that of pooled human liver, ileum, and jejunum microsomal preparations, formation of clindamycin sulfoxide correlated with measured testosterone 6-hydroxylase activity, a marker reaction for CYP3A4 (Waxman et al., 1991; Wang et al., 1997). In addition, the y-intercepts for each of these correlations are near the origin, which suggests a single enzyme being primarily responsible for the formation of metabolites (Fig. 5). In the experiment using microsomes expressing recombinant human P450 enzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5 and 4A11), only recombinant CYP3A4 and, to a lesser degree, CYP3A5 possessed measurable clindamycin S-oxidase activity (Fig. 4). Subsequent kinetic analysis of the interaction between clindamycin and CYP3A4 revealed an apparent \( K_m \) value of 99 \( \mu \)M for the reaction (Table 1).
inhibit clindamycin sulfoxide formation (Fig. 6). Interestingly, coin-
cubation with the CYP1A2 inhibitor α-naphthoflavone (Chang et al.,
1994) results in a slight increase in clindamycin sulfoxide formation
compared with control. However, given that clindamycin is for all
intents and purposes a CYP3A4 substrate and α-naphthoflavone has
been demonstrated to act as an activator of CYP3A4 oxidation (Emoto
et al., 2001; Shou et al., 2001), this observation is not surprising.
Furthermore, ketoconazole, a potent CYP3A4 inhibitor (Newton et
al., 1995; Sai et al., 2000), and cyclosporin A, a known CYP3A4
substrate (Pichard et al., 1991; Kelly et al., 1999), both markedly
inhibited the oxidation of clindamycin, which provides additional
evidence for the involvement of CYP3A4 in the biotransformation of
clindamycin. Lastly, there was marked inhibition of clindamycin
sulfoxide formation upon pretreatment with anti-CYP3A4 antibody
(Fig. 7). In addition to coincubations with select P450 inhibitors,
icubations were also carried out in the presence of the FMO inhibitor
methimazole (Dixit and Roche, 1984; Rawden et al., 2000). In this
experiment, methimazole did not inhibit clindamycin sulfoxide for-
mation (Fig. 6).
Flavin-containing monoxygenases are known to oxygenate het-
eroatom-containing compounds (Ziegler, 2002). Therefore, the poten-
tial involvement of human liver FMOs in the S-oxidation of the
candidamycin was investigated. Incubations with human liver micro-
somes, verified to possess good FMO activity (data not shown), were
conducted as a function of pH (pH 6–10). A maximum for clinda-
mycin sulfoxide formation was observed at pH 7.5, which is charac-
teristic for a P450-mediated, rather than a FMO-mediated, reaction
(Grothuesen et al., 1996). In addition, purified human FMO3, the major
FMO in human liver (Cashman and Zhang, 2002), as well as FMO1
and FMO5, did not posses clindamycin S-oxidase activity (Fig. 4).
Finally, heat-treated (50°C, 1 min) human liver microsomes still
yielded clindamycin S-oxidation rates comparable to those of control
incubations (Fig. 6). This information, coupled with the lack of
inhibition upon coincubation with methimazole, firmly establishes
that human FMOs are not involved in the S-oxidation of clindamycin.

The importance of pharmacokinetic drug interactions associated
with human immunodeficiency virus therapy is well documented
(Heylen and Miller, 1996) and oftentimes occurs as a result of
inhibition of CYP3A4 (Malaty and Kuper, 1999). The human CYP3A
subfamily plays a dominant role in the metabolic elimination of many
protease inhibitors; amprenavir (Decker et al., 1998), saquinavir (Ea-
gling et al., 2002), nefinavir (Lilijibilbridge et al., 1998), ritonavir
(Kumar et al., 1996), and indinavir (Chiba et al., 1997), as well as reverse
transcriptase inhibitors nevirapine (Erickson et al., 1999) and delavir-
dine (Voorman et al., 1998). Since Staphylococcus aureus is the most
common microorganism causing cutaneous and systemic infections in
human immunodeficiency virus-infected patients, it is possible for
c candidamycin to be coadministered as part of a polytherapy regimen
(Stryt et al., 1997; Manfredi et al., 2002). In this light, understanding
P450 enzyme interactions might allow physicians the ability to better
anticipate and manage each patient’s response to adding clindamycin
to an established drug regimen. To gather some insight into the

![Fig. 8. Summary of clindamycin metabolism in human liver microsomes.](image-url)
potential of clindamycin as an inhibitor of cytochrome P450 enzymes, clindamycin was screened for the ability to inhibit select P450 enzyme catalytic activities. The current study revealed that clindamycin had a slight inhibitory effect upon the activities of CYP3A4. Based upon the factors governing the in vitro metabolism for clindamycin, the interaction between clindamycin and CYP3A4 was not surprising. Moreover, the observed magnitude of inhibition was consistent with the apparent $K_I$ determined for clindamycin and CYP3A4 ($K_I = 99 \mu M$ and previous work described by Bohets et al. (2000). Finally, clindamycin lacked any inhibitory effect on the activities of other P450 enzymes (CYP1A2, CYP2C9, CYP2C19, and CYP2E1) tested, even at high concentrations (100 μM).

Assuming first-order kinetic, liver drug metabolism is governed by the intrinsic enzyme catalytic capacity of individual hepatocytes and the availability of drug at the site of metabolism (Rane et al., 1977). Therefore, the underlying determinants for predicting a drug’s potential to inhibit a particular P450 is its $[I]/K_I$ ratio, where $[I]$ is the concentration of the inhibitor at the site of metabolism and $K_I$ is the apparent inhibitory constant of the inhibitor (Bertz and Graneman, 1997). Administration of clindamycin as a single 600-mg tablet results in a plasma $C_{max}$ of 3.08 μg/ml at about 1.5 h (Mazur et al., 1999). Therefore, the maximum concentration of total clindamycin (protein bound and free) achieved in vivo would be about 7 μM (the molecular weight of clindamycin is 424.99 atomic mass units). Using the projected $K_I$ value determined from the inhibition study, the $[I]/K_I$ ratio for the clindamycin with respect to CYP3A4 is approximately 0.07, which suggests a nominal drug interaction with CYP3A4. Thus, as long as the presence of clindamycin does not markedly alter the disposition characteristics of a second drug (i.e., the concentration of drug available at the site of metabolism is not different in the presence or absence of clindamycin) and the enzyme affinity constant for drug is independent of inhibitor, the current data suggest that clindamycin should not substantially alter the metabolism of a second drug whose clearance is primarily mediated by CYP3A4 as well as the major hepatic P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2E1).

In conclusion, the current in vitro findings show that the antibiotic clindamycin appears to be oxidized primarily by CYP3A4 (Fig. 8). Moreover, it appears that clindamycin does not inhibit the metabolic activity of the following P450 enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2E1, and CYP2D6, and only moderately inhibited CYP3A4. Therefore, given the poor affinity toward the human hepatic cytochrome P450 enzymes tested, clinically important interactions between clindamycin and coadministered drugs, which are metabolized by these enzymes, appear unlikely.

References


