ABSTRACT:
Antiretroviral therapy for human immunodeficiency virus (HIV) infection includes treatment with both reverse transcriptase inhibitors and protease inhibitors, which markedly suppress viral replication and circulating HIV RNA levels. Cytochrome P450 (P450) enzymes in human liver, chiefly CYP3A4, play a pivotal role in protease inhibitor biotransformation, converting these agents to largely inactive metabolites. However, the protease inhibitor nelfinavir (Viracept) is metabolized mainly to nelfinavir hydroxy-t-butylamide (M8), which exhibits potent antiviral activity, and to other minor products (termed M1 and M3) that are inactive. Since indirect evidence suggests that CYP2C19 underlies M8 formation, we examined the role of this inducible, polymorphic P450 enzyme in nelfinavir t-butylamide hydroxylation by human liver. Rates of micросomal M8 formation were 50.6 ± 28.3 pmol of product formed/min/nmol P450 (n = 5 subjects), whereas kinetic analysis of the reaction revealed a K_m of 21.6 μM and a V_max of 24.6 pmol/min/nmol P450. In reconstituted systems, CYP2C19 catalyzed nelfinavir t-butylamide hydroxylation at a turnover rate of 2.2 min⁻¹, whereas CYP2C9, CYP2C8, and CYP3A4 were inactive toward nelfinavir. Polyclonal anti-CYP2C9 (cross-reactive with CYP2C19) and monoclonal anti-CYP2C19 completely inhibited microsomal M8 production, whereas monoclonal CYP2C9 and polyclonal CYP3A4 antibodies were without effect. Similarly, the CYP2C19 substrate omeprazole strongly inhibited (75%) hepatic nelfinavir t-butylamide hydroxylation at a concentration of only 12.5 μM. Our study shows that CYP2C19 underlies formation in human liver of M8, a bioactive nelfinavir metabolite. The inducibility of CYP2C19 by agents (e.g., rifampicin) often taken concurrently with nelfinavir, together with this P450’s known polymorphic nature, may thus be important determinants of nelfinavir’s antiviral potency.

Highly active antiretroviral therapy is currently the most effective type of treatment for HIV infections, and has resulted in a substantial decline in mortality and morbidity rates among patients with AIDS (Mocroft et al., 1998; Palella et al., 1998; Walsh et al., 1998). Highly active antiretroviral therapy consists of several drug regimens, including the combination of a NRTI (e.g., zidovudine or didanosine) with a protease inhibitor (e.g., ritonavir, saquinavir, indinavir, or nelfinavir), two NRTIs plus two protease inhibitors, and even a triple NRTI combination, with each possessing its own therapeutic advantages and disadvantages. Coadministration of a NRTI plus a protease inhibitor has been shown to be very effective in suppressing HIV replication and in decreasing plasma HIV RNA levels (i.e., viral load) (Tebas and Powderly, 2000). These drugs target different sites in HIV, with NRTI agents inhibiting the viral reverse transcriptase, and protease inhibitors suppressing the viral gag-pol polyprotein-cleaving protease, which results in the formation of immature and noninfectious virions.

Protease inhibitors are metabolized primarily by P450 enzymes in the liver, with P450s in the small intestine playing a secondary, albeit important, role (Vella and Florida, 1998). In fact, the capacity of intestinal P450 enzymes to metabolize protease inhibitors may, at least in part, underlie the poor bioavailability of these agents upon oral administration (Chiba et al., 1997a; Fitzsimmons and Collins, 1997; Koudriakova et al., 1998). Enzymes comprising the CYP3A subfamily [the P450 enzymes referred to in this study are designated according to the nomenclature given on Dr. David Nelson’s website for cytochromes P450 (http://dmlnson.utmem.edu/cytochromeP450.html)] are thought to be the predominant, if not exclusive, catalysts of first-pass protease inhibitor metabolism in liver. In vitro studies with recombinant human P450s and/or human liver microsomes have demonstrated that CYP3A4 has the largest capacity (lowest K_M and highest V_max) to hydroxylate and/or dealkylate indinavir (Chiba et al., 1997b; Koudriakova et al., 1998), ritonavir (Kumar et al., 1996; Koudriakova et al., 1998), and saquinavir (Fitzsimmons and Collins, 1997). In preliminary studies of nelfinavir biotransformation, CYP3A4 was found to account for nearly 50% of the oxidative nelfinavir metabolism occurring in human liver microsomes, although the possible involvement of other P450s, such as CYP2C19, CYP2C9, and CYP2D6, was also reported (Wu et al., 1996; Kerr et al., 1997; Sandoval et al., 1998). There is additional indirect evidence suggesting that P450 en-

ABBREVIATIONS: M1, 2-methoxy-3-hydroxy nelfinavir; M3, 3,4-dihydroxy nelfinavir; M8, nelfinavir hydroxy-t-butylamide; HIV, human immunodeficiency virus; NRTI, nucleoside analog reverse transcriptase inhibitor; P450, cytochrome P450; P450 reductase, NADPH:P450 oxidoreductase; D LPC, L-α-dilauroylphosphatidylcholine; HPLC, high-performance liquid chromatography.
zymes other than CYP3A4 (e.g., CYP2C19 and CYP2D6) participate in protease inhibitor biotransformation. For example, ritonavir is capable of potently inhibiting both tolbutamide methyl hydroxylation and dextromethorphan O-demethylation in human liver microsomes, which are reactions catalyzed by CYP2C9 and CYP2D6, respectively (Kumar et al., 1996). Furthermore, nelfinavir has been found to inhibit not only CYP3A4-catalyzed testosterone 6β-hydroxylation but also CYP2C19-mediated S-mephenytoin 4'-hydroxylation in a competitive fashion, with reported $K_i$ values of 4.8 μM and 126 μM, respectively (Lillibridge et al., 1998c).

Nelfinavir is metabolized in the liver to three distinct products, namely M1, M3, and M8 (see Fig. 1). Initial in vitro studies have indicated that M1 and M3 are formed by CYP3A4, whereas the predominant M8 metabolite is produced by CYP2C19 (Lillibridge et al., 1998a,b). Only M1 and M8 have been identified in plasma upon oral nelfinavir administration, with M8 again being the most abundant product (Zhang et al., 2001). M8 may be further metabolized by CYP3A4, since Baede-van Dijk et al. (2001) found that HIV patients treated concurrently with nelfinavir and rifampicin, an exemplary CYP3A-inducing antibiotic used to prevent and/or treat opportunistic mycobacterial infections, had much lower plasma levels of nelfinavir and/or M8 compared with patients treated with only nelfinavir. Since M8 is at least as potent as nelfinavir with regard to its antiviral activity, at least in vitro (Zhang et al., 2001), it is important to understand the various factors that can influence formation as well as catabolism of this $t$-butylamide-hydroxylated nelfinavir derivative.

Herein, we assessed the role of CYP2C19 in the hepatic conversion of nelfinavir to its active metabolite, nelfinavir hydroxy-$t$-butylamide or M8. The metabolism of nelfinavir was first examined in liver microsomes and in reconstituted systems containing purified human P450 enzymes. Specific polyclonal and monoclonal antibodies as well as chemical probes were then used to demonstrate that CYP2C19 is indeed the sole catalyst of M8 formation from nelfinavir in human liver.

**Materials and Methods**

**Human Liver Specimens.** Normal human liver tissue was obtained from organ donors through the Liver Transplant, Procurement and Distribution System (University of Minnesota, Minneapolis, MN). None of the subjects had any history of drug or alcohol abuse. Livers were removed within 30 min of death, cut into small pieces, frozen in liquid nitrogen, and stored at −80°C until microsomes were prepared (Raucy and Lasker, 1991). P450 content was determined from CO-reduced difference spectra (Omura and Sato, 1964), whereas protein concentrations were measured using the bicinchoninic acid procedure (Smith et al., 1985).

**Microsomal Enzymes.** CYP2C9, CYP2C8, CYP3A4, cytochrome $b_5$, and P450 reductase were purified to near electrophoretic homogeneity from human liver microsomes as previously reported (Raucy and Lasker, 1991; Jin et al., 1998; Lasker et al., 1998). Recombinant CYP2C19 and CYP2C9 were derived from *Escherichia coli* after their transfection with the appropriate CYP2C19 cDNA- and/or CYP2C9 cDNA-containing plasmid as described elsewhere (Gillam et al., 1994). The specific contents of the hemoproteins were 11.4 (CYP2C9), 3.3 (CYP2C8), 25.7 (cytochrome $b_5$), 12.7 (recombinant

![Fig. 1. Scheme for the oxidative metabolism of nelfinavir. The three major pathways of nelfinavir hydroxylation are shown. Hydroxylation at position 3 gives rise to the major bioactive metabolite M8 (nelfinavir hydroxy-$t$-butylamide). Hydroxylation at position 1 and methylation at position 2 gives rise to M1 (2-methoxy-3-hydroxy nelfinavir), whereas hydroxylation at position 1 gives M3 (3,4-dihydroxy nelfinavir).](image-url)
CYP2C19), and 4.8 (recombinant CYP2C9) nmol/mg protein, and the specific activity of P450 reductase was 19,000 units (5.6 nmol/mg). One unit of P450 reductase activity was defined as that amount catalyzing reduction of 1 nmol of ferricytochrome c/min at 22°C in 300 mM potassium phosphate buffer (pH 7.7).

Nelfinavir Metabolism Assay. The conversion of nelfinavir to M8 was assessed in incubation mixtures (0.5 ml) containing 100 mM potassium phosphate buffer (pH 7.4), 25 to 50 μM nelfinavir, 1 mM NADPH, and either human liver microsomes (protein equivalent to 250 pmol of P450) or reconstituted human P450 enzymes. Reconstituted systems consisted of 25 to 50 pmol of purified P450 (either native or recombinant), 150 to 500 pmol of P450 reductase, 7.5 to 15 μg of synthetic DLPC, and 100 to 200 pmol of cytochrome b₅. Reactions were initiated with NADPH and were terminated after 20 min at 37°C with reconstituted P450s or 30 min at 37°C with microsomes by the addition of 0.4 ml of 1 M NH₄OH and then placing the reaction tubes on ice. For kinetic studies, the concentration of nelfinavir was varied from 15 to 100 μM. For antibody inhibition studies, microsomes were first incubated with either 1.25 mg of polyclonal immune-specific or control (preimmune) IgG or with either 0.08 mg of monoclonal immune-specific or control antibody for 3 min at 37°C, and then for 10 min at room temperature, followed by addition of the remaining reaction components. With omeprazole, this chemical probe was added to the reaction mixtures at concentrations ranging from 12.5 to 100 μM. Incubation mixtures were extracted with 5 volumes of ethyl acetate, followed by evaporation of organic extracts to dryness with nitrogen gas at room temperature. The residues were resolubilized in 100 μl of methanol, and 20- to 40-μl aliquots were processed using a Waters Alliance HPLC apparatus equipped with a model 2690 Separations module and a model 2487 UV-visible detector (Waters, Milford, MA). The samples were resolved with a Waters µBondapak C₁₈ column (3.9 × 300 mm) using a linear gradient (0.175% per minute) from 44% to 47.5% acetonitrile at a flow rate of 0.7 ml/min. Column eluents were continuously monitored for UV absorbance at 210 nm. M8 eluted from the column with a retention time ranging from 8.3 to 8.6 min, whereas nelfinavir exhibited a retention time of 11.5 to 14.5 min, and removing the solvent by evaporation of organic extracts to dryness with nitrogen gas at room temperature. The residues were resolubilized in 100 μl of methanol, and 20- to 40-μl aliquots were processed using a Waters Alliance HPLC apparatus equipped with a model 2690 Separations module and a model 2487 UV-visible detector (Waters, Milford, MA). The samples were resolved with a Waters µBondapak C₁₈ column (3.9 × 300 mm) using a linear gradient (0.175% per minute) from 44% to 47.5% acetonitrile at a flow rate of 0.7 ml/min. Column eluents were continuously monitored for UV absorbance at 210 nm. M8 eluted from the column with a retention time ranging from 8.3 to 8.6 min, whereas nelfinavir exhibited a retention time of 11.5 to 14.5 min. Rates of nelfinavir M8 formation were determined from standard curves prepared by adding varying amounts of authentic M8 to incubations conducted without NADPH. Kinetic data were analyzed using the Enzyme Kinetic module of SigmaPlot (SYSTAT Software, Inc., Point Richmond, CA).

Reagents. Nelfinavir mesylate salt and M8 were generously provided by Dr. Caroline A. Lee, Pfizer Global Research and Development (La Jolla, CA). With certain lots of nelfinavir, it was necessary to further purify the agent to remove residual M8 contamination. This purification was performed by repetitively injecting aliquots of a concentrated nelfinavir solution (prepared in methanol) onto the HPLC apparatus, collecting the 210-adsorbing material eluting from the column at 11.5 to 14.5 min, and removing the solvent by vacuum evaporation. The highly purified nelfinavir was resolubilized in methanol, and its concentration was determined using an extinction coefficient of 74.5 M⁻¹ cm⁻¹ at 210 nm. Purified recombinant CYP2C9 and CYP2C19 were obtained from PanVera Corp. (Madison, WI). Polyclonal antibodies to human CYP3A4, CYP4A11, and CYP2C9 were raised in male New Zealand White rabbits as described elsewhere (Feierman and Lasker, 1996; Powell et al., 1996; Lasker et al., 1998), whereas preimmune (control) IgG was prepared from rabbit sera obtained before immunization. Monoclonal antibodies to human CYP2C19 and human CYP2C9 were obtained from Drs. Kristopher Krausz and Harry Gelboin, National Cancer Institute, National Institutes of Health (Bethesda, MD). The characteristics of these monoclonals have been described previously (Krausz et al., 2001). All other chemicals used were of the highest grade commercially available.

Nelfinavir Hydroxylation by Human Liver Microsomes. Preliminary in vitro studies, published in abstract form (Lillibridge et al., 1998a,b), have suggested that nelfinavir is converted by human liver to at least three distinct products, namely M1, M3, and M8 (Fig. 1). M1 and M3 are purportedly formed by CYP3A4, whereas M8 is generated by CYP2C19. To confirm and expand upon these observations, we initially assessed t-butylamide hydroxylation of nelfinavir to M8 by human liver microsomes. M8 was formed in a time-dependent manner, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown). Formation of M8 did not occur upon omission of NADPH from the incubation mixtures. Rates of nelfinavir t-butylamide hydroxylation among the five different subjects examined ranged from 19.4 to 78.3 pmol of M8 formed/min/nmol P450 (2.9–14.4 pmol/min/mg protein), with a mean of 50.6 ± 28 pmol of M8 formed/min/nmol P450 (10.0 ± 5.1 pmol/min/mg protein). The conversion of nelfinavir to M8 by human liver microsomes was found to exhibit simple Michaelis-Menten kinetics over the range of nelfinavir concentrations studied, with rates of product formation exhibiting linearity for at least 30 min (data not shown).
navir concentrations examined (15–100 μM) (Fig. 2A). The Eadie-Hofstee plot shown in Fig. 2B was used to derive an apparent $K_M$ of 21.6 μM and $V_{max}$ of 24.6 pmol of M8 formed/min/nmol P450. Such monophasic kinetics suggested the involvement of only a single P450 enzyme (or two P450s with highly similar $K_M$ values) in nelfinavir t-butylamide hydroxylation. Furthermore, it was obvious from HPLC analysis that metabolites other than M8 (e.g., M1 and/or M3) were formed by human liver microsomes, although attempts to identify them were not conducted here.

**Nelfinavir Hydroxylation by Purified P450 Enzymes.** Upon reconstitution with P450 reductase, cytochrome $b_5$, and DLPC, only a single P450, namely RCYP2C19, was found to convert nelfinavir to M8 at extensive rates (Table 1). Indeed, the CYP2C19-catalyzed rates noted were nearly 40-fold higher than those exhibited by intact liver microsomes. On the other hand, native CYP3A4, CYP2C9, RCYP2C9, and CYP2C8 all failed to support M8 formation in reconstituted systems. In the case of CYP3A4, this was true whether the enzyme was reconstituted with DLPC or with the phospholipid mixture (DLPC, dioleoylphosphatidylcholine, phosphatidylserine, plus 0.1 mg sodium cholate) described by Imaoka et al. (1992). That reconstituted CYP3A4 was catalytically active was indicated by the high rates of nifedipine oxidation catalyzed by the enzyme, although no difference in nifedipine oxidase activity was observed upon substitution of DLPC with the above-described phospholipid mixture (data not shown).

**Immunoinhibition of Nelfinavir Hydroxylation.** The obligatory role of CYP2C19 in M8 formation by human liver was assessed using inhibitory polyclonal and monoclonal antibodies. We initially used polyclonal antibodies to human CYP2C9, CYP3A4, and CYP4A11, the former of which cross-reacts with both CYP2C19 and CYP2C8 (Lasker et al., 1998). At an antibody/enzyme ratio of 5 mg of IgG/nmol P450, anti-CYP2C9 completely (>99%) inhibited M8 formation by liver microsomes from the two subjects examined, UCSF001 and UC6603 (Fig. 3), whereas neither anti-CYP3A4 IgG nor anti-CYP4A11 IgG elicited appreciable inhibition of microsomal M8 formation in these subjects. A monoclonal antibody to CYP2C19 gave the same marked degree of inhibition (>99%) as anti-CYP2C9 of M8 formation by liver microsomes from subject UCSF001, but at an IgG/P450 ratio of only 0.08 mg/nmol P450 (Figs. 3 and 4). With microsomes from subject UC6603, the extent of inhibition of M8 formation by monoclonal anti-CYP2C19 was at least 99% at the same time.

**TABLE 1**

*Nelfinavir t-butylamide hydroxylation by human P450 enzymes*  

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nelfinavir M8 Formation pmol M8 formed/min/nmol P450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant CYP2C19</td>
<td>2.219a</td>
</tr>
<tr>
<td>Recombinant CYP2C9</td>
<td>BD</td>
</tr>
<tr>
<td>Native CYP2C9</td>
<td>BD</td>
</tr>
<tr>
<td>Native CYP2C8</td>
<td>BD</td>
</tr>
<tr>
<td>Native CYP3A4</td>
<td>BD</td>
</tr>
<tr>
<td>UC6603 Mx</td>
<td>58</td>
</tr>
</tbody>
</table>

BD, below detection limits.  

Superscripted values indicate the average of three to six individual determinations.

Recstitution of CYP3A4 with the phospholipid mixture (DLPC, dioleoylphosphatidylcholine, and phosphatidylserine in the presence of 0.1 mg of sodium cholate) described by Imaoka et al. (1992) also gave no metabolic activity.

**Fig. 3.** Inhibition of nelfinavir t-butylamide hydroxylation by P450 antibodies.  

**ANTIBODY ADDED**  

**CONTROL**  

**ANTI-CYP2C9**  

**ANTI-CYP2C19**  

**ANTI-CYP3A4**  

**ANTI-CYP3A4 Mab**  

**ANTI-CYP4A11**  

**ANTI-CYP4A11 Mab**  

**Discussion**

We have demonstrated herein that human liver microsomes convert the potent protease inhibitor agent nelfinavir to M8, a hydroxylated t-butylamide derivative that also possesses antiviral properties. This reaction was shown to be catalyzed exclusively by CYP2C19, a P450 enzyme that not only exhibits polymorphisms among the population but is also inducible by other therapeutics. Since one of these therapeutics is rifampicin, an antibiotic commonly administered to patients with HIV to control opportunistic mycobacterial infections, the potential for drug interactions between nelfinavir and rifampicin is high among such patients. Furthermore, the polymorphic nature of CYP2C19 predicts the existence of subjects incapable of converting nelfinavir to M8, which represents a major route of elimination for the
CYP3A4 exhibited negligible nelfinavir t-butylamide hydroxylation to M8 by liver microsomes from subject UC9603 was assessed in reaction mixtures containing liver microsomes from subjects UC9603 (protein equivalent to 250 pmol of P450), 23 μM nelfinavir, 100 mM potassium phosphate buffer (pH 7.4), 1 mM NADPH, and 12.5 to 100 μM omeprazole. The reactions were initiated with NADPH and terminated after 30 min at 37°C, and M8 production was then assessed by HPLC as described under Materials and Methods. Control rates of microsomal M8 formation were 117.1 pmol of product formed/min/mmol of P450. Values denote the average of duplicate determinations that differed from each other by less than 10%.

CYP2C19 and a polyclonal antibody to CYP2C9 (which strongly cross-reacts with CYP2C19) (Lasker et al., 1998) were found to markedly inhibit (>99%) nelfinavir conversion to M8 in liver microsomes (Figs. 3 and 4). Polyclonal antibodies to CYP3A4 (and CYP4A11) had no such effect, however, at the same IgG/P450 ratios. Finally, another CYP2C19 substrate, namely omeprazole, proved capable of decreasing microsomal M8 formation in a dose-dependent manner (Fig. 5). Although omeprazole is also metabolized by CYP3A4 (Karam et al., 1996), the fact that antibodies to this P450 had no effect on nelfinavir t-butylamide hydroxylation indicates that the inhibitory effect of omeprazole stemmed from its interaction with CYP2C19. The lack of effect of anti-CYP3A4 on rates of microsomal M8 formation also implies that the corresponding P450 does not play a major role in M8 catabolism, as suggested by other workers (Bergsheoff et al., 2003) (see below). It should be noted here that each of the five subjects we studied were capable of metabolizing nelfinavir to M8, albeit at variable rates. Since Western blotting of liver microsomes from these subjects had previously revealed expression of CYP2C19 protein (data not presented), the fact that each subject catalyzed hepatic nelfinavir t-butylamide hydroxylation is not surprising.

It has been established that protease inhibitors such as nelfinavir interact in vivo with rifampicin and similar therapeutics (e.g., rifabutin) used to prevent and/or treat the opportunistic mycobacterial infections commonly found in patients with HIV. Importantly, rifampicin is capable of accelerating protease inhibitor metabolism in vivo, resulting at times in circulating levels of protease inhibitors that are no longer in the therapeutic range (Eagling et al., 1997; Vella and Floridia, 1998). A marked increase in nelfinavir elimination rates in vivo has also been observed upon rifampicin coadministration (Kerr et
References


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