CYTOCHROME P450 INVOLVEMENT IN THE BIOTRANSFORMATION OF CISAPRIDE AND RACEMIC NORCISAPRIDE IN VITRO: DIFFERENTIAL ACTIVITY OF INDIVIDUAL HUMAN CYP3A ISOFORMS

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ABSTRACT:

Identification of the human cytochrome P450 (P450) enzymes involved in the metabolism of cisapride and racemic norcisapride ([±]-norcisapride) was investigated at 0.1 and 1 μM, concentrations that span the mean plasma Cmax for cisapride. Formation of norcisapride (Nor), 3-fluoro-4-hydroxycisapride (3F), and 4-fluoro-2-hydroxycisapride (4F) from cisapride and an uncharacterized metabolite (UNK) from [±]-norcisapride in human liver microsomes (HLMs) was consistent with Michaelis-Menten kinetics for a single enzyme (Km, 6.0, 14.3, 13.9, and 107 μM; Vmax, 1350, 696, 568, and 25 pmol/mg of protein, respectively). HLMs converted cisapride to Nor at rates that were at least 3 orders of magnitude greater than those observed for [±]-norcisapride conversion to UNK. The sample-to-sample variation in the rates of Nor, 3F, 4F, and UNK formation correlated strongly (r² > 0.796) with CYP3A4/5 activity in a panel of HLMs (n = 7) and was markedly reduced by ketoconazole, a potent CYP3A inhibitor. Ketoconazole virtually eliminated [±]-norcisapride conversion to UNK (94 ± 0.5%). Studies with 10 cDNA-expressed enzymes revealed that CYP3A4 catalyzed the formation of Nor and 4F at rates >100 times those of non-CYP3A enzymes and >100- and 50-fold higher than CYP3A5 and CYP3A7, respectively. CYP3A4 was the only P450 capable of UNK formation. Therefore, CYP3A4 is the principal P450 enzyme responsible for the conversion of cisapride to Nor, 3F, and 4F and of [±]-norcisapride to UNK. Compared with cisapride, factors related to CYP3A4-mediated [±]-norcisapride metabolism (e.g., ontogeny of drug-metabolizing enzymes, inhibition, and induction) should be clinically important due to the apparent lack of dependence on cytochromes P450 for elimination.

Cisapride is a prokinetic drug that acts as a postganglionic serotonin 5-hydroxytryptamine receptor agonist (McCallum et al., 1988). It has been widely used in adults and children for the treatment of gastroparesis and symptoms associated with gastroesophageal reflux disease (Wiseman and Faulds, 1994; Vandenplas, 1998). Cisapride has also been administered frequently to neonates and young infants to facilitate oral feeding and to reduce the potential for severe adverse effects (e.g., apnea and bradycardia) associated with excessive regurgitation of gastric contents (Enríquez et al., 1998; Vandenplas, 1998).

Cisapride is a well tolerated drug with a low incidence (i.e., approximately 2%) of adverse effects that are predominantly gastrointestinal in nature (Vandenplas et al., 1996). In rare cases, cisapride has been linked to prolonged QTc intervals and the production of potentially life-threatening ventricular arrhythmias (Lewin et al., 1996; van Haarst et al., 1998). Cardiac side effects have been most commonly reported in individuals receiving accidental overdoses of cisapride or in cases in which cisapride was coadministered with other drugs that alter cisapride clearance (e.g., CYP3A inhibitors) and/or prolong QTc intervals through effects on the iKs channel (e.g., azole antifungals, erythromycin, and clarithromycin) (Michaels and Williams, 2000). Safety concerns related to the association of these rare, cardiac side effects with cisapride administration prompted a recent, voluntary withdrawal of cisapride from the U.S. market by the manufacturer.

Humans extensively metabolize cisapride to one major metabolite, norcisapride (Nor1), and to several minor metabolites, including 3-fluoro-4-hydroxycisapride (3F) and 4-fluoro-2-hydroxycisapride (4F). Norcisapride, which retains the cis-conformation present in cisapride, is slowly metabolized further to a metabolite of unknown structure and, to a lesser extent, via oxidation to two lactam metabolites, each with a molecular weight of 327 (Meuldermans et al., 1988a,b), as shown in Fig. 1. Racemic norcisapride ([±]-norcisapride) possesses approximately one-sixth of the prokinetic activity of cisapride but does not appear to be associated with cardiac side effects (Hubbard, 1994; Vandenplas et al., 1999).

The metabolism of cisapride to Nor, 3F, and 4F is attributed largely to CYP3A4, although much of the evidence to date has been indirect. Clinical investigations have reported pharmacokinetic interactions

1 Abbreviations used are: Nor, norcisapride; 3F, 3-fluoro-4-hydroxycisapride; 4F, 4-fluoro-2-hydroxycisapride; P450, cytochrome P450; HPLC, high-pressure liquid chromatography; MS, mass spectrometry; UNK, unidentified metabolite of [±]-norcisapride; Clint, intrinsic clearance.
between cisapride and compounds known to inhibit CYP3A4 (e.g., erythromycin, clarithromycin, ketoconazole, diltiazem, and grapefruit juice) (Michalets and Williams, 2000). Recent in vitro studies (Bohets et al., 2000; Desta et al., 2000) have also attributed the biotransformation of cisapride to CYP3A enzymes. These studies, however, were conducted with concentrations of cisapride (5 and 10 μM, respectively) that greatly exceeded those observed in vivo (average plasma Cmax 0.17 μM). Therefore, it was uncertain whether the results from the previous in vitro studies would reflect cisapride metabolism in vivo. Additionally, there was little or no information in the literature regarding the ability of CYP3A5 (a polymorphically expressed CYP3A enzyme present in ~25% of adult livers) or of CYP3A7 (a fetal CYP3A isoform) to catalyze the biotransformation of cisapride, although preliminary data indicate that these two CYP3A isoforms are less active than CYP3A4 in catalyzing cisapride N-dealkylation to Nor (Gotschall et al., 1999). Furthermore, there have been no published studies that identify the P450 enzymes involved in (±)-norcisapride metabolism. Given the widespread use of cisapride in the first year of life (Lacroix et al., 1997), we sought to more fully characterize the role that CYP3A isoforms play in the biotransformation of cisapride to Nor, 3F, and 4F and to identify the P450 enzymes involved in the biotransformation of (±)-norcisapride to its metabolites. To achieve these objectives, we conducted in vitro studies with cisapride (±)-norcisapride at concentrations (0.1 and 1.0 μM) that spanned the average plasma Cmax for cisapride.

Materials and Methods

Chemicals. Cisapride was purchased from Research Diagnostics (Flanders, NJ). (±)-Nor cisapride, 3-fluoro-4-hydroxycisapride, and 4-fluoro-2-hydroxycisapride were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). α-Naphthoflavone, coumarin, (S)-(−)-nicotine, orphenadrine, quercetin, sulfaphenazole, lansoprazole, quinidine, 4-methylpyrazole, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, and EDTA were purchased from Sigma/RBI (Natick, MA). All other reagents were of analytical grade.

Human Liver Microsomes and cDNA-Expressed Enzymes. Microsomes prepared from seven different human livers were purchased from GENTEST. Vials of microsomes were stored at −70°C, rapidly thawed in room temperature water, and placed on ice before use.

Incubation Conditions. Assay conditions were established for each microsomal sample so that metabolism of the parent compound did not exceed 20%. Under standard incubation conditions, human liver microsomes (2 or 200 μg of microsomal protein in reactions containing cisapride and (±)-nor cisapride, respectively) were incubated at 37 ± 1°C in 100-μl final volume incubation mixtures containing potassium phosphate buffer (50 mM; pH 7.4), MgCl2 (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (1 U/ml), glucose-6-phosphate dehydrogenase (5 mM), and cisapride or (±)-norcisapride (0.1 and 1.0 μM) at the final concentrations listed. In vitro enzyme assays were performed in 96-well microtiter plates. Reactions were initiated by the addition of the NADPH-generating system and terminated by the addition of 200 μl of ice-cold methanol containing 0.15% triethylamine. Incubation mixtures containing (±)- nor cisapride were terminated after 120 min, whereas incubation mixtures containing cisapride were terminated after 10 to 40 min, depending on the activity of each microsomal sample. When incubations with microsomes containing cDNA-expressed P450 enzymes were performed, cisapride or (±)-nor cisapride (0.1 and 1.0 μM) was incubated with 1 (CYP3A4) or 10 pmol of P450 (all other enzymes examined) for 60 min with cisapride as substrate and 20 pmol of P450 for 120 min with (±)-nor cisapride as substrate. Protein was precipitated by centrifugation at 10,000 rpm for 10 min. An aliquot (10–75 μl) of the supernatant was analyzed by HPLC/MS via direct injection, thereby alleviating the need for an internal standard. The rates of formation of Nor, 3F, and 4F (from cisapride) and of an unidentified product (UNK) (from (±)- nor cisapride) were proportional to incubation time and protein concentration (up to 60 min at 0.02 mg/ml protein with cisapride as substrate and up to 120 min at 2 mg/ml liver microsomal protein with (±)- nor cisapride as substrate) at substrate concentrations of 0.1 and 1.0 μM. Metabolite formation was also linear in incubations containing 1 (CYP3A4) or 10 pmol of P450 (all other enzymes examined) for at least 60 min with cisapride as substrate and 20 pmol of P450 for at least 120 min with (±)-nor cisapride as substrate. Experiments designed to determine kinetic parameters were performed in duplicate at substrate concentrations ranging from 0 to 100 μM. All other experiments performed with in vitro microsomes were conducted with concentrations of cisapride (5 and 10 μM, respectively) that greatly exceeded those observed in vivo (average plasma Cmax 0.17 μM). Therefore, it was uncertain whether the results from the previous in vitro studies would reflect cisapride metabolism in vivo. Additionally, there was little or no information in the literature regarding the ability of CYP3A5 (a polymorphically expressed CYP3A enzyme present in ~25% of adult livers) or of CYP3A7 (a fetal CYP3A isoform) to catalyze the biotransformation of cisapride, although preliminary data indicate that these two CYP3A isoforms are less active than CYP3A4 in catalyzing cisapride N-dealkylation to Nor (Gotschall et al., 1999). Furthermore, there have been no published studies that identify the P450 enzymes involved in (±)-nor cisapride metabolism. Given the widespread use of cisapride in the first year of life (Lacroix et al., 1997), we sought to more fully characterize the role that CYP3A isoforms play in the biotransformation of cisapride to Nor, 3F, and 4F and to identify the P450 enzymes involved in the biotransformation of (±)-nor cisapride to its metabolites. To achieve these objectives, we conducted in vitro studies with cisapride (±)-nor cisapride at concentrations (0.1 and 1.0 μM) that spanned the average plasma Cmax for cisapride.
were performed with two replicate samples per condition in triplicate (n = 6 determinations) under standard incubation conditions. Rates at which cisapride and (±)-norcisapride were converted to their respective metabolites by recombinant P450 enzymes are reported as background (control) corrected rates.

Chemical-Inhibition Experiments. Formation of metabolites from cisapride (0.1 and 1.0 μM) or (±)-norcisapride (1.0 μM) by human liver microsomes was evaluated in the presence or absence (i.e., control) of known P450 isoform-selective inhibitors. The following inhibitors were examined at concentrations previously identified as being appropriate to cause P450 isoform-selective inhibition (Madian et al., 1995; Ko et al., 1997; Stevens et al., 1997; Clarke, 1998; Desai et al., 1998): o-naphthoflavone (CYP1A2; 1 μM), coumarin (CYP2A6; 150 μM), (5S)-nicotine (CYP2A6; 200 μM), 1-naphthylamine (CYP2B6; 500 μM), quercetin (CYP2C8; 20 μM), sulfaphenazole (CYP2C9; 10 μM), lansoprazole (CYP2C19; 5 μM), quindine (CYP2D6; 0.125 μM), 4-methylpyrazole (CYP2E1; 1 μM), and ketoconazole (CYP3A4/5; 1 μM). Inhibitors were dissolved in methanol and diluted in the incubation mixtures to a final solvent concentration of 1% (v/v). Control incubations contained an equal volume of methanol.

HPLC/MS Analysis. Cisapride and its metabolites Nor, 3F, and 4F were resolved by reverse-phase HPLC with a Hewlett Packard HP1100 HPLC system equipped with a HP1100 degasser, binary pump, auto-sampler, column heater, and a HP1100 single quadrupole mass spectral detector (Hewlett Packard, Palo Alto, CA). The analytical column was a Phenomenex (Torrance, CA) Luna C18 (2) column (4.6 mm × 15 cm, 5-μm particle size) preceded by a Phenomenex C18 guard column (4-mm × 3-mm i.d., 5-μm particle size). The mobile phase was a 70:30 mixture of methanol/water containing 0.1% triethylamine. UNK was detected at an m/z of 328 (M + 1). Formation of UNK was estimated from the relationship between the amount of (±)-norcisapride analyzed and its peak area, which assumes that (±)-norcisapride and UNK ionize equally under the analysis conditions. It should be emphasized that the method used to quantify UNK provides only a rough approximation of the amounts of UNK analyzed. Under these assumptions, the limit of quantification for the assay was 2.5 fmol for UNK. The retention times of (±)-norcisapride and UNK were 2.3 and 3.1 min, respectively.

Data Analysis. Kinetic parameters for the formation of metabolites from cisapride and (±)-norcisapride were estimated from the best-fit line using least-squares linear regression analysis of Lineweaver-Burk plots. Regression coefficients (r²) between the formation of cisapride or (±)-norcisapride metabolites and the activities of cytochrome P450 enzymes were also determined using least-squares regression analysis. Significance was determined by Pearson’s regression analysis from two-tailed r tables. To be statistically significant at the 5 or 1% level of significance, r² must exceed 0.448 or 0.764, respectively.

Results

Metabolism of Cisapride and (±)-Nor cisapride by Human Liver Microsomes. In the presence of NADPH and oxygen, human liver microsomes converted cisapride to Nor, 3F, and 4F (based on retention times and positive ion mass of authentic standards). (±)-Norcisapride was converted to one apparent unidentified metabolite, UNK, which was detected at an MS setting of m/z 328, suggesting that UNK has a mass of at least 327 Da. In the absence of NADPH or human liver microsomes, no metabolites were formed from either cisapride or (±)-nor cisapride.

Correlation Experiments. Human liver microsomes prepared from seven donors were examined for their ability to metabolize cisapride and (±)-norcisapride at two substrate concentrations (0.1 and 1.0 μM). All of the microsomal samples examined converted cisapride to Nor, 3F, and 4F, and in each case, Nor was the primary metabolite formed. At a substrate concentration of 0.1 μM, the respective rates of Nor, 3F, and 4F formation varied ~5-fold (range (rates ± S.D.): Nor, 3.78 ± 0.71 to 19.5 ± 1.6 pmol/mg of protein/min; 3F, 2.29 ± 0.71 to 10.3 ± 0.4 pmol/mg of protein/min; and 4F,
The effects of various P450 inhibitors on the conversion of cisapride to UNK occurred at rates that were at least 3 orders of magnitude less than the rate of cisapride metabolism with CYP3A4/5 (\(r^2 = 0.903\)) and CYP2B6 activities (\(r^2 = 0.513\)) but not with any other cytochrome P450 activities.

Microsomes from each of the seven donors converted (±)-norcisapride to UNK. At a substrate concentration of 0.1 \(\mu M\), the rate of UNK formation varied -10-fold (0.8 ± 0.1 to 7.7 ± 0.6 fmol/mg protein/min) and was significantly correlated with CYP3A4/5 activity (\(r^2 = 0.796\)) but not with any other cytochrome P450 activities. At 1.0 \(\mu M\) (±)-norcisapride, the rate of UNK formation varied -22-fold (3.3 ± 0.4 to 73.8 ± 1.5 fmol/mg protein/min) and correlated significantly with CYP3A4/5 and CYP2B6 activities (\(r^2 = 0.887\) and 0.606, respectively) but with no other P450 activities. Note that (±)-norcisapride conversion to UNK occurred at rates that were at least 3 orders of magnitude less than rates of cisapride biotransformation to Nor.

Chemical Inhibition of Cisapride and (±)-Norcisapride Metabolism. The effects of various P450 inhibitors on the conversion of cisapride (0.1 \(\mu M\)) to Nor, 3F, and 4F and on the conversion of (±)-norcisapride to UNK (1.0 \(\mu M\)) are illustrated in Fig. 2. Because correlation studies implicated CYP3A4/5 as the dominant enzyme involved in the conversion of cisapride to Nor, 3F, and 4F, inhibitors were incubated with human liver microsomes from two donors, one with high CYP3A4/5 activity (H112) and one with low CYP3A4/5 activity (H093). Ketoconazole, a potent CYP3A4/5 inhibitor, markedly inhibited the conversion of cisapride (0.1 \(\mu M\)) to Nor, 3F, and 4F in liver microsomes with low and high CYP3A4/5 activity. The CYP2B6 inhibitor orphenadrine had little or no effect on Nor formation; however, it did cause modest inhibition of 3F formation in microsomes with high, but not low, CYP3A activity and inhibition of 4F formation in microsomes with either low or high CYP3A activity. Quercetin, a reported CYP2C8 inhibitor (Desai et al., 1998), inhibited 3F and 4F formation in microsomes with low and high CYP3A4/5 activity but had little or no effect on Nor formation. Except for modest inhibition of 4F formation by the CYP2C19 inhibitor lan-
Each point represents the mean of replicate determinations. No effect on the rate of UNK formation. The other inhibitors examined had little or no effect on UNK formation. The conversion of cisapride to norcisapride was catalyzed mainly by CYP3A4. Although CYP2C8, CYP3A5, and CYP3A7 were at least 3 orders of magnitude less than rates of cisapride biotransformation, the kinetics of cisapride metabolism were investigated in human liver microsomes with high CYP3A4/5 activity and with CYP3A4.

### Conversion of cisapride (0.1 and 1.0 μM) to UNK by heterologously expressed human P450 enzymes

<table>
<thead>
<tr>
<th>Expressed Human P450 Enzyme</th>
<th>Metabolite Formation</th>
<th>0.1 μM</th>
<th>1.0 μM</th>
<th>0.1 μM</th>
<th>1.0 μM</th>
<th>0.1 μM</th>
<th>1.0 μM</th>
<th>0.1 μM</th>
<th>1.0 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Nor</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
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<td>CYP2B6</td>
<td>0.18 ± 0.02</td>
<td>1.66 ± 0.05</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<td>&lt;0.005</td>
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<tr>
<td>CYP2C8</td>
<td>0.53 ± 0.07</td>
<td>5.38 ± 1.10</td>
<td>1.17 ± 0.54</td>
<td>15.5 ± 2.6</td>
<td>0.14 ± 0.12</td>
<td>1.51 ± 1.31</td>
<td>&lt;0.005</td>
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<tr>
<td>CYP2C9</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
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<tr>
<td>CYP2C19</td>
<td>&lt;0.1</td>
<td>0.31 ± 0.10</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.005</td>
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<tr>
<td>CYP2D6</td>
<td>&lt;0.1</td>
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<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<td>&lt;0.005</td>
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<tr>
<td>CYP2E1</td>
<td>130 ± 4</td>
<td>950 ± 164</td>
<td>59.8 ± 12.9</td>
<td>341 ± 124</td>
<td>46.8 ± 10.5</td>
<td>338 ± 39</td>
<td>0.092 ± 0.003</td>
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<tr>
<td>CYP3A4</td>
<td>0.41 ± 0.03</td>
<td>4.04 ± 0.70</td>
<td>0.19 ± 0.02</td>
<td>1.90 ± 0.89</td>
<td>0.46 ± 10</td>
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<tr>
<td>CYP3A7</td>
<td>2.26 ± 0.10</td>
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</table>

**TABLE 1**

Cisapride and (±)-norcisapride (0–100 μM) were incubated with human liver microsomes for 20 or 120 min, respectively, as described under Materials and Methods. Each point represents the mean of replicate determinations.

Cisapride and (±)-norcisapride (0–100 μM) were incubated with human liver microsomes for 20 or 120 min, respectively, as described under Materials and Methods. Each point represents the mean of replicate determinations.

and enhanced by coumarin. The other inhibitors examined had little or no effect on the rate of UNK formation.

**Cisapride and (±)-Nor cisapride Metabolism by Heterologously Expressed Human P450 Enzymes.** Microsomes prepared from baculovirus-infected insect cells expressing vector alone (control) or 1 of 10 human P450 enzymes were examined for their ability to metabolize cisapride and (±)-nor cisapride. Recombinant CYP1A2, CYP2B6, CYP2C8, CYP2C19 CYP3A4, CYP3A5, and CYP3A7 were all capable of forming Nor from cisapride (Table 1); however, CYP3A4 was at least 50 times more active at converting cisapride to Nor than the next most active enzyme (CYP3A7). CYP2C9, CYP2D6, and CYP2E1 catalyzed little or no formation of Nor.

CYP3A4 also catalyzed the highest rate of cisapride biotransformation to 3F and was 20 to 50 times more active than the next most active enzyme, which was CYP2C8 (Table 1). CYP3A5, CYP3A7, CYP2C19, and CYP2D6 also converted cisapride to 3F but at rates substantially lower than those of CYP3A4 or CYP2C8. Cisapride biotransformation to 4F was catalyzed mainly by CYP3A4. Although CYP2C8, CYP3A5, and CYP3A7 also converted cisapride to 4F, they did so at rates that were at least 100-fold less than those of CYP3A4. UNK formation from (±)-nor cisapride was solely mediated by CYP3A4 and occurred at rates that were at least 3 orders of magnitude less than rates of cisapride biotransformation to Nor.

**Determination of Kinetic Parameters for Cisapride and (±)-Nor cisapride Metabolism.** Based on the results obtained from the above studies, the kinetics of cisapride metabolism were investigated in human liver microsomes with high and low CYP3A4/5 activity and with recombinant CYP3A4, CYP3A7, and CYP2C8. Kinetic parameters for the formation of UNK from (±)-nor cisapride were determined in human liver microsomes with high CYP3A4/5 activity and with CYP3A4.

The formation of Nor, 3F, and 4F from cisapride and of UNK from (±)-nor cisapride by human liver microsomes was consistent with Michaelis-Menten kinetics. Lineweaver-Burk plots (Fig. 4) for the formation of these metabolites appeared linear over the concentration.
range examined (0.1–100 μM). This suggests that the formation of each of these metabolites is catalyzed predominantly by a single P450 enzyme (although not necessarily by the same P450 enzyme). The apparent kinetic parameters (Kₘ, Vₘₐₓ, and Vₘₐₓ/Kₘ) are contained in Table 2. The Vₘₐₓ/Kₘ ratio, a parameter reflecting in vitro intrinsic clearance (CLₖᵢᵣₜ), was ~3- to 5-fold higher for the formation of Nor than for the formation of 3F and 4F by human liver microsomes. The CLₖᵢᵣₜ for Nor formation was ~1000-fold greater than that of UNK formation from (±)-norcisapride by human liver microsomes.

Formation of Nor, 3F, and 4F by recombinant CYP3A4, CYP3A7, and CYP2C8 and of UNK by CYP3A4 conformed to typical Michaelis-Menten kinetics, based on Lineweaver-Burk plots (plots not shown). Kinetic parameters determined from the plots are contained in Table 2. Kₘ values for the formation of Nor, 3F, and 4F by recombinant CYP3A4 were similar to the corresponding values by CYP2C8 and ~3- to 5-fold lower than those by CYP3A7. The CLₖᵢᵣₜ for the formation of Nor, 3F, and 4F by CYP3A4 exceeded that of CYP3A7 by 149-, 430-, and 274-fold, respectively, and that of CYP2C8 by 33.2-, 4.45-, and 68.0-fold, respectively. Note that the Kₘ of UNK formation from (±)-norcisapride by recombinant CYP3A4 was ~14-fold greater than the corresponding Kₘ of Nor formation from cisapride. The CLₖᵢᵣₜ of Nor formation from cisapride was 1600-fold greater than that of UNK formation from (±)-norcisapride by recombinant CYP3A4.

Discussion

Consistent with in vivo data demonstrating that Nor is the major metabolite (41–45% of the administered dose) formed from cisapride in humans (Meuldermans et al., 1988b), Nor was the major metabolite formed from cisapride in these in vitro studies. Furthermore, our results indicate that at substrate concentrations that spanned the average plasma Cₘₐₓ (0.1 and 1.0 μM), the biotransformation of cisapride is catalyzed principally by CYP3A4. In vitro studies conducted with a panel of human liver microsomes demonstrated that the formation of the major metabolite Nor and the minor metabolites 3F and 4F were significantly correlated with the activity of CYP3A4/5. Ketoconazole, a potent CYP3A inhibitor, markedly reduced formation of Nor, 3F, and 4F in human liver microsomes. In human liver microsomes with high CYP3A activity, ketoconazole effectively inhibited ~80 to 90% of cisapride metabolism. Studies with a panel of cDNA-expressed enzymes confirmed that CYP3A4 is a relatively high-affinity, high-capacity enzyme capable of converting cisapride to Nor, 3F, and 4F with Kₘ values of ~3.2, 4.3, and 7.9 μM, respectively. The specific activity of CYP3A4 for the formation of Nor, 3F, and 4F was at least 50-fold greater than that of the next most active enzyme.

With the possible exception of the CYP2C8-catalyzed formation of 3F in which CLₖᵢᵣₜ mediated by CYP2C8 was ~4.5-fold lower than by CYP3A4, other P450s do not appear to contribute significantly to the metabolism of cisapride. Furthermore, if one takes into account the relative abundance of these isoforms [CYP2C8 and CYP2B6 account for ~6 and <1% (Shimada et al., 1994) of total hepatic P450 content, respectively, whereas CYP3A4 accounts for ~30% of total hepatic P450 content], the contribution of CYP2C8 or CYP2B6 to the formation of Nor, 3F, and 4F would be expected to be minimal, especially in individuals with moderate to high constitutive CYP3A activity.

Despite mild to moderate inhibition by the CYP2C9 inhibitor sulfaphenazole and the CYP2C19 substrate/inhibitor lansoprazole, CYP2C9 and CYP2C19 do not appear to contribute to the biotransformation of cisapride. In the presence of ketoconazole, which markedly reduced CYP3A activity, the relationship between the formation of cisapride metabolites and either CYP2C9 or CYP2C19 activity was poor. Furthermore, heterologously expressed CYP2C9 and CYP2C19 catalyzed little or no biotransformation of cisapride. It is likely that the inhibitory effects observed with lansoprazole are due to its ability to serve as a substrate for CYP3A4/5 (Pearce et al., 1996) and, thus, competitively inhibit CYP3A-mediated cisapride metabolism.

These results are qualitatively similar to those previously reported from in vitro studies (Bohets et al., 2000; Desta et al., 2000) conducted using higher substrate concentrations that far exceed usual plasma concentrations of cisapride associated with therapeutic administration. Although all three investigations conclude that CYP3A4 is the principal human liver P450 responsible for cisapride N-dealkylation, the role of CYP2C8 is less certain. Desta et al. (2000) observed that the CLₖᵢᵣₜ for cisapride N-dealkylation was 20 to 25% greater for CYP2C8 compared with CYP3A4. However, after correction for relative abundance of P450 isoforms in human liver microsomes, the principal role for CYP3A4 was apparent. In contrast, the results of this study and that of Bohets et al. (2000) found that the CLₖᵢᵣₜ for CYP2C8 was at least 10-fold lower than that observed with CYP3A4. In addition, we observed an approximately 8-fold greater rate of cisapride turnover compared with the previous studies (Bohets et al., 2000; Desta et al., 2000), which may be a function of the experimental conditions (e.g., lower substrate concentrations, lower protein content, and an increased substrate/enzyme ratio) used in our in vitro incubations.

We report two novel findings regarding the hepatic biotransformation of cisapride and (±)-norcisapride. First, (±)-norcisapride metabolism to an unidentified metabolite, UNK, is minimal, occurring to a very limited extent at the substrate concentrations studied. Under these conditions, UNK formation was almost exclusively CYP3A4/5-mediated and occurred at rates that were at least 3 orders of magnitude less than rates of cisapride biotransformation to Nor. Although the possibility exists that UNK may be a previously unidentified metabolite, it seems more likely that it is

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**TABLE 2**

Estimated kinetic parameters for the formation of Nor, 3F, and 4F from cisapride and UNK from (±)-norcisapride in human liver microsomes or in heterologously expressed P450 enzymes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kₘ (μM)</th>
<th>Vₘₐₓ (μmol/min/mg)</th>
<th>Vₘₐₓ/Kₘ</th>
<th>Kₘ (μM)</th>
<th>Vₘₐₓ (μmol/min/mg)</th>
<th>Vₘₐₓ/Kₘ</th>
<th>Kₘ (μM)</th>
<th>Vₘₐₓ (μmol/min/mg)</th>
<th>Vₘₐₓ/Kₘ</th>
</tr>
</thead>
<tbody>
<tr>
<td>H112</td>
<td>6.00</td>
<td>1350</td>
<td>225</td>
<td>14.3</td>
<td>696</td>
<td>48.7</td>
<td>13.9</td>
<td>568</td>
<td>40.8</td>
</tr>
<tr>
<td>H093</td>
<td>6.90</td>
<td>125</td>
<td>18.1</td>
<td>12.4</td>
<td>78.0</td>
<td>6.29</td>
<td>14.3</td>
<td>67.0</td>
<td>4.68</td>
</tr>
<tr>
<td>CYP3A4</td>
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<td>5791</td>
<td>1810</td>
<td>4.30</td>
<td>1628</td>
<td>378</td>
<td>7.90</td>
<td>2480</td>
<td>313</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>16.8</td>
<td>203</td>
<td>12.1</td>
<td>18.1</td>
<td>16.0</td>
<td>0.88</td>
<td>25.2</td>
<td>28.7</td>
<td>1.14</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>2.00</td>
<td>109</td>
<td>54.5</td>
<td>3.40</td>
<td>289</td>
<td>85.0</td>
<td>5.80</td>
<td>26.7</td>
<td>4.60</td>
</tr>
</tbody>
</table>

N.D.: not determined.

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- $V_\text{max}$: Micromolar.
- $K_\text{m}$: Picomoles per milligram of protein per minute for human liver microsomes; picomoles per nanomole of P450 per minute for cDNA-expressed enzymes.
- $V_\text{max}/K_\text{m}$: Micromolar per milligram of protein per minute for human liver microsomes; micromolar per nanomole of P450 per minute for cDNA-expressed enzymes.
one or more of the metabolites described by Meuldersmans et al. (1988a, b). We speculate that UNK may correspond to the metabolite of unknown structure, because in other mammalian species, the formation of the two lactam metabolites is catalyzed not by P450 enzymes but by cytoplasmic molybdenum hydroxylases (Lavrissen et al., 1986).

The results of this study demonstrate that CYP3A4 is the P450 enzyme primarily responsible for the metabolism of cisapride and \((\pm)-\)norcisapride. CYP3A4 is characterized by marked interindividual variability in both enzyme content and activity (up to 10- and 20-fold, respectively), and is predominantly P450 present in adult human liver (Shimada et al., 1994), and is highly expressed in human small intestine (Thummel et al., 1994). The catalytic rate of CYP3A substrates (Leeder et al., 1997) may be expected to vary between infants from 1 to 4 years of age on the basis of pharmacokinetic data for known CYP3A substrates (Leeder and Kearns, 1997). Previous clinical and the in vitro results presented here and by others (Bohets et al., 2000; Desta et al., 2000) strongly implicate CYP3A4 as the enzyme responsible for catalyzing the biotransformation of cisapride to its major metabolite, Nor. The results of this study also suggest that CYP3A4 is primarily responsible for the conversion of cisapride to the minor metabolites 3F and 4F. Also, in our in vitro results demonstrate that CYP3A7 does not catalyze the biotransformation of cisapride to Nor, 3F, or 4F to any appreciable extent. Based on these observations, it is anticipated that the developmental pattern of cisapride biotransformation would reflect CYP3A4 identity, thereby placing the least immature infants at the greatest risk for serious cardiac adverse effects associated with an accumulation of cisapride in plasma. However, this anticipated pharmacokinetic pattern was not observed in a study of 37 neonates and young infants who were given cisapride (0.8 mg/kg/day), which produced plasma concentrations following an initial dose similar to those observed in adults taking therapeutic doses of the drug (Kearns et al., 2001). It should be noted, however, that the plasma concentrations of cisapride found in neonates might be affected by differences in the relative contribution of intestinal and hepatic CYP3A isoforms to its metabolism during the first few months of life, particularly since the drug is administered orally. To date, the developmental pattern of intestinal CYP3A expression has not been characterized.

In contrast to the situation with cisapride, clinical data and our own in vitro studies have shown that the biotransformation of \((\pm)-\)norcisapride to its metabolite (or metabolites) proceeds extremely slowly. Clinical studies have further shown that \((\pm)-\)norcisapride is extensively excreted unchanged by renal mechanisms (Meuldersmans et al., 1988b). Therefore, factors related to CYP3A4-mediated metabolism (e.g., ontogeny of CYP3A enzymes, inhibition, induction, etc.) will be clinically unimportant with regard to the disposition of \((\pm)-\)norcisapride in pediatric or adult patients. It is conceivable that any differences in steady-state plasma concentrations of \((\pm)-\)norcisapride that occur between neonates and adults may be related to developmental differences in renal drug clearance and/or the apparent volume of distribution for \((\pm)-\)norcisapride. Thus, the known prokinetic activity of \((\pm)-\)norcisapride (Hubbard, 1994) and the apparent lack of dependence upon cytochrome P450 enzymes for its biotransformation in man it make it a candidate for further development as a therapeutic agent.

References


