PHARMACOKINETICS AND METABOLISM OF A CYSTEINYL LEUKOTRIENE-1 RECEPTOR ANTAGONIST FROM THE HETEROCYCLIC CHROMANOL SERIES IN RATS: IN VITRO-IN VIVO CORRELATION, GENDER-RELATED DIFFERENCES, ISOFORM IDENTIFICATION, AND COMPARISON WITH METABOLISM IN HUMAN HEPATIC TISSUE

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ABSTRACT:

CP-199,331 is a potent antagonist of the cysteinyl leukotriene-1 (LT1) receptor, targeted for the treatment of asthma. The pharmacokinetic/metabolism properties of CP-199,331 were studied in rats and compared with those in human liver microsomes/hepatocytes. In vitro biotransformation of CP-199,331 in rat and human hepatocytes was similar, consisting primarily of CP-199,331 O-demethylation. Marked sex-related differences in plasma clearance (CLp) of CP-199,331 were observed in rats: 51 and 1.2 ml/min/kg in males and females, respectively. This difference in CLp was attributed to gender differences in metabolizing capacity because Vmax and Km values for CP-199,331 metabolism were 30-fold higher and 8-fold lower, respectively, in male rat liver microsomes compared with female microsomes. Scale-up of the in vitro monosomal data predicted hepatic clearance (CLh) of 64 and 2.5 ml/min/kg in male and female rats, respectively. These values were in close agreement with the in vivo CLp, suggesting that CP-199,331 CLp in male and female rats was entirely due to hepatic metabolism. Studies with rat recombinant cytochromes P450 and anti-rat cytochrome P450 (CYP) antibodies revealed the involvement of male rat-specific CYP2C11 in the metabolism of CP-199,331. In contrast, CP-199,331 metabolism in human liver microsomes was principally mediated by CYP3A4. The projected human clearance in liver microsomes and hepatocytes varied 6-fold from low to moderate, depending on CYP3A4 activity. Considering that O-demethylation is the major route of elimination in humans, the in vivo clearance of CP-199,331 may exhibit moderate variability, depending on CYP3A4 abundance in the human population.

Cysteinyl leukotrienes (Cys-LT1s), including LTC4, LTD4, and LTE4 are products of arachidonic acid metabolism resulting from the 5-lipoxygenase pathway (Samuelsson, 1983). These arachidonate-derived metabolites mediate effects on cell membranes that are characteristic of asthma (i.e., bronchoconstriction, increased endothelial membrane permeability leading to airway edema, and enhanced secretion of mucus) (Dahlen et al., 1980; Wenzel, 1997). In human tissues, activation of Cys-LT1 receptors appears to initiate asthmatic reactions. Several Cys-LT1 receptor antagonists, including zafirlukast (accolate), pranlukast, and montelukast (Singulair) have shown clinical efficacy against asthma, thus validating intervention at the Cys-LT1 receptor as an attractive therapeutic target for the treatment of asthma. The pharmacokinetic profile of zafirlukast, a Cys-LT1 receptor antagonist, and its active metabolite, zafirlukast N-oxide, was recently reported (Yang et al., 1996). The pharmacokinetics and metabolism of zafirlukast, a selective Cys-LT1 receptor antagonist, were studied in dogs and in humans (Chambers et al., 1999). Preliminary metabolism studies of zafirlukast in dog liver microsomes revealed the presence of a lactol metabolite (CP-89,958) that covalently binds to cellular proteins (Fig. 1). To avoid the metabolic bioactivation of CP-85,898, extensive structure-activity relationship studies were undertaken to prevent hydroxylation on the chromanol ring (Masamune et al., 1995; Chambers et al., 1998a). Efforts consisted of blocking the hydroxylation site or introducing substituents prone to metabolism at an alternate reaction site (Chambers et al., 1998b). This undertaking led to the identification of CP-199,331 (R,R-trifluoro-N-[3-[6-(5-fluorobenzothiazol-2-ylmethoxy)-4-hydroxy-chroman-3-ylmethyl]benzoic acid) (Fig. 1), a potent Cys-LT1 receptor antagonist in which clinical evaluation was discontinued due to unacceptable hepatotoxicity in monkeys (Andrews et al., 1995). Analysis of monkey bile after dosing with CP-89,958 revealed the presence of significant quantities of a lactol metabolite (R,R-trifluoro-N-[3-[6-(5-fluorobenzothiazol-2-ylmethoxy)-4-hydroxy-chroman-3-ylmethyl]benzoic acid) as an optimized Cys-LT1 receptor antagonist in the series with an overall increase in antagonist potency compared with its predecessor (Chambers et al., 1999). Preliminary metabolism studies revealed that the major metabolite of CP-199,331 in rat and human liver microsomes was O-desmethyl derivative 3. Lack of formation of lactol 4 correlated well with the absence of hepatotoxic effects in monkeys and
rats after dosing with CP-199,331 at concentrations well exceeding those predicted for clinical efficacy. Overall, these observations indicated the potential utility of CP-199,331 as an agent in the treatment of asthma.

In the present report, in vitro metabolism studies were undertaken in male and female rat and human liver microsomes and male rat and human hepatocytes for elucidation of hepatic metabolism and quantitative prediction of in vivo plasma clearance (CL\text{int}). The projected rat hepatic clearance (CL\text{int}) determined in microsomes and hepatocytes was compared with the in vivo CL\text{int} in this species. The observation of sexual dimorphism in rats led us to conduct additional mechanistic studies, including the identification of the specific CYP isoform(s) responsible for the metabolism of CP-199,331 in male and female rats as well as in human liver microsomes.

Experimental Procedures

Reagents. CP-199,331-sodium salt and the O-desmethyl metabolite 3 were synthesized as described previously (Chambers et al., 1999). Isoform-specific CYP inhibitors were purchased from Sigma (St. Louis, MO) or GENTEST (Woburn, MA). All other chemicals and solvents (reagent grade or better) were obtained from Sigma. Microsomal fractions were prepared from male and female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) and human livers (IIAM, Exton, PA) using standard procedures. Protein concentrations were determined using the bicinchoninic acid assay method (Pierce Chemical, Rockford, IL). Total CYP content was measured according to published protocols (Omura and Sato, 1964), and human microsomes were characterized using CYP-specific marker substrate activities. Male Sprague-Dawley rat and human cryopreserved hepatocytes were obtained from IVT (Baltimore, MD). Rat or human CYPs and anti-rat CYP3A2 and 2C11 antibodies were obtained from GENTEST.

Microsomal and Hepatocyte Incubations. Microsomes. CP-199,331 (1–100 \( \mu \)M) was incubated with rat (pool of ~10–15 livers) or human (individual livers with low, average, and high CYP3A4 activities) liver microsomes at CYP concentrations of 0.5 to 1.5 \( \mu \)M in the presence of an NADPH-generating system consisting of NADP (0.54 mM), tri-isocitric acid (6.2 mM), isocitric dehydrogenase (0.5 U/ml), and MgCl\text{2} (11 mM). All incubations were carried out in 0.1 M potassium phosphate buffer, pH 7.4, at 37°C. Reactions were terminated by addition of ice-cold methyl-butyl ether (5:1, v/v) containing tert-butyl methyl ether (0.5% final concentration) and added to a solution of 3% acetic acid. The mixture was then passed through this mixture at every hour of incubation. Reactions were stopped by freezing the incubation aliquots in liquid nitrogen.

Kinetic Analysis for O-Demethylation and Prediction of Hepatic Clearance. To estimate the rat and human hepatic intrinsic clearance (CL\text{'int'}) for O-demethylation of CP-199,331 in liver microsomes, kinetic parameters (\( V_{\text{max}} \) and \( K_m \)) of the O-desmethyl product formation were determined at substrate concentrations of 1 to 100 \( \mu \)M for a time period associated with reaction linearity. Estimates of \( V_{\text{max}} \) and \( K_m \) were calculated using Eadie-Hofstee linearization. The CL\text{'int} was estimated as \( V_{\text{max}}/K_m \) and was expressed initially in the unit of volume per time per amount of CYP. The estimated CL\text{'int} was then scaled up to a CL\text{'int} expressed in a typical clearance unit such as milliliters per minute per kilogram. This was done using a scale-up factor based on CYP concentration in the liver and the amount of protein represented by 1 kg of body weight of rat or human (Obach et al., 1997). The CL\text{'int} was estimated using the nonrestricted well stirred model (CL\text{'int} = Q \times CL\text{'int}/Q + CL\text{'int}) where Q is hepatic blood flow) under the assumption that protein binding of CP-199,331 was similar in blood and microsomal incubation (Pang and Rowland, 1977).

To estimate the rat and human hepatic CL\text{'int} of CP-199,331 in hepatocytes, the scaling up of in vitro half-life (\( t_{1/2} \)) data, reflecting CP-199,331 depletion, was performed using the following equation (Houston, 1994; Obach et al., 1997): CL\text{'int} = (0.693/\( t_{1/2} \)) × (grams of liver/kilograms of body weight) × (milliliters of incubation/no. of cells in incubation) × (no. of cells/g of liver).

For rats, the liver and body weights were 40 g/kg of body weight and 0.25 kg, respectively, and the number of cells used in the incubation was 1.35 × 10\(^{9}\) of liver. For human, the liver and body weights were 21 g/kg of body weight and 70 kg, respectively, and the number of cells used in the incubation was 1.2 × 10\(^{10}\) of liver. Cell density in the incubations was 0.5 × 10\(^{6}\)/ml. The CL\text{'int} was estimated as described previously for microsomes.

Identification of Human and Rat CYP Isoforms Responsible for O-Demethylation of CP-199,331. Human inhibition studies. For mechanism-based CYP inhibition studies, human liver microsomes (0.5 \( \mu \)M) (from a lot with high CYP3A4, 2D6, 2C9, 2C19, and 1A2 activities) were preincubated with an NADPH-generating system at 37°C in the presence of triacetyleloandromycin (20 \( \mu \)M) (a CYP3A4 inactivator) or furafylline (10 \( \mu \)M) (a CYP1A2 inactivator) for 30 and 10 min, respectively. CP-199,331 (10 \( \mu \)M) was added and these mixtures were further incubated for 30 min at 37°C. For competitive CYP inhibition studies, liver microsomes from the same lot were incubated with CP-199,331 (10 \( \mu \)M), NADPH-generating system, and a CYP inactivator, quinidine at 1 \( \mu \)M (CYP2D6), sulfaphenazole at 10 \( \mu \)M (CYP2C9), and omeprazole at 10 \( \mu \)M (CYP2C19) for 30 min at 37°C. Incubations were conducted in duplicate. Workup and sample analysis was conducted as described previously.

Correlation analysis. CP-199,331 (10 \( \mu \)M) was incubated with human liver microsomes from six individual donors in the presence of an NADPH-generating system. Incubations were conducted in duplicate. The individual microsomal lots included in the study were previously characterized for specific CYP activities using standard marker substrates (e.g., CYP3A4 activity was exemplified by testosterone 6-hydroxylase activity). Formation rate of 3 was then correlated with CYP-specific activities (3A4, 2D6, 2C9, 2C19, and 1A2).

Metabolism by heterologously expressed CYP isoforms. CP-199,331 (20 \( \mu \)M) was incubated with microsomes from cells containing human rCYPs 3A4, 2D6, 2C9, 2C19, and 1A2 (CYP concentration of 0.05 \( \mu \)M) or rat rCYPs 3A1, 3A2, 2C11, 2C12, and 2C13 (CYP concentration of 0.05 \( \mu \)M) in the presence of an NADPH-generating system at 37°C. Incubations with human isoforms were conducted for 45 min, incubations with male rat-specific rCYP3A1, 3A2, 2C11, and 2C13, for 10 min, and incubation with female rat-specific rCYP2C12, for 40 min. Reactions were terminated by addition of ice-cold acetonitrile (2:1, v/v). The formation of 3 was assessed by liquid chromatography/mass spectrometry, as described here.

Antibody inhibition studies in rats. For the immunoinhibition study, male rat liver microsomes (200 \( \mu \)g) were mixed with various amounts of anti-rat CYP3A1 or anti-rat CYP2C11 antibodies (0–50 \( \mu \)l) and incubated at room temperature for 30 min followed by addition of CP-199,331 (10 \( \mu \)M) in phosphate buffer. After preincubation at 37°C for 2 min, the reaction was...
initiated by adding NADPH (1 mM) and terminated after a 40-min incubation by adding cold acetonitrile (2:1, v/v).

**Pharmacokinetic Analysis in Rats.** Two sets (n = 4/sex) of fasted male and female Sprague-Dawley rats (~200–220 g) with jugular vein catheters were administered CP-199,331-sodium salt in saline as an i.v. bolus solution (5 mg/kg). Rats received food (standard rodent diet) at 8 h after administration and were allowed full access to water throughout the study. Blood samples were collected from the jugular vein at appropriate time intervals and plasma samples were separated immediately by centrifugation and stored at -20°C until HPLC-UV analysis. Pharmacokinetic calculations were performed using noncompartmental analysis.

**Analysis of CP-199,331 and O-Desmethyl Metabolite 3 by HPLC-UV.** Sample analysis was performed using an HPLC-UV system consisting of an LDC Analytical ConstaMetric 4100 gradient pump (Riviera Beach, FL) and SpectroMonitor 3200 variable wavelength UV detector, HPLC membrane degasser (PerkinElmer Instruments, Norwalk, CT), and Waters 717 plus autoinjector (Waters, Milford, MA).

**Microsomes.** After centrifugation of the quenched reaction mixture, the supernatant was dried under a steady nitrogen stream and reconstituted in 150 µl of HPLC mobile phase. An autosampler was programmed to inject 70 µl on a Waters 3.9- × 150-mm Nova-Pak C18 4-µm HPLC analytical column preceded by an in-line Supelco 4.6- × 20-mm Palliguard C18 40-µm guard column. The mobile phase was a binary mixture of acetonitrile (65%) and an aqueous solution of 0.1% glacial acetic acid, 0.05% phosphoric acid, and 0.1% triethylamine (35%). A gradient pump maintained an isocratic condition at a continuous flow rate of 1.2 ml/min. A variable wavelength UV detector was operated at 293 nm. Under these conditions, CP-199,331 and 3 eluted at 3.51 and 2.11 min, respectively. HPLC data were processed by Multichrom-2 integrating software operating in the peak height mode.

**Rat plasma.** Acetonitrile (300 µl) containing internal standard was added to 100 µl of plasma. After centrifugation, the supernatant was evaporated to dryness under nitrogen, reconstituted in HPLC mobile phase (125 µl), and analyzed as described above. The dynamic range of the assay was 0.05 to 20.0 µg/ml.

**Analysis of CP-199,331 and O-Desmethyl Metabolite 3 by LC/MS/MS.** Each point represents the average of duplicate determination.
The qualitative formation of 3 in rat and human CYPs and hepatocytes was assessed using a Sciex API model 2000 LC/MS/MS triple quadrupole mass spectrometer (Thornhill, ON, Canada) in conjunction with an LDC Analytical SpectroMonitor 3200 variable wavelength UV detector. Analytes were chromatographically separated using a Hewlett Packard series 1100 HPLC system (Palo Alto, CA). After workup, the supernatant was evaporated to dryness and reconstituted in HPLC mobile phase (150 µL). An autosampler was programmed to inject 50 µL on a Zorbax Rx-C8 4.6 × 150 mm column using a binary gradient consisting of a mixture of 10 mM ammonium formate, 0.1% formic acid (solvent A), and acetonitrile (solvent B) at a flow rate of 1 ml/min. The LC gradient was programmed as follows: solvent A to solvent B ratio was held at 100:0 (v/v) for 3 min and then adjusted from 100:0 (v/v) to 10:90 (v/v) for 20 min and from 10:90 (v/v) to 100:0 (v/v) from 20 to 25 min. The column was reequilibrated for 5 min prior to the next analytical run. Postcolumn flow was split such that mobile phase was introduced into the mass spectrometer via an ion spray interface at a rate of 50 µl/min. Ionization was conducted in the positive ion mode at the ion spray interface temperature of 400°C and using nitrogen for nebulizing and heating gas. Ion spray voltage was 5.0 kV and the orifice voltage was optimized at 30 eV.

Depletion rates of CP-199,331 in hepatocytes were determined using a Sciex API model 3000 LC/MS/MS triple quadrupole mass spectrometer. Analytes were chromatographically separated using a Hewlett Packard series 1100 HPLC system. Acetonitrile was added to the hepatocyte incubation mixtures and after centrifugation, the supernatant was directly introduced to 1100 HPLC system. Analytes were chromatographically separated using a Hewlett Packard series LC/MS/MS triple quadrupole mass spectrometer. Voltages were optimized at 40 eV.

**Results**

**Pharmacokinetic and Metabolism Studies on CP-199,331 in Rats: In Vitro-In Vivo Correlations, Metabolite, and Gender-Specific Isoform Identification.** Dramatic sex-related differences in the pharmacokinetic profiles of CP-199,331 were observed in male and female rats. When CP-199,331 (5 mg/kg) was administered intravenously, the compound was cleared ~42-fold faster in male rats than in female rats (Table 1; Fig. 2). Consistent with the in vivo observations of sexual dimorphism in CLh, liver microsomes from male rats catalyzed the oxidative O-demethylation of CP-199,331 at a faster rate than those from female rats (Fig. 3). Estimates of Vmax and Km were calculated from the initial rate data shown in Fig. 3 using Eadie-Hofstee linearization (Table 1). The kinetic parameters (low Km and high Vmax) indicated that CP-199,331 displays a better affinity toward oxidation by male rat liver microsomes, resulting in a greater intrinsic clearance. Scale-up of the in vitro microsomal data, assuming that rat protein binding is similar in blood and microsomes, yielded a prediction of high CLh in male rat and low CLh in female rat (Table 1). These results were consistent with the actual CLh values in male and female rats after intravenous administration. Furthermore, scale-up of the CLh, reflecting depletion of CP-199,331 in male rat hepatocytes, resulted in a projected CLh that was in good agreement with the projected CLh using male rat liver microsomes and the actual CLh in male rats (Table 1). The excellent correlation between the projected CLh and the determined in vivo CLh suggested that in vivo CLh of CP-199,331 in male and female rats was due entirely to hepatic metabolism. Consistent with this hypothesis was the observation that the concentration of the circulating O-demethyl metabolite 3 of CP-199,331 was significantly higher in male than in female rat plasma after intravenous administration of the drug (Fig. 4).

The possibility that O-demethylation of CP-199,331 represented the principal metabolic pathway in rat was further explored by incubating CP-199,331 (20 µM) in male rat hepatocytes. Analysis of the reaction mixture by LC/MS/MS revealed extensive metabolism of the parent compound to two principal metabolites. The identity of the less polar metabolite (MH+ = 602; rt = 18.1 min) was established as O-
desmethyl CP-199,331 (3) based on comparison with HPLC retention time and the mass spectrum of the synthetic standard. The second more polar metabolite (MH\(^+\) = 778; Rt = 15.0 min) was tentatively identified on the basis of its mass spectral fragmentation as the O-glucuronide (5) (Fig. 1) formed by phase II conjugation of O-desmethyl CP-199,331 (3). Diagnostic fragments of 5 included 761 (loss of water from the parent O-glucuronide) and 585 (loss of water from O-desmethyl CP-199,331). These results implicated that the rate-limiting step in CP-199,331 metabolism in male rat hepatocytes is its corresponding O-demethylation. Although circulating O-glucuronide (5) was not detected in rat plasma (Fig. 4), a significant quantity of 5 was observed in rat bile (data not shown).

The possibility that O-desmethyl CP-199,331 generation in the male rat was mediated by a gender-specific CYP was investigated by incubating CP-199,331 (20 \(\mu\)M) with various heterologously expressed gender-specific rat rCYPs. rCYP isoforms included male rat-predominant 3A1 and male rat-specific 3A2, 2C11, and 2C13, and female rat-specific rCYP2C12. Experimental results indicated that ~80% of CP-199,331 was converted to O-desmethyl CP-199,331 (3) in the presence of male rat-specific rCYP2C11, whereas male rat-predominant CYP3A1 and male rat-specific CYP3A2 isoforms converted 14 and 9%, respectively, of CP-199,331 into the O-desmethyl metabolite. In contrast, no metabolism was discernible in male rat-specific rCYP2C13. Similar incubations with female rat-specific rCYP2C12 generated O-desmethyl CP-199,331 in trace amounts. These data strongly suggest that male rat-specific CYP2C11 is the principal CYP isoform responsible for CP-199,331 O-demethylation and male-predominant 3A1 and female-specific 3A2 also play a minor role in the metabolism. In female rats, both CYP3A1 and CYP2C12 appear to be responsible for the O-demethylation of CP-199,331.

The participation of CYP2C11 and CYP3A1 in CP-199,331 O-demethylation was further confirmed by studying the effects of anti-rat CYP2C11 and CYP3A2 antibody on CP-199,331 metabolism in male rat liver microsomes (Fig. 5). The anti-rat CYP2C11 antibody strongly inhibited the formation of O-desmethyl CP-199,331 in male rat liver microsomes in a concentration-dependent manner, whereas the anti-rat CYP3A1 antibody was not as potent of an inhibitor of O-desmethyl CP-199,331 formation under these conditions.

**Metabolism of CP-199,331 in Human Hepatic Tissue.** Biotransformation studies on CP-199,331 (20 \(\mu\)M) were also conducted in human hepatocytes. As observed in rat hepatocytes, the primary route of metabolism in human hepatocytes involved O-demethylation to 3; however, O-glucuronidation of 3 to 5 was not observed in human hepatocytes (Fig. 1). Scale-up of the CL\(_{\text{int}}\), reflecting depletion of CP-199,331 (1 \(\mu\)M) in human hepatocytes, resulted in a projected moderate CL\(_{\text{int}}\) of 6.7 ml/min/kg (Table 1).

Data were also obtained to address the identity of human CYP isoforms involved in the metabolism of CP-199,331 to O-desmethyl CP-199,331. Pretreatment of human liver microsomes with triacetyloleandomycin (3A4 inactivator) resulted in ~80% loss of activity toward metabolism of CP-199,331 at a substrate concentration of 10 \(\mu\)M. In these microsomes, triacetyloleandomycin inhibited testosterone-6β-hydroxylation formation, a marker activity for CYP3A4, by 72% (Obach, 2000). In contrast, pretreatment of human liver microsomes with furafylline (1A2 inactivator), sulfaphenazole (2C9 inhibitor), omeprazole (2C19 inhibitor), and quinidine (2D6 inhibitor) did not prevent CP-199,331 O-demethylation. Furthermore, only heterologously expressed human rCYP3A4 catalyzed the O-demethylation of CP-199,331, whereas other recombinant CYP isoforms did not. Finally, rates of CP-199,331 O-demethylation in liver microsomes from six individual humans were measured at substrate concentration of 10 \(\mu\)M and plotted versus rates of metabolism of CYP-specific marker substrates. A high correlation (\(r^2 = 0.95\)) between CYP3A4 activities of individual human livers for 6β-hydroxytestosterone formation and CP-199,331 O-demethylation was discernible (Fig. 6). In contrast, no correlation was found between overall CP-199,331 metabolism and activities specific to CYP1A2, CYP2C9, CYP2C19, or CYP2D6 (data not shown). These results suggested that CYP3A4 was the principal isoform involved in CP-199,331 O-demethylation in human.

Based on the observation that CYP3A4 catalyzes the O-demethylation of CP-199,331 in humans, CP-199,331 (1–100 \(\mu\)M) was incubated with human liver microsomes from individual livers with low, average, and high CYP3A4 activities, and the rates of formation of O-desmethyl CP-199,331 were measured. \(V_{\text{max}}\) and \(K_{\text{m}}\) values were determined using the Eadie-Hofstee linearization of the initial rate data for human liver microsomes with low, average, and high CYP3A4 activity (Fig. 7). The \(V_{\text{max}}\) value increased with in-
creasing CYP3A4 activity in the liver, whereas the \( K_{\text{m}} \) value remained similar across liver microsomes with varying CYP3A4 activities. Scale-up of the in vitro microsomal data, assuming that human protein binding is similar in blood and microsomes, projected a human CL\( \text{h} \) that varied \( \sim 6 \)-fold from low to moderate, depending on the CYP3A4 activity in the liver microsomes (Table 1).

**Discussion**

The superfamily of CYP isoforms has been extensively characterized in rats (for review, see Mugford and Kedderis, 1998). There are \( \sim 40 \) genes coding for specific isoforms in the rat genome with four major subfamilies of CYP isoforms in the rat liver (for review, see Wrighton and Stevens, 1992; Guengerich, 1994). Female rats have \( \sim 10 \) to 30\% less total CYP compared with male rats, and male rats generally exhibit distinctly higher activities than females. There are instances, however, when female rats have higher activities than males (Skeett, 1989). This gender-related discrepancy stems from the fact that CYP isoforms can be expressed specifically or preferentially in either males or females. It is now well established that sexual dimorphism in rat drug metabolism is due to the differential expression of various gender-dependent CYP isoforms mediated by hormonal regulation (Waxman et al., 1985; Legraverend et al., 1992a; Lin et al., 1996; Thompson et al., 1997). For example, CYP2C11 is expressed only in male rats, whereas CYP2C12 is limited to female rats (Bandiera, 1990; Waxman et al., 1990; Kobliakov et al., 1991; Legraverend et al., 1992b). In contrast, no sex-dependent differences have been reported in any of the CYP isoforms expressed in human liver (Wolff and Strecke, 1992). Other key differences between rat and human CYP isozymes include the species-specific expression of CYP isoforms. For instance, the CYP2C subfamily, in particular CYP2C11 and CYP2C12, the major constitutively expressed isoforms in rat liver is not found in human liver (Nelson et al., 1996). Similarly, CYP3A4, the major CYP isoform detected in human liver is in relatively low concentration in rat liver.

In the present study, significant sex-related differences were observed in the metabolism of a cyssteinyl receptor antagonist, CP-199,331, in rats. The observation that male rats metabolized CP-199,331 to \( O \)-desmethyl CP-199,331 more rapidly than females, was consistent with the results that CP-199,331 was preferentially metabolized by the male rat-specific CYP2C11 and to a lesser extent by the male rat-predominant CYP3A isoforms. Overall, these results strongly suggest that the sexual dimorphism observed in CP-199,331 metabolism may result from the gender-related differential expression of CYP2C and/or CYP3A isoforms in rats. Consistent with the in vitro microsomal data that predicted significantly higher CL\( \text{h} \) of CP-199,331 in male versus female rat, males demonstrated a greater CL\( \text{h} \) than females. In addition, a good correlation also was discernible in the predicted high CL\( \text{h} \) of CP-199,331 in male rat hepatocytes and the in vivo CL\( \text{h} \) in male rats. These results strongly suggest that CL\( \text{h} \) of CP-199,331 in rats is mediated primarily by hepatic metabolism. If so, these results also implicate that gender-related differences in hepatic metabolism in vivo can be predicted accurately by in vitro metabolic data using liver microsomes and/or hepatocytes.

Also of particular interest is the in vivo finding that CP-199,331 was cleared \( \sim 40 \)-fold faster in male rats than in female rats. Although several literature studies have addressed gender-dependent differences in pharmacokinetics of drug candidates, few demonstrate gender differences in CL\( \text{h} \), as dramatic as that observed with CP-199,331. For instance, the anti-acquired immunodeficiency virus drug indinavir is cleared \( \sim 2 \)-fold faster in male rats than in female rats (Lin et al., 1996). Such a dramatic difference in CL\( \text{h} \) between male and female rats can be attributed to the predominant contribution of male rat-specific CYP2C11 in the metabolism of CP-199,331. Overall, these observations suggest that CP-199,331 and related analogs may represent a useful class of selective CYP2C11 substrates that could be used in probing the active site of the enzyme.

Although gender-specific CYP2C and to lesser extent 3A were responsible for metabolism of CP-199,331 in rats, subsequent studies in human liver microsomes revealed that CYP3A4 was the principal isoform responsible for metabolism of CP-199,331 in this species. This finding was further substantiated when a high correlation \( (R^2 = 0.95) \) between CYP3A4 activities of individual human livers for CP-199,331 \( O \)-demethylation, and \( 6 \)-hydroxysterosterone formation was observed. In contrast, CYP1A2, CYP2C9, CYP2C19, and CYP2D6 activities of individual human livers did not correlate with rates of CP-199,331 \( O \)-demethylation. Although there are some gender-dependent metabolic differences between men and women, it is noteworthy to point out that these differences are not related to differential expression of CYP isoforms (e.g., CYP3A4), and intradividual differences in metabolism can outweigh any gender-dependent differences in metabolism (Wolff and Strecke, 1992). Metabolic studies in human liver microsomes revealed that the projected human CL\( \text{h} \) of CP-199,331 varied \( \sim 6 \)-fold from low to moderate, depending on CYP2C11 activity (Table 1). Assuming that \( O \)-demethylation is the rate-limiting step in the elimination of CP-199,331 in human, the in vivo CL\( \text{p} \) of CP-199,331 may exhibit moderate variability, depending on the abundance of CYP3A4 in individual livers. Studies are currently underway to determine whether CP-199,331 exhibits gender-dependent hepatic metabolism/pharmacokinetics in other preclinical species relevant for prephase I toxicological assessments.

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**References**


