DISPOSITION OF THE ANTIPSYCHOTIC AGENT CI-1007 IN RATS, MONKEYS, DOGS, AND HUMAN CYTOCHROME P450 2D6 EXTENSIVE METABOLIZERS

Species Comparison and Allometric Scaling

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ABSTRACT:
The disposition of CI-1007 (I), an antipsychotic dopamine agonist, was studied after iv or po administration to rats, monkeys, and dogs and po administration to human cytochrome P450 2D6 extensive metabolizers (EMs). I was extensively metabolized after po administration, with high hepatic clearance (CL) values and negligible urinary excretion. Values for systemic plasma CL (28–40 ml/min/kg) suggested hepatic plasma flow-limited CL. The oral CL of I was similar among the species. Strong correlation was achieved in interspecies scaling for CL. After oral administration of [14C]I, the major route of 14C elimination in rats was in the bile (64%), followed by feces (29%) and urine (3.2%). Fecal excretion (64%) was the major route of 14C elimination in monkeys, followed by urine (14%). Three hydroxy metabolites, i.e. PD 147693 (II), PD 149394 (III), and PD 155144 (IV), and two sulfates, i.e. PD 163637 (V) and PD 163639 (VIII), were identified in monkey plasma, urine, or feces. VIII was the major metabolite excreted in monkey urine, and VI was the major component in feces. Trace amounts of II, VI, and VIII were detected in the plasma and urine of human EMs but not in rats or dogs. II is an active metabolite that was present in all species. After oral administration, observed maximal plasma concentration and AUC values for II were higher than the corresponding values for I in dog plasma, approximately 20–40% of the values for I in monkeys and human EMs, and <5% of the values for I in rat plasma. Although the metabolic profiles differ among species, strong correlation was achieved in allometric scaling because the elimination of I from the body is mainly limited by hepatic blood flow.

CI-1007 (PD 143188, I) [1,2,3,6-tetrahydro-4-phenyl-1-[3-phenyl-3-cyclohexen-1-yl)methyl]pyridine, R(+)-enantiomer] is a dopamine D2 and D3 partial agonist (Pugsley et al., 1995; Meltzer et al., 1995; Feng et al., 1997a) undergoing clinical trials as an antipsychotic agent. Similar to known antipsychotic drugs, CI-1007 produces potent inhibitory effects on locomotor activity in rats and continuous avoidance in squirrel monkeys. I causes only mild extrapyramidal side effects in monkeys. A positive correlation has been established between the plasma concentration and the effects of I in animal models (Feng et al., 1997a). I is highly bound to plasma proteins (>98%), with blood/plasma concentration ratios ranging from 0.75 to 0.85 (Feng et al., 1993, 1997a). Several metabolites were identified in our previous work. Two monohydroxy derivatives (PD 147693, II, and PD 149394, III) and one dihydroxy derivative (PD 155144, IV) were found in monkey plasma (Feng et al., 1993, 1994, 1995; Wright et al., 1995). Like its parent drug, II produces antipsychotic-like behavioral effects in both rats and monkeys (Feng et al., 1997a); the other two hydroxy metabolites appear inactive in behavioral tests. The purpose of the present work was to compare the metabolism and disposition of I in rats, monkeys, dogs, and humans. Results from an in vitro metabolism study (Sinz et al., 1995) indicated that I is a substrate for CYP2D6, and initial human trials were conducted in healthy subjects demonstrating the normal/wild-type CYP2D6 genotype (CYP2D6 EMs). The proposed partial metabolic pathway of I is shown in fig. 1.

Materials and Methods

Chemicals. I, [14C]I, II, III, IV, and the sulfate conjugates PD 163249 (V), PD 163637 (VI), PD 163638 (VII), PD 163639 (VIII), and PD 163640 (IX) were synthesized by Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co. (Ann Arbor, MI).

Rat Study. Mass Balance and Metabolic Profiling. Six male Wistar rats with bile duct cannulas were used. After an overnight fast, rats received a single oral dose of 10 mg/kg (290 μCi/kg) [14C]I in 0.5% methylcellulose. Animals were housed individually in stainless steel metabolism cages for collection of urine and feces. Rats were allowed access to food 4 hr after dosing. Water was provided ad libitum. Urine, bile, and feces samples were collected before dosing and at different times up to 192 hr after dosing.

On a separate occasion, one male Wistar rat received a single oral dose of 50 mg/kg (1100 μCi/kg) [14C]I in 0.5% methylcellulose, after an overnight fast. Blood samples were collected at 2 and 4 hr after dosing, and plasma samples were obtained by centrifugation and stored frozen until analysis.

Abbreviations used are: CYP, cytochrome P450; CL, clearance; Vss, steady-state volume of distribution; MRT, mean residence time; EM, extensive metabolizer; DMSO, dimethylsulfoxide.

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Plasma Pharmacokinetics. Male Wistar rats received a single iv dose of 1 mg/kg I (N = 4) in a mixture of 5% dextrose/ethyl alcohol (90:10, v/v) or an oral dose of 100 mg/kg I (N = 4) in 0.5% methylcellulose, after an overnight fast. Blood samples were collected before dosing and at different times up to 32 hr after dosing; plasma was obtained by centrifugation and stored frozen until analysis. The pharmacologically active metabolite II was only a minor component in rat plasma, as demonstrated in previous tests (Feng et al., 1994, 1997a), and a 100 mg/kg oral dose was used in the current study to determine the pharmacokinetic profile of this metabolite in rats. I showed linear pharmacokinetics in rats, over the dose range of 10–100 mg/kg, in our previous studies.

Monkey Study. Mass Balance and Metabolic Profiling. Four male cynomolgus monkeys were used. After an overnight fast, four monkeys received a single oral dose of 25 mg/kg (19.3 µCi/kg) [14C]I in 0.5% methylcellulose. Animals were housed individually in stainless steel metabolism cages for collection of urine and feces. Monkeys were allowed access to food 4 hr after dosing. Water was provided ad libitum. Urine and feces were collected before dosing and at different times up to 10 days after dosing.

On a separate occasion, two male and two female cynomolgus monkeys received a single oral dose of 74 mg/kg (111 µCi/kg) [14C]I in 0.5% methylcellulose, after an overnight fast. Blood samples were collected at 5 or 24 hr after dosing, and plasma was obtained by centrifugation and stored frozen until analysis.

Pharmacokinetics. After an overnight fast, four male cynomolgus monkeys received an iv dose of 5 mg/kg I in a mixture of 5% dextrose/ethyl alcohol (90:10, v/v) and an oral dose of 25 mg/kg I in 0.5% methylcellulose, with a 2-week washing period between doses. Blood samples were collected before dosing and at different times up to 28 hr after dosing. Plasma was obtained by centrifugation and stored frozen until analysis.

Dog Study Evaluating Plasma Pharmacokinetics. Male beagle dogs received a single iv dose of 2.5 mg/kg I (N = 4) in a mixture of 5% dextrose/ethyl alcohol (90:10, v/v) or an intraduodenal dose of 15 mg/kg I (N = 1) in labrosol, after an overnight fast. Blood samples were collected before dosing and at different times up to 28 hr after dosing, and plasma was obtained by centrifugation and stored frozen until analysis. Oral (gavage) administration of I was avoided because this route might cause emesis in the dogs.

Human Study Evaluating Plasma and Urine Pharmacokinetics. Healthy subjects (EMs, N = 4) received a single 15-mg oral capsule dose of I, after an overnight fast. Blood and urine samples were collected before dosing and at different times after dosing, and plasma samples were obtained by centrifugation and stored frozen until analysis.
Scintillation Counting. The volumes of rat urine and bile and monkey urine samples were measured. Aliquots (0.100 ml) were combined with 10.0 ml of scintillation cocktail, and total radioactivity was quantitated by liquid scintillation counting. Feces samples were weighed and homogenized in 9 volumes of water/methanol (50:50, v/v). Aliquots (0.500 ml) of the homogenate were analyzed by combustion and liquid scintillation counting, as described above.

Radioisometric-HPLC Assay of Rat Urine and Bile or Monkey Urine and Feces. HPLC was performed using a Perkin-Elmer (Norwalk, CT) system, including a LC-410 pump, an ISS-200 autosampler, and an LC-240 fluorescence detector. Plasma, urine, bile, and feces were analyzed using reverse-phase HPLC with dual radioisometric (Flow-One A-280; Packard Instruments Co., Meriden, CT) and fluorescence detection. Separation was achieved on a Zorbax Rx C8 column (5-µm particle size, 250 × 4.6 mm; MAC-MOD Analytical, Chadds Ford, PA). The mobile phase consisted of solvent A (0.02 M ammonium acetate, pH 4.0/acetonitrile, 90:10, v/v) and solvent B (0.02 M ammonium acetate, pH 4.0/acetonitrile, 25:75, v/v), at a flow rate of 1.0 ml/min. The proportion of solvent A was 100% at 0 time, and the proportion of solvent B was increased linearly from 0% to 85% in 90 min. The metabolite peaks were compared with the chromatograms of synthetic standards, i.e. I (CI-1007), II (PD 147693), III (PD 149394), IV (PD 151544), V (PD 163249), VI (PD 163637), VII (PD 163638), VIII (PD 163639), and IX (PD 163640) (fig. 1).

The 0–24-hr bile and 0–48-hr urine samples were used for metabolic profiling in rats, because most of the radioactivity was eliminated during these periods. The 0–72-hr urine and 0–96-hr feces samples were used for metabolic profiling in monkeys, because most of the radioactivity was eliminated during these periods.

Aliquots (5 ml) of rat or monkey urine were mixed with 1.0 ml of DMSO and evaporated under a gentle N₂ stream, in a 50°C water bath, to a final volume of approximately 1 ml. Aliquots (200 µl) of the concentrated urine samples were mixed with 200 µl of 1 N acetic acid; 200 µl of this mixture was injected for radioisometric-HPLC analysis.

Aliquots (200 µl) of rat bile were mixed with 100 µl of methanol, 250 µl of DMSO, and 375 µl of 1 N acetic acid and were then centrifuged at 1600g for 10 min. Aliquots (200 µl) of the supernatant were injected for radioisometric-HPLC analysis.

Monkey feces samples were weighed and homogenized in 9 volumes of water/methanol (50:50, v/v). Aliquots (200 µl) of the homogenate were mixed with 50 µl of DMSO and 200 µl of 1 N acetic acid; 200 µl was injected for radioisometric-HPLC analysis.

HPLC Analysis of Rat, Monkey, Dog, and Human Plasma. Metabolism. Aliquots (500 µl) of the plasma samples from rats or monkeys receiving [¹⁴C] and evaporated under a gentle N₂ stream, in a 50°C water bath, to a final volume of approximately 1 ml. Aliquots (200 µl) of the concentrated urine samples were mixed with 200 µl of 1 N acetic acid; 200 µl of this mixture was injected for radioisometric-HPLC analysis.

HPLC samples were assayed for I and II using validated HPLC assays, as reported previously (Feng et al., 1995). The minimal quantitation limit was 3 ng/ml for I in rat and monkey plasma, 5 ng/ml for I in dog plasma, and 5 ng/ml for II in plasma from all species. Linearity was demonstrated up to 300 ng/ml.

Quantitative of I and II in Rat, Monkey, and Dog Plasma Using HPLC. Samples were assayed for I and II using validated HPLC assays, as reported previously (Feng et al., 1995). The minimal quantitation limit was 3 ng/ml for I in rat and monkey plasma, 5 ng/ml for I in dog plasma, and 5 ng/ml for II in plasma from all species. Linearity was demonstrated up to 300 ng/ml.

Quantification of I and II in Human Plasma and Urine Using LC/MS/MS. Extracts of plasma or urine samples were analyzed by LC/MS/MS with a Perkin-Elmer Sciex API III mass spectrometer, with an ion-spray interface, operating in the positive-ion mode. Samples were chromatographed on a Waters NovaPak C₁₈ column (2.0 mm × 15 cm; Waters Chromatography, Milford, MA) under isocratic conditions, at a flow rate of 0.4 ml/min. The mobile phase was a mixture of acetonitrile/20 mM ammonium acetate, pH 4.5 (8:2, v/v). The minimal quantitation limit was 0.10 ng/ml in both plasma and urine. Linearity was demonstrated up to 15 ng/ml (Feng et al., 1998; Sramek et al., 1998).

Pharmacokinetic Analysis. Pharmacokinetic parameters were calculated using noncompartmental methods (WinNONLIN, version 1.1; SCI Software, Lexington, KY).

Interspecies Scaling. Interspecies scaling was performed according to the equation $P = aW^b$, where $P$ is a pharmacokinetic parameter, $W$ is body weight, and $a$ and $b$ are constants.

Results

Excretion. After oral administration of [¹⁴C]I, biliary excretion was the major route of [¹⁴C] elimination in rats, followed by elimination in feces and urine, for 192 hr after dosing (fig. 2, table I). Fecal excretion was the major route of [¹⁴C] elimination in monkeys, followed by excretion in urine, for 10 days after dosing. I was not detected in rat urine or bile, monkey urine or feces, or human urine, suggesting negligible urinary excretion of unchanged drug. Approximately 30% of the dose was excreted as unchanged drug in rat feces (Feng et al., 1993).

Metabolic Profile in Rat Plasma, Urine, and Feces. HPLC radiochromatograms obtained from rat plasma, urine, and feces are shown in fig. 3. The unchanged drug and two major unknown components were detected in rat plasma. The chromatographic peak corresponding to the monohydroxy metabolite II was small and was not detected by radioisometric-HPLC. II was detected in rat plasma by an HPLC-fluorescence assay in the pharmacokinetic study.

The 0–48-hr urine and bile samples were used for profiling because the majority of [¹⁴C] radioactivity was excreted during this period. Several polar components were detected in rat urine and bile. The monohydroxy metabolite II was identified in rat bile as a minor component (fig. 3). No radioactive peaks corresponding to other hydroxy (III and IV) or sulfate (V, VI, VII, VIII, and IX) metabolites were detected in rat plasma, urine, or feces. Retention times were 73.0, 58.0, 61.0, 47.0, 30.0, 46.0, 49.5, 37.5, and 38.5 min for standards I, II, III, IV, V, VI, VII, VIII, and IX, respectively.
Metabolic Profile in Monkey Plasma, Urine, and Feces. HPLC radiochromatograms obtained for monkey plasma, urine, and feces are presented in fig. 4. The monosulfates VI and VIII were identified as major metabolites in monkey plasma. VI was excreted predominantly in monkey feces and VIII in monkey urine. Chromatographic peaks corresponding to the parent drug and the hydroxy metabolites II, III, and IV were relatively small. The structures of I, II, III, IV, and the sulfate metabolites were confirmed by HPLC with fluorescence detection and by MS in a previous study with monkey kidneys (Feng et al., 1994, 1997b, 1998; Wright et al., 1995). Several minor radioactive peaks were also detected in monkey plasma, urine, and feces.

Pharmacokinetics. Plasma concentration-time profiles for I and II after administration of single iv or po doses of I to rats, monkeys, dogs, and humans are presented in figs. 5 and 6. After iv administration, plasma concentrations of I declined in a multiexponential manner (fig. 5). Elimination t1/2 values were 9.9, 19.4, and 14.3 hr for rats, monkeys, and dogs, respectively (table 2). Systemic plasma CL values (27.8–40 ml/min/kg) approximate the liver plasma flow in these species (Altman and Dittmer, 1974; Bernareggi and Rowland, 1991; Davis and Morris, 1993; Dedrick and Forrester, 1973; Mordenti, 1986; Ohnhaus and Locher, 1975; Yates et al., 1978). This finding, in combination with negligible urinary excretion, suggests a strong first-pass effect after oral administration of I. Oral CL values were similar for rats, monkeys, dogs, and humans (table 3). The Vss values (14.6–20.5 liters/kg) far exceeded the total plasma volume (<0.1 liter/kg) for rats, monkeys, and dogs, suggesting that I, as a lipophilic drug, is distributed substantially into tissue. I is highly (≥98%) bound to plasma proteins across species, with blood/plasma concentration ratios of 0.75–0.85 (Feng et al., 1993, 1997a). The large Vss values also indicate binding to tissue proteins. Pharmacokinetic parameters for I and II, obtained after administration of oral doses, are summarized in table 3, and results of interspecies scaling are shown in fig. 7. Strong correlation was achieved in the scaling of CL/F (r² = 0.99), CL (r² = 1.0), and Vss (r² = 1.0).

Discussion

After an oral dose of [14C]I, the excretion of radioactivity was moderately fast in rats, with most of the radiolabeled dose appearing in bile within the first 48 hr (fig. 2). Excretion of 14C radioactivity was slower in monkeys. Most of the radiolabeled dose was recovered in 0–120-hr feces samples after an oral dose. I was metabolized extensively after oral administration and was not detected in rat urine or bile or in monkey urine or feces. Approximately 30% of the dose was
excreted as unchanged drug in rat feces (Feng et al., 1993). Total po recovery of $^{14}$C radiolabel was 96% in rats and 80% in monkeys. Approximately 70% of the dose was absorbed in rats, as demonstrated by the radioactivity excreted in urine (3.2%) and bile (64%). Total urinary and fecal $^{14}$C recovery was 78%, which may be considered as the percentage of the dose absorbed, with the assumption that no metabolism occurred in the gastrointestinal tract. As shown in fig. 4, I was almost completely metabolized after oral administration to monkeys, and no unchanged drug was detected in urine or feces.

The proposed partial metabolic pathway in rats, monkeys, dogs, and humans is presented in fig. 1. Phenyl ring hydroxylation and sulfate conjugation appear to be the major metabolic routes in cynomolgus monkeys after oral administration of I. The two monohydroxy metabolites (II and III) and the dihydroxy metabolite (IV) were identified in our previous work, using LC/MS (Wright et al., 1995). In the current study, VI and VIII (the monosulfate conjugates of II and IV, respectively) were identified as major components in monkey plasma by comparison with synthetic standards. VIII was excreted predominantly in monkey urine, whereas the less polar sulfate VI was mainly recovered in monkey feces (possibly via bile) (fig. 4). VI, VIII, and three other sulfates, i.e. V, VII, and IX, were identified in monkey kidney by LC/MS, in a separate study (Feng et al., 1998). The sulfates VI and VIII were also identified by LC/MS/MS in plasma and urine of human EmS (Feng et al., 1998; Sramek et al., 1998), but not rats or dogs. These findings suggest similar metabolic profiles in monkeys and humans, which is consistent with the results from an in vitro study. II, III, IV, and four sulfate conjugates (V, VI, VIII, and IX) were formed when $^{14}$C-I was incubated with monkey or human hepatocytes (Sinz et al., 1995).

Several polar metabolite peaks were also detected in rat urine and bile. Further identification was not pursued because the in vitro human metabolic profile was similar to that for monkeys but different from that for rats (Sinz et al., 1995). I was incubated with rat liver microsomes in that study, and a small amount of II was detected. This is similar to in vivo findings that II is a minor component in rat bile and plasma. III and VI were not detected in rat liver microsomal incubation mixtures, whereas several unknown metabolites were formed.

As shown in figs. 5 and 6, plasma concentrations of II, the active monohydroxy metabolite, were quantitatively different across species. Observed maximal plasma concentration and AUC values for II were comparable to those for I in dog plasma, <5% of those for I in rat plasma, and approximately 20–40% of those in the plasma of monkeys and human EmS. II appears to be a major metabolite in dogs. The plasma AUC value for II was approximately 50% of that for I in dog plasma after iv administration.

I is primarily eliminated by the liver, with negligible urinary excretion. Hence, its CL from the body is mainly limited by hepatic blood flow, although the metabolic pathway varies among species. Because hepatic blood (or plasma) flow was shown to have an allometric relationship with body weight (Boxenbaum, 1980), it was
expected that the CL of I would show an allometric relationship with body weight. In fact, strong correlation was achieved in the allometric scaling of CL/F (r² = 0.98), CL (r² = 1.0), and Vss (r² = 1.0). Four species (rats, monkeys, dogs, and human EMs) were used for the scaling of CL/F, and three species (rats, monkeys, and dogs) were used for the scaling of systemic CL measured after iv administration. The slopes for CL/F (0.98) and CL (0.88) vs. body weight were similar, suggesting similar systemic bioavailability among species and indicating that the predicted human CL (20.8 ml/min/kg) value is reliable. The calculated oral bioavailability was 13, 6.1, 4.7, and 4.2% for rats, monkeys, dogs, and humans, respectively. The predicted CL approximates human liver plasma flow, and Vss exceeds plasma volume (Bernareggi and Rowland, 1991; Davis and Morris, 1993; Mordenti, 1986), indicating high CL values and substantial tissue distribution in human EMs. The correlation was weakened when CL was corrected by maximal lifespan. Elimination t1/2 and MRT values did not correlate well with body weight. It is generally difficult to predict t1/2 values in humans by allometric scaling, because t1/2 is determined by both Vss and CL. Similar examples have been reported in the literature (Evans et al., 1973; Mordenti, 1985; Reuning and Gerates, 1986). Propranolol is mainly eliminated by metabolism. It showed similar t1/2 values of 30–40 min in rats, monkeys, and dogs, but the CL values were 90, 18, and 34 ml/min/kg, respectively.

An alternative way to obtain t1/2 or MRT values for humans is to calculate the values using allometrically estimated Vss and CL values for humans (Obach et al., 1997). When the MRT (10.8 hr) of I was calculated from the predicted human CL and Vss, its value suggested a twice- or thrice-daily dosing regimen for clinical trials, because approximately 67% of the drug would be eliminated within 11 hr. The predicted value is comparable to the results from clinical trials with human EMs, after single or multiple doses of I, where an accumulation t1/2 of 9.2 hr was determined (Feng et al., 1998).

In summary, I was extensively metabolized after oral administration, with predominant hepatic CL and negligible urinary excretion. Although metabolic profiles differed among species, strong correlation was achieved in allometric scaling, because the CL of I from the body is mainly limited by hepatic blood flow. Significant amounts of the active metabolite II were detected in human plasma, suggesting that II may contribute to the antipsychotic effect.

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References