Absorption, Metabolism, and Excretion of Paliperidone, a New Monoaminergic Antagonist, in Humans

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ABSTRACT:

Absorption, metabolism, and excretion of paliperidone, an atypical antipsychotic, was studied in five healthy male subjects after a single dose of 1 mg of [14C]paliperidone oral solution (~16 μCi/subject). One week after dosing, 88.4 to 93.8% (mean 91.1%) of the administered radioactivity was excreted: 77.1 to 87.1% (mean 79.6%) in urine and 6.8 to 14.4% (mean 11.4%) in the feces. Paliperidone was the major circulating compound (97% of the area under the plasma concentration-time curve at 24 h). No metabolites could be detected in plasma. Renal excretion was the major route of elimination with 59% of the dose excreted unchanged in urine. About half of the renal excretion occurred by active secretion. Unchanged drug was not detected in feces. Four metabolic pathways were identified as being involved in the elimination of paliperidone, each of which accounted for up to a maximum of 6.5% of the biotransformation of the total dose. Biotransformation of the drug occurred through oxidative *N*-dealkylation (formation of the acid metabolite M1), monohydroxylation of the alicyclic ring (M9), alcohol dehydrogenation (formation of the ketone metabolite M12), and benzisoxazole scission (formation of M11), the latter in combination with glucuronidation (M16) or alicyclic hydroxylation (M10). Unchanged drug, M1, M9, M12, and M16 were detected in urine; M10 and M11 were detected in feces. The monohydroxylated metabolite M9 was solely present in urine samples of extensive CYP2D6 metabolizers, whereas M10, another metabolite monohydroxylated at the alicyclic ring system, was present in feces of poor metabolizers as well. In conclusion, paliperidone is not metabolized extensively and is primarily renally excreted.

Paliperidone (R076477, Invega; Johnson & Johnson Pharmaceuticals, L.L.C., Titusville, NJ) is an atypical antipsychotic (Fig. 1) that belongs to the chemical class of benzisoxazole derivatives. The molecular formula is $C_{23}H_{27}FN_4O_3$ and the molecular weight is 426.49. Paliperidone (or 9-hydroxyrisperidone) is the major and active metabolite of risperidone (Risperdal; Johnson & Johnson Pharmaceuticals, L.L.C.) (Mannens et al., 1993; Megens and Awouters, 1994), a second-generation antipsychotic, that is registered worldwide for the treatment of schizophrenia. Paliperidone is

a centrally active dopamine D_2 and serotinergic 5-HT$_{2A}$ antagonist, as demonstrated in both in vitro and in vivo animal and human studies. Paliperidone is also active as an antagonist at α_1- and α_2-adrenergic receptors and H$_1$ histaminergic receptors. Paliperidone has no affinity for cholinergic muscarinic or β_1- and β_2-adrenergic receptors (Megens and Awouters, 1994; Schotte et al., 1996; Karlsson et al., 2005). Paliperidone is a racemate. The pharmacologic profiles of the racemate and the two enantiomers are similar in in vitro binding assays, in vitro receptor occupancy studies, and in vivo functional interaction studies (Schotte et al., 1996). Experiments in humans using positron emission tomography showed that paliperidone occupies central D_2 and 5-HT$_2$ receptors. The in vivo apparent dissociation constant ($K_D{}^{app}$) for central D_2 receptors is estimated to be 4.9 ng/ml (Karlsson et al., 2005).

Paliperidone extended-release (3–15 mg once daily) has been shown to be effective in reducing symptoms of schizophrenia and in improving personal and social performance in short- and long-term studies in the treatment of schizophrenia (Davidson et al., 2007; Kane et al., 2007; Kramer et al., 2007; Marder et al., 2007). Paliperidone extended-release has a slowly ascending plasma concentration-time

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ABBREVIATIONS: paliperidone, R076477, (±)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4*H*-pyrido[1,2-a]-pyrimidin-4-one; HT, 5-hydroxytryptamine, serotonin; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; UGT, UDP glucuronosyltransferase; MS/MS, tandem mass spectrometry.
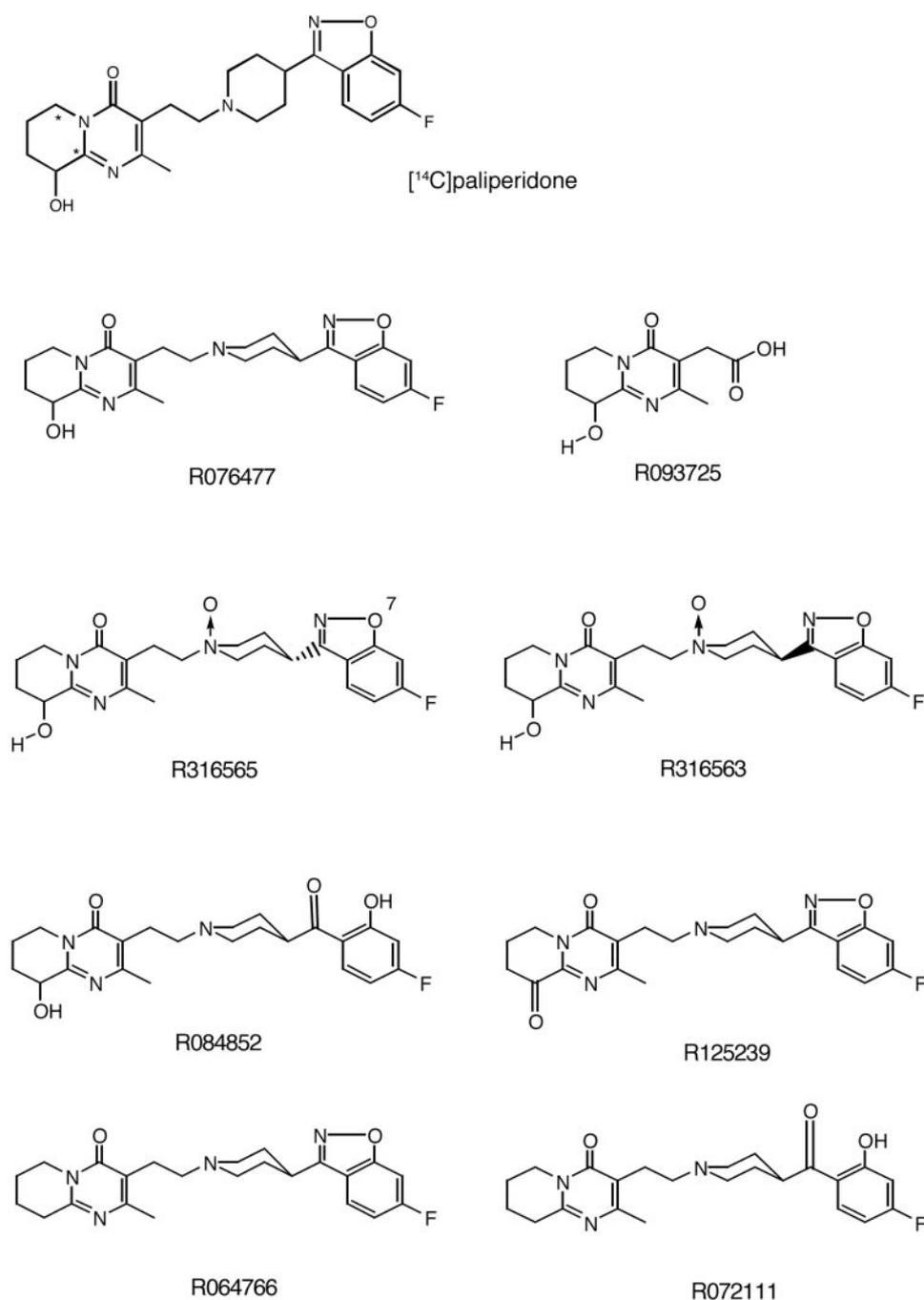
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FIG. 1. Chemical structure of [¹⁴C]paliperi-done, with the position of the [¹⁴C] labels indi-cated with an asterisk (∗), and of the synthetic reference compounds.

profile reaching maximum concentrations approximately 24 h after dosing (Yang and Plosker, 2007).

In vitro metabolism studies using recombinant human cytochrome P450 enzymes and correlation studies using a panel of human liver microsomes has revealed that both CYP2D6 and CYP3A4 are involved in the 9-hydroxylation of risperidone to paliperidone (Fang et al., 1999). Metabolism experiments on paliperidone with heterologous organisms expressing human CYP3A4 and CYP2D6 suggested the possible involvement of these forms in the metabolism of the drug (unpublished data).

Here we report the absorption, metabolism, and excretion of pali-peridone in healthy male subjects after a single dose of [¹⁴C]paliperi-done oral solution. The objective of the study was to characterize the excretion and metabolism of paliperidone and to elucidate the meta-bolic pathways and the structure of the metabolites. As there are indications that CYP2D6 is involved to some extent in the metabolism of paliperidone, both poor and extensive metabolizers for this isozyme were included in the study.

Materials and Methods

Paliperidone and Reference Compounds. Unlabeled paliperidone (R076477) was synthesized by the Chemical and Manufacturing Department of Johnson & Johnson Pharmaceutical Research and Development (Beerse, Belgium). [¹⁴C]Paliperidone, synthesized by the Radiochemistry group of Johnson & Johnson Pharmaceutical Research and Development, was labeled with ¹⁴C at the 6- and 10-positions of the 6,7,8,9-tetrahydro-2-methyl-4H-pyrido [1,2-a]-pyrimidin-4-one ring system (Fig. 1). It showed a specific activity of 841 MBq/mmol (22.7 mCi/mmol or 53.3 μCi/mg) and a radio-chemical purity of 99% [radio-high-performance liquid chromatography

(HPLC)]. [^{14}C]Paliperidone was diluted with unlabeled paliperidone and dissolved in an aqueous solution of hydroxypropyl-β-cyclodextrin and citric acid to obtain a final paliperidone concentration of 0.0984 mg/ml and specific activity of 592 kBq/mg ($\sim$16 μCi/mg). The pH was adjusted to 4.0 with NaOH. The radiochemical purity of [^{14}C]paliperidone in the drug formulation at the time of dosing was 99.5%, as determined by radio-HPLC. The following nonradiolabeled reference compounds were synthesized by the Radiochemistry group of Johnson & Johnson Pharmaceutical Research and Development: R064766, R076477, R072111, R084852, R093725, R125239, R316563, and R316565 (Fig. 1). The purities of the reference compounds were examined separately by liquid chromatography (LC)-mass spectrometry (MS). Solvents and reagents were all of analytical grade and were purchased from commercial sources.

Subjects and Dosing. The clinical part of the study was conducted at SGS Biopharma (Antwerp, Belgium) in accordance with good clinical practice guidelines, the Declaration of Helsinki (1964 and subsequent revisions), and the Administration of Radioactive Substances Advisory Committee. The protocol was approved by an independent ethics committee and by the Department of Biomedical Physics and Radiation Protection, Ghent University (Ghent, Belgium). All participating subjects gave written informed consent before participation. Healthy Caucasian males aged between 40 and 63 years with a body mass index of 20 to 28 kg/m^2 were enrolled in this single-center, single-dose, open-label study (study R076477-P01-103). Subjects were healthy on the basis of medical history and a prestudy physical examination, electrocardiogram, and clinical laboratory tests. Subjects were nonsmokers and had no history of alcohol and drug abuse. Subjects were phenotyped with respect to CYP2D6 using a dextromethorphan metabolic ratio. Two poor (dextromethorphan metabolic ratio >0.345) and three extensive metabolizers (dextromethorphan metabolic ratio <0.0255) participated in the study. Subjects were genotyped with respect to CYP2D6, CYP3A4, CYP3A5, UGT1A1, and UGT1A6. No medication other than the study drug was allowed from 14 days before dosing of the study drug until the end of the study, with the exception of emergency medication to treat adverse events. Subjects remained in the study center from at least 10 h before dosing until 168 h after dosing. Subjects fasted from at least 10 h before dosing until 4 h after dosing. Each subject received a single 1-mg dose of [^{14}C]paliperidone as 10 ml of a 0.1 mg/ml oral solution with a total radioactivity of $\sim$16 μCi, along with 200 ml of water.

Sample Collections. Blood samples ($\sim$10 ml) were obtained before dosing and at 0.5, 1, 1.5, 3, 6, 12, 16, 36, 48, 72, 96, 120, 144, and 168 h after dosing. In addition, $\sim$20 ml of blood for the purpose of metabolite profiling and $\sim$3 ml for the determination of ^{14}C radioactivity in whole blood were collected at 2, 4, 8, and 24 h after dosing. Samples for complete urinary output were collected over the following intervals: 0 to 4, 4 to 8, 8 to 12, 12 to 16, 16 to 24, 24 to 36, 36 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after dosing and were stored at 2–6°C until liquid scintillation counting and at $\leq$–18°C thereafter. Stool samples were collected once before dosing and from 0 to 168 h after dosing as for voided samples. All other samples were kept at $\leq$–18°C until analysis.

Determination of Radioactivity. Radioactivity in blood, plasma, urine, and feces was measured using a Packard Tri-Carb 1900TR or 2100TR liquid scintillation spectrometer (PerkinElmer Life and Analytical Sciences, Boston, MA).

Blood and plasma. Blood radioactivity concentrations were measured after combustion of quadruplicate dried 0.25-ml aliquots in a Packard Sample Oxidizer model 307 (PerkinElmer Life and Analytical Sciences). Carbosorb (9 ml; PerkinElmer Life and Analytical Sciences) was used to absorb the ^{14}CO$_2$ and Permafluor (11 ml; PerkinElmer Life and Analytical Sciences) was used as a scintillation cocktail. Duplicate 250-μl aliquots of plasma were diluted with distilled water up to 1 ml and mixed with 10 ml of Ultima Gold (Packard BioScience) as a scintillation cocktail. The total radioactivity levels were expressed as nanogram equivalents per milliliter using the specific activity of [^{14}C]paliperidone in the drug formulation. The limit of quantification was 72 dpm/ml (2.0 ng-Eq/ml).

Urine. Duplicate 0.25-ml aliquots of urine were diluted with distilled water up to 1 ml and mixed with 10 ml of Ultima Gold as a scintillation cocktail.

Feces. Fecal samples were homogenized in methanol with an Ultra-Turrax and centrifuged, and residues were then extracted another two times with methanol, followed by filtration of the suspensions through a Büchner funnel.

The methanolic extracts of each fecal sample were combined, and duplicate 0.25-ml aliquots were diluted with distilled water up to 1 ml and mixed with 10 ml of Ultima Gold as a scintillation cocktail. The fecal residues were air-dried and weighed. The dried residues were ground to a fine powder in an Ultra Centrifugal Mill ZM100. Four weighed subsamples of approximately 100 mg of each residue sample were combusted and measured in a manner similar to that for the blood samples.

Determination of Paliperidone in Plasma. Paliperidone and risperidone plasma concentrations were determined by an LC-MS/MS assay, based on Method A published by Remmerie et al. (2003), except for the deviations indicated below. The chromatography conditions were optimized: 10-μl aliquots of the processed samples were injected on a 3-μm C18-A Polaris column (50 × 4.6 mm i.d.). The mobile phase, a mixture of 0.01 M ammonium formate (adjusted to pH 4.0 with formic acid) as elution solvent A, spectrophotometric acetonitrile as elution solvent B and spectrophotometric methanol as elution solvent C, was delivered at 1.2 ml/min. Isocratic elution of the analytes was achieved using a mixture of 70% A, 20% B, and 10% C for 3.1 min, after which a step gradient was applied with 5% A, 10% B, and 85% C until 4.1 min; finally, the column was reequilibrated at 70% A, 20% B, and 10% C until 5.5 min. The eluent was split in a ratio of 1:11 to introduce 0.1 ml/min into the mass spectrometer. Quantitation was achieved by MS/MS detection in the positive ion mode, using a PerkinElmer Sciex (Foster City, CA) API 3000 mass spectrometer, equipped with a TurboIonSpray interface. As internal standard, a structural analog, R068808, was used instead of the stable isotope-labeled internal standards to avoid cross-talk from the [^{14}C$_2$]paliperidone test compound in the multiple reaction monitoring channel of the internal standard. Detection of the internal standard was performed in the multiple reaction monitoring mode, monitoring the transition of the m/z 421.2 precursor ion to the m/z 201.0 product ion.

In line with existing bioanalytical guidelines (Shah et al., 2000; U.S. Food and Drug Administration, 2001), the optimized method was revalidated by a partial validation, demonstrating performance equivalent to that of the original assay. The data support the accurate and precise quantitation of paliperidone and risperidone in 500 μl of heparin plasma over a concentration range of 0.1 to 250 ng/ml with a lower limit of quantification of 0.1 ng/ml and with acceptable accuracy and precision.

Pharmacokinetic Analysis. Pharmacokinetic parameters were calculated using noncompartmental analysis. Actual blood sampling times and target urine sampling times were used. Results are given as mean ± S.D., except for the time of maximum concentration (t_{max}) for which medians and range are given. The area under the plasma concentration-time curve (AUC$_\infty$) values were calculated by linear trapezoidal summation and extrapolation to infinity, calculated as AUC$_\infty$ = AUC$_{last}$ + C_{last}/λ_z, where C_{last} is the last measurable concentration and concentration and λ_z is the elimination rate constant, estimated by linear regression of the terminal points of the ln-linear plasma concentration-time curve. The terminal half-life, $t_{1/2}$, was calculated as 0.693/λ_z. The apparent plasma clearance (CL/f) was calculated as dose divided by AUC$_\infty$. The renal clearance (CL$_R$) of paliperidone was calculated as the total amount excreted unchanged in urine divided by AUC$_\infty$. Furthermore, the clearance by glomerular filtration (CL$_{GFR}$) was calculated as the creatinine clearance (CL$_{CR}$) multiplied by the fraction unbound of paliperidone (f_u). CL$_{CR}$ (milliliters per minute) was calculated as CL$_{CR}$ = Ae$_{creat}$ · 1000/C$_{CR}$ · 1440, where Ae$_{creat}$ is the total cumulative amount of creatinine excreted in urine over 24 h (micromoles) and C$_{CR}$ is the serum creatinine concentration at 12 h after drug intake (micromoles per liter). The f_u value used (0.23) was determined in a study published previously, in which the protein binding of paliperidone in human plasma from healthy male volunteers was assessed using equilibrium dialysis (Mannens et al., 1994). Active renal clearance (CL$_{act}$) was calculated as the difference between CL$_R$ and CL$_{GFR}$.

Metabolite Profiling in Plasma, Urine, and Fecal Extracts. Urine samples of the collection periods between 0 and 12, 12 and 24, 24 and 48, 48 and 96, and 96 and 168 h, as well as selected methanolic fecal extracts from each subject (representative for the major part of the excreted radioactivity) were pooled by mixing constant fractions of the respective urine and methanolic fecal extract samples.

No radio-HPLC analysis was performed on plasma samples because of their low level of radioactivity (<413 dpm/ml). Urine samples of up to 1.9 ml were injected after centrifugation onto the radio-HPLC system. For feces, 10-ml
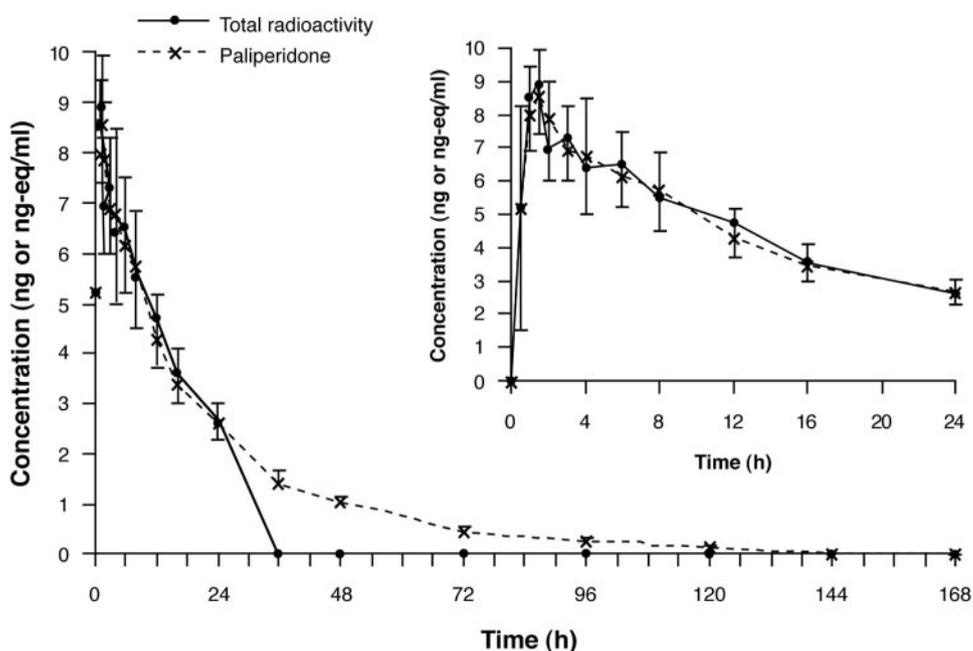
Fig. 2. Mean ($\pm$ S.D.) plasma concentration-time profiles of paliperidone and total radioactivity in healthy male subjects ($n = 5$) after a 1-mg single dose of [^{14}C]paliperidone. Total radioactivity concentrations were below the limit of quantification of 2.0 ng-eq/ml from 24 h after dosing onward; the limit of quantification for paliperidone was 0.1 ng/ml.

samples of the pooled methanolic extracts were evaporated under nitrogen, and the residues were reconstituted in 300 μl of dimethyl sulfoxide. Aliquots of 200 μl of these samples were injected onto the radio-HPLC system.

The HPLC apparatus consisted of a Waters Alliance 2695 system, equipped with an automatic injector. The samples were chromatographed on a stainless steel column (30 cm $\times$ 4.6 mm i.d.) packed with Kromasil C-18 (5 μm; Akzo Nobel, Amsterdam, The Netherlands). The columns were packed by a balanced density slurry procedure (Haskel DSTV 122-C pump, 7 $\times$ 10^7 Pa). UV detection was performed at 230 nm using a Waters 996-diode array detector. On-line radioactivity detection of HPLC eluates was performed with a Berthold Radioactivity Monitor LB 509 system equipped with a flow-through cell of 1000 μl. The eluates were mixed with Ultima Flo AP (Packard BioScience) as a scintillation cocktail delivered by a Berthold LB 5035-3 pump at a flow rate of 8.0 ml/min. Detector outputs were connected to the Millennium (Waters, Milford, MA) chromatography data system. Elution was started with a linear gradient at a flow rate of 1 ml/min from 100% of an aqueous solution of 0.1 M ammonium acetate adjusted to pH 8.5 (solvent system A) to 50% of solvent system A and 50% of solvent system B composed of an aqueous solution of 1 M ammonium acetate (adjusted to pH 8.5)-methanol-acetonitrile (10:10:80, by volume) over 30 min. This solvent composition was held for 5 min. Subsequently, a linear gradient over 1 min to 100% of solvent system B was applied, and this solvent composition was held for another 2 min before returning to the starting conditions. The concentrations of paliperidone and its major metabolites in urine and fecal extracts were calculated on the basis of the recovery of the radioactivity in the samples, as well as on the areas of the radioactivity peaks obtained after reversed-phase radio-HPLC of appropriate aliquots of these samples.

Structural Characterization of Metabolites. Metabolites were identified by LC-MS/MS and by HPLC cochromatography of a mixture of the parent compound and synthetic metabolites (see Fig. 1 for structures). The chromatographic part of the apparatus was the same as that outlined above. The LC-MS detector was a Finnigan LCQ (Thermo Electron Corporation, Waltham, MA) or a Q-TOF Ultima (Waters). For the LCQ mass spectrometer, electrospray ionization was used in the positive mode, and the settings were optimized for maximum intensity for paliperidone by using the auto-tune function within the LCQ Tune program. The Q-TOF mass spectrometer was equipped with a dual electrospray ionization probe and was operated in the positive ion and negative ion modes.

Glucuronic acid and sulfate conjugates excreted in urine were characterized by a comparison of radio-HPLC chromatograms of samples with and without treatment with β-glucuronidase-arylsulfatase from *Helix pomatia* (10 μl/ml of acetate-buffered sample, pH 5.0; Boehringer Ingelheim USA, Ridgefield, CT),

β-glucuronidase from *Escherichia coli* (10 μl/ml of phosphate-buffered sample, pH 7.0; Boehringer Ingelheim) and arylsulfatase from *Aerobacter aerogenes* (10 μl/ml of phosphate buffered sample, pH 7.0; Sigma-Aldrich, St. Louis, MO). Incubations were performed overnight (16–24 h) or longer at 37°C. The metabolites in methanolic extracts of human fecal samples were identified by cochromatography of selected samples with methanolic extracts of fecal samples from an excretion and metabolism study of paliperidone in rats. The chromatographic part of the apparatus was the same as that outlined previously.

Results

Demographics, Safety, and Tolerability. Five healthy male Caucasian subjects received the study drug and completed the study. Subject age ranged from 40 to 63 years (mean: 51.2 years), body weight ranged from 68.7 to 78.6 kg (mean: 73.38 kg), and body mass index ranged from 24 to 28 kg/m^2 (mean: 25.5 kg/m^2). None of the subjects used concomitant medication during the study. Paliperidone was well tolerated, no serious adverse events occurred, and no subjects discontinued the study because of an adverse event. Electrocardiograms, clinical laboratory tests, and vital signs showed no clinically relevant changes.

Genotyping and Phenotyping. Subjects were phenotyped for CYP2D6 using dextromethorphan and genotyped for CYP2D6, CYP3A4, CYP3A5, UGT1A1, and UGT1A6. On the basis of both phenotyping and genotyping, three subjects were extensive CYP2D6 metabolizers, whereas one subject (105) was a poor CYP2D6 metabolizer. On the basis of the dextromethorphan metabolic ratio, another subject (103) was phenotyped as being a poor CYP2D6 metabolizer (i.e., having a dextromethorphan metabolic ratio >0.345) but was genotyped to be a heterozygous extensive CYP2D6 metabolizer (CYP2D6 composite genotype: *1/*5). The subject's phenotype value was 0.357, which is close to the antimode (Schmid et al., 1985). The dextromethorphan metabolic ratio for metabolic characterization of the subjects was used, leading to a total of three extensive CYP2D6 metabolizers and two poor CYP2D6 metabolizers.

Pharmacokinetics of Radioactivity and Paliperidone. The mean plasma concentration-time profiles and pharmacokinetic parameters of total radioactivity and unchanged paliperidone after a single oral dose of 1 mg of [^{14}C]paliperidone to five healthy male subjects are

TABLE 1

Pharmacokinetic parameters of paliperidone and total radioactivity in plasma

Data are mean ± S.D. [except for t_{max}: median (minimum–maximum)] obtained from healthy male subjects ($n = 5$) receiving a 1-mg single oral dose of [^{14}C]paliperidone.

Parameter	Total Radioactivity [^{14}C]	Paliperidone
C_{max} (ng/ml)[a]	9.54 ± 1.35	8.85 ± 1.31
t_{max} (h)	1.5 (1.0–1.5)	1.5 (1.0–1.5)
$AUC_{24 h}$ (ng · h/ml)[a]	114 ± 19.9	111 ± 22.0
AUC_{∞} (ng · h/ml)[a]	175 ± 30.7	187 ± 29.3
$t_{1/2}$ (h)	15.2 ± 2.15	24.8 ± 4.35
CL/f (ml/min)	97.9 ± 17.6	91.0 ± 15.0
$Ae_{0–168 h}$ (% dose)		
Urine	79.6 ± 4.20	59.4 ± 7.12
Feces	11.4 ± 3.07	Not detected
CL_{CR} (ml/min)		113 ± 10.3
CL_R (ml/min)		53.1 ± 9.47
CL_{GFR} (ml/min)[b]		25.9 ± 2.36
CL_{act} (ml/min)		27.2 ± 7.50

C_{max}, maximum peak plasma concentration; t_{max}, time to maximum peak plasma concentration; $AUC_{24 h}$, area under the plasma concentration-time curve from 0 to 24 h; AUC_{∞}, area under the plasma concentration-time curve from zero to infinity; $t_{1/2}$, terminal half-life; CL/f, total plasma clearance; Ae (% dose), amount of unchanged drug excreted in the urine/feces; CL_{CR}, creatinine clearance; CL_R, renal clearance; CL_{GFR}, average clearance by glomerular filtration rate; CL_{act}, active renal clearance.

[a] For total radioactivity, the units for C_{max} and AUC were nanogram-equivalents per milliliter and nanogram-equivalents per milliliter per hour per milliliter, respectively.

[b] For the calculation of clearance by glomerular filtration (CL_{GFR}), the fraction unbound of paliperidone was 0.23.

shown in Fig. 2 and Table 1, respectively. The results indicate that paliperidone was absorbed rapidly after oral administration and that unchanged drug (paliperidone) accounted for almost all of the circulating radioactivity. Maximum plasma concentrations of total radioactivity and unchanged paliperidone were observed at approximately 1.5 h (median) after dosing. The maximum concentration of total radioactivity (mean C_{max} of 9.54 ng-Eq/ml) was only slightly higher than that of unchanged drug (mean C_{max} of 8.85 ng/ml) (Table 1). The terminal half-lives of total radioactivity and unchanged drug were on average 15.2 and 24.8 h, respectively. During the first 24 h after dosing, the percentage of unchanged drug versus total radioactivity in plasma on average was 97.0%, indicating that the unchanged drug accounted for almost the entire radioactivity in plasma. Renal clearance accounted for about half of the apparent plasma clearance of unchanged drug (on average, CL/f of 53.1 and 91.0 ml/min, respectively). Approximately 50% of the renal clearance of unchanged drug occurred by filtration (average CL_{GFR}: 25.9 ml/min), and the other half occurred by active processes (average CL_{act}: 27.2 ml/min) (Table 2).

There were no differences in the overall plasma pharmacokinetics of paliperidone between poor ($n = 2$) and extensive ($n = 3$) CYP2D6 metabolizers as illustrated in Fig. 3. All subjects belonged to the same CYP3A4 and CYP3A5 genotypes; hence, genotyping was not explored further. There was no relationship between the genotypic expression of metabolizing enzymes UGT1A1 and UGT1A6 and the primary pharmacokinetic parameters of exposure (C_{max}, $AUC_{24 h}$, and AUC_{∞}).

Urinary and Fecal Excretion and Mass Balance. The individual cumulative recoveries of radioactivity in urine and feces over 0 to 168 h are depicted in Fig. 4. At 7 days after dosing, on average 91.1% (range: 88.4–93.8%) of the administered radioactivity had been excreted. Radiolabeled material was excreted largely in the urine [77.1–87.1% (mean: 79.6%) over 7 days]. A minor part was excreted with the feces [6.8–14.4% (mean 11.4%) over 7 days]. Unchanged drug accounted for most of the radioactivity excreted in urine from 0 to 168 h (on average 59.4 ± 7.12% of the dose). No unchanged drug was excreted with the feces (Table 1). There were no differences between extensive and poor CYP2D6 metabolizers in urinary and fecal excretion of ^{14}C-labeled moiety or unchanged paliperidone (Fig. 4).

Blood Distribution of Paliperidone. The distribution of paliperidone between human blood and plasma was determined in samples collected at 2, 4, and 8 h after dosing. The blood/plasma ratio ranged on average from 0.78 ± 0.13 to 0.83 ± 0.05, indicating that the radioactivity in blood is mainly distributed to plasma. The level of radioactivity in the 24-h postdose blood samples was below the limit of detection.

Metabolite Profile of Paliperidone. No metabolite profiling could be performed in plasma samples, owing to the low level of radioactivity (<413 dpm/ml) in those plasma samples. However, as mentioned previously, unchanged paliperidone accounted for 97% of the total plasma radioactivity over the first 24 h after dosing.

Table 2 presents the individual and mean percentages of urine metabolites of paliperidone, expressed as a percentage of the dose, after oral administration of 1 mg of [^{14}C]paliperidone to male human subjects. A representative chromatogram is shown in Fig. 5. Unchanged drug accounted for 74.6 to 90.0% of the sample radioactivity when normalized to the percentage of the sum of unchanged drug and the detected metabolites. Unchanged drug represented 51.4 to 67.5% (59.4% on average) of the administered dose, indicating that paliperidone was metabolized to a limited extent. Besides the parent drug, a total of four metabolites were detected in urine: M1, M9, M12, and M16. These metabolites represented relatively small amounts of the dose, on average ~5, ~4, ~3, and ~4%, respectively. With respect to the formation of metabolites M1, M12, and M16, no substantial differences were observed between the extensive (subjects 101, 104, and 106) and the poor (subjects 103 and 105) CYP2D6 metabolizers. However, M9 was only detected in the urine of the extensive metabolizers and was not present in the urine samples of the poor metabolizers. The percentages of parent drug recovered in urine samples of the extensive CYP2D6 metabolizers did not differ substantially from those of the poor metabolizers. On the other hand, metabolite M10, which is also a monohydroxylated metabolite at the alicyclic ring system, was present in feces of poor metabolizers as well.

The overall recovery of the radioactivity in the methanolic extracts of the stool samples was rather low (mean: 56.8%; range: 50–65%). For the stool samples fortified with [^{14}C]paliperidone, the extraction recovery was 92% of the theoretical value. Methanolic extracts from pooled stool samples of the different subjects were analyzed for metabolite profiling. Representative radio-HPLC chromatograms of an extensive (subject 101) and a poor (subject 105) CYP2D6 metabolizer are depicted in Fig. 6. Unchanged paliperidone could not be detected in fecal samples of any of the subjects. Two metabolites, M10 and M11, were present in the fecal extracts of each subject. Another metabolite eluting just behind metabolite M10 was detected in a few fecal samples. Each of the fecal metabolites accounted for approximately 0.4 to 0.9% of the dose.

Metabolite Identification. The structures of the metabolites identified are shown in the metabolic scheme (Fig. 7). The metabolites were given a numerical code based on the retention time of metabolites detected in previously conducted in vitro and in vivo metabolism studies (rats and dogs).

Paliperidone was metabolized to a limited extent; most of the drug was excreted unchanged in the urine. No unchanged drug was detected in the feces. Besides the parent drug, metabolites M1, M9, M12, and M16 could be identified in the urine by LC-MS/MS, and metabolites M10 and M11 were identified in the feces. In addition, an unidentified metabolite was found in the feces of one subject. All metabolites represented small amounts of the total dose administered, with a maximum of 6.48% of the dose (metabolite M1) excreted into urine (Table 2) and inappreciable amounts in feces.

The mass fragmentation behaviors of radioactive paliperidone (**UD**)

TABLE 2

Excretion of paliperidone and its metabolites in urine

Healthy male subjects were treated with 1 mg of oral [^{14}C]paliperidone. Urine was analyzed from 0 to 168 h after dosing, and data are expressed as individual and mean $\pm$ S.D. percent of the total dose administered ($n = 5$).

| Compound | % Total Dose (0–168 h) | | | | | % Total Dose |
| | Extensive CYP2D6 Metabolizers | | | Poor CYP2D6 Metabolizers | | |
	Subject 101	Subject 104	Subject 106	Subject 103	Subject 105	
M1	4.52	2.48	6.48	4.44	4.83	4.55 $\pm$ 1.42
M9	2.70	3.19	5.37	N.D.	N.D.	3.75 $\pm$ 1.42
M12	2.42	4.29	0.55	N.D.	3.70	2.74 $\pm$ 1.66
M16	4.77	4.40	5.06	2.51	3.57	4.06 $\pm$ 1.03
UD	52.4	63.4	51.4	62.3	67.5	59.4 $\pm$ 7.12

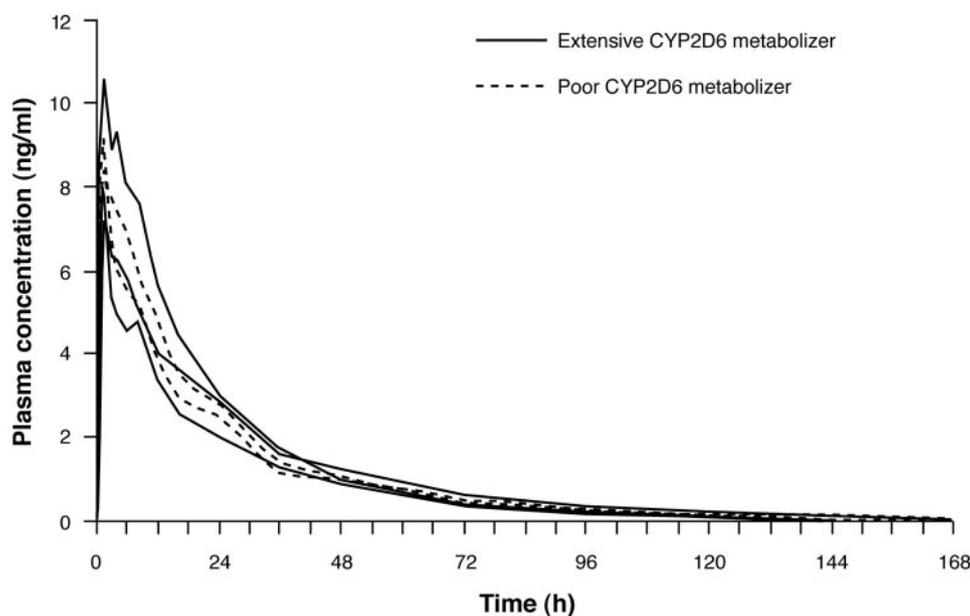N.D, below the quantification limit; UD, unchanged drug.

FIG. 3. Individual plasma concentration-time profiles of paliperidone in healthy male subjects, poor ($n = 2$) and extensive ($n = 3$) CYP2D6 metabolizers after a 1-mg single dose of [^{14}C]paliperidone.

and drug substance R076477 were similar (protonated molecular weights of 427). MS/MS fragmentation of the protonated molecular ions was characterized by fragment ion *m/z* 207, corresponding to a five-ring closure at the pyrimidine-4-one position of the molecule. MS/MS fragmentation of *m/z* 207 resulted in fragments at *m/z* 179, 165, and 110, which were observed for both UD and drug substance R076477 (Table 3).

Identification of **M1** in urine by cochromatography with a mixture of unlabeled authentic compounds was hampered by the interference of endogenous components in urine with the authentic substances, as the low radioactivity levels in the urine necessitated the injection of relatively large quantities of urine. M1 had the same retention time as authentic substance R093725 in overlay plots for urine samples and a separately injected mixture of unlabeled authentic compounds, providing supplementary evidence for the chemical identity of M1. Moreover, the fragmentation behaviors of the authentic compound R093725 and M1 were identical. The exact mass of the protonated molecular ion of R093725 was 239.1032, and that of M1 was 239.1041 (-3.76 ppm) (Table 3). The acid metabolite of paliperidone (M1) was formed by oxidative *N*-dealkylation.

The protonated molecular ions *m/z* 443 in the electrospray ionization mass spectra of **M9** and the base peak in the MS/MS spectra at *m/z* 223 were shifted 16 mass units compared with the drug substance, indicating hydroxylation of the hydroxymethylpyrimidine-4-one part of the molecule. The minor fragment ion *m/z* 205, corresponding to

the loss of water, absent in the spectrum of the drug substance, suggested hydroxylation of the saturated six-ring part. Therefore, M9 was formed by monohydroxylation of the alicyclic ring and the proposed structure is depicted in Fig. 7. This was confirmed by exact mass analysis.

M12 had mass fragmentation behavior similar to that authentic substance R125239 (Fig. 7; Table 3). The protonated molecular ions *m/z* 425 in the electrospray ionization mass spectra of M12 and the base peak in the MS/MS spectra at *m/z* 205 were shifted 2 mass units compared with the parent drug, indicating reduction of the hydroxy group on the hydroxymethylpyrimidine-4-one part of the molecule to a keto function. Quadrupole time-of-flight exact mass analysis confirmed the proposed structure. Metabolite 12, identified in urine, could be formed by alcohol dehydrogenation and also nonenzymatically. For example, it was present in urine samples incubated under conditions for enzymatic hydrolysis with β-glucuronidase/arylsulfatase in the absence of these hydrolyzing enzymes.

M16, which was identified in urine, had a protonated molecular ion *m/z* at 606. This was shifted 176 mass units compared with the authentic compound R084852, indicating that M16 could be a glucuronide of R084852 (Fig. 7; Table 3). A neutral loss of 176 in MS^2 confirmed this proposition. The MS^3 fragment ion *m/z* 207 further proved this identification. Therefore, M16 could be identified in both studies as a glucuronide of R084852 (M11). Furthermore, the disappearance of M16 in the chromatograms after enzymatic hydrolysis
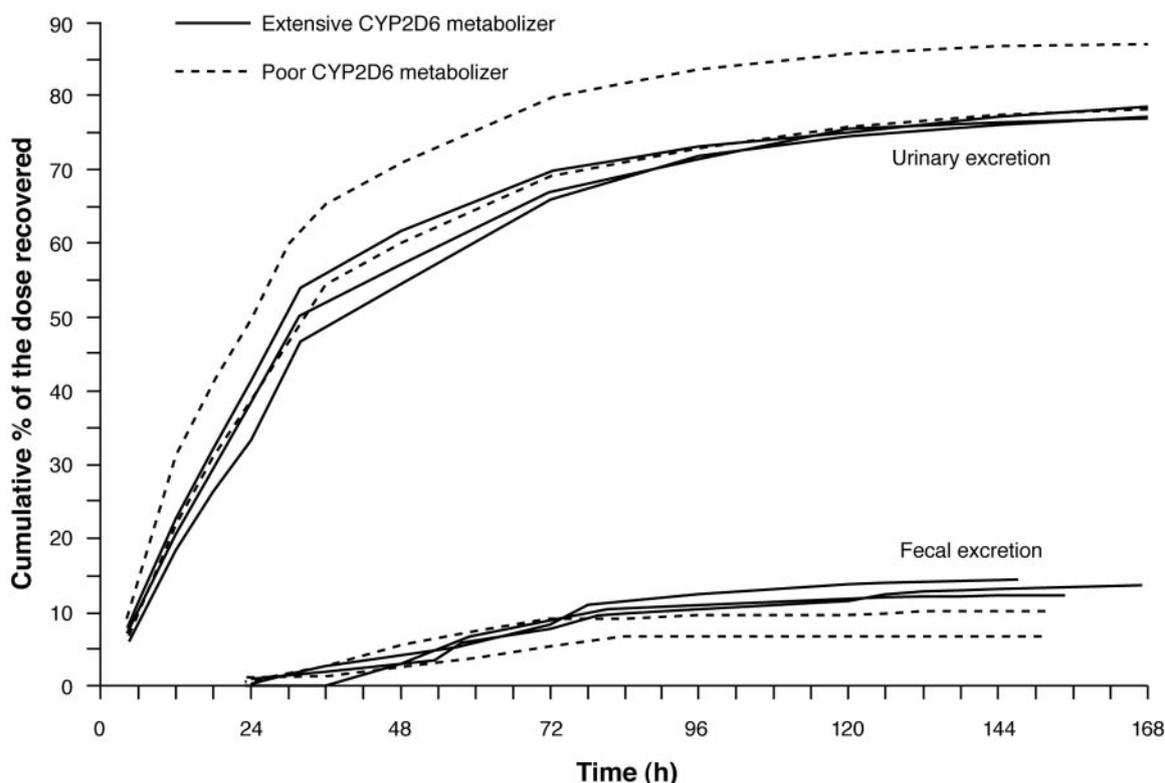
FIG. 4. Individual cumulative urinary and fecal excretion of radioactivity after a single oral dose of 1-mg of [^{14}C]paliperidone to healthy male subjects ($n = 5$).

with β-glucuronidase/arylsulfatase from *H. pomatia* and with β-glucuronidase from *E. coli* was in accordance with the LC-MS/MS findings that M16 is a glucuronide conjugate (Fig. 5).

As metabolites in methanolic fecal extracts from rats were present to a significantly larger amount than in human fecal extracts, the metabolites in methanolic extracts of human fecal samples from the present study were identified by cochromatography of selected samples with methanolic extracts of fecal samples from rats obtained from an in vivo metabolism study in the rat, and LC-MS/MS analysis of the rat feces metabolites that coeluted with those in human feces. The latter approach was followed because **M10** and **M11**, which were present in the fecal extracts of each subject, could be identified as hydroxylated R084852 (M10) and R084852 (M11) (Fig. 7). M11 was formed by benzisoxazole scission, whereas M10 was formed by benzisoxazole scission in combination with alicyclic hydroxylation. Another metabolite eluting just behind M10 was detected in a few fecal samples including the pooled fecal extracts of an extensive metabolizer (subject 101) (Fig. 6), as mentioned before. It was not given a code as it could not be identified.

Discussion

The aim of the present study was to characterize the excretion and metabolism of paliperidone in humans and to identify the metabolic pathways and the structures of the metabolites. One week after administration of an oral dose of 1 mg of [^{14}C]paliperidone to healthy male subjects, on average 91.1% of the administered radioactivity had been excreted. The majority of the radioactivity was recovered in the urine (79.6 ± 4.20%); only a small part was excreted into the feces (11.4 ± 3.07%). On average, 59% of the oral dose was excreted unchanged in urine within 7 days, indicating that renal clearance of unchanged drug is the predominant route of elimination for paliperidone. No unchanged drug was detected in fecal extracts. The absolute bioavailability of the instant-release formulation of paliperidone is

106% (Cleton et al., 2006a). These data also suggest that the metabolism of paliperidone is limited.

Renal clearance of paliperidone ranged from 51.4 to 67.5 ml/min, which is approximately 2-fold higher than the clearance by glomerular filtration, which ranges from 17.0 to 36.5 ml/min. The clearance by glomerular filtration is calculated by multiplying the creatinine clearance with the fraction unbound of paliperidone, which amounts to 0.23 (Mannens et al., 1994). This result indicates that active tubular secretion probably plays a significant role in the renal clearance of paliperidone. As paliperidone is a cation at physiologic pH, the organic cation transporter may be involved in this active transport. However, data from a human drug-drug interaction study with trimethoprim, an inhibitor of the organic cation transporter, do not indicate clinically relevant changes in renal excretion with coadministration (Cleton et al., 2006b).

Paliperidone represents the major circulating compound after oral administration of [^{14}C]paliperidone as only minor differences were observed between paliperidone and total radioactivity concentrations during the first 24 h after administration. The apparent half-life of total radioactivity appeared to be shorter than that of paliperidone (15.2 and 24.8 h, respectively). Because of differences in the lower limit of quantification of the bioanalytical methods, the total radioactivity concentrations were below the limit of quantification of 2.0 ng-Eq/ml from 24 h after dosing onward, whereas paliperidone could be quantified until 168 h after dosing (limit of quantification 0.1 ng/ml) (Fig. 2). This probably explains the lower half-life and the AUC values extrapolated to infinity of total radioactivity compared with those of unchanged drug.

Paliperidone metabolites were identified using radio HPLC and LC/MS-MS. Interpretation of these data resulted in four metabolites identified in urine (M1, M9, M12, and M16), each of which accounted for up to a maximum of 6.48% of the dose. In feces, only two metabolites were identified (M10 and M11) and one metabolite re-
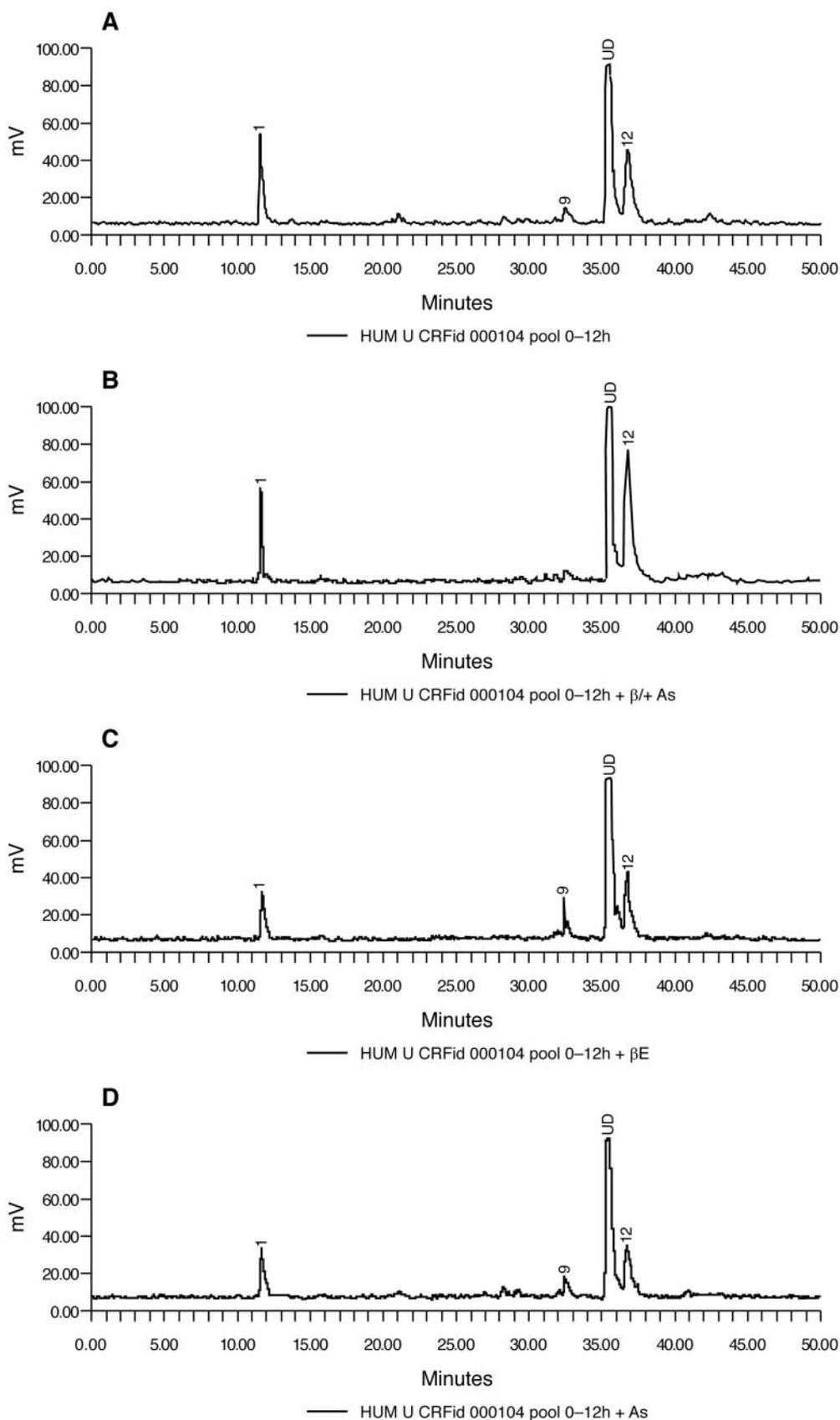
FIG. 5. Representative radio-HPLC chromatograms of paliperidone metabolites in urine (obtained for the 0- to 12-h individual pooled urine sample of subject 104) after a 1-mg single oral dose of [^{14}C]paliperidone. A, untreated sample. B, sample hydrolyzed with β-glucuronidase/arylsulfatase from *H. pomatia* ($+\beta/+$As). C, sample hydrolyzed with β-glucuronidase from *E. coli* ($+\beta$E). D, sample hydrolyzed with arylsulfatase from *A. aerogenes* ($+$As).

mained unidentified. These metabolites each represented only a small fraction of the administered dose (0.4–0.9%). The following biotransformation pathways were proposed (Fig. 7): 1) oxidative *N*-dealkyla-

tion with formation of the acid metabolite R093725 (M1: 2.48–6.48% of the dose); 2) monohydroxylation of the alicyclic ring (M9: 2.7–5.37%), this metabolite was solely present in urine samples of exten-
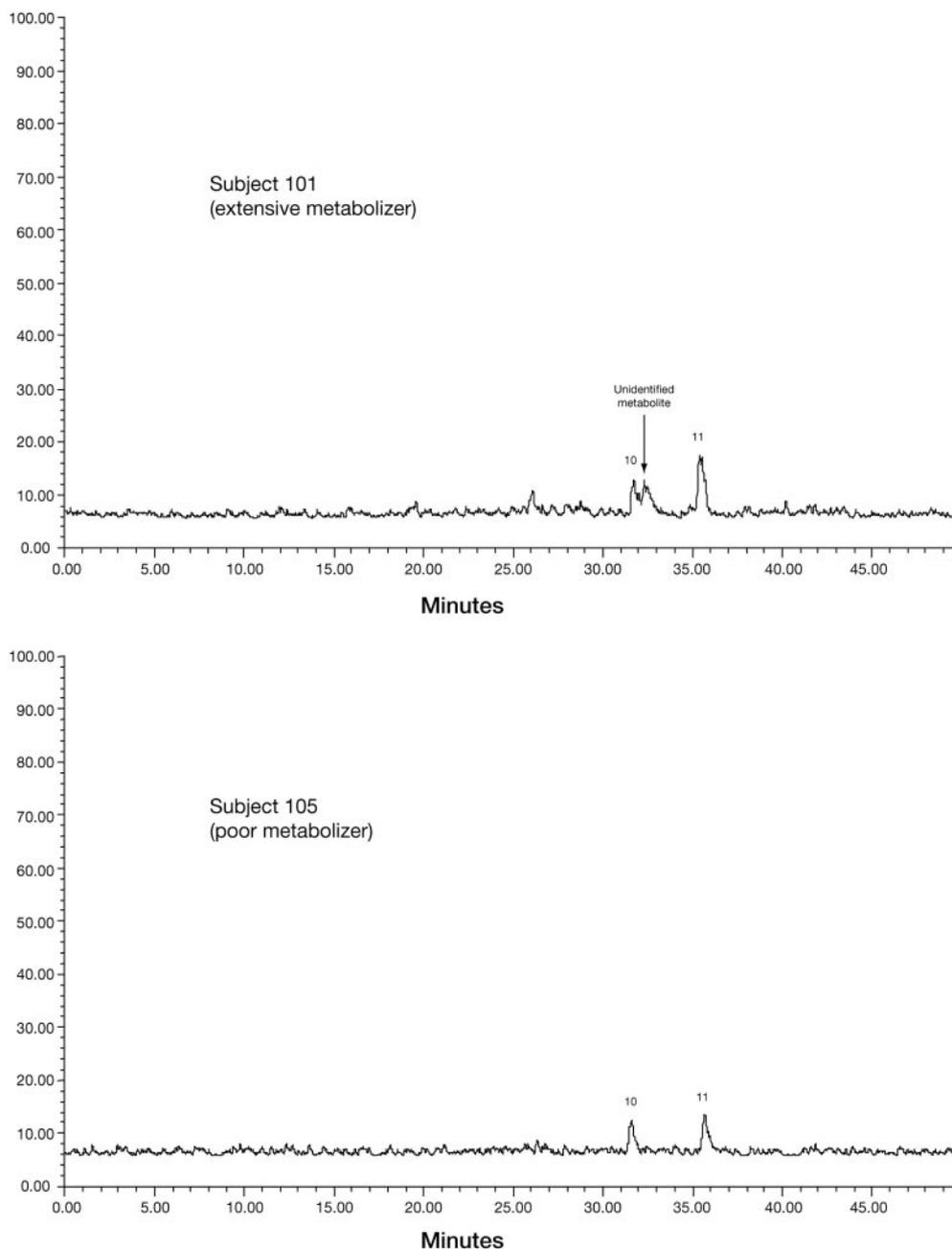
FIG. 6. Representative radio-HPLC chromatogram of paliperidone metabolites in the extracts of fecal samples of male subjects after a 1-mg single oral dose of [^{14}C]paliperidone.

sive CYP2D6 metabolizers; 3) alcohol dehydrogenation at the 9-hydroxy function (formation of the ketone metabolite R125239, M12: 0.55–4.29%), benzisoxazole scission (formation of R084852, M11), the latter in combination with alicyclic hydroxylation (M10), or glucuronidation (M16: 2.51–5.06%). On average, 7% of the total radioactivity found in urine samples was not identified.

In animal models, paliperidone was mostly metabolized by alicyclic hydroxylation, oxidative *N*-dealkylation, and benzisoxazole scission in rats and dogs, with the additional pathway of alcohol dehydrogenation observed in dogs (unpublished data). Comparison of profiles showed that all metabolites detected in humans were also found in at least one animal species.

In rats, paliperidone and its metabolites were rapidly excreted after p.o. administration; at 96 h after dosing, the radioactivity was completely excreted (102.3% of the dose in males, 102.4% of the dose in females) (unpublished data). Excretion occurred predominantly in the feces, comprising ~86% of the dose; ~15% of the dose was excreted in the urine. Paliperidone was extensively metabolized: unchanged paliperidone accounted for only 3.19 and 6.42% of the dose in male and female rats, respectively (unpublished data). In dogs, the excretion of total radioactivity was somewhat slow; at 168 h after dosing, the major part of the [^{14}C]paliperidone-related radioactivity was excreted in urine (59.8%), and 32.4% of the dose was excreted in feces. Unchanged paliperidone accounted for a major part of the total radioactivity in urine (unpublished data).

In incubations with human liver cells and subcellular fractions, the extent of paliperidone metabolism was very limited, which complicated the identification of the cytochrome P450 isoenzymes involved in the metabolism of paliperidone (unpublished data). No definitive conclusions could be drawn from correlation experiments using a panel of human liver microsomes or from experiments with diagnostic chemical inhibitors. Minor metabolism was observed in incubates of paliperidone with
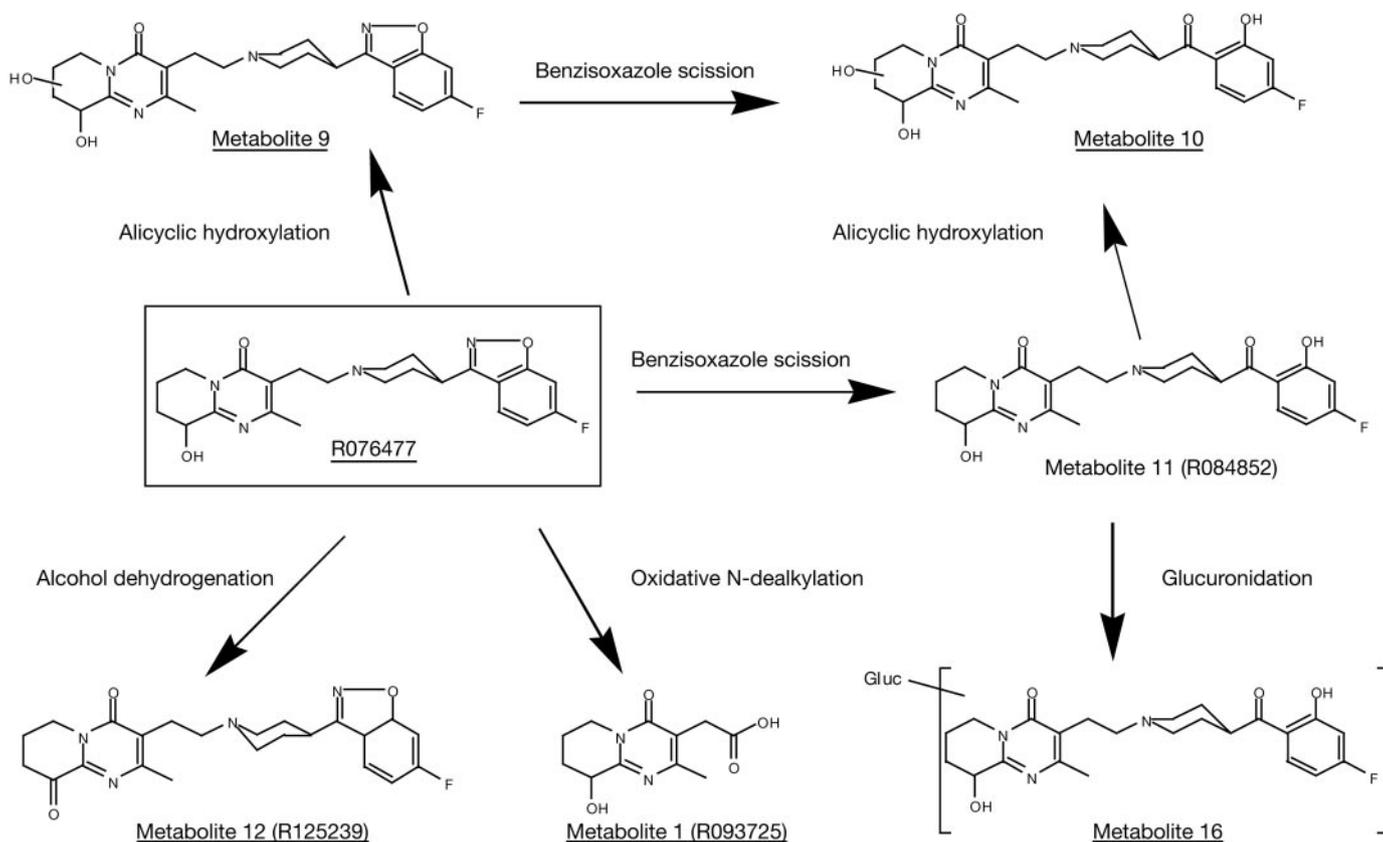
FIG. 7. Proposed metabolic scheme for paliperidone (R076477) in humans.

TABLE 3

Identification of paliperidone metabolites in humans

Metabolite	Identification Method	[M + H]⁺	Characteristic Product Ions (*m/z*)	Identification	Plasma	Urine	Feces
UD[a]	LC-MS/MS cochromatography	427	207, 179, 165, 110	Paliperidone	+	+	N.D.
M1[a]	LC-MS/MS	239	175, 177	Oxidative *N*-dealkylation-acid metabolite	N.D.	+	N.D.
M9	LC-MS/MS	443	223	Monohydroxylation of alicyclic ring of paliperidone	N.D.	+	N.D.
M10	Coelution with rat feces metabolite LC-MS/MS	446	205, 223	Benzisoxazole scission and monohydroxylation of alicyclic ring	N.D.	N.D.	+
M11[a]	Coelution with rat feces metabolite LC-MS/MS	430	207	Benzisoxazole scission	N.D.	N.D.	+
M12[a]	LC-MS/MS cochromatography	425	205, 233	Alcohol dehydrogenation-ketone metabolite at the 9-hydroxy function of paliperidone	N.D.	+	N.D.
M16	LC-MS/MS enzymatic hydrolysis	606	207, 430	Benzisoxazole scission and glucuronidation (glucuronide of M11)	N.D.	+	N.D.
		N.D.	No spectrum available	Unknown metabolite	N.D.	N.D.	+

N.D., not detected.
[a] Identification was confirmed by comparison with authentic standard.

heterologous organisms expressing human CYP3A4 and CYP2D6, suggesting the possible involvement of these two cytochrome P450 forms in the metabolism of paliperidone (unpublished data).

Both CYP2D6 poor ($n = 2$) and extensive ($n = 3$) metabolizers were included in the present study as the involvement of CYP2D6 in paliperidone metabolism was suggested by in vitro experiments. No differences were observed in the overall plasma pharmacokinetics of paliperidone between poor and extensive CYP2D6 metabolizers. It should be noted that the sample size of this study was too small to draw definite conclusions. This is in line with the observations from a human drug–drug interaction study with paroxetine, an inhibitor of CYP2D6, which indicate that there are no clinically relevant interactions based on metabolic inhibition of CYP2D6 (unpublished data). Although the overall pharmacokinetics were similar between the

CYP2D6 poor and extensive metabolizers, the metabolite profile showed minor differences. Metabolite M9, formed by monohydroxylation of the alicyclic ring, was only observed in extensive metabolizers, indicating that CYP2D6 is involved in this metabolic pathway. On the other hand, metabolite M10, which is also formed by monohydroxylation of the alicyclic ring, was present in feces of a poor metabolizer. Metabolite M12 was detected in one of the two poor CYP2D6 metabolizers. The influence of other metabolizing isozymes on the pharmacokinetics of paliperidone was explored by genotyping subjects for CYP3A4, CYP3A5, UGT1A1, and UGT1A6. As all subjects had the same CYP3A4 and CYP3A5 genotype, genotyping was not explored further. It appeared there is no relationship between the genotypic expression of metabolizing enzymes UGT1A1 and UGT1A6 and paliperidone pharmacokinetics.

In summary, paliperidone was not metabolized extensively in the liver, and renal excretion was the major route of elimination, with 59% of an oral liquid dose excreted unchanged in urine. About half of the renal excretion occurred by active secretion. Other than renal excretion, four metabolic pathways were identified as being involved in the elimination of paliperidone, each of which accounted for up to a maximum of 6.5% of the administered dose.

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