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Absorption, Distribution, Metabolism, and Elimination of the Direct Renin Inhibitor Aliskiren in Healthy Volunteers

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ABSTRACT:

Aliskiren (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamid hemifumarate) is the first in a new class of orally active, nonpeptide direct renin inhibitors developed for the treatment of hypertension. The absorption, distribution, metabolism, and excretion of [^{14}C]aliskiren were investigated in four healthy male subjects after administration of a single 300-mg oral dose in an aqueous solution. Plasma radioactivity and aliskiren concentration measurements and complete urine and feces collections were made for 168 h postdose. Peak plasma levels of aliskiren (C_{max}) were achieved between 2 and 5 h postdose. Unchanged aliskiren represented the principal circulating species in plasma, accounting for 81% of total plasma radioactivity ($AUC_{0-\infty}$), and indicating very low exposure to metabolites. Terminal half-lives for radioactivity and aliskiren in plasma were 49 h and 44 h, respectively. Dose recovery over 168 h was nearly complete (91.5% of dose); excretion occurred almost completely via the fecal route (90.9%), with only 0.6% recovered in the urine. Unabsorbed drug accounted for a large dose proportion recovered in feces in unchanged form. Based on results from this and from previous studies, the absorbed fraction of aliskiren can be estimated to approximately 5% of dose. The absorbed dose was partly eliminated unchanged via the hepatobiliary route. Oxidized metabolites in excreta accounted for at least 1.3% of the radioactive dose. The major metabolic pathways for aliskiren were *O*-demethylation at the phenyl-propoxy side chain or 3-methoxy-propoxy group, with further oxidation to the carboxylic acid derivative.

Hypertension is a major risk factor for cardiovascular and kidney diseases, and affects more than 25% of adults worldwide (Kearney et al., 2005). Despite the known risks associated with hypertension and the availability of a range of antihypertensive drug therapies, the majority of patients with hypertension do not have their blood pressure controlled to recommended target levels (<140/90 mm Hg for most patients). Indeed, data from the National Health and Nutrition Examination Surveys for 1999 to 2002 showed that blood pressure was uncontrolled in more than 70% of patients with hypertension in the United States (Centers for Disease Control and Prevention, 2005).

The renin system plays a key role in the physiological regulation of blood pressure and intravascular volume through the actions of the peptide angiotensin II. Excessive renin system activity may lead to hypertension and associated target organ damage (Weir and Dzau, 1999). Drugs that inhibit the renin system, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, have proven to be highly successful treatments for hypertension and related cardiovascular diseases (Sleight and Yusuf, 2003). However, all currently available agents that inhibit the renin system stimulate compensatory renin release from the kidney, which results in an increase in plasma renin activity that may ultimately lead to increased levels of angiotensin II (Mooser et al., 1990; Azizi and Menard, 2004).

Therefore, targeting the renin system at its point of activation by directly inhibiting renin activity has long been proposed as the optimal means of suppressing the renin system (Skeggs et al., 1957). However, previous efforts to develop clinically effective direct renin inhibitors have been thwarted by the low potency and/or poor pharmacokinetic profiles of peptide-like compounds (Fisher and Hollenberg, 2005). Previous generation renin inhibitors have exhibited an oral bioavailability of around 1%, because of low intestinal absorption and/or considerable hepatic first-pass metabolism (Rongen et al., 1995).

Aliskiren (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamid hemifumarate) is the first in a new class of orally effective, nonpeptide direct renin inhibitors developed for the treatment of

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ABBREVIATIONS: P450, cytochrome P450; HPLC, high performance liquid chromatography; AMS, accelerator mass spectrometry; $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity; AUC_{0-t}, area under the concentration-time curve from time zero to t (last measured time point above LOQ); CL/*F*, apparent plasma clearance; LOQ, limit of quantification; LSC, liquid scintillation counting; MS, mass spectrometry; MS/MS, tandem mass spectrometry; ADME, absorption, distribution, metabolism, and excretion; $t_{½}$, elimination half-life; t_{max}, time to maximum concentration.

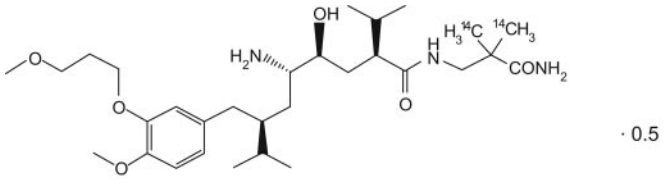


FIG. 1. Structure of [^{14}C]aliskiren

hypertension. The design of aliskiren resulted from a combination of crystallographic structure analysis and computational molecular modeling of the binding of putative inhibitor compounds to human renin. Crystallographic structure analysis of subsequent inhibitors revealed a hitherto uncharacterized nonsubstrate subpocket within the human renin active site (Wood et al., 2003). This allowed the addition of further substituents to fill this subpocket and thus increase affinity for the enzyme, leading to the synthesis of aliskiren, a potent (in vitro IC_{50} 0.6 nM) and highly specific inhibitor of human renin (Wood et al., 2003). Pharmacokinetic studies in healthy volunteers have demonstrated that aliskiren is rapidly absorbed (t_{max} 1–3 h) and exhibits a long plasma half-life ($t_{½}$ 30–40 h) (Vaidyanathan et al., 2006a,b) suitable for once-daily dosing. Aliskiren demonstrates dose-proportional pharmacokinetics at doses of up to 600 mg once daily in healthy volunteers (Vaidyanathan et al., 2006b). Clinical trials have shown that once-daily treatment with aliskiren lowers blood pressure at least as effectively as angiotensin receptor blockers (Stanton et al., 2003; Gradman et al., 2005; Pool et al., 2006) and angiotensin-converting enzyme inhibitors (Uresin et al., 2006) in patients with hypertension.

Studies investigating the disposition of oral doses of [^{14}C]aliskiren in rats and marmosets indicated that excretion of an oral dose occurred almost exclusively in the feces, mainly as unchanged aliskiren; a small proportion of the absorbed dose was excreted in the form of oxidized metabolites, probably derived from oxidation by CYP3A4 (Novartis, data on file). However, no interaction of aliskiren with cytochrome P450 (P450) isoenzymes was found in human liver microsomes in vitro (Vaidyanathan et al., 2005), suggesting a low potential for clinically significant drug interactions of aliskiren. Indeed, no clinically relevant pharmacokinetic interactions have been observed between aliskiren and the P450 substrates celecoxib, digoxin, lovastatin, or warfarin, or the P450 inhibitor cimetidine, in healthy volunteers (Dieterle et al., 2004, 2005; Dieterich et al., 2006). Animal studies indicate that aliskiren is a substrate for the efflux transporter P-glycoprotein, which may play a role in the hepatobiliary/intestinal excretion of the drug; however, the lack of pharmacokinetic interaction between aliskiren and the P-glycoprotein substrate digoxin indicates that aliskiren does not inhibit P-glycoprotein activity (Dieterich et al., 2006). The aim of the present study was to characterize the absorption, distribution, metabolism, and excretion of a single 300-mg oral dose of [^{14}C]aliskiren in healthy male subjects.

Materials and Methods

Clinical Study and Subjects. The study was performed at Swiss Pharma Contract (SPC) Ltd. (Allschwil, Switzerland). Four healthy, nonsmoking male subjects, aged 26 to 47 years, with normal medical history, vital signs (body temperature, blood pressure, and heart rate), 12-lead electrocardiograph, and laboratory tests participated in this open-label study. All patients had body weight within ±20% of normal for their height and frame size according to Metropolitan Life Insurance Tables.

Exclusion criteria included exposure to radiation greater than 0.2 mSv in the 12 months before the start of the study; use of any prescription drug, over-the-counter medication (except paracetamol), grapefruit juice, St John's wort, and/or herbal remedies in the 2 weeks before the study; and a history of any condition known to interfere with the absorption, distribution, metabolism, and excretion of drugs.

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1964 and subsequent revisions), and all patients gave written informed consent before participation. The subjects were exposed to a radiation dose <1 mSv, which was calculated according to the guidelines of the International Commission on Radiological Protection and Swiss regulations. The protocol and the dosimetry calculation were approved by the local ethics committee and by the Swiss Federal Health Authority (Bundesamt für Gesundheit) Radiation Protection Department.

Study Medication. Aliskiren was specifically labeled with ^{14}C in the 2-methyl groups (Fig. 1); this position is metabolically stable. The radioactive label had a specific activity of 9.27 kBq/mg (0.25 μCi/mg) as 300 mg free base and 55.56 kBq/ml in 50 ml of drink solution, and a radiochemical purity of >99%. The established solid dosage form of aliskiren could not be manufactured with ^{14}C-radiolabeled drug substance because of radiochemical instability. The radiolabeled drug was stable in aqueous solution, frozen at −20°C. Subjects therefore received a single 300-mg oral dose of [^{14}C]aliskiren (331.5 mg of hemifumarate salt, prepared by the Isotope Laboratory, Novartis Pharma AG, Basel, Switzerland), containing a mean dose of radioactivity of 2.8 MBq (75 μCi), in the form of an oral solution (in 50 ml of water). After dose administration, the solution container was rinsed twice with 50 ml of water, which was also swallowed by the subjects.

Study Protocol. After a screening period of up to 21 days, eligible subjects reported to the study center at least 16 h before dosing for baseline safety evaluations and were domiciled in the study center for the 168-h postdose observation period. Safety and pharmacokinetic assessments were performed for up to 336 h postdose.

A single 300-mg oral dose of aliskiren was administered to all subjects in the morning, after an overnight fast of at least 10 h. Blood samples were collected by direct venipuncture or an indwelling catheter into heparinized tubes predose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, and 168 h postdose. Three aliquots of 0.3 ml each were taken from each sample and frozen immediately at ≤−20°C for subsequent radiometry. Plasma was prepared from the remaining blood by centrifugation at 4°C for 10 min at 2000*g*. Urine was collected predose and at 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h postdose, in a total of 10 fractions. Fecal samples were collected predose and thereafter up to 168 h postdose; each portion was diluted with 2 to 3 volumes of water and homogenized. Blood plasma, urine, and feces were stored at ≤−20°C until required for analysis. Blood samples collected on days 10, 12, and 15 were not analyzed for radioactivity because the terminal elimination phase for aliskiren could be characterized sufficiently with the samples collected in the time period 48 to 144 h.

Throughout the study, subjects were not permitted to perform strenuous physical exercise (for 7 days before dosing until after the end of study evaluation) or to take alcohol (for 72 h before dosing until the end of the study) or citrus fruit or fruit juices (for 48 h before dosing throughout the domiciled period). Intake of xanthine-containing food or beverages was also not permitted from 48 h before dosing until 48 h postdosing. Consumption of other foods that might lead to interactions with study drug or lead to technical problems in the analysis of excreta was also not permitted during the domiciled period.

Analysis of Unchanged Aliskiren. *Plasma sample preparation.* Plasma samples were cleaned by automated solid-phase extraction using a 96-well plate and Oasis MCX 10-mg extraction cartridges (Waters Corporation, Milford, MA) on a Multiprobe II (PerkinElmer Life and Analytical Sciences, Boston, MA). After the conditioning steps [500 μl of methanol/water (90:10 v/v) containing 1% acetic acid, 500 μl of 1% acetic acid in water], 600 μl of acidified sample was transferred to the well. The sample was washed twice with acetic acid (1% in water), and once with methanol/acetonitrile (40:60 v/v). After the elution step [300 μl of methanol/water (90:10, v/v) containing

2% ammonia], the extract was partially evaporated (concentration by approximately 2-fold) and then diluted with 150 μl of 1% acetic acid in water.

Urine sample preparation. Urine samples were cleaned by automated solid-phase extraction using a 96-well collection plate and Oasis MCX 10-mg extraction cartridges, on a Multiprobe II. After the conditioning steps (200 μl of methanol, then 200 μl of pH 12 buffer), 200 μl of alkalinized sample was transferred to the well. The sample was washed with 400 μl of methanol/water (25:75, v/v). After the elution step [300 μl of acetonitrile/water (90:10 v/v) containing 1% acetic acid], the extract was partially evaporated (concentration by approximately 2-fold) and then diluted with 200 μl of 1% acetic acid in water.

HPLC-MS/MS analysis. HPLC was performed using a MetaSil Basic 5-μm column (50 × 2.0 mm; column temperature 40°C, flow rate 0.25 ml, injection volume 10 μl; Metachem, Palo Alto, CA) with gradient elution from 10 mM aqueous ammonium acetate/acetonitrile (75:25 v/v) to 10 mM aqueous ammonium aetate/acetonitrile (40:60 v/v) over 0.4 min.

An API 3000 (Applied Biosystems, Foster City, CA) was used for mass spectrometry. The general settings used were selected reaction monitoring, positive ion mode, and electrospray ionization interface; temperature 500°C, mass resolution 0.7 atomic mass unit, scan time 0.50 s. The lower limit of detection for the HPLC-MS/MS assay was 0.5 ng/ml for plasma and 5 ng/ml for urine. A derivative of aliskiren (gem-dimethyl d_6-aliskiren) was used as an internal standard.

Total Radioactivity Measurement. Total ^{14}C radioactivity in blood and plasma was measured at Novartis Pharma AG using liquid scintillation counting (LSC). Blood and plasma samples (triplicates of 300 μl each, weighed) were counted after solubilization in Biolute *S*-isopropanol (1:1 v/v; Zinsser Analytic, Frankfurt, Germany), and LSC used RiaLuma (Lumac-LSC, Groningen, the Netherlands). LSC was performed using a Tri-Carb 3170 TR/SL liquid scintillation counter ("low-level counter"; PerkinElmer Life and Analytical Sciences). Counting was performed for 60 or 180 min per sample in low level counting mode.

Total ^{14}C radioactivity in urine and feces was measured at RCC Ltd. (Itingen, Switzerland) using LSC with a typical counting time of 10 min. Fecal samples (quadruplicates of 400 mg each, weighed) were counted after homogenization in 2 to 3 volumes of water and solubilization with Soluene 350 (PerkinElmer Life and Analytical Sciences); LSC used Irga-Safe Plus (PerkinElmer Life and Analytical Sciences, Boston, MA). Urine samples (duplicates of 1 ml each) were measured directly with scintillation cocktail (Irga-Safe Plus). LSC was performed using a Tri-Carb 2500 TR, 2550TR/LL, or 2900TR liquid scintillation counter (Packard Biosciences).

Quench correction was performed by the external standard method. The background for blood and plasma was determined and subtracted from the measurements of study samples. The limit of quantification (LOQ) of LSC was determined as described previously (Jost et al., 2006) and was defined as the minimal number of sample disintegrations that are statistically significant above background and that show a relative statistical uncertainty equal to or smaller than 20%. Thus, the LOQ was 17 ng-Eq/ml (2.8 dpm) for blood (counting time 60 min), 11.4 ng-Eq/ml (1.8 dpm) for plasma (counting time 180 min), and approximately 0.01% of dose for urine and feces.

Radioactivity levels in plasma samples collected at 16 and 144 h postdose were below the LOQ of LSC and were therefore analyzed using accelerator mass spectrometry (AMS) by Xceleron Ltd. (York, UK). Samples were thawed and centrifuged at 4000*g* for 5 min at 10°C; 60-μl aliquots of plasma were then dried under a vacuum with copper oxide, combusted (at 900°C for 2 h), reduced to graphite, and analyzed using AMS, which separates the carbon isotopes and determines specifically the ^{14}C isotope (Garner, 2000).

Biologic sample preparation for metabolite profile analysis. For the following sample preparation processes, radioactivity was traced by quantitative radiometric measurements of aliquots using a Tri-Carb 2500TR liquid scintillation counter as described previously (Botta et al., 1985).

Plasma. A plasma sample of 2 ml was mixed with 2 ml ice-cold acetonitrile. After 30 min on ice, the sample was centrifuged (17,500 g, 15 min) and the supernatant was withdrawn. The extract was then concentrated in a rotary evaporator to a volume of 0.7 to 1.1 ml. An aliquot was taken for determination of total radioactivity by LSC; the rest of the sample (0.6–1 ml) was analyzed by HPLC to obtain the metabolite profile. The overall recovery from sample processing and analysis was 88%.

Urine. Individual urine samples were centrifuged and 1 ml supernatant directly injected for HPLC analysis. The recovery from sample processing and analysis was complete.

Feces. From each subject, the two samples of feces homogenate that contained the most of the applied radioactivity were pooled. Thus more than 98% of the radioactivity excreted with bile/feces was covered. Approximately 2 g of pooled feces homogenate were mixed with 2 ml water and 4 ml acetonitrile and shaken for 30 min. After centrifugation at 10,000 g for 15 min, the supernatant was withdrawn and a 200 μl sample was directly injected for HPLC analysis. The overall recovery from sample processing and analysis was 90%.

Metabolite analysis by HPLC-radiometry. Samples of plasma, urine and feces extract were chromatographed by reversed-phase HPLC with subsequent radioactivity detection. HPLC analysis was performed on an Agilent 1100 HPLC chromatographic system (Agilent Technologies, Palo Alto, CA) incorporating a capillary pump G1376A, a degasser G1379A, a thermostat sample holder G1329A (set at 15°C), a column thermostat G1316A (set at 40°C), and a diode array multiwavelength UV detector G1315B (set at 235 nm). Chromatographic separation was performed on a LiChrospher 100-5 RP-18 ec column (5 μm, 250 × 2 mm; Macherey-Nagel, Düren, Germany) protected by a guard filled with the same material.

Gradient elution using mobile phase solvent A (50 mM ammonium acetate adjusted to pH 6.0 with acetic acid) and solvent B (acetonitrile) was applied at a flow rate of 0.25 ml/min as follows: 0 to 35 min, 10 to 30% solvent B; 35 to 45 min, 30% solvent B; 45 to 50 min, 30 to 40% solvent B; 50 to 65 min, 40 to 90% solvent B; 65 to 70 min, 90% solvent B. Samples of 200 to 1000 μl were injected via a 1-ml loop into the HPLC system. Radioactivity was detected offline by collecting the eluate in 0.25-min fractions into three 96-well Deepwell LumaPlates (PerkinElmer Life and Analytical Sciences) by means of an Agilent 1100 fraction collector (Agilent Technologies). After solvent evaporation in a SpeedVac Plus SC210A vacuum centrifuge (Thermo Fisher Scientific, Waltham, MA), radioactivity was determined (counting time 20 min, three times) on a TopCount NXT microplate scintillation and luminescence counter (Packard Biosciences).

Metabolite Characterization by HPLC-MS. Selected pooled extracts of urine and feces from individual subjects were analyzed directly by LC-MS with simultaneous radioactivity detection. For confirmation of proposed structures of metabolites of aliskiren, the retention times in the radiochromatograms and mass spectral data obtained in the current study were compared with those obtained for reference compounds and samples from a parallel study in rabbits (see below, *^{1}H NMR analysis*).

HPLC-MS was run using an Agilent 1100 or Alliance HT 2795 HPLC instrument (Waters Corporation), and a triple stage quadrupole mass spectrometer (Finnigan model TSQ7000; Thermo Fisher Scientific) with an ESI II electrospray ion source, in positive ion mode. Alternatively, for exact mass determination, a time-of-flight (LCT) mass spectrometer (Micromass, Manchester, UK) was used, in positive ion mode. For hydrogen/deuterium exchange experiments, water in the mobile phase was replaced by D_2O.

LC-MS analysis. MS spectra with unit mass resolution were determined by LC-MS using an Agilent 1100 HPLC system, equipped with a binary capillary pump model G1376A, a degasser model G1379A, and a UV-visible diode array detector model G1315B with a standard flow cell model G1315-60012. UV spectra were monitored in the range 200 to 800 nm. Chromatographic separation was performed using the same column and gradient as described for *Metabolite analysis by HPLC-radiometry*, with a flow rate of 250 μl/min.

For LC-MS analysis of metabolites **M12** to **M14**, a column CC 8/3 Nucleodur C18 Pyramid was used (5 μm, 250 mm × 4.6 mm i.d.; Macherey-Nagel), protected by a guard filled with the same material. At a flow rate of 750 μl/min, the following solvent gradient was formed: 0 to 10 min, 10 to 30% solvent B; 10 to 65 min, 30 to 90% solvent B; 65 to 70 min, 90% solvent B; 70 to 71 min, 90 to 10% solvent B; 71 to 80 min, 10% solvent B.

Samples were injected using a PAL autosampler (CTC, Zwingen, Switzerland). The column effluent was used for UV-visible (diode array detector) monitoring and then split in a ratio of 1:6; the smaller fraction was passed into the electrospray LC-MS interface, and the remainder was used for radioactivity monitoring. If radioactivity concentrations were sufficient, online radiomonitoring was performed using a Berthold model LB507A (Berthold, Munich, Germany) with a model Z-200 flow cell, after mixing with 2.8 ml/min

Flo-Scint A liquid scintillation cocktail (PerkinElmer Life andAnalytical Sciences). For off-line radiomonitoring, 0.1- or 0.2-min fractions were collected into 96-well LumaPlates and counted on a TopCount NXT microplate scintillation counter as described above.

MS was performed using a model TSQ7000 triple stage quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an ESI II ion source, in positive ion mode; settings were: manifold temperature 70°C, spray voltage 4.0 kV, capillary temperature 250 or 275°C, in-source collision-induced dissociation offset 0, 30, or 40, and 60 V.

Exact mass determination. Exact masses were determined by LC-MS using a Waters Alliance HT 2795 HPLC system, equipped with a diode array detector model PDA996 and with chromatographic conditions as described under *Metabolite analysis by HPLC-radiometry* above. After the column, the effluent was used for UV-visible (diode array detector) monitoring. The effluent was then split in a ratio of approximately 1:6; the smaller fraction was passed through a valve, allowing temporary diversion of the flow to waste, and then into the electrospray LC-MS interface. The remainder of the effluent was used for online radioactivity monitoring using a Berthold model LB506 C-1 radiomonitor equipped with a flow cell model Z-100-4. Before entering the radiomonitor, the effluent was mixed with 1.2 ml/min RiaLuma liquid scintillation cocktail.

MS was performed using a time-of-flight (LCT) mass spectrometer, in positive ion mode, employing a Z-spray interface with LockSpray option. The reference channel of the LockSpray interface was operated with a solution of sulfadimethoxine (0.78 μg/ml) and reserpine (0.19 μg/ml) in acetonitrile at a flow rate of 10 μl/min. During data acquisition from the reference channel, the cone voltage was set to 30 V. For the MS run, cone voltages of 20, 60, and 80 V were applied. The source block temperature was 80°C, and the desolvation temperature was 160°C, using nitrogen as the desolvation gas.

^{1}H NMR analysis. Since the metabolites in human urine were available only at very low concentrations, metabolites were obtained in a parallel study from New Zealand rabbits, after a single oral dose of 200 mg/kg. Urinary metabolites from rabbits that were shown to be identical in LC-MS with the human metabolites were subjected to ^{1}H NMR analysis. ^{1}H NMR spectra were recorded on a Bruker DMX-500 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) equipped with a 4-mm selective inverse ^{1}H/^{13}C LC probe (flow cell, 120 μl). The amounts of metabolites used for analysis were 5 to 15 μg. NMR spectra were obtained in the stop-flow mode using XWINNMR software, version 3.1, and HyStar software, version 2.3 (Bruker BioSpin AG). The stop-flow NMR measurement of single peaks in the chromatogram was controlled by the UV signal from a diode array detector (280 nm). The ^{1}H NMR pulse program *lc1pncwps* from Bruker BioSpin AG was applied. Signals from the HPLC solvents were suppressed using a multiple solvent suppression with ^{13}C-decoupling.

Pharmacokinetic Analysis. Pharmacokinetic parameters ($AUC_{0-\infty}$, AUC_{0-t}, C_{max}, t_{max}, $t_{½}$, and CL/*F*) for total radioactivity in blood and plasma and unchanged aliskiren in plasma, and the amount of unchanged drug excreted into urine from time 0 to time *t* (Ae_{0-t}) were determined by noncompartmental methods using WinNonlin Pro (Version 4.1; Pharsight Corp., Mountain View, CA).

Blood and plasma concentrations of radioactivity, parent drug, and metabolites are expressed in mass units (ng-Eq/ml) or in molar units (μM). One gram of plasma or blood was taken as 1 ml. Plasma concentrations of metabolites were derived from the radiochromatograms (metabolite patterns) by calculating the proportion (percentage) recovered and the proportions (percentages) of individual metabolites from the peak areas and total radioactivity eluting from the column. The latter were converted to concentrations (ng-Eq/ml) using the total plasma radioactivity concentrations. Plasma concentrations of parent drug were derived from the radiochromatograms in the same way, but these values are to be considered as semiquantitative only (as opposed to those determined by the validated quantitative LC-MS/MS assay).

Results

Subjects and Tolerability. Four Caucasian male subjects took part in this study; all completed the study. The subjects had a mean age of 35 years (range 26–47) and a mean weight of 72 kg (range 60–88). No adverse events or clinically relevant changes in vital signs, clinical chemistry, hematology, or urinalysis were observed during the course of the study.

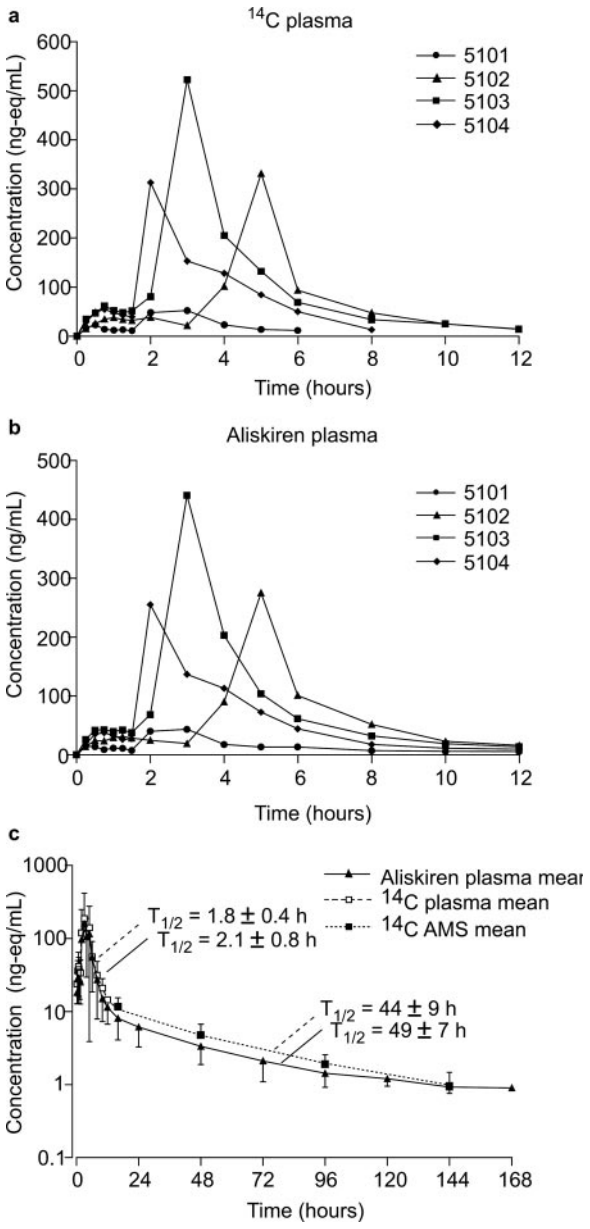


FIG. 2. Individual plasma concentration-time profiles for radioactivity (a) and aliskiren (b) in each subject, and semilogarithmic plot of plasma concentration-time profile for radioactivity and aliskiren (c). Values are presented as absolute concentration in a and b, and as mean ± S.D. (n = 4) in c.

Blood and Plasma Concentrations of Radioactivity and Aliskiren. Concentration-time profiles for plasma radioactivity and aliskiren in each of the four study subjects after single oral doses of 300 mg of [^{14}C]aliskiren are presented in Fig. 2, a and b, respectively. Interindividual variability was substantial, and one subject exhibited a considerably lower exposure than did the other subjects. Peak concentrations (C_{max}) of radioactivity in blood and plasma and for aliskiren in plasma were reached between 2 and 5 h after dosing. Concentration-time curves for radioactivity and aliskiren were parallel throughout the entire observation period (Fig. 2c). Radioactivity in plasma was detected typically for 8 to 12 h after dosing but, thereafter, was below the limit of quantification of conventional LSC and was therefore assessed using the more sensitive AMS method.

The key pharmacokinetic parameters for radioactivity and aliskiren

TABLE 1

Pharmacokinetic parameters for aliskiren in plasma and total radioactivity concentrations in blood and plasma

Values are shown as mean ± S.D. ($n = 4$) with the exception of t_{max} values, which are presented as the median (range).

Parameter	Plasma Aliskiren	Blood Total Radioactivity	Plasma Total Radioactivity
t_{max} (h)	3 (2–5)	2.5 (2–5)	3 (2–5)
C_{max} (ng/ml)[a]	254 ± 163	171 ± 88	305 ± 193
$t_{\lambda 1}$ (h)	2.1 ± 0.8	1.9 ± 0.3[b]	1.8 ± 0.4
AUC_{0-t} (ng · h/ml)[c,d]	1051 ± 547	427 ± 226	1310 ± 614
$t_{\lambda z}$ (h)	48.7 ± 6.9	N.A.	44.3 ± 9.4
$AUC_{0-\infty}$ (ng · h/ml)[c,d]	1108 ± 546	591 ± 93[b]	1374 ± 638
% of ^{14}C-$AUC_{0-\infty\ plasma}$	81 ± 2	61 ± 12[b]	(100)
CL/*F* (l/h)	380 ± 310	520 ± 860[b]	290 ± 210

N.A., not applicable.
[a] ng-Eq/ml for radioactivity.
[b] $n = 3$.
[c] ng-Eq · h/ml for radioactivity.
[d] AUC_{0-t} was calculated using the linear trapezoidal rule. t (= t_{last}) was 96 to 168 h for plasma aliskiren, 4 to 12 h for blood radioactivity, and 144 h for plasma total radioactivity. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + AUC_{t-\infty}$, where $AUC_{t-\infty} = C_t \times t/\ln(2)$.

in blood and plasma are summarized in Table 1. Early apparent half-lives for elimination from plasma (by noncompartmental analysis) were 1.8 h for radioactivity and 2.1 h for aliskiren (difference not significant). Terminal half-lives of radioactivity and aliskiren were 49 h and 44 h, respectively. Approximately 81% of total plasma radioactivity ($AUC_{0-\infty}$; 86% for AUC_{0-10h}) was accounted for by unchanged aliskiren, indicating very low exposure to metabolites. Radioactivity in blood was detected up to 4 to 12 h after dosing and was subsequently below the LOQ. The mean ratio of AUC_{0-10h} blood/plasma was 0.61, indicating that radioactivity was largely present in plasma.

Excretion and Mass Balance in Urine and Feces. Radioactivity was excreted almost completely via the biliary/fecal route, with only 0.6% of the radioactive dose recovered in urine (Table 2; Fig. 3). The majority of fecal excretion of radioactivity (approximately 80% of dose) occurred within 72 h of dosing. Total excretion (mass balance) over the 168-h collection period was 91.5 ± 4.5% of dose, with moderate interindividual variability (range 85–95%). Unchanged aliskiren accounted for 0.4% of dose in urine (approximately 70% of the recovered radioactivity) and for 77.5% of dose in feces (probably >85% of radioactivity); overall, the sum of oxidized metabolites in excreta amounted to approximately 1.4% of the radioactive dose.

Metabolism of Aliskiren. *Plasma.* Metabolite patterns in plasma were determined only at t_{max} because of the low levels of radioactivity in plasma. At t_{max}, unchanged aliskiren accounted for most of the radioactivity (Fig. 4a). In addition, minor proportions of metabolites **M2** (carboxylic acid, oxidized side chain; ≤1% of aliskiren C_{max}) and **M3** (alcohol, *O*-demethylated; 1–5% of aliskiren C_{max}), and trace levels of **M1** (phenol, *O*-demethylated) were detected. These data are semiquantitative because of incomplete ^{14}C extraction recovery (88%). AUC fractions represented by these metabolites in plasma could not be determined accurately because of the low radioactivity at time points after t_{max}.

Urine. Urine samples containing sufficient radioactivity were analyzed for metabolic patterns; thus, one to four urine samples per volunteer were measured and the sum of the urinary metabolites was calculated. Unchanged [^{14}C]aliskiren accounted for the major part of radioactivity (approximately 70%) in all analyzed urine samples (Fig. 4b). Using a sensitive, validated HPLC-MS/MS assay for aliskiren, the amount of unchanged aliskiren excreted in urine was determined to be 0.4% of dose.

In addition, trace amounts of the metabolites **M2**, **M3**, **M4** (phenol, *O*-dealkylated), and **M6** (*O*-glucuronide conjugate of **M4**) were detected in urine (Fig. 4, b and c). The unlabeled metabolite **M9** (lactone) was also detected by LC-MS. Because of low radioactivity levels, only early urine fractions could be analyzed and the results extrapolated to total amounts excreted in 7 days; in total, **M3** amounted to <0.1% of the dose and all other metabolites to trace amounts (Table 2).

Feces. The major proportion of the administered radioactive dose was excreted with the feces. For metabolite analysis, a single feces pool was prepared for each volunteer containing at least 78% of the applied radioactivity dose, and by solvent extraction, 90% of the radioactivity was extracted for HPLC analysis. No major differences in fecal metabolite pattern were observed between individual subjects. Unchanged [^{14}C]aliskiren was the predominant compound in the feces; metabolites **M2** and **M3** were found typically in amounts of 0.7 to 1.2% of the dose (Fig. 4d). In addition, traces of **M1** (0.1%) and other peaks were detected (Table 2). LC-MS also detected **M4** and the unlabeled metabolite **M9** in feces extracts.

Feces extracts contained an additional distinct peak close to the aliskiren peak, designated **P62**, which accounted for approximately 1% of the dose. LC-MS runs under chromatographic conditions identified three separate peaks within **P62**, corresponding to metabolites **M12** (*N*-acetylated), and **M13** and **M14** (structural isomers containing an additional $C_3H_4O_2$ moiety in the central part of the molecule). The fact that **P62** was only observed in feces extracts suggested that the components of **P62** were not systemic metabolites but were formed in gut or feces. This hypothesis was supported by the observation that ^{14}C-plasma concentrations in subject 5101 were distinctly lower than those in the other three volunteers, but the feces extract contained the same proportion of **P62** (i.e., 1% of dose) as the other subjects.

Metabolite Structure Elucidation. The chemical structures of the metabolites were elucidated essentially based on LC-MS data (Table 3), although in some cases, for complete elucidation, analysis by ^{1}H NMR was required. However, ^{1}H NMR analysis of the human samples was not feasible because of low metabolite concentrations. Therefore, ^{1}H NMR analysis was performed with urinary metabolites, which had been obtained from a parallel rabbit study and which, based on LC-MS data, were identical with the respective human metabolites (Table 4). The combined data provided unambiguous metabolite identification.

The mass spectrum of the parent compound aliskiren and its proposed interpretation are provided in Fig. 5. Major signals observed were the protonated intact molecule $M + H^+$ (*m*/*z* 552) and four key fragments (*m*/*z* 436, 209, 137, and 117; Fig. 5a). These ions or the mass difference between them can be related to several substructures of the molecule (Fig. 5b). The fragment ions *m*/*z* 436 and *m*/*z* 117 were formed after cleavage of the central amide bond, whereas the fragment ions *m*/*z* 209 and *m*/*z* 137 represent substructures of the

TABLE 2

Metabolite balance in urine and feces

Values are shown as the percentage of dose (mean ± S.D., $n = 4$).

Metabolite	Functional Group/Metabolic Change	Urine	Feces	Total
Front peak	Not identified[a]	<0.1	0.1	0.1 ± 0.1
M1	Phenol, *O*-demethylated		0.1	0.1 ± 0.1
M2	Carboxylic acid (oxidized side chain)	<0.1	0.5	0.6 ± 0.5
M3	Alcohol, *O*-demethylated	<0.1	0.6	0.6 ± 0.4
M4	Phenol, *O*-dealkylated	<0.1		<0.1
M6	*O*-Glucuronide of **M4**	<0.1		<0.1
Aliskiren	(Parent compound)	0.4	77.5	79.8 ± 3.0
P62	**M12**: *N*-acetyl derivative		1.0	1.0 ± 0.1
	M13, **M14**: partly characterized ($+C_3H_4O_2$)			
Traces	Unidentified	<0.1	1.5	1.6 ± 0.7
Not analyzed			9.7	9.7 ± 3.1
Total ^{14}C		0.6	90.9	91.5 ± 4.5

[a] Not unambiguously identified; possible 3-amino-2,2-dimethylpropionamide, or a hydrolysis or oxidation product thereof.

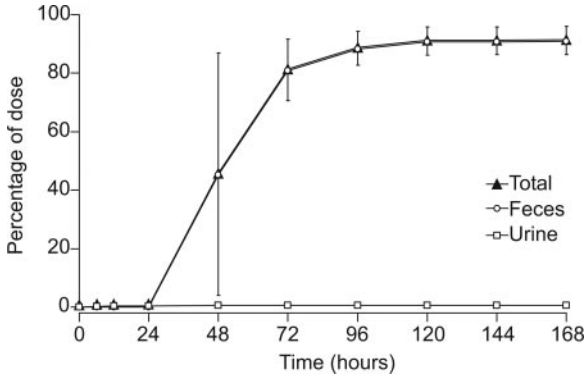


FIG. 3. Cumulative excretion of radioactivity in human urine and feces. Values are presented as mean ± S.D.

fragment ion *m/z* 436 and comprise the 1-methoxy-2-(3-methoxypropoxy)-4-methylene benzene moiety or parts of it. Fragment ions of the metabolites analogous to those of the parent compound allowed biotransformations to be assigned to these substructures as described below.

The mass spectra of metabolites **M1** and **M3** showed molecular ions $M + H^+$ at *m/z* 538, indicating that they are demethylated metabolites. *O*-Demethylation was assigned to the region of fragment C (mass difference 209 − 14 = 195). The mass spectra did not allow further differentiation between the two possible positions for *O*-demethylations (methoxy groups). ^1H NMR analysis of metabolite **M1** showed absence of the signal at approximately 3.7 ppm. Comparison of ^1H NMR data of aliskiren with metabolites (Table 4) had shown that the signal at 3.7 ppm was due to the phenolic methoxy group (C-11, scheme in Table 4), whereas the signal of the propoxymethyl group (C-10) was at approximately 3.2 ppm. Hence, metabolite **M3** was concluded to be *O*-demethylated at the phenyl-propoxy side chain.

Metabolite **M2** was isobaric to the parent drug ($M + H^+$ at *m/z* 552). On the basis of exact mass measurements of the protonated molecular ion and key fragment ions, and hydrogen/deuterium exchange, it was considered that **M2** is most likely formed by demethylation of the 3-methoxy-propoxy group and further oxidation of the resulting hydroxyl group to a carboxylic acid.

The mass spectrum of metabolite **M4** showed a molecular ion $M + H^+$ at *m/z* 480. *O*-Dealkylation was assigned to the region of fragment C (mass difference 209 − 72 = 137), indicating oxidation of **M2** or **M3** or aliskiren, with loss of the propoxy side chain. It was therefore concluded that **M4** was the methoxy phenol derivative. Metabolite

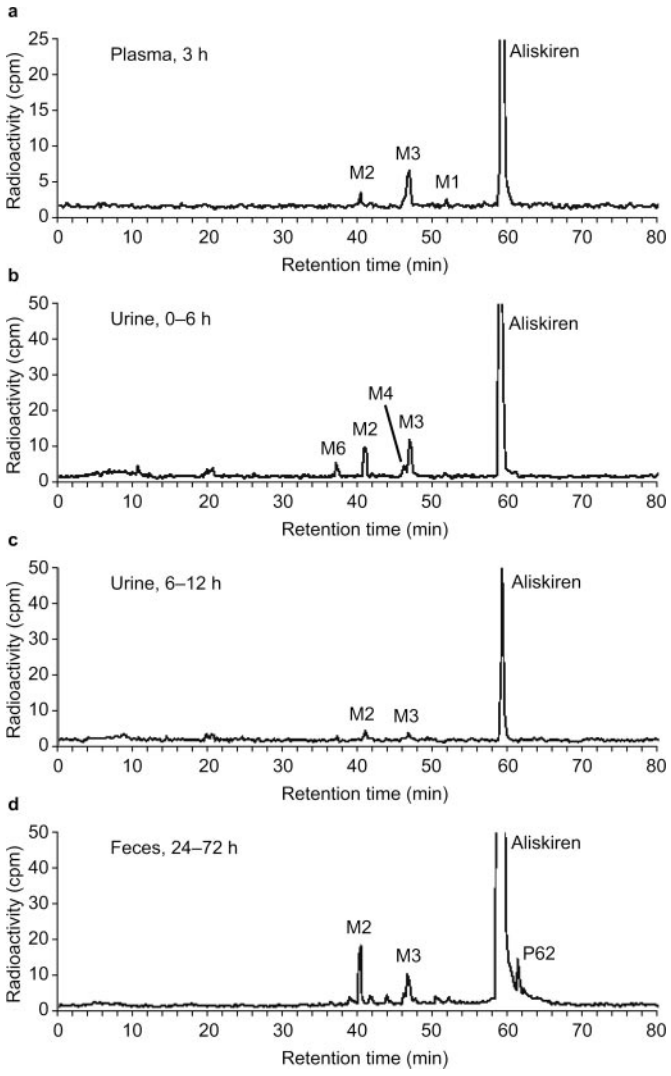


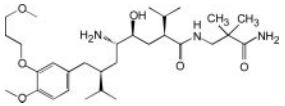
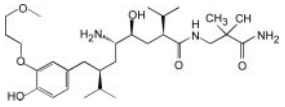
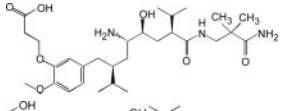
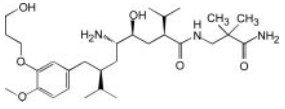
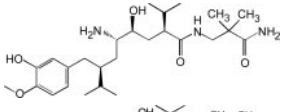
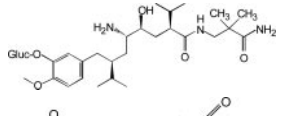
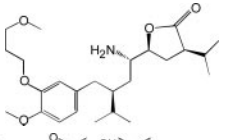
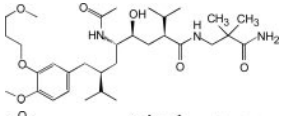
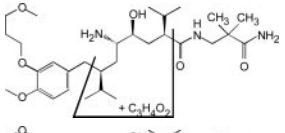
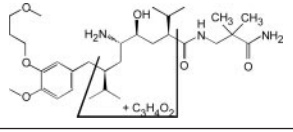
FIG. 4. Representative aliskiren metabolite patterns in plasma (a), urine (b and c), and feces (d) under HPLC analysis with radiodetection. Metabolite patterns under HPLC analysis with off-line radiodetection were determined as follows: a, plasma sample at t_{max} (3 h) from subject 5103; b, urine fractions 0 to 6 h (0.39% of dose) from subject 5103; c, urine fractions 6 to 12 h (0.12% of dose) from subject 5102; and d, feces fraction pool 24 to 72 h (91.1% of dose) from subject 5104.

M6 was identified as a glucuronic acid conjugate of **M4**. ^1H NMR analysis of **M6** showed the presence of the C-11 methoxy group (see scheme in Table 4), and a typical chemical shift for the anomeric

TABLE 3

Mass spectral data and structures of aliskiren and metabolites in urine and feces

The table presents summarized data from LC-MS runs (electrospray ionization, positive ion mode) of urine and feces extracts after an oral dose of 300 mg of [^{14}C]aliskiren.

Component	Proposed Formula of $M + H^+$	Proposed Structure	Mass Shift[a]	$M + H^+$	$M + H^+ - H_2O$	A[b]	B[b]	$B^b - H_2O$	C[b]	D[b]	Additional Major Signals
Aliskiren	$C_{30}H_{54}N_3O_6$		+7	552	534	117	436	418	209	137	517 ($M + H^+ - H_2O - NH_3$), 500 ($M + H^+ - H_2O - 2NH_3$), 401 ($B - H_2O - NH_3$), 346, 334, 317, 285, 177, 100, 73
M1	$C_{29}H_{52}N_3O_6$		+8	538	520	117	422	404	195		576 ($M + K^+$), 560 ($M + Na^+$), 73
M2	$C_{29}H_{50}N_3O_7$		+8	552	534	117	436	418	209	137	590 ($M + K^+$), 574 ($M + Na^+$), 55, 500, 400, 346, 334, 100
M3	$C_{29}H_{52}N_3O_6$		+8	538	520	117	422	404	195		576 ($M + K^+$), 560 ($M + Na^+$)
M4	$C_{26}H_{46}N_3O_5$		+8	480	462	117	364	346	137	137	518 ($M + K^+$), 141
M6	$C_{32}H_{54}N_3O_{11}$		+11	656	638		364[c]				480 ($M + H^+ - C_6H_8O_6$), 462 ($M + H^+ - C_6H_8O_6 - H_2O$)
M9	$C_{25}H_{42}NO_5$		+3	436			436		209	137	419 ($M + H^+ - NH_3$), 346, 285, 268, 163, 73
M12 (ATG045)	$C_{32}H_{56}N_3O_7$		+6	594	576		478		209		632 ($M + K^+$), 616 ($M + Na^+$), 559 ($M + H^+ - H_2O - NH_3$), 500, 472, 418, 401, 373, 317, 285, 100, 73
M13	$C_{33}H_{58}N_3O_8$		+7	624	606	117	508	490	209	137	662 ($M + K^+$), 646 ($M + Na^+$), 589 ($M + H^+ - H_2O - NH_3$), 588 ($M + H^+ - 2H_2O$), 571 ($M + H^+ - 2H_2O - NH_3$), 562, 500, 472, 265, 177, 163, 73
M14	$C_{33}H_{58}N_3O_8$		+7	624	606	117	508	490	209	137	662 ($M + K^+$), 646 ($M + Na^+$), 589 ($M + H^+ - H_2O - NH_3$), 588 ($M + H^+ - 2H_2O$), 500, 472, 373, 265, 177, 163, 73

[a] of $[M + H^+]$ after H/D exchange.
[b] Figure 5b describes the formation of fragment ions A, B, C, and D.
[c] Fragment was formed after loss of glucuronic acid moiety ($C_6H_8O_6$).

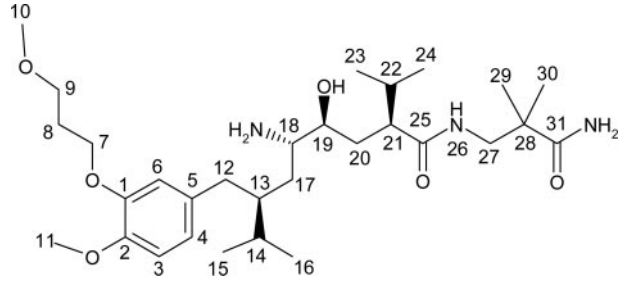
TABLE 4

^{1}H NMR spectral data of aliskiren and metabolites

Chemical shifts of characteristic signals in ^{1}H NMR spectra collected from aliskiren and from metabolites **M1** and **M6** obtained from a parallel rabbit study. The metabolites were identical with the respective human metabolites based on LC-MS. For carbon atom numbers, see structure of aliskiren below. The signals of protons attached to carbon atoms 8, 12, 13, 14, 17, 20, 21, and 22 were in the chemical shift range 1.3 to 2.6 ppm. The signals of the protons 1′ to 5′ refer to the glucuronic acid moiety in **M6**, where the 1′-signal is due to the anomeric proton.

Index Carbon Atom	^{1}H Shift		
	Aliskiren	**M1**	**M6**
		ppm	
3	6.84	6.71	6.96
4	6.72	6.62	6.91
6	6.77	6.75	6.93
7	3.97	3.98	
9	3.48	3.50	
10	3.22	3.22	
11	3.69		3.79
15,16	0.77	0.77	0.82
18	*a*	*a*	2.84
19	3.14	3.14	3.24
23,24	0.77	0.77	0.82
27	3.18,3.25	3.18,3.25	3.25,3.33
29, 30	1.04,1.05	1.04,1.05	1.11,1.12
1′			5.07
2′,3′,4′			3.59,3.57,3.57
5′			4.03

[a] Broad or overlapping signal, not assigned.



1′-proton. Thus, the glucuronic acid moiety was concluded to be attached to the phenol 1-O position (Table 4).

The unlabeled metabolite **M9** eluted at 62.0 min, relative to a retention time for aliskiren of 53.4 min (metabolite analysis by HPLC radiometry). **M9** was identified as the lactone derivative of aliskiren, based on the mass spectral data, chromatographic retention time, and comparison with synthetic reference compound. Lactone formation occurs after cleavage of the central amide bond.

A further trace metabolite was observed in several metabolite patterns as a front peak. It was not unambiguously identified, but is likely to represent 3-amino-2,2-dimethylpropanamide (β-amino-isobutyramide), or a hydrolysis or oxidation product thereof. It is assumed to be formed, in addition to **M9**, as a second product of hydrolysis at the central amide bond in aliskiren.

As described above, radiochromatograms of fecal extracts showed a peak **P62** eluting on the tail edge of the parent drug. LC-MS runs performed under different chromatographic conditions revealed three chromatographic peaks (**M12**, **M13**, and **M14**) accounting for 1% of the dose. The ratios of the three components varied as follows: **M12**, 0 to 0.2% of dose; **M13**, 0.2 to 0.7% of dose; **M14**, 0.3 to 0.7% of dose. The mass spectra of **M12** indicated either an *O*- or *N*-acetylation of aliskiren, as deduced from the proposed elemental composition of the protonated molecular ion $M + H^+$ at *m*/*z* 594, the fragment ions 478, 209, and 117, and the number of exchangeable protons (six). A comparison of retention time and mass spectral data with the reference

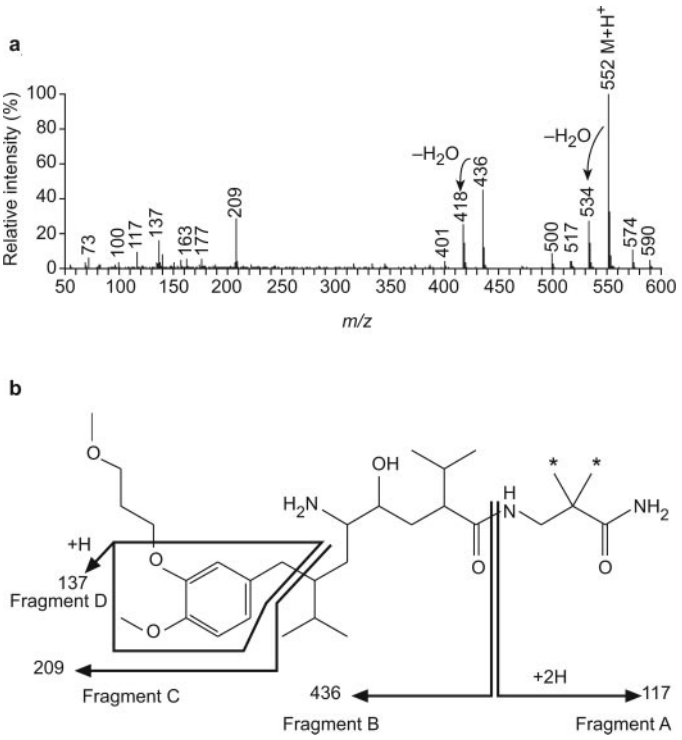


FIG. 5. Electrospray mass spectrum of aliskiren: a, averaged spectrum; and b, proposed interpretation. a shows averaged and background subtracted spectrum after electrospray ionization carried out in positive ion mode, with in-source collision-induced dissociation offset (APICID) 40 V and 80 V. Proposed interpretation of the spectrum in b is in agreement with exact mass measurements: difference between measured and calculated masses was ≤4.8 mDa for all fragments with a given interpretation. Asterisks indicate positions of ^{14}C-labels.

standard ATG045 confirmed the presence of an *N*-acetylated metabolite (**M12**) in the feces extracts of subjects 5102, 5103, and 5104.

The two other metabolites **M13** and **M14** showed identical mass spectra, exhibiting the same protonated molecular ions at *m*/*z* 624 and key fragment ions A to D (see Fig. 5b). The proposed elemental composition and the number of labile protons determined by hydrogen/deuterium exchange experiments were also identical. Because the fragment ions C (*m*/*z* 209) and A (*m*/*z* 117) remained unchanged in comparison with the mass spectrum of aliskiren, the metabolites are proposed to be structural isomers containing an additional $C_3H_4O_2$ moiety in the central part of the molecule; however, final structure elucidation was not achieved because of the low amounts available for analysis.

A proposed scheme of the biotransformation pathways for aliskiren is provided in Fig. 6a, and a detailed metabolic pathway for aliskiren is presented in Fig. 6b. For metabolites **M12** to **M14**, which appear to be formed in the gut, see Fig. 6c.

Discussion

A single 300-mg oral dose of [^{14}C]aliskiren was well tolerated, with no adverse events reported during the course of the study. After oral administration of [^{14}C]aliskiren as the hemifumarate salt in an aqueous drink solution, peak plasma concentrations of both aliskiren and radioactivity were reached between 2 and 5 h after dosing. Unchanged aliskiren accounted for 81% of plasma radioactivity, indicating very low exposure to metabolites. The concentration-time curves for ^{14}C radioactivity and aliskiren were approximately parallel, and terminal half-lives of radioactivity and aliskiren were 49 h and 44 h, respectively.

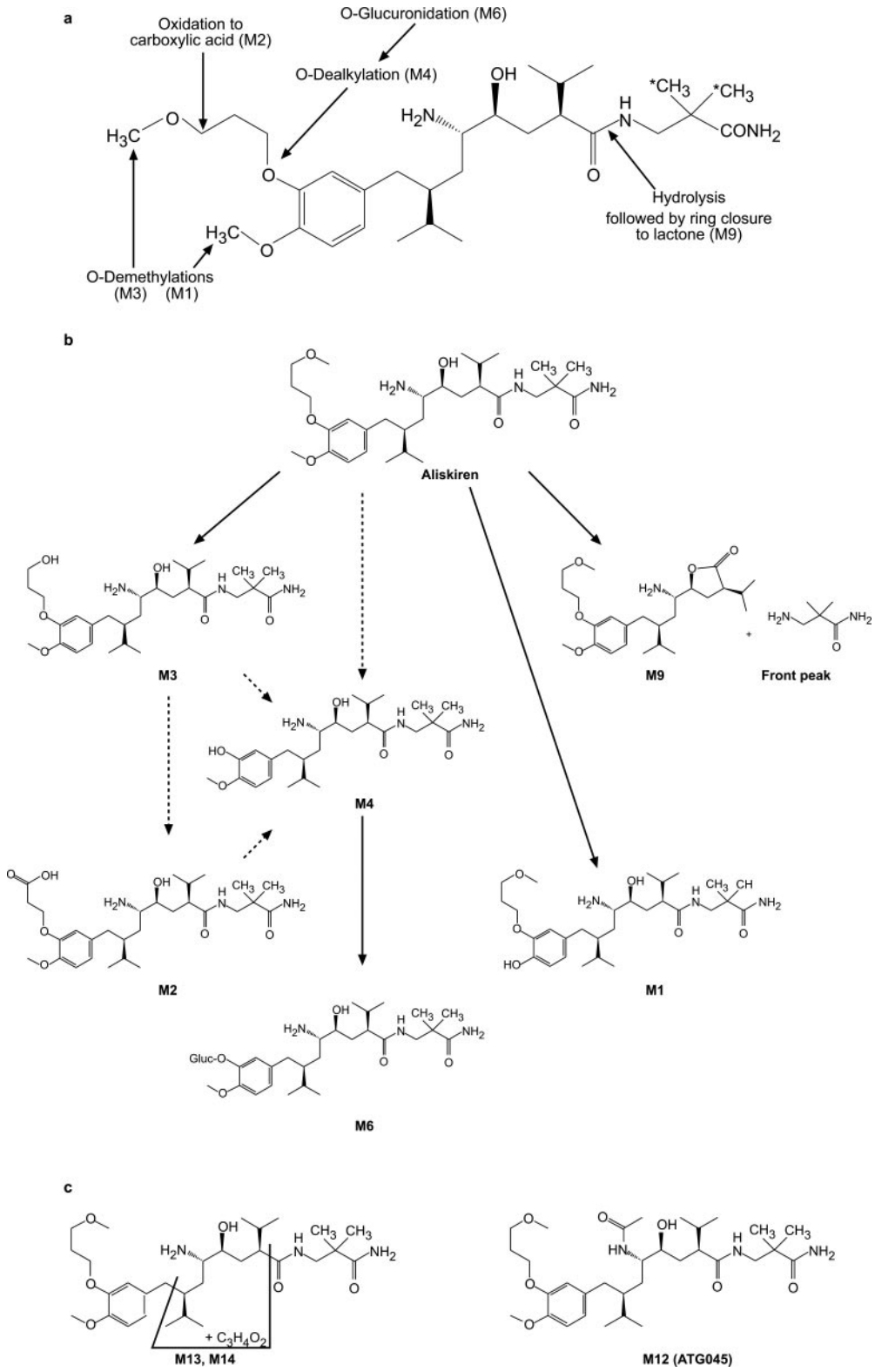


FIG. 6. Metabolism of aliskiren in humans. a, condensed scheme of metabolism; b, detailed metabolism pathways and metabolite structures; c, proposed derivatives of aliskiren presumably formed in intestine. Dotted arrows indicate potential alternative pathways leading to formation of metabolites **M2** and **M4**.

Radioactivity was detectable using conventional LSC for up to 12 h. At later time points, analysis required the highly sensitive AMS technique. Single samples were analyzed both with LSC and AMS, with AMS giving 10 to 20% higher values. Therefore, aliskiren accounted for approximately 86% of the plasma radioactivity AUC_{0-10h}, versus 81% of radioactivity $AUC_{0-\infty}$. The difference between LSC and AMS was within common analytical accuracy ranges and thus was not significant. Since AMS has been validated as a quantitative method for ^{14}C radioactivity (Garner et al., 2000), no systematic method cross-check was performed.

The low levels of metabolites of aliskiren in the plasma, urine, and feces suggest a minor role for metabolism in the elimination of aliskiren, but the observed metabolite profile indicates that oxidative processes represent the major pathway for the proportion of aliskiren

that is metabolized. The two major metabolites, the oxidized derivatives **M3** (*O*-demethylated alcohol derivative) and **M2** (carboxylic acid derivative) accounted for approximately 3% and 1%, respectively, of the radioactivity in the plasma (at t_{max}). An additional oxidized metabolite, **M1**, was also detected in plasma, and **M1** to **M3** plus a further oxidized metabolite **M4** and traces of its glucuronic acid conjugate (**M6**), and an unlabeled hydrolysis product (lactone derivative **M9**) were observed in the urine. With the exception of **M6**, all of these metabolites were also detected in the feces. Further phase II conjugation was only observed for the oxidized metabolite **M4** (by glucuronic acid conjugation to **M6**), and there was no evidence for direct glucuronic acid conjugation of aliskiren.

The terminal metabolites **M1** to **M4** accounted for 1.4% of the excreted dose and were all formed by oxidation at the side chain by *O*-demethylation, *O*-dealkylation, and/or alcohol oxidation, probably by CYP3A4 (Novartis, data on file). It is not known whether any aliskiren metabolites exhibit pharmacological activity. However, the very low concentration levels of metabolites as compared with unchanged aliskiren suggest that the metabolites are unlikely to contribute to the biological activity of aliskiren. The trace metabolites **M12** (*N*-acetyl derivative), and **M13** and **M14** (which could be characterized only partially) were found only in the feces (in peak **P62**). Taken together with the observation that the proportions of these metabolites found in the feces were similar in all four subjects (despite notably lower ^{14}C-plasma concentrations in one subject), it seems likely that **M12**, **M13**, and **M14** are a fecal artifact produced from unabsorbed aliskiren, probably by the intestinal microflora. Indeed, acetylation (which would produce **M12**) is a metabolic pathway that is known to occur under the anaerobic conditions of the gut (Goldin, 1990).

Aliskiren undergoes oxidative metabolism by P450 isoenzymes to a low degree. Aliskiren is not an inhibitor of P450 activity and is unlikely to exhibit pharmacokinetic interactions with drugs that are P450 isoenzyme substrates. An in vitro study showed no notable effects of aliskiren at a concentration of 20 μM (approximately 5-fold higher than the mean C_{max} of aliskiren observed in the present study) on the activity of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (Vaidyanathan et al., 2005). Moreover, studies in healthy volunteers have demonstrated no drug interactions between aliskiren and celecoxib, cimetidine, digoxin, lovastatin or warfarin, all of which are known to interact with P450 isoenzymes (Dieterle et al., 2004, 2005; Dieterich et al., 2006).

Apart from detailed investigation of pathways for metabolism, the major objectives of a human ADME study are assessment of the extent of absorption and identification of the key elimination processes. The minimal extent of absorption after oral dosing can be estimated as the radioactivity dose proportion excreted in urine, plus the dose proportion excreted in the form of metabolites in feces. However, in the present case, this is not adequate since renal excretion and metabolism are minor, and biliary elimination of unchanged drug is neglected. Therefore, other available data on aliskiren should be considered. 1) In an absolute bioavailability study in humans (Azizi et al., 2006), based on plasma AUC, the oral bioavailability of aliskiren was determined to be 2.6%. 2) In the same study, the renal excretion of unchanged aliskiren after an intravenous dose of 20 mg was 7.5% of dose. Thus, elimination occurred predominantly via nonrenal processes (ratio of nonrenal/renal approximately 12), including transport with bile and possibly through gut wall, and/or metabolism. 3) In ADME studies in rats and marmosets with oral and intravenous dosing (Novartis, data on file), biliary/fecal dose elimination was predominant; e.g., up to 90% and 78% of intravenous doses were recovered in the feces of rat and marmoset, respectively, largely in the form of unchanged aliskiren. Furthermore, aliskiren has been found to be a substrate for P-glycoprotein, thus intestinal P-glycoprotein might contribute to elimination. On the basis of the absolute bioavailability study, the oral absorption in humans would be at least 2.6% of dose. In the present oral human ADME study, the renal excretion of aliskiren, determined using a sensitive method, was 0.4% of dose, approximately 20 times less than after an intravenous dose. Combined with the results of the intravenous study, an extent of absorption of approximately 5% can be estimated.

In the present human ADME study, metabolites accounted for 0.2% of dose in urine. The amount of metabolites formed after absorption and excreted in feces (excluding the fecal metabolite **P62**, which appears to be formed from unabsorbed aliskiren in the intestine) appears to be at least 1.3% of dose. With various unidentified trace peaks in the fecal metabolite pattern (near detection limit), the total amount of metabolites may have been in the range 1.5 to 3%. Thus, only part of the absorbed aliskiren was eliminated through metabolism. A similar or larger dose fraction, recovered in the feces in unchanged form, must have been due to aliskiren elimination via the hepatobiliary route, and thus, hepatobiliary elimination is concluded to be a main elimination process. Nevertheless, it should be noted that the bulk of the dose excreted in feces is due to unabsorbed drug.

Consistent with our findings regarding the elimination of absorbed aliskiren, the pharmacokinetics of aliskiren are not significantly altered by renal impairment (Vaidyanathan et al., 2007a). No significant effect on aliskiren pharmacokinetics was found in patients with impaired hepatic function; thus, no dosage adjustment for aliskiren is required (Vaidyanathan et al., 2007b).

In the present study, the pharmacokinetics of ^{14}C radioactivity and aliskiren showed large interindividual variability. Indeed, one subject (5101) exhibited a considerably lower exposure to aliskiren than did the other three subjects. The reason is unknown. High variability in aliskiren pharmacokinetic parameters has also been described in clinical studies with solid drug administration (Vaidyanathan et al., 2006a). Since aliskiren is a substrate for P-glycoprotein, interindividual variations in intestinal P-glycoprotein expression might contribute to the observed variability in pharmacokinetics (Cascorbi, 2006).

In summary, aliskiren is absorbed to a low extent after an oral dose. Excretion of aliskiren is nearly complete within 168 h, with the majority of an oral dose of aliskiren excreted unchanged in the feces. Parent drug represented the principal circulating species in plasma. Absorbed drug appears to be eliminated via the hepatobiliary route and, to some degree, through oxidative metabolism.

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