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

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Received November 10, 2006; accepted May 16, 2007

ABSTRACT:

Aliskiren \((2\text{S},4\text{S},5\text{S},7\text{S})-N\text{-carbamoyl-2-methylpropyl}-5\text{-amino}-4\text{-hydroxy}-2,7\text{-disopropyl-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 \([^{14}\text{C}]\)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_{\text{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 \((\text{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 (Kearney et al., 2005). 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).

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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 IC₅₀, 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 (tₘᵢₙᵦ = 1–3 h) and exhibits a long plasma half-life (t₁/₂ = 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., 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., 2006b) in patients with hypertension.

Studies investigating the disposition of oral doses of [¹⁴C]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 citriveau, 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 [¹⁴C]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 [¹⁴C] 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) 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., 2006b) in patients with hypertension.

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% 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 MultiPrep II. After the conditioning steps (200 µl of methanol, then 200 µl of pH 12 buffer), 200 µl of alkalized sample was transferred to the well. The sample was washed with 400 µl of methanol/water (25:75, 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/min, injection volume 10 µl; Metachem, Palo Alto, CA) with gradient elution from 10 mM aqueous ammonium acetate/acetoni trite (75:25 v/v) to 10 mM aqueous ammonium acetate/acetoni trite (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 prostaglandin E2/PGD2) was used as an internal standard.

**Total Radioactivity Measurement.** Total 14C 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 ItaLuma (Lumac-ItaLuma, Groningen, the Netherlands). LSC was performed using a Tri-Carb 3170 TR/TR 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 14C 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 showed 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 4000g 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 14C 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 (0.6–1.1 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 acetoni trite 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 extracts 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 LiChrophor 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 Fischer 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, 1H NMR analysis).

**LC-MS analysis.** LC-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 Nuclear C18 Pyramyd was used (5 µm, 250 µm × 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 of water.
Flo-Scint A liquid scintillation cocktail (PerkinElmer Life and Analytical 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 reserpin (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.

**1H 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 1H NMR analysis. 1H NMR spectra were recorded on a Bruker DMX-500 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) equipped with a 4-mm selective inverse 1H/13C 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 1H NMR pulse program lc1pncwps from Bruker BioSpin AG was applied. Signals from the HPLC solvents were suppressed using a multiple solvent suppression with 1H-decoupling.

**Pharmacokinetic Analysis.** Pharmacokinetic parameters (AUC, Cmax, tmax, t1/2, 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 (AUC0–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.

**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 [14C]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 (Cmax) 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
in blood and plasma are summarized in Table 1. Early apparent half-lives for elimination from plasma (by noncompartamental 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 (AUC0→∞; 86% for AUC0→104 h) 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 AUC0→104 h to radioactivity in fecal metabolites (approximately 80% of dose) occurred within 72 h of dosing. Total excretion (mass balance) over the 168-h collection period was 91.5% of dose. Unchanged [14C]aliskiren accounted for the major part of radioactivity (approximately 70%) in all analyzed urine samples (Fig. 4a). The mass spectrum of the parent compound aliskiren and its metabolites were elucidated essentially based on LC-MS data (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
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/H11001 at m/z 538, indicating that they are demethylated metabolites. O-Demethylation was assigned to the region of fragment C (mass difference 209/H11002 14/H11005 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 propoxy-methyl 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/H11001 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/H11001 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 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>
<thead>
<tr>
<th>Metabolite</th>
<th>Functional Group/Metabolic Change</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front peak</td>
<td>Not identified*</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>M1</td>
<td>Phenol, O-demethylated</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>M2</td>
<td>Carboxylic acid (oxidized side chain)</td>
<td>0.5</td>
<td>0.6</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>M3</td>
<td>Alcohol, O-demethylated</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>M4</td>
<td>Phenol, O-dealkylated</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>M6</td>
<td>O-Glucuronide of M4</td>
<td>0.4</td>
<td>77.5</td>
<td>79.8 ± 3.0</td>
</tr>
<tr>
<td>Aliskiren</td>
<td>(Parent compound)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P62</td>
<td>M12: N-acetyl derivative</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>M13, M14: partly characterized (+C3H4O2)</td>
<td>1.5</td>
<td>1.6 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Traces</td>
<td>Unidentified</td>
<td>&lt;0.1</td>
<td>1.5</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Not analyzed</td>
<td></td>
<td></td>
<td>9.7</td>
<td>9.7 ± 3.1</td>
</tr>
<tr>
<td>Total 14C</td>
<td></td>
<td>0.6</td>
<td>90.9</td>
<td>91.5 ± 4.5</td>
</tr>
</tbody>
</table>

* 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.

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 tmax (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.
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 [14C]aliskiren.

<table>
<thead>
<tr>
<th>Component</th>
<th>Proposed Formula of $\text{M} + \text{H}^+$</th>
<th>Proposed Structure</th>
<th>Mass Shift</th>
<th>$\text{M} + \text{H}^+$</th>
<th>$\text{M} + \text{H}^+-\text{H}_2\text{O}$</th>
<th>$\text{A}^a$</th>
<th>$\text{B}^b$</th>
<th>$\text{C}^c$</th>
<th>$\text{D}^d$</th>
<th>Additional Major Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliskiren</td>
<td>C$<em>{29}$H$</em>{54}$N$_3$O$_6$</td>
<td>+7</td>
<td>552</td>
<td>534</td>
<td>117</td>
<td>436</td>
<td>418</td>
<td>209</td>
<td>137</td>
<td>517 ($\text{M} + \text{H}^+-\text{H}_2\text{O}-\text{NH}_3$), 500 ($\text{M} + \text{H}^+-\text{H}_2\text{O}-2\text{NH}_3$), 401 ($\text{B}^b$-H$_2$O-NH$_3$), 346, 334, 317, 285, 177, 100, 73</td>
</tr>
<tr>
<td>M1</td>
<td>C$<em>{29}$H$</em>{52}$N$_3$O$_6$</td>
<td>+8</td>
<td>538</td>
<td>520</td>
<td>117</td>
<td>422</td>
<td>404</td>
<td>195</td>
<td>576 (M + K$^+$), 560 (M + Na$^+$), 73</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>C$<em>{29}$H$</em>{52}$N$_3$O$_7$</td>
<td>+8</td>
<td>552</td>
<td>534</td>
<td>117</td>
<td>436</td>
<td>418</td>
<td>209</td>
<td>137</td>
<td>590 (M + K$^+$), 574 (M + Na$^+$), 55, 500, 400, 346, 334, 100</td>
</tr>
<tr>
<td>M3</td>
<td>C$<em>{29}$H$</em>{52}$N$_3$O$_6$</td>
<td>+8</td>
<td>538</td>
<td>520</td>
<td>117</td>
<td>422</td>
<td>404</td>
<td>195</td>
<td>576 (M + K$^+$), 560 (M + Na$^+$)</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>C$<em>{29}$H$</em>{52}$N$_3$O$_6$</td>
<td>+8</td>
<td>480</td>
<td>462</td>
<td>117</td>
<td>364</td>
<td>346</td>
<td>137</td>
<td>518 (M + K$^+$), 141</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>C$<em>{32}$H$</em>{56}$N$_3$O$_7$</td>
<td>+11</td>
<td>656</td>
<td>638</td>
<td>364$^c$</td>
<td>480 (M + H$^+-$C$_6$H$_8$O$_6$), 462 (M + H$^+-$C$_6$H$_8$O$_6$-H$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M9</td>
<td>C$<em>{25}$H$</em>{42}$NO$_5$</td>
<td>+3</td>
<td>436</td>
<td>436</td>
<td>209</td>
<td>137</td>
<td>419 (M + H$^+$-NH$_3$), 346, 285, 268, 163, 73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M12 (ATG045)</td>
<td>C$<em>{32}$H$</em>{56}$N$_3$O$_7$</td>
<td>+6</td>
<td>594</td>
<td>576</td>
<td>478</td>
<td>209</td>
<td>632 (M + K$^+$), 616 (M + Na$^+$), 559 (M + H$^+$-H$_2$O-NH$_3$), 500, 472, 418, 401, 373, 317, 285, 100, 73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M13</td>
<td>C$<em>{33}$H$</em>{58}$N$_3$O$_8$</td>
<td>+7</td>
<td>624</td>
<td>606</td>
<td>117</td>
<td>508</td>
<td>490</td>
<td>209</td>
<td>137</td>
<td>662 (M + K$^+$), 646 (M + Na$^+$), 589 (M + H$^+-\text{H}_2\text{O}$-NH$_3$), 588 (M + H$^+-$2H$_2$O), 571 (M + H$^+-\text{H}_2\text{O}$-NH$_3$), 562, 500, 472, 265, 177, 163, 73</td>
</tr>
<tr>
<td>M14</td>
<td>C$<em>{33}$H$</em>{58}$N$_3$O$_8$</td>
<td>+7</td>
<td>624</td>
<td>606</td>
<td>117</td>
<td>508</td>
<td>490</td>
<td>209</td>
<td>137</td>
<td>662 (M + K$^+$), 646 (M + Na$^+$), 589 (M + H$^+-\text{H}_2\text{O}$-NH$_3$), 588 (M + H$^+-$2H$_2$O), 500, 472, 373, 265, 177, 163, 73</td>
</tr>
</tbody>
</table>

$^a$ 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$_6$H$_8$O$_6$).
$^d$ Fragment was formed after loss of glucuronic acid moiety (C$_6$H$_8$O$_6$-H$_2$O).
The mass spectra of M12, M13, and M14 were 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.

<table>
<thead>
<tr>
<th>Index Carbon Atom</th>
<th>1H Shift: Aliskiren</th>
<th>M1</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.84</td>
<td>6.71</td>
<td>6.96</td>
</tr>
<tr>
<td>4</td>
<td>6.72</td>
<td>6.62</td>
<td>6.91</td>
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<tr>
<td>6</td>
<td>6.77</td>
<td>6.75</td>
<td>6.93</td>
</tr>
<tr>
<td>7</td>
<td>3.97</td>
<td>3.98</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.48</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.22</td>
<td>3.22</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.69</td>
<td></td>
<td>3.79</td>
</tr>
<tr>
<td>15, 16</td>
<td>0.77</td>
<td>0.77</td>
<td>0.82</td>
</tr>
<tr>
<td>18</td>
<td>a</td>
<td>a</td>
<td>2.84</td>
</tr>
<tr>
<td>19</td>
<td>3.14</td>
<td>3.14</td>
<td>3.24</td>
</tr>
<tr>
<td>23, 24</td>
<td>0.77</td>
<td>0.77</td>
<td>0.82</td>
</tr>
<tr>
<td>27</td>
<td>3.18, 3.25</td>
<td>3.18, 3.25</td>
<td>3.25, 3.33</td>
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<tr>
<td>29, 30</td>
<td>1.04, 1.05</td>
<td>1.04, 1.05</td>
<td>1.11, 1.12</td>
</tr>
<tr>
<td>1’</td>
<td></td>
<td></td>
<td>5.07</td>
</tr>
<tr>
<td>2’, 3’, 4’</td>
<td></td>
<td></td>
<td>3.59, 3.57, 3.57</td>
</tr>
<tr>
<td>5’</td>
<td></td>
<td></td>
<td>4.03</td>
</tr>
</tbody>
</table>

* Broad or overlapping signal, not assigned.

A single 300-mg oral dose of [14C]aliskiren was well tolerated, with no adverse events reported during the course of the study. After oral administration of [14C]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 14C radioactivity and aliskiren were approximately parallel, and terminal half-lives of radioactivity and aliskiren were 49 h and 44 h, respectively.

**Discussion**

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.

![Diagram](image-url)
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\(_{0-10}\)h, versus 81% of radioactivity AUC\(_{0-110}\)h. 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\text{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 (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 14C-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\text{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 investigations 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 excretion is negligible. 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/nonrenal approximatively 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, 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 the 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 14C radioactivity and aliskiren showed large interindividual variability. Indeed, one subject (S1101) exhibited a considerably lower exposure to aliskiren than 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 (CASCOBRI, 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.

Acknowledgments. We are grateful for the excellent technical assistance of Robert Nufer, Daniel Pierroz, Ronald Freiburghaus, and Matthias Frommherz. Furthermore, we acknowledge the important contributions by Drs. I. Ottinger and B. Leboulanger (Pharmaceutical Development, Novartis), Dr. R. Rhys (Isotope Laboratory, Novartis), Prof. R. C. Garner (Xceleron Ltd.), and Dr. R. Burri (RCC Ltd.). We also thank Dr. R. White for editorial assistance in the preparation and submission of the final manuscript.

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