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-disopropyl-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 hypertension. The absorption, distribution, metabolism, and excretion (ADME) of aliskiren was previously studied (Rongen et al., 2005). Aliskiren is rapidly absorbed after oral administration, with peak plasma levels of aliskiren reaching 49 ng/mL within 2 h of dosing. The half-life of aliskiren in plasma is approximately 49 h, representing significantly reduced elimination compared to other renin inhibitors such as the angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (Rongen et al., 2005).
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_{1/2} 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-converting enzyme inhibitors (Uresin et al., 2006b). The aim of the present study was to characterize the absorption, metabolism, and excretion of oral doses of [14C]aliskiren in rats and marmosets in order to provide pharmacokinetic data for subsequent clinical studies.

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 normal results of routine laboratory evaluations and to take alcohol (for 72 h before dosing until the end of the study) or to take xanthine-containing food or beverages was also not permitted in the time period 48 to 144 h.

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) 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 14C-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 [14C]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 −70°C for subsequent radiometry. Plasma was prepared from the remaining blood by centrifugation at 4°C for 10 min at 2000g. 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 [300 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...
% 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 Multiport 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), 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 0 to 10 mM aqueous ammonium acetate/acetonic (75:25 v/v) to 10 mM aqueous ammonium acetate/acetonic (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 alisiken (gem-dimethyl d₄-alisiken) 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 Biotone S-isopropanol (1:1 v/v; Zinsser Analytic, Frankfurt, Germany), and LSC used RiaLuma (Lumac-LSC, Groeningen, the Netherlands). LSC was performed using a Tri-Carb 3170 TR/S 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 ¹³C 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), and then diluted with 150 μl of 1% acetic acid in water. Approximately 2-fold) and then diluted with 150 μl of 1% acetic acid in water.

**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 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 multichannel 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 alisiken, 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, ’H NMR analysis).

**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 a position ion mass spectrometer. For hydrophilic-lipophilic exchange experiments, water in the mobile phase was replaced by D₂O.

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 radio monitoring was performed using a Berthold model LB507A (Berthold, Munich, Germany) with a model Z-200 flow cell, after mixing with 2.8 ml/min
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.

Results
Subjects and Tolerability.

The key pharmacokinetic parameters for radioactivity and aliskiren

\[
\begin{align*}
\text{AUC}_{0-\infty}^\text{[14C]} & \quad \text{AUC}_{\text{pl, max}}^\text{[14C]} \\
\text{C}_{\text{max, max}} & \quad \text{t}_{1/2, \text{pl}}
\end{align*}
\]

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.
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 (AUC0–t; 86% for AUC0–t0h) 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–t0h/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 excreted 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) was excreted almost completely via the biliary/fecal route, with only 0.6% of dose, with 85% of radioactivity); overall, the sum of oxidized metabolites in the recovered radioactivity accounted for 0.4% of dose in urine (approximately 70% of 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–t0h/blood/plasma was 0.61, indicating that radioactivity was largely present in plasma.

**Metabolism of Aliskiren.** Plasma. Metabolite patterns in plasma were determined only at tmax because of the low levels of radioactivity in plasma. At tmax, 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 Cmax) and M3 (alcohol, O-demethylated; 1–5% of aliskiren Cmax), and trace levels of M11 (phenol, O-demethylated) were detected. These data are semiquantitative because of incomplete 14C 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 tmax.

Urine. Urine samples containing sufficient radioactive data 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 [14C]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 unlabelled 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 radioactive dose, and by solvent extraction, 90% of the radioactivity was extracted for HPLC analysis. No major differences in fecal metabolite patterns were observed between individual subjects. Unchanged [14C]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 CH2O 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 14C-plasma concentrations in subject S101 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 1H NMR was required. However, 1H NMR analysis of the human samples was not feasible because of low metabolite concentrations. Therefore, 1H 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma Aliskiren</th>
<th>Blood Total Radioactivity</th>
<th>Plasma Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>tmax (h)</td>
<td>3 (2–5)</td>
<td>2.5 (2–5)</td>
<td>3 (2–5)</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>254 ± 163</td>
<td>171 ± 84</td>
<td>305 ± 193</td>
</tr>
<tr>
<td>AUC0–t0h (ng · h/ml)</td>
<td>1051 ± 547</td>
<td>427 ± 226</td>
<td>1310 ± 614</td>
</tr>
<tr>
<td>% of 14C-AUC0–tmax</td>
<td>81 ± 2</td>
<td>61 ± 12b</td>
<td>(100)</td>
</tr>
<tr>
<td>CL/F (h)</td>
<td>380 ± 310</td>
<td>520 ± 866b</td>
<td>290 ± 210</td>
</tr>
</tbody>
</table>

N.A., not applicable.

* ng-Eq/ml for radioactivity.

* ng-Eq·h/ml for radioactivity.

* AUC0–t0h was calculated using the linear trapezoidal rule. tmax was 96 to 168 h for plasma aliskiren, 4 to 12 h for blood radioactivity, and 144 h for plasma total radioactivity. AUC0–t was calculated as AUC0–t + AUC0–t0h, where AUC0–t0h = C0 × tmax/2.
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 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+ 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 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>&lt;0.1</td>
<td>0.5</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>M3</td>
<td>Alcohol, O-demethylated</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>M4</td>
<td>Phenol, O-dealkylated</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>M6</td>
<td>O-Glucuronide of M4</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Aliskiren</td>
<td>(Parent compound)</td>
<td>0.4</td>
<td>77.5</td>
<td>77.9 ± 3.0</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>M13, M14</td>
<td>partly characterized (+C3H4O2)</td>
<td>&lt;0.1</td>
<td>1.5</td>
<td>1.6 ± 0.7</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>1.5</td>
<td>1.6 ± 0.7</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.
### 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 $M + H^+$</th>
<th>Proposed Structure</th>
<th>Mass Shift of $M + H^+$</th>
<th>$M + H^+$ - H$_2$O</th>
<th>$M + H^+$ - H$_2$O - NH$_3$</th>
<th>Additional Major Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliskiren</td>
<td>$C_{30}H_{54}N_3O_6$</td>
<td><img src="image" alt="Structure" /></td>
<td>+7</td>
<td>552</td>
<td>534</td>
<td>117</td>
</tr>
<tr>
<td>M1</td>
<td>$C_{32}H_{52}N_3O_6$</td>
<td><img src="image" alt="Structure" /></td>
<td>+8</td>
<td>538</td>
<td>520</td>
<td>117</td>
</tr>
<tr>
<td>M2</td>
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<td>552</td>
<td>534</td>
<td>117</td>
</tr>
<tr>
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<tr>
<td>M4</td>
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<td>117</td>
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<tr>
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<td>638</td>
<td>364$^c$</td>
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<tr>
<td>M9</td>
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<td>+3</td>
<td>436</td>
<td>436</td>
<td>209</td>
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<tr>
<td>M12 (ATG045)</td>
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<td><img src="image" alt="Structure" /></td>
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<tr>
<td>M13</td>
<td>$C_{33}H_{58}N_3O_8$</td>
<td><img src="image" alt="Structure" /></td>
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<td>624</td>
<td>606</td>
<td>117</td>
</tr>
<tr>
<td>M14</td>
<td>$C_{33}H_{58}N_3O_8$</td>
<td><img src="image" alt="Structure" /></td>
<td>+7</td>
<td>624</td>
<td>606</td>
<td>117</td>
</tr>
</tbody>
</table>

$^a$ of [M + H$^+$] after H/D exchange.

$^b$ Fragment was formed after loss of glucuronic acid moiety ($C_6H_8O_6$).

$^c$ Fragment was formed after loss of glucuronic acid moiety ($C_6H_8O_6$).
The mass spectra of M12, 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 C3H4O2 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 [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.
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<sub>0-10h</sub>, versus 81% of radioactivity AUC<sub>0-10h</sub>. 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 <sup>14</sup>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 unlabelled 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 $^{14}$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 isoforms 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 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 study, 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/nonrenal 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 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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References


Centers for Disease Control and Prevention (2005) Racial/ethnic disparities in prevalence, development, Novartis), Dr. R. Rhys (Isotope Laboratory, Novartis), 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.