Pharmacokinetics, Distribution, and Metabolism of $[^{14}C]$Sunitinib in Rats, Monkeys, and Humans


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ABSTRACT:

Sunitinib is an oral multitargeted tyrosine kinase inhibitor approved for the treatment of advanced renal cell carcinoma, imatinib-refractory gastrointestinal stromal tumor, and advanced pancreatic neuroendocrine tumors. The current studies were conducted to characterize the pharmacokinetics, distribution, and metabolism of sunitinib after intravenous and/or oral administrations of $[^{14}C]$sunitinib in rats (5 mg/kg i.v., 15 mg/kg p.o.), monkeys (6 mg/kg p.o.), and humans (50 mg p.o.). After oral administration, plasma concentration of sunitinib and total radioactivity peaked from 3 to 6 h. Plasma terminal elimination half-lives of sunitinib were 8 h in rats, 17 h in monkeys, and 51 h in humans. The majority of radioactivity was excreted to the feces with a smaller fraction of radioactivity excreted to urine in all three species. The bioavailability in female rats was close to 100%, suggesting complete absorption of sunitinib. Whole-body autoradiography suggested radioactivity was distributed throughout tissues, with the majority of radioactivity cleared within 72 h. Radioactivity was eliminated more slowly from pigmented tissues. Sunitinib was extensively metabolized in all species. Many metabolites were detected both in urine and fecal extracts. The main metabolic pathways were $N$-de-ethylation and hydroxylation of indolylidine/dimethylpyrrole. $N$-Oxidation/hydroxylation/desaturation/deamination of $N,N'$-diethylylamide and oxidative defluorination were the minor metabolic pathways. Des-ethyl metabolite M1 was the major circulating metabolite in all three species.

Introduction

Sunitinib L-malate (N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide, Sutent; Pfizer Inc., New York, NY) is an oral multitargeted tyrosine kinase inhibitor targeting platelet-derived growth factor receptors (α and β), stem cell factor receptor, vascular endothelial growth factor receptors (1, 2, and 3), and Fms-like tyrosine kinase-3 receptor (Abrams et al., 2003; Mendel et al., 2003; Murray et al., 2003; O’Farrell et al., 2003; Pfizer Inc., data on file). In the clinic, sunitinib demonstrated a significant improvement in progression-free survival compared with those receiving interferon-α (11 versus 5 months) in patients with metastatic renal-cell carcinoma (Motzer et al., 2007). For patients with imatinib-refractory gastrointestinal stromal tumor (Demetri et al., 2006), sunitinib prolonged time to tumor progression of 6.3 months for patients treated with sunitinib compared with 5 months for patients dosed with placebo. Recently, an improvement in progression-free survival with sunitinib was observed in patients with pancreatic neuroendocrine tumors: median of 11.4 months compared with 5.5 months with placebo (Raymond et al., 2011). The U.S. Food and Drug Administration and the European Commission have granted approval for the use of sunitinib in advanced renal-cell carcinoma, imatinib-refractory gastrointestinal stromal tumor, and advanced pancreatic neuroendocrine tumor. In addition, the compound is in development for the treatment of patients with other solid tumors, including neuroendocrine, breast, lung, colorectal, pancreatic, and prostate tumors (Kulke et al., 2005; Brahmer et al., 2007; Saltz et al., 2007; Zurita et al., 2007; Burstein et al., 2008; Socinski et al., 2008).

In the nonclinical species studied (mice, rats, and monkeys), sunitinib exhibited good oral pharmacokinetic and dispositional properties: linear kinetics at clinically relevant doses; rapid and extensive absorption; long half-lives; and a large volume of distribution without metabolic induction (Haznedar et al., 2009). In cancer patients (Britten et al., 2008), sunitinib displayed a long half-life and a large volume of distribution with moderate interpatient variability. In the present study, we investigated pharmacokinetics, distribution, metabolism, and excretion of sunitinib in rat, monkey, and human after a single administration of $[^{14}C]$sunitinib.

ABBREVIATIONS: HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography/tandem mass spectrometry; AUC, area under the concentration; IS, internal standard; ESI, electrospray ionization; P450, cytochrome P450; SU012487, N,N-diethyl-2-[(5-[(Z)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carbonyl]amino]ethanamine oxide; SU012662, N-[2-(ethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide; SU014335, N-[2-aminoethoxy]ethyl]-5-[(Z)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide; PHA-782584, N-[2-diethylaminoethyl]oxy]ethyl]-5-[(Z)-(5-hydroxy-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide; PHA-77473, N-[2-diethylaminoethyl]oxy]ethyl]-5-[(5-fluoro-2-oxo-indolin-3-yl)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide.
**Materials and Methods**

**Chemicals and Reagents.** \(^{[14C]}\)Sunitinib \(t\)-maleate (Fig. 1) had a specific activity of 458.8 MBq/mmol and a radiochemical purity >99% (by radio-HPLC). Sunitinib and metabolite standards \(N,N,N\)-diethyl-2-[(5\([Z]\)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1\(H\)-pyrrole-3-carboxylamino]ethanamine oxide (SU012487), \(N\)-(2-ethylaminoethyl)-[5\([Z]\)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1\(H\)-pyrrole-3-carboxamide (SU012662), \(N\)-(2-aminoethoxy)-5-[\(Z\)-(5-fluoro-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1\(H\)-pyrrole-3-carboxamide (SU014335), \(N\)-(2-diethylaminoethyl)-5-[\(Z\)-(5-hydroxy-2-oxo-indolin-3-ylidene)methyl]-2,4-dimethyl-1\(H\)-pyrrole-3-carboxamide (PHA-782584), \(N\)-(2-diethylaminoethyl)-5-[5-(5-fluoro-2-oxo-indolin-3-yl)methyl]-2,4-dimethyl-1\(H\)-pyrrole-3-carboxamide (PHA-774731), and M11 were synthesized by Pfizer. General purpose reagents and solvents were of analytical grade (or a suitable alternative), obtained principally from Carlo Erba Reagents (Val de Rueil, France) and Sigma-Aldrich S.r.l. (Milan, Italy). Ultima Gold, Phanfluor E, and Carbo-Sorb E from Canberra Industries (Meriden, CT) were used as liquid scintillation cocktails. Ultima Flo, used as scintillant for the HPLC radiodetector, was obtained from LabLogic Systems Ltd. (Brandon, FL).

All animal husbandry and in-life procedures conducted in this study complied with the Animal Welfare Act Regulations (nine Code of Federal Regulations parts 1, 2, and 3) and the Guide for Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996) and were approved by Pfizer’s Institutional Animal Care and Use Committee.

**Rat Studies.** Eight female and three male Sprague-Dawley rats were obtained from Charles River Italy (weight range 224–268 g; Calco, Italy). The rats were acclimated for 1 week before the start of the study. A single bolus intravenous administration of \(^{[14C]}\)sunitinib \(t\)-maleate was given to five female rats at a nominal dose level of 5 mg/kg (free base equivalents, 50 \(\mu\)Ci/kg) via the tail vein injection. Oral doses of \(^{[14C]}\)sunitinib were given to three female and three male rats at a nominal dose level of 15 mg/kg (free base equivalents, 50 \(\mu\)Ci/kg) by gastric gavage. Blood samples of approximately 0.5 ml were taken from rats that were canulated into the superior vena cava 1 h before dose and 1, 3, 6, 8, 24, 48, and 72 h after oral administration and 2 h before dose and 5 and 20 min and 1, 3, 6, 24, 48, and 72 h after intravenous dosing. The blood samples were transferred immediately into precooled tubes placed in an ice/water bath protected from light. An aliquot of blood was transferred to a sample vial containing 3 to 4 kg were obtained from the Centre de Rehabilitation des Primates de Lwiro (Port-Louis, Mauritius). The monkeys were acclimated for more than 40 days before start of the study. An oral dose of \(^{[14C]}\)sunitinib \(t\)-maleate was given to each animal at a nominal dose level of 6 mg/kg (37 \(\mu\)Ci/kg) by gastric gavage. Monkey blood samples of approximately 2 ml were taken from femoral vein and collected into heparinized tubes at the following time points: before dose and 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168, 240, and 336 h after dose. Preparation of monkey plasma samples was same as described for rats. Monkey urine samples were collected into weighed dry ice-cooled containers protected from light before dose; at 0 to 4, 4 to 8, and 8 to 24 h after dose; and then daily until 14 days after dose. Feces were collected into weighed containers before dose; at intervals of 0 to 8 and 8 to 24 h after dose; and then daily until 14 days after dose. The excreta samples were stored at \(-80^\circ\mathrm{C}\) until analysis.

**Human Study.** This was an open-label, single-dose, single-center, phase I study to evaluate the mass-balance and pharmacokinetics of sunitinib in healthy male subjects. All of the subjects provided written, informed consent before participation in the study. This study was conducted in compliance with the ethical principles originating in or derived from the World Medical Association Declaration of Helsinki (2000) and in compliance with the institutional review board, informed consent regulations, and International Congress of Harmonization Good Clinical Practices Guidelines and in compliance with the U.S. Food and Drug Administration regulations for informed consent and protection of patient rights. Eight subjects with a mean age of 33 years (range 20–45) and mean weight of 78.1 kg (range 64.1–92.2) were enrolled and screened for safety. Six subjects were included for pharmacokinetic and mass balance evaluation (two subjects were excluded because of possible incomplete collection of feces). All subjects were healthy given that no clinically relevant abnormalities were identified by a detailed medical history, full physical examination, 12-lead electrocardiogram, and clinical laboratory tests. On day 1, each subject received a single capsule (50 mg p.o.) of sunitinib containing approximately 100 \(\mu\)Ci of \(^{[14C]}\)sunitinib. Serial blood samples were taken before dose; at 1, 2, 4, 8, 12, 16, 24, 36, and 48 h after dose; and then daily until 21 days. Urine and feces were collected daily over 21 days or longer until two consecutive samples from urine and feces had <1% of the total administered radioactivity. Human dosimetry projections were based upon the tissue distribution data obtained from a whole-body autoradioluminography study in pigmented male and female Lister Hooded rats administered 20 mg/kg \(^{[14C]}\)sunitinib \(t\)-maleate (equivalent to 15 mg freebase/kg, 100 \(\mu\)Ci/kg). On the basis of the assumption that \(^{[14C]}\)sunitinib-associated radioactivity distributes into and is eliminated from tissues in a comparable manner in humans and Lister Hooded rats on a body weight basis (microcurie per kilogram), the projected exposure to \(^{14}\)C radioactivity for any tissue in human research subjects administered 100 \(\mu\)Ci of \(^{[14C]}\)sunitinib (or 1.43 \(\mu\)Ci/kg for a human weighing 70 kg) falls below the single dose limits of 3000 or 5000 milligrams (Loevevinger and Berman, 1968; Hendee, 1973; U.S. Food and Drug Administration, 2011).

**Radioactivity Analysis.** Duplicate aliquots of rat urine (50–1000 mg) or monkey urine (500–1000 mg), triplicate aliquots of human urine (1 ml) or human plasma (0.25 ml), and an aliquot of rat plasma (50–100 mg) were each mixed with Ultima Gold scintillation cocktail before liquid scintillation counting. Duplicate aliquots of homogenized rat or monkey feces and an aliquot of homogenized human feces (250–500 mg) were combusted, respectively, in oxygen using an Oxidizer Packard System 387 (PerkinElmer Life and Analytical Sciences, Waltham, MA). The CO\(_2\) produced in the combustion process was absorbed into Carbo-Sorb E and mixed with Permafluor E scintillant. The efficiency of the combustion process was checked by combustion of radiochemical standards (\(^{14}\)C-Spec-Chec; PerkinElmer Life and Analytical Sciences) every 10–15 samples and was between 96 and 98%. A correction factor was used accordingly. All samples prepared in scintillation fluid were subjected to liquid scintillation counting for 5 min, together with representative blank samples, using a Packard TR 2100 Liquid Scintillation Analyzer (PerkinElmer Life and Analytical Sciences) with automatic quench correction by an external method.

**Quantitative Whole-Body Autoradiography.** Five male and five female Sprague-Dawley rats and four male and four female Lister Hooded rats, age \(-7\) to 8 weeks at dosing (body weight 179–310 g), were supplied by Charles River (Margate, Kent, UK). The animals were acclimated to the experimental unit for 7 days before use on the study. The dose was administered by gastric gavage at a target dose level of 20 mg/kg (\(^{[14C]}\)sunitinib \(t\)-maleate (equivalent to 15 mg freebase/kg, 100 \(\mu\)Ci/kg). Albino rats (one male and one female) were killed by CO\(_2\) narcosis at each of the following time points: 3, 6, 24, 72, and 168 h after dose. Pigmented rats (one male and one female) were killed by CO\(_2\) at each of the following time points: 24, 72, 168, and 336 h after dose. Immediately after sacrifice, the right eye from each animal was removed, and...
the carcass was frozen by immersion in a mixture of solid CO₂ in hexane for 30 min. The frozen carcass was then embedded in a block of cellulose, which was frozen in the same way. After equilibration at approximately −20°C, sagittal sections (30 μm thick) were taken through each animal using a whole-body cryomicrotome (Leica, Wetzlar, Germany). The samples were freeze-dried before storage at ambient temperature. Duplicate aliquots of liver were removed from each frozen block after sectioning. The whole eyes and freeze-dried before storage at ambient temperature. Duplicate aliquots of liver were protein-precipitated with methanol (300 μl), sonicated, and centrifuged (this step was repeated twice). All of the supernatants were collected in one vial, and the solvent was removed under a stream of nitrogen. The residue was redissolved in 200 μl of methanol, followed by 200 μl of water; 150 μl of this solution was injected in the HPLC system (see "Radioactive Metabolite Profiling" for detail) for profiling. Monkey plasma samples collected at 2, 4, 8, and 72 h after dosing were pooled for each sex and protein-precipitated with five volumes of ice-cold methanol. After centrifugation at 14,000 rpm, the supernatant was evaporated under a stream of nitrogen. The residues were redissolved in a mixed solvent consisting of 10 mM ammonium formate aqueous solution at pH 4 and acetonitrile (95.5 by volume) and injected into the HPLC system for profiling. An equal volume (5 ml) of each human plasma sample collected at 1, 2, 4, 8, 12, 16, 24, 36, and 48 h after dose from each subject was pooled to generate a plasma pool. The levels of radioactivity in plasma samples beyond 48 h were too low to allow for meaningful profiling of metabolites. Each pooled sample was precipitated by the addition of 4 volumes of acetonitrile/methanol mixture (1:1 v/v), vortexed, and centrifuged. The supernatant was removed and retained. The pellet was re-extracted as above, and the supernatants were combined and transferred into glass tubes for evaporation to dryness under nitrogen at 40°C. The residues were reconstituted in 1 ml of 30:70 (v/v) methanol/20 mM ammonium acetate, pH 4, and 900 μl was injected into the HPLC system for profiling.

Representative rat urine samples with radioactivity levels corresponding to 4 to 5% recovery of dose were analyzed by injection of neat samples (200 μl). Because monkey urine 8 to 24 h after dose contained the largest proportion of the dose excreted in urine, these were chosen for analysis. Urine (5.5 ml) was basified by the addition of 275 μl of 25% ammonia solution. An aliquot (5 ml) of the basified urine was loaded on an solid-phase extraction column (Oasis hydrophilic-lipophilic-balanced 6 ml, 0.2-g phase; Waters, Milford, MA) previously conditioned with 5 ml of methanol and equilibrated with water containing 5% of ammonia solution. After washing with 5 ml of water, the retained radioactivity was eluted with methanol (2 × 4 ml). The elution phase
was evaporated under a stream of nitrogen at 40°C. The residues were redissolved in a mixture (550 μl) of 10 mM ammonium formate at pH 4 and acetonitrile (95:5 by volume), and 450 μl of this solution was injected onto the HPLC system for metabolite profiling and identification. Human urine samples collected at 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, and 168 to 192 h after dosing were pooled on a percentage weight basis to generate a urine pool (10 ml). Each pooled sample was mixed with acetonitrile (1:1 v/v), vortexed, and centrifuged. The supernatants were transferred into glass tubes for evaporation to dryness under nitrogen at 40°C. The residues were reconstituted in 1 ml of 30:70 (v/v) methanol/20 mM ammonium acetate, pH 4, and 900 μl was injected into the HPLC system for profiling.

Aliquots (1 g) of the homogenized rat or monkey fecal samples containing significant levels of radioactivity were accurately weighed into centrifuge tubes. To remove fats, each sample was extracted twice with 2 ml of n-hexane, sonicated for 15 min, and centrifuged for 20 min at 20,000 rpm. The hexane extract was verified to be free of radioactivity and discarded. Three milliliters of methanol then were added to each sample, sonicated for 15 min, and centrifuged at 20,000 rpm for 20 min. This step was repeated an additional three times, and the supernatants were combined. The supernatants from the extractions were then evaporated under a stream of nitrogen at 40°C. The residues were redissolved in 600 μl of methanol. Of the redissolved extracts, 150 μl was diluted 1:1 with water, and 200 μl of the resulting solution was injected in the HPLC system for profiling. The supernatants from the extractions were then evaporated under a stream of nitrogen at 40°C. The residues were redissolved in 600 μl of methanol. Of the redissolved extracts, 150 μl was diluted 1:1 with water, and 200 μl of the resulting solution was injected in the HPLC system for profiling. The supernatants from the extractions were then evaporated under a stream of nitrogen at 40°C. The residues were redissolved in 600 μl of methanol. Of the redissolved extracts, 150 μl was diluted 1:1 with water, and 200 μl of the resulting solution was injected in the HPLC system for profiling.

Because no data were collected for the extraction and HPLC recoveries of radioactivity from the samples, we assumed either 100% extraction and HPLC recoveries of radioactivity or equal efficiency of extraction and HPLC recoveries for the parent drug and its metabolites.

FIG. 3. Plasma concentration-time profiles of total radioactivity, sunitinib, and M1 after a single oral administration of 6 mg/kg [14C]sunitinib to monkeys. A, female. B, male. The plasma levels of radioactivity are expressed as nanogram equivalents per milliliter.

FIG. 4. Plasma concentration-time profiles of total radioactivity, sunitinib, and M1 after a single oral administration of 50 mg of [14C]sunitinib to human males. The plasma levels of radioactivity are expressed as nanogram equivalents per milliliter.
Determination of Concentrations of Sunitinib and Its Desethyl Metabolite (M1, SU012662) in Plasma. Aliquots of rat or monkey plasma (50 μl) were extracted with 500 μl of methanol containing 2.42 nM [14C]sunitinib as internal standard (IS) in a 96-well polypropylene dual membrane microplate. The methanolic phase was filtered and collected into a second 96-well plate through a vacuum. The organic phase was dried under nitrogen at 37°C, and the residue was reconstituted with 200 μl of 15 mM ammonium formate, pH 3.25. After vortex mixing for 15 s, the samples were centrifuged at 4000 rpm for 10 min. Aliquots of 20 μl of the resulting solution were injected into the LC-MS/MS system. A Symmetry Shield C8 column (2.1 x 3.0 mm; Phenomenex, Torrance, CA) was used to perform the chromatographic analysis. The mobile phase was ammonium formate buffer solution (15 mM, pH 3.25), acetonitrile (75:25, v/v) with a flow rate of 0.35 ml/min. Retention times of M1 and sunitinib were approximately 2.5 and 4 min, respectively. Total cycle time was 5 min.

For MS detection with electrospray ionization (ESI) in a positive mode (LCQ-Deca Tetra, RAM; In-Us Systems, Inc., Tampa, FL, for animal sample analysis) and (0.069 – 241 ng/ml) for sunitinib and 0.178 and 595 nM (0.066 – 220 ng/ml) for M1. Higher concentrations could be analyzed after appropriate dilution of samples.

Radioactive Metabolite Profiling. All radioactivity profiling and metabolite structure elucidation were performed using HPLC (Agilent 1100; Agilent Technologies, Santa Clara, CA; column: YMC J’s ODS-H80, 5-μm, 150 × 4.6 mm; guard cartridge C18, 4.0 × 3.0 mm; Phenomenex, Torrance, CA) coupled in-line with radiochemical detection (ARC StopFlow System Model C; AIM Research Company, Newark, DE, for human sample analysis; model 3 β-RAM; In-Us Systems, Inc., Tampa, FL, for animal sample analysis) and MS detection with electrospray ionization (ESI) in a positive mode (LCQ-Deca XP; Thermo Fisher Scientific for human sample analysis; and Micromass Q-Tof II; Waters for animal sample analysis). The mobile phase consisted of 10 mM ammonium formate, pH 4.0/acetoniitrite (95:5, v/v) as solvent A and 10 mM ammonium formate, pH 4.0/acetoniitrite (5:95, v/v) as solvent B. The flow rate was maintained at 1.0 ml/min. The column temperature was maintained at 40°C. The drug and metabolites were eluted using a linear gradient in mobile phase composition summarized as follows: equilibration for 2 min at 0% B, linear gradient to 5% B from 2 to 3 min, linear gradient to 10% B from 3 to 5 min, linear gradient to 15% B from 5 to 18 min, linear gradient to 25% B from 18 to 33 min, held at 25% B from 33 to 39 min, linear gradient to 100% B from 39 to 40 min, held at 100% B from 40 to 50 min, linear gradient to 0% B from 50 to 51 min, held at 0% B from 51 to 60 min.

When MS was used, the HPLC effluent was split so that 20% of the flow was introduced into the mass spectrometer via the ESI source, whereas 80% was diverted to the β-RAM detector. When MS was not used, the total HPLC effluent was introduced into the β-RAM detector. The analog outputs from the ARC/β-RAM and MS detectors were recorded in real time by the ARC data system version 2.4 (AIM Research Company, Newark, DE). The major operating parameters for the ion-trap ESI-MS methods were as follows: spray voltage 5.0 kV; capillary voltage 5.0 V; tube lens offset 55 V; capillary temperature 200°C; sheath gas flow rate 80 (arbitrary); and auxiliary gas flow rate 20 (arbitrary). LC-MS spectra were acquired over a mass range of m/z 200 to 1400 for all samples in an ion-trap mass spectrometer. Ion-trap LC-MS* (n = 2–4) experiments were performed to generate multistage mass spectra for selected molecular ions representing possible metabolites of sunitinib. At a constant pressure of 40 psi, helium was used as the damping and collision gas for all MS* experiments. Precursor isolation window, activation window, activation Q, and activation time were set at 1.8 atomic mass units, 30 to 50%, and 0.25 and 30 ms, respectively. Additional accurate mass data were obtained using a Micromass Q-Tof II spectrometer, which was manually tuned in the positive ion mode. A 100 μM solution (50:50/solvent A:B) of a freshly prepared sunitinib standard was infused (~100 μM, ~10 μl/min) into the spectrometer using an ESI ion source. Optimal tune parameters were as follows: capillary 3 kV; cone voltage 25 V; extractor 6 V; source 150°C; desolvation 150°C. The scan range was set from 100 to 1000. To obtain accurate mass measurements, the TOF was calibrated daily using the known accurate masses for the fragment ions of [Glu1]fibrinopeptide B in the selected range.

### Table 2

Pharmacokinetic parameters obtained in female Sprague-Dawley rats after an administration of 5 mg/kg i.v. and in male and female Sprague-Dawley rats after a single administration of 15 mg/kg p.o. [14C]sunitinib

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female (i.v.)</th>
<th></th>
<th></th>
<th>Female (p.o.)</th>
<th></th>
<th></th>
<th>Male (p.o.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Radioactivity</td>
<td>Sunitinib</td>
<td>M1</td>
<td>Radioactivity</td>
<td>Sunitinib</td>
<td>M1</td>
<td>Radioactivity</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>2148 ± 642</td>
<td>1823 ± 551</td>
<td>450 ± 174</td>
<td>2926 ± 570</td>
<td>1253 ± 87</td>
<td>1412 ± 392</td>
<td>5220 ± 958</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>NA</td>
<td>3.0 ± 0</td>
<td>6.0 ± 3</td>
<td>4.0 ± 2.0</td>
<td>7.0 ± 1.0</td>
<td>5.0 ± 3.0</td>
<td>3.0 ± 0</td>
</tr>
<tr>
<td>AUC0-t (ng × h/ml)</td>
<td>17,427 ± 178</td>
<td>7202 ± 802</td>
<td>6724 ± 1620</td>
<td>69,949 ± 22,358</td>
<td>23,994 ± 4726</td>
<td>37,225 ± 13,979</td>
<td>82,039 ± 20,986</td>
</tr>
<tr>
<td>Cl/F (ml/h kg)</td>
<td>10.3 ± 0.9</td>
<td>7.4 ± 0.3</td>
<td>8.4 ± 1.0</td>
<td>9 ± 0.9</td>
<td>7.2 ± 0.4</td>
<td>9.0 ± 1.1</td>
<td>9.0 ± 0.5</td>
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<tr>
<td>Vss (ml/kg)</td>
<td>NA</td>
<td>3361 ± 940</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Table 3

Pharmacokinetic parameters obtained in humans after a single oral administration of 50 mg of [14C]sunitinib and in male and female Cynomolgus monkeys after a single oral administration of 6 mg/kg [14C]sunitinib

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Human</th>
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<th>Female Monkey</th>
<th></th>
<th></th>
<th>Male Monkey</th>
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<tbody>
<tr>
<td></td>
<td>Radioactivity No.*</td>
<td>Sunitinib</td>
<td>M1</td>
<td>Radioactivity</td>
<td>Sunitinib</td>
<td>M1</td>
<td>Radioactivity</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>674 ± 19.4</td>
<td>24.4 ± 4.0</td>
<td>6.2 ± 1.8</td>
<td>214 ± 26</td>
<td>83 ± 12</td>
<td>54 ± 5</td>
<td>243 ± 13</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>8.0 ± 0</td>
<td>8.0 ± 0</td>
<td>6.7 ± 3.3</td>
<td>4.0 ± 0</td>
<td>4.0 ± 0</td>
<td>7.0 ± 2.0</td>
<td>8.0 ± 0</td>
</tr>
<tr>
<td>AUC0-t (ng × h/ml)</td>
<td>2537.1 ± 858.4</td>
<td>1063 ± 262</td>
<td>592.6 ± 91.4</td>
<td>5692 ± 1151</td>
<td>1099 ± 87</td>
<td>1272 ± 137</td>
<td>9095 ± 432</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>28.2 ± 13.2</td>
<td>50.9 ± 6.7</td>
<td>93.2 ± 15.4</td>
<td>121 ± 2.0</td>
<td>16.0</td>
<td>19.0 ± 6.0</td>
<td>222.0 ± 40.0</td>
</tr>
<tr>
<td>CL/F (lh/kg)</td>
<td>25.3 ± 14.2</td>
<td>49.9 ± 14.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Calculated from data up to 24 h after dose.

NA, not applicable; CL, clearance; Vss, volume of distribution at steady state.
solution of warfarin (50 μg/ml in 1:1 solvent A:B, m/z 309.1127) was infused into the reference source of the LockSpray at a rate of 2 μl/min, and a reference scan was obtained every 2 s. All of the data were acquired in centroid mode to minimize the size of the data files. Samples were analyzed in MS mode, and the data were searched for the accurate masses of possible metabolites.

Pharmacokinetic Analysis. Pharmacokinetic calculations were performed using a noncompartmental approach (linear trapezoidal rule for AUC calculation and linear regression of natural log-transformed plasma concentration versus time data) with the aid of WinNonLin (Pharsight, Mountain View, CA). Maximal concentrations (Cmax) and the time of occurrence of maximal concentrations (tmax) were read directly from the raw data. The half-life of the terminal decay phase, t1/2, was determined by linear regression analysis of the natural-log concentration versus time curve, where t1/2 = ln(2)/slope of the regression line. The choice of the number of points in the terminal phase

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<thead>
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<th>Tissue</th>
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—, Not detected.
was based on visual inspection of the data; the regression range was recorded and reported together with the half-life. The area under the plasma concentration versus time curve, AUC_{0,\infty}, was determined by the linear trapezoidal rule up to the last detectable concentration C_{last} at time t_{last}, and denoted as AUC_{0,\text{last}}. Beyond this time, the AUC_{0,\text{last}} was determined by extrapolation from the observed C_{last} assuming monoexponential decline and using the formula:

\[ \text{AUC}_{(0-\infty)} = \text{AUC}_{(0-\text{last})} + C_{\text{last}} \times \frac{t_{1/2,z}}{\ln(2)} \]

Results

Excretion of Radioactivity. After single oral nominal dosing of 15 mg/kg (free base equivalents) of [14C]sunitinib to rats, the amount of radioactivity eliminated in urine and feces in the period 0 to 72 h after oral dosing accounted for 9.3 and 71.1% of the dose, respectively, in female rats and 8.5 and 75.2% of the dose, respectively, in male rats (Table 1). After intravenous administration to female rats, the radioactivity in urine and feces was 9.1 and 77.2% of the dose. In all treatments, mean total recovery of radioactivity, including cage washings within 72 h, was in the range of 82 to 87%. The radioactivity in expired air was less than 0.1% after intravenous administration to female rats, thus demonstrating that the carbon-14 was on a metabolically stable position of the molecule. More than 70% of total radioactivity was recovered in the feces after intravenous or oral administration, indicating biliary clearance as the major excretion route of the compound in rats.

After a single oral administration of [14C]sunitinib to male and female Cynomolgus monkeys at a nominal dose of 6 mg/kg, the amount of radioactivity eliminated in urine and feces over the period of 0 to 336 h accounted for 4.8 and 87.3% of the dose, respectively, in male monkeys and 6.1 and 84.1% of the dose, respectively, in female monkeys (Table 1). In all animals, mean total recovery of radioactivity, including cage washings within 336 h, was in the range 91 to 94%. More than 84% of total radioactivity was recovered in the feces after oral administration to both sexes.

After a single oral administration of [14C]sunitinib to human subjects at a nominal dose of 50 mg, the mean cumulative radioactivity (% dose) recovered in both feces and urine over 504 h was 77.0% for the six evaluable subjects. Fecal recovery accounted for 61.0% of the administered dose, whereas urinary recovery accounted for 16% of the dose (Table 1). The major portion of the recovery occurred in the first 168 h; total, fecal, and urinary recoveries were 61.4, 48.2, and 13.2%, respectively.

Plasma Pharmacokinetics of Total Radioactivity, Sunitinib, and M1 Metabolite. Mean plasma concentration-time profiles of total radioactivity, sunitinib, and M1 after intravenous and oral dosing of sunitinib in rats and oral dosing in Cynomolgus monkeys and humans are shown in Figs. 2 through 4. The pharmacokinetic parameters of total radioactivity, sunitinib, and M1 for rats, monkeys, and humans are summarized in Tables 2 and 3, respectively.

After intravenous administration of [14C]sunitinib to female rats, the plasma clearance of sunitinib was low, accounting for ~16% of the hepatic blood flow, the volume of distribution was high, and the average apparent terminal half-life was 7.4 h. The metabolite M1 reached maximal plasma concentrations at 3 h after dosing and then declined with a terminal half-life (8.4 h) similar to that calculated for the parent compound. The plasma levels of total radioactivity showed maximal values just after the administration and then declined more slowly compared with sunitinib and M1. Parent compound and its main metabolite M1 accounted for 80% of the total radioactivity. After oral administration of [14C]sunitinib to female and male rats, plasma levels of sunitinib achieved the maximal concentration at

### Table 5

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of total radioactivity (μg freebase equivalent per g tissue) in female rat tissues following a single oral administration of [14C]sunitinib</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
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<td>Spinal cord</td>
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<tr>
<td>Uveal</td>
<td>5.07</td>
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—, Not detected.
approximately 3 to 4 h after dosing and then declined with a terminal half-life similar to that observed after intravenous administration (Fig. 2). The bioavailability in females was close to 100%. The plasma levels of total radioactivity reached their maximal values at 5 to 6 h after dosing and then seemed to decline with the same apparent terminal half-life as the parent compound and its main metabolite, M1. After both intravenous and oral administration, the metabolite M1 showed a similar terminal half-life as the parent compound, suggesting that its disposition is formation-rate limited. In male rats, plasma AUC ratio of M1/sunitinib was 6, whereas the ratio in females was 1.5, suggesting that the pharmacokinetics in rats were sex-dependent and that occurrence of first-pass formation of M1 in males was more extensive than in females. Approximately 90% of plasma total radioactivity was associated with sunitinib and M1.

After oral administration of \([1^4C]\)sunitinib to Cynomolgus monkeys, plasma levels reached, on average, maximal concentrations at 5 and 4 h after dosing in males and females, respectively (Fig. 3). The mean terminal half-life of the parent compound was 18 and 16 h in males and females, respectively. In both genders, the metabolite M1 reached maximal concentrations later than the parent compound and then declined with an apparent terminal half-life similar to that calculated for the parent compound. The plasma levels of radioactivity reached their maximal values between 4 and 8 h after dosing and then declined slowly and had a correspondingly long half-life (122–222 h). Sunitinib and M1 accounted for 31 and 42% of total plasma radioactivity in male and female monkeys, respectively.

After oral dosing with 50 mg of \([1^4C]\)sunitinib to human subjects, plasma levels of total radioactivity peaked at approximately 8 h and were undetectable after 36-h postdosing (Fig. 4). Maximal plasma sunitinib and M1 concentrations were observed at approximately 8 and 6 h after dose, respectively. After that, both sunitinib and M1 declined slowly with a long half-life (50.9 h for sunitinib and 93.2 for M1). In contrast to total radioactivity, sunitinib and M1 were quantifiable by LC-MS/MS analysis in plasma up to 288 to 480 h after dose. Because of the large differences in the time when levels of plasma radioactivity, sunitinib, and M1 fell below their respective lower limits of quantitation, a meaningful comparison of AUC of total radioactivity and of sunitinib and M1 in human subjects could not be made.

**Tissue Distribution of Radioactivity in Rats.** After oral administration of \([1^4C]\)sunitinib to male and female albino and pigmented rats, radioactivity was distributed throughout multiple tissues (Fig. 5). The absorption, distribution, and elimination patterns between the sexes and strains were very similar, although female rats generally had higher concentrations of radioactivity at each time point compared with the males (Tables 4 and 5). After peak concentrations were observed at the first time point after dose (3 h for albino animals and 24 h for pigmented animals), the radioactivity was eliminated so that by 72 h after dose, the radioactivity in the majority of tissues was below the limit of quantitation (0.04–0.06 \(\mu g\) freebase equivalent/g tissue). Exceptions to this in both males and females were the radioactivity levels in the pigmented tissues, eye, and uveal tract. In both sexes of the pigmented groups, the radioactivity levels in the whole
eye were still markedly elevated at 336 h after dose in comparison with other tissues. Likewise, in both groups, radioactivity levels in the uveal tract increased over the course of the study. There seemed to be some differences between sexes in that the observed peak uveal tract concentrations in pigmented male rats were at 72 h after dose compared with 336 h in the females. The actual concentrations of radioactivity in the eye and uveal tract at each time point were higher in the uveal tract increased over the course of the study. There seemed to be no obvious sex difference in radioactivity in the brain and spinal cord. In both albino and pigmented male rats, the concentrations of radioactivity in the testis were relatively high at each measured time point.

Metabolite Profiling. A typical radio-HPLC profile of pooled rat plasma samples from 3 and 6 h after dose is shown in Fig. 6B. The parent and des-ethyl metabolite M1 were clearly present, representing 14.4 and 83.9% plasma radioactivity, respectively, in male and 34.3 and 53.2% plasma radioactivity, respectively, in female based on AUCs of sunitinib, M1, and total radioactivity (Table 2). The presence of other metabolites could not be clearly determined because of low radioactivity. The radio-HPLC profile of an 8-h plasma sample of male monkey is shown in Fig. 6A. No differences were observed between the metabolite profiles of male and female monkeys. The parent and M1 were the major components in the 2-, 4-, and 8-h samples; small amounts of other metabolites were also detected, most notably, the carboxylic acid (M11) and a metabolite putatively identified as being hydroxylated on the ethyl function (M2F, identified based on MS data but not associated with measurable radioactivity). No metabolites could be detected at 72 h because of low levels of radioactivity. Sunitinib and M1 represented 16.0 and 15.2% plasma radioactivity, respectively, in male monkeys and 9.3 and 22.3% plasma radioactivity, respectively, in female monkeys based on AUCs of sunitinib, M1, and total radioactivity (Table 3). In pooled human plasma, only two components were quantifiable by radiochemical detection corresponding to unchanged parent and M1 (Fig. 6C).

A summary of metabolite abundance in urine samples across the species expressed as percentage of administered dose is shown in Table 6, and the urinary metabolite profiles are shown in Fig. 7. The parent compound was detected in all of the rat urine samples analyzed, accounting for ~0.6 and 0.3% of dose after oral and intravenous administration, respectively. The major metabolites detected in rat urine were M1 and M2E, corresponding to ~1.2 and 0.8% dose, respectively. Combined M7A/B/C and combined M8/9 each accounted for 0.6% dose. There was no obvious sex difference in metabolite profiles in the rats. In monkey urine samples, the parent compound accounted for ~0.2% dose in both male and female animals. M1 was the major metabolite present in the monkey urine corresponding to ~1.3% of the dose. A number of unidentified metabolites were also present corresponding to ~1.4% dose. In the pooled human urine, parent drug and the metabolite M1 also represent the major radioactive components accounting for 6.4 and 6.9% dose, respectively.

A summary of metabolite abundance in fecal samples expressed as percentage of radioactivity is shown in Table 6, and the fecal metabolite profiles are shown in Fig. 8. The majority of the fecal radioactivity in rat was recovered between 8 and 48 h after dose; therefore, only 24- and 48-h rat fecal samples were profiled. In both 24- and 48-h rat fecal samples after intravenous administration, the parent compound accounted for 22.6% dose, whereas after oral administration, it accounted for 11.5% dose in females and only 5.8% dose in males. In all samples, the major metabolite was M1, accounting for 18.5% dose in the analyzed intravenous samples, and 32.1 and 21.0% dose in male and female oral samples, respectively. Four radioactive peaks were found in rat fecal samples corresponding to isomers of monohydroxylated metabolites of sunitinib (M2A, B, C, and D) for an average total amount of 9.4% dose. M2D is particularly abundant in male rats, accounting alone for ~6.5% dose. M8/9 and M6 were minor rat fecal metabolites accounting for 4.6 and 2.2% dose, respectively. The fecal metabolite profiles were similar from a qualitative and quantitative point of view both in male and female monkeys and also at the different time intervals investigated. The parent compound accounted for 9% dose. The major fecal metabolite were the M1 and M1iso (E-isomer of the exocyclic double bond of M1), accounting for 30% dose. The E/Z-isomerization of M1 was not observed in rat and human fecal samples. The exocyclic double bond (Z-isomer) in sunitinib and other analogs of sunitinib has been reported to undergo isomerization to form E-isomer when exposed to light (Sistla et al., 2006). Although all of the sample handling was done protected from direct light, E/Z-isomerization of the exocyclic double bond was observed for M1 in monkey feces (M1iso in Fig. 8A). M11 and M8 were two other major radioactive peaks in monkey feces, each representing 6.2% dose. Four peaks were found corresponding to isomers of monohydroxylated metabolites of the parent (M2A, B, C, and D) for an average combined amount of 12.5% dose. M2A was particularly abundant both in males and females accounting alone for ~7.7% dose. Minor monkey fecal metabolites included M2E (2.9% dose), M4A (1.1% dose), and an unknown metabolite (2.9% dose). In the pooled human fecal homogenates, four radioactive components were quantifiable, including two major components, the metabolite M1 (25.0% dose) and the parent drug (13.6% dose), as well as two minor metabolites, M2D (3.7% dose) and an unknown metabolite (5.8% dose). These minor metabolites were not detectable in human plasma samples. The unknown metabolite.

<table>
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<th>Rat</th>
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<th>Human</th>
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<tr>
<td></td>
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</tr>
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<td>3.8</td>
<td>—</td>
</tr>
<tr>
<td>M3</td>
<td>1.7</td>
<td>—</td>
<td>1.1</td>
</tr>
<tr>
<td>M4E</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M2D</td>
<td>0.7</td>
<td>6.5</td>
<td>0.4</td>
</tr>
<tr>
<td>M10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M5A/B</td>
<td>0.9</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>M1</td>
<td>32.1</td>
<td>21.0</td>
<td>18.5</td>
</tr>
<tr>
<td>M1L</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>11.5</td>
<td>5.8</td>
<td>22.6</td>
</tr>
<tr>
<td>UK</td>
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<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
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<td>—</td>
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</tr>
<tr>
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<tr>
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<tr>
<td>M1</td>
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<td>0.5</td>
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<tr>
<td>Sunitinib</td>
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<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>M2E</td>
<td>1.5</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>M7A/B/C</td>
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<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>M8/9</td>
<td>0.5</td>
<td>0.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

UK, unknown metabolite. |
—, Not detected. |
* Sum of M1 and M1iso (E-isomer of the exocyclic double bond of M1).
identified in human feces seemed to be the same unknown metabolite observed in monkeys (2.9% dose) based on the comparison of chromatographic retention times. Several other minor metabolites were also observed in excreta: M3, M5A/B, M4A/C/D/E, and M10.

**Metabolite Identification.** The identity of metabolites for which cold standards were available (M2E, SU012487; M1, SU012662; M3, SU014335; M8, PHA-782584; and M9, PHA-774731; and M11) were confirmed by comparing their chromatographic retention times and MS/MS spectra with those of the standards spiked in a blank fecal extract. The identity of metabolites for which standards were not available was proposed based on their accurate mass with a mass accuracy of 5 to 10 ppm. MS-MS fragmentation analysis was also performed to verify that peaks were drug related and to obtain structural information from the fragment ions. The structures and MS fragment ions of sunitinib and its metabolites are compiled in Table 7.

Two fragment ions at $m/z$ 283 and 326 were generated from sunitinib ($m/z$ 399) via cleavage of the aliphatic amide bond and loss of the diethylamine moiety, respectively. M1 gave a molecular ion at $m/z$ 371.1883 (1.3 ppm mass deviation of a calculated mass), 28 Da less than sunitinib, indicating loss of an ethyl group, and consistent with $N$-dealkylation. The same fragment ions at $m/z$ 283 and 326 observed in parent were also found in product ions of M1.

M2A, B, C, and D all had the same molecular ion at $m/z$ 415.2067, consistent with a series of mono-oxygenated metabolites. All four oxygenated metabolites gave the same product ion spectra. The fragment ions of M2 metabolites at $m/z$ 342 and 299 were 16 Da higher than the corresponding fragment ions of sunitinib, indicating the oxidation on indolylidene/dimethylpyrrole moiety. M2E also had a molecular ion at $m/z$ 415.2067, 16 Da higher than that of sunitinib; however, it fragmented to two ions at $m/z$ 326 and 283, suggesting that oxidation occurred at the diethylamine group. M2E was proposed to be an $N$-oxide and was confirmed with a synthetic standard (SU012487). M2E was a major metabolite in rat urine and monkey feces, representing approximately 1 and 3% of the dose, respectively. However, it was not detected in human excreta. M2F was another mono-oxygenated metabolite with a molecular ion at $m/z$ 415. Upon fragmentation of the molecular ion, two fragment ions at $m/z$ 326 and 283 were formed, suggesting that oxidation occurred at the diethylamine group. Because α-hydroxyl structure (carbinolamine) would be unstable and spontaneously decompose to M1, we propose that the site of oxidation is on the terminal carbon of the ethyl group.

M3 was a minor metabolite excreted into urine and feces. It gave a molecular ion at $m/z$ 343, 28 Da less than that of M1, indicating further $N$-dealkylation from M1. The existence of fragment ions at $m/z$ 326 and 283 confirmed that M3 was the bis-desethyl metabolite.
M4A, C, and D were all minor metabolites observed in feces. All three metabolites showed a protonated molecular ion at $m/z$ 387.1832, 16 Da higher than that of M1, suggesting a mono-oxygenated metabolite of M1 (1.3 ppm from the theoretical mass). The presence of fragment ions at $m/z$ 342 and 299 indicated oxidation on the indolylidene/dimethylpyrrole moiety in M4, but the exact position of the oxidations could not be determined based on MS data alone. M4E gave an identical molecular ion to M4A, C, and D; however, the major MS/MS fragment ion was 16 Da lower at $m/z$ 283 with another ion at $m/z$ 324, suggesting oxidation to the amide nitrogen with subsequent dehydration to form the 324 ion (see Table 7 for proposed structure). If hydroxyl group is to the amide nitrogen, the carbinolamine would decompose spontaneously to cleave the diethyl amine. Therefore, we propose that carbon to the amide nitrogen was hydroxylated.

Minor metabolites M5A and B were excreted in both rat urine and feces. Both metabolites gave identical MS spectra. The molecular ion at $m/z$ 495.1713 (80 Da higher than that of M2) was 1.0 ppm from the theoretical value of a sulfate of a hydroxylated metabolite. After loss of sulfate (−80), M5 further fragmentated to ions at $m/z$ 341 and 299, similar to that of M2. Thus, M5A and B were identified as sulfate conjugates of mono-oxidized metabolites M2.

M6 was a minor metabolite detected in rat and monkey feces. It had a molecular ion at $m/z$ 575.2517 (0.9 ppm from the theoretical value of parent glucuronide), 176 Da higher than that of sunitinib, consistent with a sunitinib glucuronide. The fragment ions of M6 at $m/z$ 502 and 459 were 176 Da higher than the corresponding fragment ions of sunitinib, indicating the glucuronidation occurred on a nitrogen atom of indolylidene/dimethylpyrrole moiety.

M8 was a major metabolite in rat excreta and monkey feces. The molecular ion of M8 at $m/z$ 397.2239 was 2 Da less than that of sunitinib, and the fragment ions of M8 at $m/z$ 324 and 281 were also 2 Da less than the corresponding fragment ions of sunitinib, indicating a desaturation metabolite of sunitinib. However, the accurate mass was 51 ppm from the mass corresponding to the loss of two hydrogen atoms. Instead, it was 1.2 ppm from the theoretical value of an oxidative des-fluoro metabolite of sunitinib. The structure of M8 is illustrated in Table 7 and was confirmed with a synthetic standard (PHA-782584). This metabolic transformation has previously been reported in Park et al. (2001).

M9 was a minor metabolite excreted in rat and monkey feces. The molecular ion of M9 at $m/z$ 401 was 2 Da higher than that of sunitinib, and the fragment ions of M9 at $m/z$ 328 and 285 were also 2 Da higher than the corresponding fragment ions of sunitinib, indicating a saturation metabolite of sunitinib. The structure of a saturated exocyclic double-bond in M8 was confirmed with a synthetic standard (PHA-774731).

Metabolite M10 was a minor metabolite only observed in monkey feces. It had a molecular ion at $m/z$ 397.2040, consistent with a loss of two hydrogen atoms resulting from desaturation of sunitinib (1.5 ppm from theoretical value). The presence of fragment ions at $m/z$ 326 and 283 suggested that one of the terminal ethyl groups was desaturated, possibly resulting from oxidation and subsequent dehydration.

Metabolite M11 was a major radioactive component in monkey urine and feces. The molecular ion ($m/z$ 358.1203) corresponds to
a loss of 41 Da from sunitinib and was initially identified from its accurate mass and MS/MS fragmentation as a carboxylic acid resulting from loss of the diethylamine function (accurate mass within 1.5 ppm from theoretical value). A fragment ion at m/z 283, but no ion at m/z 326, indicated a modification of the diethylamine moiety. The identification was further confirmed by the increase in response obtained for this metabolite, compared with parent, when analyzed in negative ion mode in comparison to positive ion mode. The retention time of this metabolite was also highly influenced by small changes in the pH of the buffer and eluted either slightly earlier, slightly later or, in some cases, coeluted with the peak of sunitinib. The identification was confirmed by comparison with a synthetic standard of M11. The proposed metabolic scheme of sunitinib in humans and animals is shown in Fig. 9.

**Discussion**

The disposition and metabolism of sunitinib were investigated in rats, monkeys, and humans. After single oral administration of [14C]sunitinib, the

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Structure</th>
<th>[M + H]⁺</th>
<th>Fragment Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunitinib</td>
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<td>326, 283, 255, 238</td>
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<tr>
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<td>326, 283, 255, 238</td>
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<td>M2A,B,C,D</td>
<td><img src="image" alt="Structure" /></td>
<td>415.2067</td>
<td>342, 299, 271, 254, 205</td>
</tr>
<tr>
<td>M2E</td>
<td><img src="image" alt="Structure" /></td>
<td>415.2067</td>
<td>326, 283</td>
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TABLE 7—Continued

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Structure</th>
<th>[M + H]$^+$</th>
<th>Fragment Ions</th>
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<td>326, 283, 255</td>
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<tr>
<td>M3</td>
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<td>326, 283</td>
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<td>M4A/C/D</td>
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<tr>
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<tr>
<td>Metabolite</td>
<td>Structure</td>
<td>([M + H]^+)</td>
<td>Fragment Ions</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
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<td><img src="image" alt="M6 Structure" /></td>
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<td>502, 459</td>
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<tr>
<td>M7A/B/C</td>
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<tr>
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<td>324, 281, 253, 236</td>
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<tr>
<td>M9</td>
<td><img src="image" alt="M9 Structure" /></td>
<td>401</td>
<td>328, 285, 177, 134</td>
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<tr>
<td>M10</td>
<td><img src="image" alt="M10 Structure" /></td>
<td>397.2040</td>
<td>326, 283, 255, 238</td>
</tr>
</tbody>
</table>
The majority of radioactivity was excreted in the feces with a smaller fraction of radioactivity excreted in urine in all three species. More than 70% total radioactivity was recovered in the feces after intravenous and oral administration to both female and male rats, indicating bile as the major excretion route of sunitinib in rats. The bioavailability in female rats was close to 100%, suggesting complete absorption of sunitinib.

**Table 7—Continued**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Structure</th>
<th>[M + H]$^+$</th>
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<td>283, 255, 238</td>
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</table>

*a* No information on the fragment ions of M7A/B/C.

![Proposed metabolic scheme of sunitinib](image)
From whole-body autoradiography studies, it was determined that radioactivity was distributed throughout tissues after oral administration of [14C]-sunitinib to male and female albino and pigmented rats. There was minimal sex difference in the distribution and elimination of radioactivity in male and female albino rats, although the actual concentrations were generally higher at each time point in the females. Maximal concentrations occurred at the first time point (3 h) in the majority of tissues, and by 72 h in both male and female rats, the concentrations were below the limit of detection in the majority of tissues. Concentrations of radioactivity in most tissues of pigmented rats decreased with time between 24 and 336 h and were below the limit of detection by 72 h. However, radioactivity was eliminated more slowly from pigmented tissues (eye and uveal tract), such that they were still markedly elevated at 336 h after dose in comparison with the remaining tissues. These results indicate a high degree of melanin-associated binding of radioactivity in both male and female rats, concomitant with an extended half-life of elimination, which occurs beguinly with many basic drugs (Zane et al., 1990). There were no toxicological concerns related to pigment binding discovered during preclinical testing, and likewise, there have been no pigment-binding related toxicological impacts observed in patients to date. In all groups, there was little evidence of transfer of radioactivity to the brain and spinal cord in rats. However, the extent of distribution into central nervous system appeared to vary among the species (Patyna and Peng, 2006). In mice, concentrations of sunitinib were higher in brain (7-fold) than in plasma. In monkeys, brain concentrations of sunitinib and SU12662 were similar to the concentrations in plasma.

Plasma levels of sunitinib and its desethyl metabolite (M1) were assayed using a fully validated LC-MS/MS method. Total plasma radioactivity was also measured. In female rats, the parent compound showed low clearance, high volume of distribution, and an apparent plasma half-life of approximately 7 h after intravenous administration of sunitinib. A similar terminal half-life was observed after oral administration of sunitinib to female and male rats. A long terminal half-life of sunitinib was observed in both humans (51 h) and monkeys (16–18 h). Human plasma pharmacokinetics in the study subjects were consistent with the results observed in previous single-dose studies with nonradiolabeled drug (Bello et al., 2007; Britten et al., 2008). In all three species, the metabolite M1 showed similar or longer terminal half-life compared with the parent compound, suggesting that its disposition is formation-rate limited. The ratio between the plasma levels of radioactivity and the sum of sunitinib and M1 plasma levels in rats was close to unity, suggesting that sunitinib and M1 represented the major circulating species in rats. Sunitinib and M1 were also the most abundant drug-related components in plasma of humans and monkeys. Radiochemical metabolite profiles of plasma samples from the three species showed two major radioactive peaks corresponding to sunitinib and M1.

After oral dosing, the levels of sunitinib were lower in male rats compared with females with corresponding higher levels of M1, suggesting that the pharmacokinetics in rats is sex dependent and that the occurrence of first-pass formation of M1 in males is more extensive than in females. In NADPH-supplemented rat liver microsomal incubation of 25 µM sunitinib, M1 was the major metabolite. Furthermore, CYP3A4 was demonstrated to be the major human P450 isoform to catalyze the formation of M1 (manuscript in preparation). Sex-specific metabolism often results from differences in expression of hepatic microsomal P450 enzymes and other drug-metabolizing enzymes (Shapiro et al., 1995). For example, rat CYP3A2 is predominantly expressed in male rats, which may explain the lower level of sunitinib and concurrent higher level of M1 observed in male rats. However, sex differences in P450 expression in humans and monkeys are less pronounced (Bulloch et al., 1995; Scandlyn et al., 2008). Long-lived radioactivity was observed in monkey plasma, which cannot be accounted for by sunitinib and M1. M11 is a monkey-specific metabolite and was observed as a minor metabolite in plasma. However, because of low radioactivity content as well as limited amount of sample after 72 h, we could not confirm whether the radioactivity at later time points was attributed to M11.

Sunitinib was extensively metabolized in all species. Many metabolites were detected both in urine and feces extracts, and in the majority of cases, putative structures were proposed. The main metabolic pathways were N-de-ethylation and hydroxylation of indolylidene/dimethylyprrole. Minor metabolic routes were oxidative defluorination that replaced the fluorine with a hydroxy group, N-oxidation/hydroxylation/desaturation of the N,N-diethylamine moiety, and a monkey-specific route that involved oxidation to a carboxylic acid through loss of the N,N-diethylamine moiety. No major differences in metabolite profiles were observed between the different routes of administration or between sexes. Some quantitative differences between sexes were observed in rats, and metabolism seemed to be more extensive in male rats.

In summary, sunitinib was extensively metabolized in rats, monkeys, and humans primarily via oxidative metabolism pathways thought to be modulated by P450s (manuscript in preparation). Fecal excretion was the major route of elimination of the dosed radioactivity in the three species, whereas renal elimination was a minor route of clearance. Sunitinib-related radioactivity was distributed throughout rat tissues, with a high degree of melanin-associated binding.

Acknowledgments

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Authorship Contributions

Participated in research design: Speed, Bu, Pool, Peng, Wu, Patyna, Bello, and Kang.
Conducted experiments: Speed, Bu, and Kang.
Contributed new reagents or analytic tools: Speed, Bu, and Kang.
Wrote or contributed to the writing of the manuscript: Speed, Wu, and Kang.

References
