The chemokine receptor, CCR5, acts as the major coreceptor involved in viral entry into the cell in the case of primary HIV infection. Therefore, blocking the CCR5 receptor prevents viral entry into host cells and should thus decrease viral load in HIV-1 infected individuals. This theory is supported by genetic evidence which shows that individuals lacking a functional CCR5 gene are highly resistant to HIV-1 infection (Liu et al., 1996; Samson et al., 1996; Chantry, 2004). This theory is supported by genetic evidence which shows that individuals lacking a functional CCR5 gene are highly resistant to HIV-1 infection (Liu et al., 1996; Samson et al., 1996; Chantry, 2004).

SPECIES DIFFERENCES IN THE DISPOSITION OF THE CCR5 ANTAGONIST, UK-427,857, A NEW POTENTIAL TREATMENT FOR HIV

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ABSTRACT:

UK-427,857 ([4, 4-difluoro-N-((1S)-3-[exo-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)cyclohexanecarboxamide) is a novel CCR5 antagonist undergoing investigation for use in the treatment of human immunodeficiency virus (HIV) infection. Pharmacokinetic and metabolism studies have been performed in mouse, rat, dog, and human after single and multiple administration by oral and intravenous routes. The compound has physicochemical properties that are borderline for good pharmacokinetics, being moderately lipophilic (log D7.4 2.1) and basic (pKb 7.3), possessing a number of H-bonding functionalities, and with a molecular weight of 514. The compound was incompletely absorbed in rat (20–30%) but well absorbed in dog (>70%). Based on in vitro studies in Caco-2 cells, UK-427,857 has relatively poor membrane permeability, and transcellular flux is enhanced in the presence of inhibitors of P-glycoprotein. Further evidence for the involvement of P-glycoprotein in restricting the oral absorption of UK-427,857 was obtained in P-glycoprotein null mice (mdr1a/mdr1b knockout). In these animals, AUC after oral administration was 3-fold higher than in control animals. In oral dose escalation studies in humans, the compound demonstrated nonlinear pharmacokinetics, with increased dose-normalized exposure with increased dose size, consistent with saturation of P-glycoprotein. The oral dose-exposure relationship of UK-427,857 in humans was not reflected in either rat or dog. In animal species and humans, UK-427,857 undergoes some metabolism, with parent compound and major metabolites present in the systemic circulation and excreta. Elimination of radioactive dose was primarily via the feces. In rat, parent compound was secreted via bile and directly into the gastrointestinal tract. Metabolites were products of oxidative metabolism and showed a high degree of structural consistency across species.

The chemokine receptor, CCR5, acts as the major coreceptor involved in viral entry into the cell in the case of primary HIV infection. Therefore, blocking the CCR5 receptor prevents viral entry into host cells and should thus decrease viral load in HIV-1 infected individuals. This theory is supported by genetic evidence which shows that individuals lacking a functional CCR5 gene are highly resistant to HIV-1 infection (Liu et al., 1996; Samson et al., 1996; Chantry, 2004).

UK-427,857 ([4, 4-difluoro-N-((1S)-3-[exo-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)cyclohexanecarboxamide) is a novel CCR5 receptor antagonist designed through a rational drug discovery program and is currently undergoing clinical evaluation (Bayes et al., 2003). UK-427,857 has a molecular weight of 514 and is a moderately lipophilic (log D7.4 2.1) and basic (pKb 7.3) molecule. The empirical formula is C35H41F3N7O2 and the structure is shown in Fig. 1.

Pharmacokinetic studies with UK-427,857 were undertaken in rat and dog during the discovery program leading to the identification of this compound as a development candidate. These studies were performed to characterize the general pharmacokinetic properties of the compound and to predict the potential pharmacokinetic profile in humans as part of the compound selection process. The animal pharmacokinetic studies and early in vitro permeability assessments indicated that the membrane permeability of UK-427,857 was restricted, leading to incomplete absorption. Additional in vitro studies in Caco-2 monolayers with inhibitors of P-glycoprotein and studies in P-glycoprotein null mice were undertaken to understand the absorption mechanism of the compound and to aid in the prediction of the likely clinical pharmacokinetic profile. Further pharmacokinetic data in animal species have been obtained as part of the general toxicology program and have allowed characterization of the dose-exposure relationships in both rat and dog. The dose-exposure relationship after oral administration in humans was studied over a wide dose range during early clinical trials. A major objective of this paper is to characterize the disposition properties of UK-427,857 in humans and to relate these to our knowledge of the disposition in animal species, to identify species differences and similarities. Such understanding provides valuable information for the prediction of human pharmacokinetic properties for future drug candidates. In addition, thorough
characterization of the disposition and metabolic fate of UK-427,857 has been performed in the animal species used within the toxicology program and in humans to confirm the suitability of the animal species in the safety assessment of the compound. These studies used 14C-labeled compound and mass spectrometry to facilitate the identification of metabolic products.

Materials and Methods

Chemicals. UK-427,857; internal standards, UK-377,461 (structural analog) and UK-462,015 (D2, UK-427,857); and authentic metabolite standards, UK-408,027 (metabolite 2) and UK-437,719 (metabolite 3), were synthesized at Pfizer Global Research and Development (Sandwich, UK). [14C]UK-427,857 (see Fig. 1) was prepared by Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK) with a radiochemical purity of 98% (by HPLC) and a specific activity of 33 Ci/mmol.

FIG. 1. Structure of UK-427,857. Position of carbon-14 label is indicated by the asterisk.

Toxicokinetic Study in P-glycoprotein Null Mice. Dose solutions were freshly prepared on the day of use. UK-427,857 (4 mg/ml) was dissolved in water containing 5% DMSO and 0.5% 1 N HCl. Male mice (25 g, 10 per strain, wild-type b6v and mdrla/mdrlb knockout) received oral doses of 16 mg/kg by gavage. Blood samples (0.1 ml, n = 2 per strain per time point) were collected under terminal isoflurane anesthesia from the inferior vena cava. All blood samples were transferred to lithium heparin tubes, mixed, and centrifuged. Plasma samples were stored frozen before analysis.

Radiolabeled Studies in Animals. [14C]UK-427,857 was administered orally to CD1 male mice (Charles River, UK; n = 11, 25, 200 mg/kg, −2 μCi per mouse), male (n = 6) and female (n = 6) Sprague-Dawley rats (250 g, 100 mg/kg, −20 μCi per rat), and male (n = 1) and female (n = 1) beagle dogs (15 kg, 5 mg/kg, −100 μCi per dog). All doses were prepared in 0.2 M lactate buffer, pH 3.0, at nominal concentrations of 5 to 50 mg/ml. Daily urine and feces output was collected from mice (n = 3), rats (n = 2 per sex), and dogs (n = 2) for up to 7 days depending on recovery of radioactivity. Serial blood samples were collected from the two dogs (0–48 h). Blood samples were collected at individual time points (1, 3, 7, and 24 h) from four female and four male rats (n = 1 per time point) and eight mice (n = 2 per time point) under terminal anesthesia. Feces was homogenized with water (feces/water 1:1 for rat and mouse, 4:1 for dog). Urine and fecal homogenates were stored frozen before analysis. Aliquots of whole blood were retained and remaining samples centrifuged for preparation of plasma. Whole blood, plasma, and red blood cell samples were stored frozen before analysis. Radioactivity in urine, cage washes (approximately 1 ml), and plasma (0.1–1.0 ml) was measured by liquid scintillation counting of duplicate weighed samples in 4 ml of Scintiverse (Perkin-Elmer Life Sciences, Meriden, CT). Radioactivity in fecal homogenate and whole blood (approximately 0.5 g) was measured by combustion of triplicate weighed samples in an Oximate Sample Oxidizer (Canberra Industries) and liquid scintillation counting of evolved 14CO2 in 10 ml of Permafluor E+ (Canberra Industries).

Biliary and Intestinal Secretion Study in Rat. Two male rats (Sprague-Dawley, 340 g) were anesthetized with isoflurane, and bile ducts were cannulated following laparotomy. Anesthesia was maintained with isoflurane for the duration of the experiment. [3H]UK-427,857 (3 mg/kg, −65 μCi per rat) was administered by i.v. injection into the caudal vein in a vehicle of 5 mM HCl in DMSO/saline (5:95). Total bile output was collected at intervals up to 6 h after dose administration, and the gastrointestinal tract and contents were collected at the end of the experiment. Total radioactivity in bile (10 μl) was measured by liquid scintillation counting of radioactive aliquots in Scintiscint. Radioactivity in gut contents and homogenized gastrointestinal tract (1:1 w/w with water) was measured by combustion of triplicate weighed aliquots (−200 mg), and the evolved 3H2O was measured by liquid scintillation counting in Monophase S (Canberra Industries). Metabolite profiling of bile and gastrointestinal tract contents was performed as described for animal excreta samples.

Radiolabeled Studies in Humans. Written consent and ethical approval were obtained as detailed above for this study, which was carried out at LCG.
DISPOSITION OF UK-427,857 IN ANIMALS AND HUMANS

Bioscience (Cambridge, UK). Healthy male volunteers (n = 3, aged 45–65 years, weight 80–90 kg) were given single 300-mg [14C]UK-427,857 (~48 μCi per subject) solution doses. Urine and feces were collected daily for 7 days, and serial blood samples were collected at time points up to 120 h postdose. Urine, feces, and blood samples were treated, stored, and analyzed for total radioactivity as previously described for animal species.

Analysis of Excreta and Plasma Samples for Metabolites. For all species, excreta samples were pooled to represent >90% of the total administered dose. For the animal species, one fecal and one urine sample per sex (where applicable) were generated, representing >90% of the total dose eliminated in that matrix. In humans, single pooled samples for feces and urine were profiled for each subject separately. In animal species, plasma samples were pooled in a time-normalized manner (Hop et al., 1998) to generate a single sample representing >80% of the total plasma AUC. Fecal homogenates were extracted with methanol (20 ml), followed by a mixture of methanol (19 ml) and 0.1 M Tris buffer (pH 6; 1 ml) and, finally, a mixture of methanol (18 ml) and 0.1 M Tris buffer (pH 9; 2 ml). The combined extracts were reduced to dryness under nitrogen at 37°C on a TurboVap (Zymark). Urine samples were centri-fuged before HPLC profiling. Plasma samples were treated with 3 volumes of methanol and centrifuged, and the supernatants were reduced to dryness under nitrogen at 37°C. HPLC profiling of excreta and plasma samples was achieved with an HRPB column (250 × 7.76 mm; Hitachi, Theale, Berkshire, UK) using a linear binary solvent gradient involving methanol:0.1 M ammonium acetate at a flow rate of 2 ml/min. For excreta samples, on-line radiochemical detection (β-Ram; LabLogic, Brodhill, Sheffield, UK) was used to monitor the drug-related components, with LAURA 3 (LabLogic) for data analysis. The drug-related components were isolated separately for identification and reduced to dryness under nitrogen at 37°C. For plasma, fractions were col-lected into 96-well Scintiplates (PerkinElmer Life and Analytical Sciences, Boston, MA), which were dried on a DD4 vacuum centrifuge (Genevac, Ipswich, Suffolk, UK) and analyzed on a Microbeta 1450 scintillation counter (PerkinElmer Life and Analytical Sciences). The drug-related components were initially analyzed by direct infusion mass spectrometry at a flow rate of 5 μl/min following reconstitution in methanol/water (70:30 v/v; both contain-ing 2 mM ammonium acetate) and appropriate dilution. Subsequently, liquid chromatography-tandem mass spectrometry was used to confirm assignments by comparison with authentic standards and resolve the components of mixed regions. Mass spectrometric identification used either an API4000 or 4000QTRAP instrument (both Applied Biosystems/MSD Sciex, Foster City, CA), using TurboS婴pro in positive ion mode. The mass spectrometer was optimized primarily by variation of the declustering potential, the collision energy, the TurboS婴pro temperature, and (for the QTRAP) the linear ion trap fill time.

Caco-2 Monolayer Studies. Caco-2 cells were seeded in 24-well Falcon Multwell plates (polyethylene terephthalate membranes, pore size 1.0 μm) at 4.0 × 10^4 cells per well. The cells were grown as previously described (Hamill et al., 2004): monolayer permeability was determined after 15 to 17 days of culture. Cells were used between passage 34 and 41. The study was initiated by the addition of UK-427,857 [25 μM in Hanks’ balanced salt solution, pH 7.4] to the donor well, 250 μl to the apical chamber, or 1 ml to the basolateral chamber, with control buffer in the opposing acceptor well. The effects of P-glycoprotein inhibitors on transport in both apical to basolateral (A to B) and basolateral to apical (B to A) directions were assessed by inclusion of verapamil (100 μM) or CP-100,356 (10 μM) in both donor and acceptor well solutions. Membrane integrity was assessed at the end of the 2-h incubation using Lucifer yellow added to the apical wells at a concentration of 100 μM in Hanks’ balanced salt solution. Membrane integrity was assessed at the end of the 2-h incubation using Lucifer yellow added to the apical wells at a concentration of 100 μM. All liquid handling procedures were conducted using a Tecan Genesis robot (Tecan, Durham, NC). The concentrations of UK-427,857 in each sample were determined by a HPLC system with mass spectrometric detection. The analytical column was a 40-μm Opti-Lynx Reliasil C18 (3 × 15 mm; Optimize Technologies, Oregon City, OR). The Hanks’ balanced salt solution was directly injected onto the column, and salts were washed to waste by back flushing with mobile phase consisting of 90:10 water/methanol containing 0.027% formic acid. Analyte was then eluted from the column with mobile phase comprising 10:90 water/methanol containing 0.027% formic acid. Flow rate was 1.5 ml/min for loading and 1.2 ml/min for elution. UK-427,857 was detected by an API 2000 mass spectrometer with an ionspray interface (PerkinElmerSciex Instruments, Boston, MA) using specific mass transition.

Quantitation was performed against a calibration line constructed over the concentration range 0.5 to 5 μM from known standards. Apparent permeability (P_app) was calculated as previously described (Harrison et al., 2004).

P-gp Binding Affinity. The apparent binding affinity of UK-427,857 for recombinant human P-glycoprotein expressed in insect cell membranes was determined as previously described (Abel et al., 2001). Apparent kinetic parameters Kd and Vmax were obtained by Michaelis-Menten analysis of the data.

Plasma Protein Binding Determinations. Samples of mouse, rat, dog, and human plasma from toxicology or clinical studies were used for the determination of plasma protein binding. Composite samples were prepared for mouse (n = 12) and rat (n = 9) by pooling Cmax samples according to sex and dose level. Individual Cmax samples were analyzed for dog (n = 54) and human (n = 54). [14C]UK-427,857 was added to samples at a concentration that allowed accurate radiochemical detection but did not exceed 10% of the measured concentration in any sample. Aliquots of plasma samples (0.15 ml) were dialyzed (Spectropor membrane strips, 120 × 22 mm, molecular weight cutoff 12,000–14,000; Spectrum Medical Industries, Rancho Dominguez, CA) against isotonic Krebs-Ringer buffer (0.15 ml, pH 7.4) for 4 h at 37°C on an oscillating platform in a 96-well equilibrium dialysis apparatus (HTDialysis, Gales Ferry, CT; Banker et al., 2003). Following dialysis, concentrations of drug in plasma and buffer were measured by liquid scintillation counting. The free fraction in plasma was calculated from the ratio of the concentration in buffer to plasma after dialysis.

Blood to Plasma Partitioning of UK-427,857. The partitioning of UK-427,857 between red blood cells and plasma in rat and dog was determined by the addition of approximately 100 ng/ml [3H]UK-427,857 (0.1 mg/ml solution in methanol) to triplicate 3-ml aliquots of freshly collected control rat and dog whole blood. Samples were incubated at room temperature for 20 min. Triplicate weighed aliquots (ca. 100 μg) of whole blood were removed and analyzed for radioactivity by liquid scintillation counting of evolved H2O after combustion by an Oximate automatic sample oxidizer. Plasma was prepared by centrifugation of the remaining whole blood and radioactivity determined in duplicate weighed aliquots (ca. 100 μg) by liquid scintillation counting. The blood/plasma partition was expressed as a ratio of concentration in blood relative to plasma. The blood to plasma partitioning of UK-427,857 in human whole blood was similarly determined using fresh whole blood from six individual donors, spiked with approximately 100 ng/ml [14C]UK-427,857, with radioactivity in whole blood determined by liquid scintillation counting of evolved 14CO2.

Analysis of UK-427,857 in Animal and Human Plasma. The analysis of UK-427,857 in plasma from mice, rats, and dogs was carried out by solvent extraction followed by HPLC with mass spectrometric detection. The analytical procedure was validated by the analysis of quality control samples. The method involved addition of internal standard (UK-377,461, 25 ng) to plasma samples (0–0.5 ml) followed by the addition of borate buffer (1 ml, 0.2 M, pH 9) to precipitate proteins. After mixing and centrifugation, the organic layer was transferred using a Genesis robot (Tecan, Maennedorf, Switzerland) into a 96-deep well block (Porvair Filtronics Ltd., Sherrerton, UK) and evaporated to dryness under a stream of nitrogen using a Techne Dri-Block DB.3A sample concentrator fitted with a 96-needle well array (Fischer Scientific Ltd., Loughborough, UK). Samples were reconstituted in 200 μl of 70% methanol (v/v) in water (containing 2 mM ammonium acetate). Aliquots of these samples (180 μl) were injected onto the HPLC system. The HPLC system comprised an HS100 C18 column (5 μm, 50 × 6.4 mm; Thermo Electron, Waltham, MA) and mobile phase 90% methanol (v/v) in water (containing 2 mM ammonium acetate) at a flow rate of 1 ml/min, split 50:1 postcolumn using an Acurate flow splitter (Preseach Ltd., Hitchin, Hertfordshire, UK). Detection was by multiple reaction monitoring for the transitions m/z 514 → 389 (UK-427,857) and 464 → 216 (UK-377,461) using a Scieix API2000 mass spectrometer with ionspray interface (PerkinElmerSciex Instruments). The limit of quantitation was 2.5 to 20 ng/ml, depending on sample volume. Quality control samples were found to be within 15% of the nominal concentration throughout the calibration range, satisfying acceptance criteria for the method.

The analysis of UK-427,857 in human plasma was performed using protein precipitation followed by turbulent flow chromatography (Chassaing et al., 2001) with mass spectrometric detection. The analytical procedure was again

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Results

Single-Dose Intravenous Pharmacokinetics of UK-427,857 in Rat and Dog. Single-dose intravenous pharmacokinetic data in rat and dog are shown in Table 1. Half-life values after intravenous administration are short in both rat and dog, at 0.9 and 2.3 h, respectively. The blood/plasma partition of UK-427,857 was determined at 1.1 and 0.9 for rat and dog, respectively. UK-427,857 exhibits high clearance in the rat, with a value close to liver blood flow (blood clearance calculated at 67 ml/min/kg). Clearance in the dog is moderate, at around 50% of liver blood flow in this species (blood clearance calculated at 23 ml/min/kg). UK-427,857 demonstrated large volumes of distribution in both rat and dog, at 0.9 and 2.3 h, respectively. The blood/plasma partition of UK-427,857 was broadly similar on day 1 and at steady state. The relationship between dose and systemic exposure (AUC and Cmax) is shown in Fig. 2 using data from day 1 of these studies.

Caco-2 Permeability and P-gp Affinity. Permeability of UK-427,857 across Caco-2 monolayers in the apical to basolateral (A to B) direction was limited, with Papp values of 1 × 10⁻⁵ cm/s (n = 3). Markedly higher permeability was observed in the basolateral to apical (B to A) direction, with a mean Papp value of 12 × 10⁻⁵ cm/s (n = 3), providing an efflux ratio (B to A/A to B) of >10, demonstrating polarized transport in the Caco-2 system. Such a profile is indicative of transporter-mediated efflux (Zhang and Benet, 1998). Verapamil and CP-100,356, which are both known inhibitors of P-glycoprotein (Doige and Sharom, 1992; Wandel et al., 1999), markedly reduced the efflux ratio of UK-427,857 across Caco-2 monolayers in the apical to basolateral (A to B) direction, with a mean

Table 1. Pharmacokinetic parameters (mean ± S.D. where available) for UK-427,857 after single i.v. and oral doses to rat, dog, and human.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat (n = 2)</th>
<th>Dog (n = 4)</th>
<th>Human (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous dose (mg/kg)</td>
<td>1.0</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>0.9</td>
<td>2.3 ± 0.6</td>
<td>–</td>
</tr>
<tr>
<td>Clearance (ml/min/kg)</td>
<td>74</td>
<td>21 ± 3</td>
<td>–</td>
</tr>
<tr>
<td>Volume of distribution (l/kg)</td>
<td>6.5</td>
<td>4.3 ± 1.3</td>
<td>–</td>
</tr>
<tr>
<td>Plasma protein binding (%)</td>
<td>51.0 ± 2.1</td>
<td>63.7 ± 7.3</td>
<td>75.5 ± 4.3</td>
</tr>
<tr>
<td>Blood/plasma partitioning, n = 3–6</td>
<td>1.1</td>
<td>0.9</td>
<td>0.59</td>
</tr>
<tr>
<td>Oral dose (mg/kg)</td>
<td>10.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>_</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>5.5</td>
<td>82</td>
<td>128</td>
</tr>
<tr>
<td>AUC (ng·h/ml)</td>
<td>12.4</td>
<td>335</td>
<td>292</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.0</td>
<td>1.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>6.0</td>
<td>42</td>
<td>40</td>
</tr>
</tbody>
</table>

a n = 4 for intravenous dose, n = 2 for oral dose.

b Human doses of 30 and 300 mg assuming body weight of 70 kg for comparative purposes.

c Insufficient data to allow calculation.

d Cmax and AUC values were normalized to 1 mg/kg dose.
427,857 transport in Caco-2 cells (Fig. 3). The apparent $K_m$ for UK-427,857 in the ATP hydrolysis assay for P-gp binding was $37 \pm 6.4 \mu M$ ($n = 5$), with $V_{\text{max}}$ of $55 \pm 3.4 \text{nmol/mg/min}$.

**Pharmacokinetics of UK-427,857 in P-gp Knockout Mice.** After oral doses of 16 mg/kg UK-427,857 to wild-type fvb mice, the average $C_{\text{max}}$ ($n = 2$) was 536 ng/ml and the AUC derived from average data was 440 ng·h/ml. The elimination half-life was 0.7 h. In mdr1a/1b knockout animals, following the same dose, the average $C_{\text{max}}$ and AUC were increased by 108% and 183%, respectively, at 1119 ng/ml and 1247 ng·h/ml. The elimination half-life was 1.0 h.

**Excretion Studies.** The recoveries of radioactivity in urine and feces following single oral doses of [14C]UK-427,857 to animal species and humans are shown in Table 2. The predominant route of excretion in all species is fecal, accounting for between 72 and 94% of recovered radioactivity. The extent of urinary excretion ranged from 4.5% in male rat to 19.6% in human. Excretion of dosed radioactivity occurred rapidly in rodent species, with >95% of the recovered radioactivity obtained within the first 24 h. Excretion occurred more slowly in dog and humans, but in all cases, >90% of the recovered radioactivity was obtained within 96 h. In rats and mice, only 0.1 and 0.2%, respectively, of dosed radioactivity was recovered in the carcasses at 96 h postdose (by alkali digestion and scintillation counting).

**Urinary and Fecal Metabolites.** Methanolic extraction of fecal homogenates and centrifugation of urine resulted in the recovery of >80% of the drug-related material in all cases. HPLC profiling of excreta samples showed that in all species, a significant fraction of the dose was eliminated unchanged (see, for example, human feces and urine profiles, Fig. 4, panels A and B), with parent compound representing between 33% and 79% of the total excreted radioactivity (human, $n = 3$; and rat, $n = 4$, respectively). A high degree of commonality was observed in the metabolism of UK-427,857 across all species. The major metabolic pathways in humans were oxidation of the triazole moiety, oxidation in the difluorocyclohexyl ring, and
TABLE 2

Excretion of radioactivity (as percentage of administered dose) in mouse, rat, dog, and human following single oral doses of [3H]-UK-427,857

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>Urine Excretion</th>
<th>Feces Excretion</th>
<th>Total Excretion</th>
<th>Total Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–t</td>
<td>0–t</td>
<td>0–24 h</td>
<td>0–t</td>
<td></td>
</tr>
<tr>
<td>Mouse (n = 3)</td>
<td>200</td>
<td>10.0</td>
<td>86.9</td>
<td>94.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Male rat (n = 2)</td>
<td>100</td>
<td>4.5</td>
<td>93.7</td>
<td>93.2</td>
<td>98.3</td>
</tr>
<tr>
<td>Female rat (n = 2)</td>
<td>100</td>
<td>7.5</td>
<td>88.4</td>
<td>93.5</td>
<td>95.9</td>
</tr>
<tr>
<td>Male dog (n = 1)</td>
<td>5</td>
<td>6.8</td>
<td>86.0</td>
<td>94.4</td>
<td>93.2</td>
</tr>
<tr>
<td>Female dog (n = 1)</td>
<td>5</td>
<td>16.5</td>
<td>72.0</td>
<td>47.3</td>
<td>89.3</td>
</tr>
<tr>
<td>Human (n = 3)</td>
<td>3.2</td>
<td>19.6</td>
<td>76.4</td>
<td>19.6</td>
<td>96.0</td>
</tr>
</tbody>
</table>

a Includes cage washes in the case of animal species.

N-dealkylation adjacent to the tropane ring, whereas in some animal species, aromatic hydroxylation at the para position of the phenyl ring was a major pathway (Fig. 5). The major excreted metabolites in humans were a product of hydroxylation of the methyl group of the triazole moiety (Met 8), accounting for 10% of the total dose (mean of three subjects), four products of mono-oxidation in the difluoroclohexyl ring (Mets 4–7; together accounting for 29%), and the secondary amine resulting from N-dealkylation adjacent to the tropane ring (Met 2; UK-408,027; 7%) (Fig. 5). All were identified in the excreta of the toxicology species. The structures of Met 2 and Met 3 were further confirmed by comparison of mass spectral data with authentic standards.

Circulating Metabolites. Protein precipitation of plasma by treatment with methanol resulted in the recovery of >80% of the drug-related material in all cases. In human plasma, the major components were unchanged UK-427,857, accounting for 42% of the plasma AUC (mean of three subjects), the secondary amine resulting from N-dealkylation adjacent to the tropane ring (Met 2, UK-408,027; 22%), and an analog of the amine involving oxidation of the methyl group of the triazole ring (Met 1; 11%). A number of minor circulating components were also observed in humans (Fig. 4, panel C). Unchanged UK-427,857 was also the major circulating component in animal species (mean values, 58% mouse, 67% rat, and 58% dog), whereas the secondary amine resulting from N-dealkylation (Met 2) was a major plasma metabolite (>5% circulating radioactivity) in all species.

Biliary and Intestinal Metabolites in Rat. Following intravenous administration of [3H]-UK-427,857 to bile duct-cannulated rats, 64% of the administered dose was excreted in bile in 6 h. A further 15% was associated with the gastrointestinal tract (3.5%) and its contents (11.5%). Parent compound was the major component present in bile, accounting for approximately 40% of dosed radioactivity. Profiling of the radioactivity in gastrointestinal tract contents showed only a single component present that corresponded to parent compound.

Discussion

UK-427,857 has high clearance in rat (estimated in excess of 80% liver blood flow) and moderate clearance in dog (estimated at around 50% of liver blood flow). In the dog, where oral bioavailability is in excess of 40%, absorption would appear to be near-complete, whereas in the rat, exposure in the hepatic portal vein indicates absorption of only around 20 to 30%. Such species differences in intestinal availability between rat and dog are quite common (van de Waterbeemd et al., 2001) and may reflect the larger aqueous pores present in the gastrointestinal tract of dogs (He et al., 1998) facilitating absorption of UK-427,857 via the paracellular route. Given the good aqueous solubility of UK-427,857, oral formulation changes were not considered to impact the extent of absorption. Aqueous pores in the gastrointestinal tract of humans are generally considered more similar in size to rat than to dog. At the time of candidate nomination, UK-427,857 was expected to have moderate absorption in human, similar to the rat, and moderate clearance, similar to the dog. Experience with previous compounds showing both variability in intestinal availability between species and apparent affinity for P-glycoprotein indicated the potential for nonlinear pharmacokinetics in humans.

The oral dose-exposure relationship in rat and dog is shown by the C_{max}/Dose and AUC/Dose values for day 1 toxicology data and single-dose data in Fig. 2, and contrasted with single oral-dose values for human. On this dose-normalized basis, exposure in rat is clearly much lower (approximately 1 order of magnitude) than in both dog and human, and is in keeping with the higher clearance observed in this species after intravenous administration. Excluding the lowest dose in rats (10 mg/kg), where only partial pharmacokinetic profiles were obtained, the dose-exposure relationship is broadly linear in terms of AUC but shows some subproportionality in terms of C_{max} at the highest dose (1500 mg/kg). In the dog, dose-normalized AUC and C_{max} show limited variability (2- to 3-fold) across the dose range 1 to 150 mg/kg. In contrast, the clinical dose-exposure relationship in humans shows marked nonproportionality in terms of both AUC and C_{max}, with superproportional increases observed up to doses of 300 mg. Dose-normalized exposure is approximately 10-fold higher at a dose of 300 mg compared with a dose of 3 mg. Based on an estimated fluid volume of 500 ml (Dressman et al., 1998) in the small intestine, a dose of 3 mg of UK-427,857 would result in a concentration of about 12 μM, which is below the P-glycoprotein apparent K_{m} of 37 μM and, hence, would not be expected to saturate P-glycoprotein-mediated efflux. This superproportional dose-exposure relationship is similar to that previously observed for a number of compounds including the NK2 antagonist, UK-224,671 (Beaumont et al., 2000), the α-antagonist, UK-294,315 (Harrison et al., 2004), and the phosphodiesterase 5 inhibitor, UK-343,664 (Abel et al., 2001). All of these compounds have arisen out of drug discovery programs that have either targeted enzyme inhibitors with subtype selectivity or are nonaminergic 7-transmembrane spanner receptor antagonists. The physicochemical requirements of such targets requires the incorporation of H-bonding functionalities, which, in itself, provides molecules with properties that are not considered ideal in terms of absorption and pharmacokinetics (Lipinski et al., 1997). Indeed, it would appear that these drug discovery programs tend to result in compounds of borderline membrane permeability that are subject to transport by P-glycoprotein and hence show nonproportional oral pharmacokinetics as a result of saturation of the efflux transport when given at relatively high doses (Harrison et al., 2004). The ability of P-glycoprotein to affect the membrane permeation of UK-427,857 has been demonstrated in the Caco-2 cell model using inhibitors of the transport protein. The role of P-glycoprotein is also supported by the studies in P-glycoprotein knockout mice, in which the absence of the transporter resulted in limited variability (2- to 3-fold) across the dose range 1 to 150 mg/kg. However, the clinical dose-exposure relationship in humans shows marked nonproportionality in terms of both AUC and C_{max}, with superproportional increases observed up to doses of 300 mg. Dose-normalized exposure is approximately 10-fold higher at a dose of 300 mg compared with a dose of 3 mg. Based on an estimated fluid volume of 500 ml (Dressman et al., 1998) in the small intestine, a dose of 3 mg of UK-427,857 would result in a concentration of about 12 μM, which is below the P-glycoprotein apparent K_{m} of 37 μM and, hence, would not be expected to saturate P-glycoprotein-mediated efflux. 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All possess a number of H-bond donors or acceptors, are moderately lipophilic (Log D_{7.4} values 1.4–2.6) and basic (calculated pKa 8.7 to 10.6), and have molecular weights above 500. Although other compounds have similar physicochemical properties they have not shown non-linear absorption profiles, it would appear that a high molecular weight results in an increased risk of this phenomenon. Indeed, a molecular weight above 500 has been shown to be associated with poorly absorbed compounds (Lipinski et al., 1997), and such relatively large molecules may be considered relatively poor membrane transporters.
permeators and, hence, more liable to be affected by P-glycoprotein. The limited permeability of UK-427,857 through cell membranes is supported by the studies in Caco-2 cells where, even in the presence of transport inhibitors, the rate of permeation of the compound is relatively slow. The size of UK-427,857 and the other molecules cited (mol. wt. >500) would indicate that the transcellular pathway will not be a significant route of absorption in humans. It would appear that restricted membrane permeability per se is more critical in the sensitivity of these compounds to efflux than their affinity for the transport protein which, itself, appears to have a wide structure-activity relationship (Stouch and Gudmundsson, 2002) and is thus able to bind to a variety of xenobiotics. These moderately lipophilic bases meet the structural requirements previously assigned to P-glycoprotein substrates, possessing, as they do, multiple hydrophobic regions and H-bond acceptor functions (Ekins et al., 2002). The failure of the P-glycoprotein inhibitors to completely inhibit the polarized transport

**Fig. 4.** Representative HPLC radiochromatograms for human feces (A, 0–120 h) and human urine (B, 0–36 h) and a reconstituted HPLC radiochromatogram for plasma (C, 0–18 h) following single oral (300 mg) administration of [14C]UK-427,857.
of UK-427,857 in Caco-2 cells may indicate a role of additional transport proteins in the efflux of this compound. Current knowledge on the clinical significance of such transporters prevents any conclusion as to their potential relevance at this time.

It would appear, therefore, that neither rat nor dog provides pharmacokinetic data that are predictive of the nonproportional oral pharmacokinetics of UK-427,857 in human. It is likely that multiple factors contribute to the species differences that are observed, including the generally higher permeability of dog gastrointestinal tract relative to other species, resulting in consistently high absorption throughout the dose range. It could be argued that the dose range in dog does not extend low enough to make this conclusion; however, such data are not available for UK-427,857, and previous experiences would indicate that no nonproportionality would be observed. In rat, incomplete bioavailability is apparent, based on hepatic portal vein exposures at relatively low oral doses. Gut wall metabolism is not thought to contribute significantly to the incomplete intestinal availability, given the overall low abundance of metabolites in the rat; incomplete absorption is therefore considered most likely. The high systemic clearance of compound in rat makes accurate assessment of bioavailability complicated, but there is no evidence that high oral doses saturate transporter (including P-glycoprotein) efflux, with pharmacokinetics appearing to remain reasonably proportional over an extended range. Overall, the most direct and valuable preclinical indicator of nonproportional clinical pharmacokinetics appears to be the in vitro data obtained in Caco-2 cells. Although neither rat nor dog is entirely predictive of the absorption of UK-427,857, it should be noted that the animal pharmacokinetic data in these two animal species were essential to demonstrate that the compound possessed oral bioavailability. In vitro data alone indicated very poor membrane
permeability, which, in other drug discovery programs, might have been taken as an indication of no oral bioavailability potential (Walker et al., 2001). Only the combination of in vitro and in vivo data provide good representation of the bioavailability properties of UK-427,857.

UK-427,857 undergoes a degree of metabolism in animals or humans; however, parent compound is the major circulating and excreted component in all species. The metabolism of UK-427,857 that is observed involves oxidation at a number of positions in the molecule, resulting in hydroxylation or N-dealkylation. Additional studies (not reported here) have indicated a role for cytochrome P450 in the metabolism of UK-427,857. All biotransformation pathways observed in humans are also observed in the toxicology species reported here. Limited renal excretion of UK-427,857 is observed in all animal species (2–6% of the dose), with highest levels of unchanged drug in urine in humans accounting for 8% of the administered dose. High levels of unchanged drug in the feces of all species (25% human, 74% rat, 36% mouse, and 39% dog) may in part reflect unabsorbed dose passing through the gastrointestinal tract unchanged. Data from the bile duct-cannulated rat study demonstrate that a high amount of unchanged drug is secreted via the bile. This study also provides evidence for direct secretion of drug into the gastrointestinal tract by the presence of parent compound in the gut contents after intravenous administration when bile has been diverted. Secretion of xenobiotics from the systemic circulation into the gut lumen across the gut wall may be due to efflux by transporters including P-glycoprotein. The ability of the P-glycoprotein inhibitor, verapamil, to reduce the systemic clearance of the β-blocker, talinolol, has provided evidence of this phenomenon (Spahn-Langguth et al., 1998). In the case of talinolol, there is evidence to show that this process of small intestinal secretion also occurs in humans (Gramatté et al., 1996). Excretion of UK-427,857 in bile may again involve P-glycoprotein-mediated transport, since this protein has been shown to be associated with the canalicular membrane (Müller and Jansen, 1997). The high systemic clearance and extensive biliary excretion observed in rat would also indicate that uptake into the liver may be facilitated by carrier proteins to achieve the high hepatic extraction observed in this species. A number of transporter proteins have been identified that might perform this role; in particular, the sinusoidal membrane transporters Oatp1 and Oatp2 in the rat have been shown both in vitro and in vivo to be involved in active uptake of drug substrates into the liver (Aytorn and Morgan, 2001). It is interesting to speculate as to whether the incorporation of metabolic stability into drug design tends to result in molecules more susceptible to transporter-mediated processes or whether the property of relative metabolic stability makes the transporter-mediated processes more visible. In the case of UK-427,857, transporter-mediated processes form an important element of drug disposition and would appear to be a major factor in the species differences that are observed. The clinical consequences, in terms of drug interaction potential, at the level of drug absorption will depend upon the clinical dose and the associated degree of saturation of the transport protein.

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