THE DISPOSITION OF VORICONAZOLE IN MOUSE, RAT, RABBIT, GUINEA PIG, DOG, AND HUMAN


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ABSTRACT:

Voriconazole is a new triazole antifungal agent with potent, wide-spectrum activity. Its pharmacokinetics and metabolism have been studied in mouse, rat, rabbit, dog, guinea pig, and humans after single and multiple administration by both oral and intravenous routes. Absorption of voriconazole is essentially complete in all species. The elimination of voriconazole is characterized by nonlinear pharmacokinetics in all species. Consequently, pharmacokinetic parameters are dependent upon dose, and a superproportional increase in area under the curve is seen with increasing dose in rat and dog toxicology studies. Following multiple administration, there is a decrease in systemic exposure. This is most pronounced in mouse and rat, less so in dog, and not observed in guinea pig or rabbit. Repeat-dose toxicology studies in mouse, rat, and dog have demonstrated that induction of cytochrome P450 by voriconazole (autoinduction of metabolism) is responsible for the decreased exposure in these species. Autoinduction of metabolism is not observed in humans, and plasma steady-state concentrations remain constant with time. Voriconazole is extensively metabolized in all species. The major pathways in humans involve fluoropyrimidine N-oxidation, fluoropyrimidine hydroxylation, and methyl hydroxylation. Also, N-oxidation facilitates cleavage of the molecule, resulting in loss of the fluoropyrimidine moiety and subsequent conjugation with glucuronic acid. Major pathways are represented in animal species. The major circulating metabolite in rat, dog, and human is the N-oxide of voriconazole. It is not thought to contribute to efficacy since it is at least 100-fold less potent than voriconazole against fungal pathogens in vitro.

Voriconazole [VEND, UK-109,496,1 (2R,3S)-2-(2,4-difluorophenyl)-3-[(S-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yi)-2-butanol; Fig. 1] is a new antifungal agent that is a derivative of fluconazole, having one triazole moiety replaced by a fluoropyrimidine ring and a methyl group added to the propanol backbone (Richardson et al., 1995). This change in structure results in potent, wide-spectrum activity in vitro and a fungicidal action against various mold species, including Aspergillus (Barry and Brown, 1996; Murphy et al., 1997). In common with other azole antifungal agents, such as fluconazole and itraconazole, its primary mode of action is inhibition of fungal cytochrome P450-dependent 14α-sterol demethylase, an essential enzyme in ergosterol biosynthesis (Sanati et al., 1997). Voriconazole shows a greater selectivity for the fungal enzyme than for the corresponding rat liver enzyme compared with both ketoconazole and itraconazole (Pye et al., 1995). Voriconazole is moderately lipophilic (log D7.4 = 1.8) and a single diastereomer with R- and S-stereochemistry by virtue of two chiral centers (2R, 3S) as shown in Fig. 1.

Pharmacokinetic and metabolism studies have been performed in preclinical species as part of the voriconazole development program. The primary reasons for conducting these studies during the discovery and development process are: 1) to determine the likely pharmacokinetics in humans by prediction from animal data; 2) to confirm that the selected preclinical efficacy and safety models are appropriate; and 3) to guide the selection of appropriate dosage regimens for toxicology studies.

Within the preclinical evaluation of voriconazole, pharmacokinetic studies were conducted following intravenous and oral administration in mouse, rat, rabbit, guinea pig, and dog as single or multiple doses. In addition, the excretion and metabolic fate of14 C-labeled voriconazole has been determined in mouse, rat, rabbit, guinea pig, dog, and human following intravenous and oral administration. The results from these studies are discussed and compared with published human pharmacokinetic data for voriconazole (Purkins et al., 2002). The properties of voriconazole are also compared and contrasted with other antifungal azoles, such as fluconazole and itraconazole.

Materials and Methods

Chemicals. Voriconazole, internal standards UK-101,608 and UK-103,446 (Fig. 1), UK-121,265 (N-oxide metabolite of voriconazole), UK-51,060, and UK-215,364 (for structures see Fig. 4) were synthesized at Pfizer Global Research and Development (Sandwich, UK). 14C-Voriconazole was prepared by Amersham Biosciences UK, Ltd. (Cardiff, UK) with a radiochemical purity >98% (by thin-layer chromatography and HPLC) and a specific activity of 46.3 or 54.7 μCi/mg. The identity of [14C]voriconazole was confirmed by mass spectrometry. The chemical purity of all compounds was >95%.

1 Abbreviations used are: UK-109,496, (2R,3S)-2-(2,4-difluorophenyl)-3-[(S-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yi)-2-butanol; HPLC, high-performance liquid chromatography; AUC, area under the curve; CL, clearance; API, atmospheric pressure ionization.

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Single- and Multiple-Dose Voriconazole Studies in Animals. All animal work was carried out in compliance with UK or French law and was approved by a local ethical review process as appropriate. The intravenous formulation of voriconazole contained hydroxypropyl-β-cyclodextrin, at a concentration of 160 mg/ml in water, as an excipient to improve solubility. The concentration of voriconazole was in the range to 30 mg/ml, depending on species and final dose. Dose levels were in line with those used in toxicology. For oral administration, voriconazole was suspended in either an aqueous solution of 0.5% (w/v) methycellulose containing 1% (v/v) Tween 80 (rat or dog) or an aqueous polyethylene glycol 400 solution (50–100%) for the remaining species (mouse, rabbit, or guinea pig). All animals had free access to food and water, and were kept in experimental facilities maintained with a 12-h light/dark cycle. After dosing, blood samples were collected into heparinized tubes at selected time points. Plasma was obtained by centrifugation and stored at approximately −20°C before analysis.

Male mice (CD1 strain; approximately 30 g; Charles River, Margate, Kent, UK) received seven daily oral (30 mg/kg) or intravenous (10 mg/kg) doses of voriconazole. Blood samples were collected on days 1 and 7 (three animals per time point). For the excretion study, male mice (n = 3) received seven daily oral doses of voriconazole (30 mg/kg) followed by a [14C]voriconazole oral dose on day 8 and then another four daily doses of voriconazole. After the radiolabeled dose, animals were housed individually in metabolism cages designed for separate collection of urine and feces.

For the pharmacokinetic study, male and female Sprague-Dawley rats (CD strain; approximately 170–250 g; Charles River, UK) received once daily oral doses (30 mg/kg) of voriconazole by gavage tube for 6 days. On day 7, either a final oral gavage dose or a final intravenous dose (10 mg/kg) was administered via the tail vein. Blood samples were collected from two animals per time point. For the excretion study, after the radiolabeled dose, rats were housed individually in metabolism cages designed for the separate collection of urine and feces. Animals (n = 3 per sex per route) received 9 once daily doses of voriconazole by the oral (30 mg/kg) or intravenous (10 mg/kg) dose routes followed by a dose of [14C]voriconazole on day 10. Another four daily doses of voriconazole (oral or intravenous as appropriate) were then administered.

Female New Zealand White rabbits (approximately 2.5 kg; Interfauna Ltd., Bath, UK) received seven daily oral (10 mg/kg) or intravenous (3 mg/kg) doses of voriconazole. Blood samples were collected from three animals per time point. For the excretion study, animals (n = 3) received seven daily oral doses of voriconazole (30 mg/kg), followed by a [14C]voriconazole dose on day 8, and then another four daily doses of voriconazole. Animals were housed singly in cages designed for separate collection of urine and feces.

Male Dunkin Hartley guinea pigs (approximately 350 g; Charles River, UK) received a single oral (10 mg/kg) gavage dose of voriconazole. Female animals received three times daily (every 8 h) oral (10 mg/kg) doses for 5 days, or a single intravenous (10 mg/kg) dose followed by once daily oral (10 mg/kg) administration for 6 days, with a final intravenous dose (10 mg/kg) on day 7. Blood samples were collected from one animal per time point (except the single-dose oral study, which used three animals per time point). For the excretion study, female animals (n = 3) received twice daily oral doses of voriconazole (10 mg/kg) for 5 days, followed by a [14C]voriconazole dose on day 6, and then additional twice daily doses of voriconazole for 4 days.

Animals were housed singly in metabolism cages designed for the separate collection of urine and feces.

Two male and two female beagle dogs (weight range 10–14 kg; Interfauna Ltd.) received a single oral solution dose (6 mg/kg) via gavage tube, followed 48 h later by eight daily oral doses of voriconazole at 6 mg/kg. The same animals received an intravenous bolus dose of voriconazole at 3 mg/kg, followed 48 h later by another eight daily intravenous doses. [14C]Voriconazole was administered on day 10, followed by another four daily doses (oral or intravenous as appropriate) of voriconazole. Blood samples were collected on days 1 and 10 following oral and intravenous administration. The intravenous radiolabeled dose was administered to the same dogs 48 days after the oral radiolabeled dose. Animals were housed individually in cages designed for separate collection of urine and feces.

Single-Dose Pharmacokinetics of UK-121,265. Intravenous doses of UK-121,265 were formulated in the excipient aqueous sulfobutyl ether β-cyclodextrin (160 mg/ml) to a final concentration of 1 mg/ml (rats) and 2 mg/ml (dogs). Male and female Sprague-Dawley rats (CD strain, n = 10 per sex; Charles River, Lyon, France) received a single intravenous dose (1 mg/kg) of UK-121,265. Blood samples (0.5 ml) were collected from five animals per sex at each time point. A female beagle dog (14 kg; Pfizer colony, Sandwich, UK) received a single intravenous dose (1 mg/kg) of UK-121,265 administered by infusion at a rate that delivered the intended dose in 7.5 min. Blood samples (5 ml) were collected at selected time points after dosing.

Toxicology Studies in Rat and Dog. Within the toxicology program for voriconazole (which was performed by Pfizer Global Research and Development, Amboise, France), Sprague-Dawley rats (Charles River, France), and beagle dogs (Marshall Farms USA, Inc., North Rose, NY) received multiple daily oral doses of voriconazole for 1 month. Toxicokinetic data were obtained from groups of 10 male and 10 female rats receiving oral doses of 3, 10, 30, and 80 mg/kg and from groups of 3 male and 3 female dogs receiving oral doses of 3, 6, and 12 mg/kg voriconazole. Doses were prepared in 0.5% (w/v) aqueous methylcellulose containing 1% (v/v) Tween 80 and were administered at 10 ml/kg (rats) or 1 ml/kg (dogs) by oral gavage. Blood samples (1 ml) were collected from rats at 1, 3, 5, and 24 h after dosing on days 1 and 26 of the study. Blood samples (2.5 ml) were collected from dogs at 2, 5, 8, and 24 h after dosing on days 1 and 16 of the study.

After necropsy, liver samples from rats and dogs were retained and washed with saline. A 20% (w/v) homogenate was prepared in 0.25 M sucrose, 0.1 mM Tris/HCl buffer (pH 7.4). Successive ultracentrifugation steps yielded microsomes that were resuspended in 0.1 M phosphate buffer (pH 7.4) containing 20% (v/v) glycerol. Microsomes were stored at −60°C before analysis. Cytochrome P450 content was assayed spectrophotometrically (Omura and Sato, 1964), and protein concentration was determined using a commercially available kit (Pierce BCA Protein Assay Reagent; Chemical, Rockford, IL) using bovine serum albumin as a standard. Relative liver weights were calculated from (liver/body weight ratio × 100).

Administration to Humans. Three healthy male volunteers received twice daily oral doses of voriconazole (200-mg capsules) for 10 days. On day 6, the morning dose was replaced by an oral solution dose of [14C]voriconazole (170 mg in 0.1 M hydrochloric acid; 90 μCi or 3.33 MBq). Three additional healthy male volunteers received twice daily intravenous doses of voriconazole (3 mg/kg given as 60-min infusions in 160 mg/ml hydroxypropyl-β-cyclodextrin)
for 10 days. On day 6, the morning dose contained [14C]voriconazole (75 µCi or 2.78 MBq). The clinical study was conducted at Pharma Bio-research (Zuidlaren, The Netherlands). The six volunteers provided written consent were between ages 23 and 25 years, and weighed between 66 and 92 kg. They showed no evidence of any clinically significant disease or abnormality following review of laboratory data and full physical examination. Blood samples (15 ml) were taken at specified time points after the radiolabeled dose into heparinized tubes. After centrifugation, plasma samples were transferred to polypropylene tubes and stored at −20°C until analysis. Urine and plasma were collected into plastic containers and stored at −20°C.

**Plasma Protein Binding.** The extent of plasma protein binding was determined in mouse, rat, rabbit, guinea pig, dog, and human control plasma. [14C]Voriconazole was added to plasma (4 ml) at a concentration of 1 µg/ml (n = 3 per species), and aliquots (1 ml) were dia lyzed (Spectrapor 1 dialysis membrane, 6000- to 8000 Da cutoff, Spectrum Laboratories, Inc., Rancho Domingas, CA) against 0.1 M phosphate buffer, pH 7.4 (1 ml). Dialysis was carried out for 2 h at 37°C using a rotating dialyzer (DuNorm M.S.E. Ltd., Crawley, UK). Drug concentrations in buffer and plasma were measured by liquid scintillation counting.

**Analysis of Voriconazole and UK-121,265 in Plasma Samples.** The concentration of voriconazole was determined in plasma from mouse, rat, rabbit, guinea pig, dog, and human pharmacokinetic studies and from rat and dog toxicology studies using a liquid-liquid extraction method followed by reverse-phase HPLC with UV detection. The method involved addition of internal standard (1 µg of UK-101,608) to 0.1 ml (except rat, 0.5 ml, and human, 1 ml) plasma samples that had previously been appropriately diluted with control plasma (obtained from the same species). Voriconazole (and internal standard) was extracted from plasma samples using diethyl ether or ethyl acetate (4 ml), following addition of 0.2 M sodium borate buffer, pH 9.0 (1 ml). After evaporation of the organic solvent under a stream of nitrogen, the extracts were taken up in 100 µl of mobile phase [0.1 M N,N,N′N′-tetramethylethylenediamine (pH 7.4)/methanol, 30:70, v/v], Chromatography was performed using a Spherisorb 5-µm ODS2 column (25 cm × 4.6 mm; Hichrom, Berkshire, UK) with detection by UV absorbance at 254 nm. The flow rate was 1 ml/min. On this system the retention times of voriconazole and internal standard were 6 and 8 min, respectively. The lower limit of detection was 10 ng/ml (human), 50 ng/ml (mouse, rat, or rabbit), or 100 ng/ml (dog or guinea pig). To satisfy acceptance criteria, analysis of quality control samples provided results within 20% of the correct value at the lower end of the calibration range and within 15% of the correct value at the middle and top of the calibration range (top 1000 ng/ml).

A combined assay was later developed for analysis of voriconazole and UK-121,265 in rat and dog plasma using a specific validated HPLC method with multiple reaction monitoring detection. Before analysis, plasma samples (0.1 ml) were diluted with appropriate control plasma, and internal standard (UK-103,446) was added followed by 20 mM formic acid buffer, pH 3 (0.5 ml). Samples were loaded into an activated 96-well solid-phase extraction block (50-ng C15, Porvair plc, Norfolk, UK) and washed with 20 mM formic acid, pH 3 (1 ml). Analytes were eluted using methanol (1 ml), and the eluates were evaporated to dryness under a stream of nitrogen. Residues were reconstituted in 250 µl of 2 mM ammonium acetate buffer, pH 4, in methanol/water (40:60, v/v). Samples (200 µl) were injected onto the HPLC column (Hypersil C18, 50 × 4.6 mm) with a mobile phase consisting of 2 mM ammonium acetate buffer, pH 4, in methanol/water (90:10, v/v) (eluant A) and 2 mM ammonium acetate buffer, pH 4, in methanol (eluant B) (65:35 eluant A/B) at 1 ml/min. Detection was by multiple reaction monitoring for the transitions [M′+H]+ → m/z 224 (voriconazole), 366 to 224 (UK-121,265), and 384 to 224 (UK-103,446) using an API 365 mass spectrometer (MDs Sciex, Concord, ON, Canada) in positive TurboIonSpray mode. On this system the retention times were 5 min (UK-121,265), 6.5 min (voriconazole), and 7.7 min (UK-103,446). The limit of detection was 10 ng/ml for voriconazole and UK-121,265. To satisfy acceptance criteria, analysis of quality control samples provided results within 20% of the correct value at the lower end of the calibration range and within 15% of the correct value at the middle and top of the calibration range (1000 ng/ml in rat; 5000 ng/ml in dog).

**Analysis of Radioactivity.** Radioactivity in samples of protein binding buffer (0.5 ml), plasma (0.25–0.5 ml), and urine (1 ml) was measured by liquid scintillation counting in scintillation cocktail. Daily fecal samples were mixed with water (approximately 1:4, v/v) before analysis. Radioactivity in triplicate-weighed homogenized feces samples (0.4 g) and whole blood (0.2 g) was determined by liquid scintillation counting of evolved 14CO2 after combustion of the samples in an Oximate Sample Oxidizer (PerkinElmer Life Sciences, Boston, MA).

**Analysis of Urine for Metabolites.** For each species, gender, and route of administration, composite urine samples were prepared by mixing proportionate amounts to account for at least 85% of radioactivity excreted by that route (range 85–97% across species). Urine was analyzed by direct injection onto a gradient HPLC system comprising a Spherisorb 5-µm ODS2 column (25 cm × 4.6 mm; Hichrom) eluted by a gradient of 0 to 100% methanol in 0.1 M ammonium acetate over 40 min at 1 ml/min. The eluate was monitored for UV absorbance (254 nm) and by an on-line radioactivity detector (β-Ram; Lablogic Systems Ltd., Sheffield, UK) or by fraction collection (0.25 min), mixing with 2 ml of scintillation fluid followed by liquid scintillation counting. Metabolites were isolated from a 1-ml composite urine sample for all species except human (4 ml). These metabolites were further purified where necessary by isocratic HPLC prior to mass spectral analysis.

**Analysis of Feces for Metabolites.** For each species (except rabbit, where there was no significant fecal excretion), following and route of administration, composite feces samples were prepared by mixing proportionate amounts of homogenate to account for at least 85% of radioactivity excreted by that route (range 88–98% across species). Fecal homogenates were extracted twice using methanol/ethyl acetate (20:80, v/v). The centrifuged extracts were combined, evaporated to dryness under nitrogen, and resuspended in methanol (0.5 ml) for injection onto the gradient HPLC system (conditions as described for urine). Metabolites were isolated from similarly prepared extracts of pooled fecal homogenate (5 g) for all species. Initially isolated metabolites were further purified wherever necessary by isocratic HPLC prior to mass spectral analysis.

**Analysis of Plasma for Metabolites.** Drug-related material was isolated from rat plasma (1 h; n = 5 per sex) and dog plasma (1 h; n = 5 per sex) following daily oral administration of voriconazole for 6 days followed by [14C]voriconazole on day 7 (rats, 50 mg/kg/day; dogs, 8 mg/kg/day). Solid phase extraction on C18 cartridges was used, followed by analysis using the same gradient HPLC system described for urine. Human plasma from the radiolabeled study was analyzed in all subjects at 1 h after the radiolabeled dose. Drug-related material was isolated using liquid-liquid extraction into a mixture of ethyl acetate and 1-butanol (90:10, v/v) followed by analysis using the same gradient HPLC system as for urine. Recovery of radioactivity from plasma was similar by either solid-phase or liquid-liquid extraction.

**Metabolite Identification.** Where possible, metabolite characterization was performed initially by the comparison of retention times on the gradient HPLC system with authentic standards that had been added to the relevant biological matrix. To confirm metabolite identities, or to identify components for which no reference standard was available, mass spectral analysis was performed. The radioactive components were analyzed by liquid chromatography/mass spectrometry using a Supelcosil 3-µm LC18-DB column (3.3 × 0.46 cm) with a flow rate of 0.2 ml/min and an isotropic mobile phase (50% methanol, 50% 2 M ammonium acetate) or by direct infusion of cut HPLC fractions into an API III triple quadrupole mass spectrometer (MDS Sciex) fitted with an IonSpray interface utilized in positive ion mode. Where possible, metabolite characterization was performed by comparison of retention times on the gradient HPLC system with authentic standards that had been added to the relevant biological matrix. To confirm metabolite identities, or to identify components for which no reference standard was available, mass spectral analysis was performed.

**Pharmacokinetic Analysis of Data.** When more than one sample was obtained for each time point, mean concentration data were used for pharmacokinetic analysis. The maximum observed plasma concentrations (Cmax) were obtained directly from the recorded data. Terminal elimination rate constant (kdes) was determined by linear regression of the log plasma concentrations. The area under the plasma concentration-time curve (AUCt) values were calculated between time 0 and the final time point at which measurable drug concentrations were observed, using the trapezoidal rule. AUCinf was calculated by extrapolation to infinity with kdes. Clearance (CL) was calculated by the relationship dose/AUCinf, and the volume of distribution was calculated by the relationship CL/kdes. The relative apparent bioavailability was calculated from dose-corrected AUC values after oral and intravenous administration.

**Pharmacokinetic Modeling.** Rat and dog plasma concentration data were fitted to a one-compartment model using weighted nonlinear least-squares regression. Elimination rates were described by the Michaelis-Menten equa-
Voriconazole was absorbed with a nuous administration is shown in Table 1. After oral administration, subjects.

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protein binding at 1/H9262 investigated in both genders; therefore, only pooled data are presented for

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volume of distribution. Consequently, only AUC following intravenous administration is shown in Table 1. After oral administration, voriconazole was absorbed with a T9262/10 mean of 78% in all animal species.


Results

Plasma Protein Binding. Voriconazole exhibits moderate plasma protein binding at 1 μg/ml with values ranging from 45 to 67% in animals (Table 1). Protein binding in human plasma was 58%.

Single-Dose Pharmacokinetics. Plasma concentration versus time profiles of voriconazole following single oral or intravenous administration for each of the five animal species used are shown in Fig. 2. Voriconazole intravenous pharmacokinetics are not linear and show a “hockey-stick” log-concentration versus time profile. Attempts to fit the data using conventional first-order elimination did not give a good

fit and could not be used to derive parameters such as clearance and volume of distribution. Consequently, only AUC following intravenous administration is shown in Table 1. After oral administration, voriconazole was absorbed with a T9262/10 mean of 8 h or less (Table 1). Data for AUC following both oral and intravenous administration show that voriconazole pharmacokinetics in the rat are gender-dependent, with a lower systemic exposure in male animals (Table 1). This phenomenon was not observed in dogs, the only other species investigated in both genders; therefore, only pooled data are presented for this species. Comparison of dose-normalized AUC values following oral and intravenous administration shows that the apparent bioavailability of voriconazole is high (>75%) in all animal species.

WinNonlin software allowed a fit of the single-dose rat (male) and dog (mean of male and female) plasma data to be made using a one-compartment model with elimination based on the Michaelis-Menten equation. The use of this model gave a better fit to the data and allowed calculation of Vmax, Km and C0h (extrapolated concentration at time 0) values (Table 2). From these parameters, a value for apparent clearance (Vmax/Km) and a value for Vd (dose/C0h) under first-order elimination conditions were calculated (Table 2). Due to the wide discrepancy in the in vivo Vmax and Km values between species, any prediction of human pharmacokinetic parameters is likely to be inaccurate. The range of these Km values spans the therapeutic plasma concentration (rat above, dog below), and the uncertain effect of nonlinearity destroys any correlation attempt. However, the animal data do allow an alert that nonlinear clearance should be expected in humans and that appropriate dosage increments and plasma sampling strategies should be employed during the initial exposure of human subjects.

Multiple-Dose Pharmacokinetics. Multiple administration of voriconazole resulted in an appreciable decrease in AUC compared with single administration in most animal species (Table 1). After oral administration, this effect is most pronounced in mouse and rat, is less so in dog, and is not observed in guinea pig or rabbit. A sex difference in exposure is still apparent in the rat, with females having a 2-fold higher exposure than males.

Toxicokinetic Studies. The effect of increasing dose on exposure to voriconazole was investigated in rat and dog toxicology studies. Following single oral administration, there was a greater than proportional increase in AUC, with increasing dose (Fig. 3). Between the lowest (3 mg/kg) and highest (80 mg/kg) oral dose given to rats, there was a 68-fold increase in exposure for this 26-fold increase in dose. In dog, there was a 9-fold increase in exposure for a 4-fold increase in oral dose. The nonlinear behavior was expected since plasma concentrations achieved in these studies parallel and exceed the in vivo Km values reported above.

Following multiple oral administration, there was a decrease in exposure at all dose levels compared with day 1 data. This was more marked in rat than in dog. A greater than proportional increase in AUC was still evident after multiple oral administration of voriconazole in both species (Fig. 3). There was an increase in hepatic cytochrome P450 content in livers from both rats and dogs treated with multiple doses of voriconazole (Tables 3 and 4). The enzyme induction appeared to be dose-related. There was an associated 1.4- to 1.7-fold increase in relative liver weight at the highest dose compared with control animals in both rat and dog.

Excretion Studies. The recoveries of radioactivity in urine and feces following multiple intravenous and oral doses of [14C]voriconazole to animal species and human are shown in Table 5. The predominant route of excretion in rat, rabbit, and dog was the urine, which accounted for 55 to 87% of the dose, in comparison to 7 to 37% for feces. By contrast, in mouse and guinea pig, the feces contained a slightly higher proportion of the dose (53 and 54%, respectively) than the urine. In humans, the urine was the most important route of excretion following either oral or intravenous administration, accounting for a mean 78% of the dose, compared with 23% in feces. In all species the majority (>90%) of total radioactivity was recovered in the first 48 h.

Urineary and Fecal Metabolites. In all species except rabbit, urine and feces were important routes of excretion; therefore, both were profiled for metabolites. Chromatography revealed a complex mixture of metabolites of [14C]voriconazole in both matrices. In the rabbit,

<table>
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<th>Parameter</th>
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- a Male animals were used for single oral dose study.
- b Number of animals per time point, except for dog and rabbit studies, which involved serial bleeding.
- c n = 3 per time point for single oral dose study in guinea pigs.
- d Once daily for up to 10 days (minimum 5 days), except guinea pig multiple oral (three times daily).
only urine was examined, and the profile was less complex with only two components being detected. The metabolism of voriconazole across species is summarized in Fig. 4, which shows total excreted metabolites as a percentage of the administered dose. Unchanged voriconazole recovered in excreta accounted for 9% (mouse), 5% (rat, guinea pig, and dog), and 2% (human) of total dosed radioactivity. The major urinary component in humans accounted for 21% of the dose (Fig. 4, pathway A). This component cochromatographed with authentic standard UK-121,265, the N-oxide of voriconazole, and identity was confirmed by API mass spectrometry. UK-121,265 was also observed in the urine of mouse, rat, guinea pig, and dog. Another major pathway of metabolism involved hydroxylation of the fluoropyrimidine ring or adjacent methyl group (Fig. 4, pathways B and C) followed by glucuronide conjugation. In humans, the hydroxylated fluoropyrimidine metabolite was present in both urine and feces, accounting for 12% of the dose. This metabolite was also identified in animal species (mouse, rat, guinea pig, and dog). In the rabbit, 78% of the dose was attributable to the hydroxylated fluoropyrimidine metabolite, which was excreted only in the urine. Chromatographic analysis revealed the presence of two major components in rabbit urine, which accounted for 31 and 47% of the dose, respectively. These were further characterized by proton NMR analysis and found to be isomeric products of hydroxylation on the fluoropyrimidine ring.

The glucuronide conjugate of the hydroxylated fluoropyrimidine
metabolite represented 16% of the dose in human urine. Identification was based upon API mass spectrometry of the aglycone. This metabolite was also identified in mouse urine.

The hydroxymethyl metabolite of voriconazole (Fig. 4, pathway C) was identified in rat, dog, and guinea pig. The glucuronide conjugate of this metabolite was identified in human, rat, and dog urine by API mass spectrometry of the aglycone.

Cleavage of the N-oxide metabolite, UK-121,265, results in loss of the fluoropyrimidine moiety (Fig. 4, pathway D) to form UK-51,060. This metabolite was identified in rat, dog, and guinea pig. A glucuronide conjugate (presumed to be formed via UK-215,364; Fig. 4, pathway E) was a major metabolite in human urine (20% of the dose). Identification was by glucuronide hydrolysis and cochromatography of the aglycone with an authentic standard for UK-215,364. This glucuronide metabolite was also identified in urine from mouse, rat, dog and guinea pig. The aglycone was only present in guinea pig urine.

A minor human glucuronide-conjugated metabolite (5% of the dose in urine) arose through oxidative defluorination of UK-215,364 (Fig. 4, pathway F). Several minor human metabolites arose from a combination of pathways A and B to form multiple oxidized metabolites. In total, these metabolites accounted for 14% of the dose.

Circulating Metabolites. Following multiple oral administration of voriconazole in rat (50 mg/kg/day) and dog (8 mg/kg/day), there was significant exposure to the N-oxide metabolite UK-121,265 in both species. In these studies the plasma concentration-time profile of UK-121,265 paralleled that of voriconazole (dog, Fig. 5), and the AUC ratios of UK-121,265 compared with voriconazole were 0.3 and 0.4 in rat and dog, respectively.

In rat, dog, and human, the profile of radioactive components was investigated in plasma 1 h after oral administration of [14C]voriconazole (Fig. 6). All identification in this matrix was made upon the basis of chromatographic retention time and cochromatography. The major component was voriconazole in all species (Fig. 6, region 9). In dog and human, one other radioactive component was identified as the N-oxide UK-121,265 (Fig. 6, region 7), accounting for 7 and 15%, respectively, of total radioactivity in plasma. In rat, two other radioactive components were identified as UK-121,265 and UK-51,060 (23 and 8% of total plasma radioactivity, respectively).

Pharmacokinetics of the N-Oxide Metabolite. To determine its pharmacokinetic profile, a single intravenous dose (1 mg/kg) of the N-oxide metabolite UK-121,265 was administered to rats and dogs (Fig. 7). In rats, elimination of UK-121,265 was biphasic with a terminal elimination half-life of 1.8 h. There was evidence for a sex difference in clearance in this species, with females having a lower mean clearance (10.7 ml/min/kg) than males (22.4 ml/min/kg). Reduction of UK-121,265 back to voriconazole was evident in both sexes. In the dog, UK-121,265 showed linear elimination with a half-life of 0.6 h and a plasma clearance of 12.2 ml/min/kg. Volume of distribution was 0.7 l/kg, and there was evidence for reduction of UK-121,265 back to voriconazole.

Discussion
Elimination of voriconazole from plasma does not follow simple linear pharmacokinetics in animals. The convex plasma profiles observed following intravenous administration of voriconazole (Fig. 2)
are characteristic of compounds that show capacity-limited elimination. This type of profile is also seen for drugs such as phenytoin (Arnold and Gerber, 1970; Gerber and Wagner, 1972), theophylline (Dunn and Rybak, 1984), and fluoxetine (Hiemke and Hartter, 2000). Since voriconazole is eliminated predominantly by metabolism, it is likely that saturation of metabolic clearance is the cause of the nonlinearity. Consequently, pharmacokinetic parameters are dependent upon dose, and a greater than proportional increase in exposure is seen with increasing dose in rat and dog toxicology studies (Fig. 3). Similar capacity-limited pharmacokinetics are observed in humans (Purkins et al., 2002), and for a 2-fold increase in oral dose there was a 2.8-fold and 3.9-fold increase in (Purkins et al., 2002), and for a 2-fold increase in oral dose there was a similar capacity-limited pharmacokinetics are observed in humans (Fig. 3). Consequently, pharmacokinetic parameters are dependent upon dose, and a greater than proportional increase in exposure is seen with increasing dose in rat and dog toxicology studies (Fig. 3). Similar capacity-limited pharmacokinetics are observed in humans (Purkins et al., 2002), and for a 2-fold increase in oral dose there was a 2.8-fold and 3.9-fold increase in exposure.

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Despite their structural similarity, the capacity-limited pharmacokinetic profile of voriconazole contrasts markedly with that of fluconazole, the latter being eliminated predominantly by renal clearance of unchanged drug. Consequently, the pharmacokinetic parameters of voriconazole are more in keeping with other azole antifungals, such as itaconazole, which is also eliminated by metabolism and shows evidence for a nonlinear pharmacokinetic profile in humans. The properties of voriconazole are more in keeping with other azole antifungals, such as itaconazole, which is also eliminated by metabolism and shows evidence for a nonlinear pharmacokinetic profile in humans (Grant and Clissold, 1989).

Absorption of voriconazole is essentially complete in all species studied, which is in keeping with its moderately lipophilic nature (log D7.4 = 1.8). Voriconazole was absorbed after an oral dose with a T<sub>max</sub> of 8 h or less in toxicology species and ca. 2 h in humans (Purkins et al., 2002). [14C]Voriconazole excretion studies revealed unchanged drug in feces to be less than 7% of the dose in all toxicology species and undetectable in humans. Moreover, voriconazole displays a similar recovery of radioactivity in urine after intravenous or oral dosing to rat, dog, and human. In addition, comparison of AUC values obtained following oral or intravenous administration shows that apparent oral bioavailability is high in all animal species (>75%). Apparent bioavailability values of >100% obtained by comparison of dose-normalized AUC values reflect the capacity-limited pharmacokinetics are observed in humans (Purkins et al., 2002), and for a 2-fold increase in oral dose there was a 2.8-fold and 3.9-fold increase in exposure. Consequently, pharmacokinetic parameters are dependent upon dose, and a greater than proportional increase in exposure is seen with increasing dose in rat and dog toxicology studies (Fig. 3).
kinetic profile of voriconazole. Therefore, these values are not absolute bioavailability values, but they do indicate that a large proportion of an oral voriconazole dose is systemically available. Oral bioavailability in human is estimated to be greater than 90% (Purkins et al., 2002). By contrast, the absorption of itraconazole is variable and only improved when the drug is administered with food (Grant and Clissold, 1989; Bailey et al., 1990). Itraconazole is extremely insoluble except under very acidic conditions, whereas voriconazole has an

**Fig. 4.** Proposed major metabolites of voriconazole (as percentage of dose) following multiple administration of $^{14}$C voriconazole to animal species and humans.
aqueous solubility of 0.7 mg/ml, a property that contributes to its excellent absorption profile.

Multiple oral administration of voriconazole results in an appreciable decrease in AUC in mouse and rat (3- to 6-fold), leading to lower systemic concentrations of the drug. This effect was less pronounced in the dog, and not observed in the guinea pig or rabbit, the latter already being a high-clearance species on single dose. Repeat-dose toxicology (30-day) studies with voriconazole in rats and dogs have demonstrated up to 3-fold induction of liver cytochrome P450 concentrations compared with control animals. This is associated with a dose-related liver enlargement in both species. These observations are consistent with autoinduction of voriconazole metabolism, thereby leading to a significant increase in clearance upon multiple dosing. These effects are completely reversible, and cytochrome P450 concentrations in rats that underwent a 1-month recovery (untreated) period returned to levels that were similar to those of control animals (data not shown).

The pharmacokinetic behavior of a compound can usually be predicted from single-dose data, particularly if the pharmacokinetics are linear. In the case of a saturated elimination process with zero-order elimination occurring within the dose range, supralinear accumulation would be expected when dose input approaches \( V_{\text{max}} \). Hence, modeling of the data from dog (both sexes) for multiple intravenous doses of 3 mg/kg predicted that accumulation would occur with no attainment of steady state (data not shown). However, in reality, both dog and rat show a reduction in AUC following multiple oral and intravenous administration. This is due to the unexpected autoinduction of metabolism upon multiple dosing, and consequently, nonlinear modeling of the pharmacokinetic data is still not predictive. Although preclinical studies predicted nonlinear pharmacokinetics of voriconazole, the complexity of this clearance prevented accurate estimation of clinical pharmacokinetic parameters based on allometry prior to clinical studies. This complexity also indicated that in vitro systems would be unlikely to provide a useful extrapolation tool at the preclinical stage of the program.

Autoinduction of voriconazole metabolism is not observed in humans. At steady state in humans, plasma concentrations of voriconazole remain constant with time. There is no evidence for induction of CYP3A4 activity since the ratio of excreted cortisol and 6-β-hydroxycortisol in urine remains unchanged following multiple administration (200 mg b.i.d.) (data not shown). It is not unusual to see induction of metabolism in preclinical species that is not subsequently observed in humans. The clinical doses used are normally lower than doses in the toxicology studies and, therefore, do not cause an induction response. Those drugs that are enzyme inducers in the clinic (e.g., troglitazone, rifampicin) are administered at high doses resulting in total drug concentrations in the 10 to 100 \( \mu \)M range (Smith, 2000). The mean steady-state (200 mg b.i.d.) plasma voriconazole concentration in humans is 1.7 \( \mu \)g/ml (5 \( \mu \)M), giving a free drug concentration of 0.7 \( \mu \)g/ml (2 \( \mu \)M), which overlaps with exposures causing induction of cytochrome P450 in animal species (Tables 3 and 4). This indicates that other factors (such as species differences in receptors) are involved in the observed induction profile of voriconazole.

Significant autoinduction of metabolism in mouse and rat upon multiple dosing compromises the use of these species for efficacy testing since steady-state plasma levels are difficult to maintain. Autoinduction of metabolism appears less significant in the dog, and is absent in the guinea pig and rabbit. However, the short terminal elimination half-life of less than 1 h in the rabbit means that this species is less than optimal for evaluation of voriconazole efficacy, although some success was obtained with frequent dosing of immuno-suppressed animals, using doses up to 45 mg/kg/day (George et al., 1996). The absence of significant autoinduction of metabolism in the
As a consequence of its nonlinear pharmacokinetic profile, voriconazole metabolism has been investigated following oral or intravenous administration of radiolabeled material at steady state in animals and humans. Generally such studies are performed after single doses, but given the autoinduction observed for voriconazole, this is unlikely to be representative of the metabolite profile at steady state, and as a result, the findings from steady-state studies are more relevant to the safety program. Metabolism of voriconazole is complex in all species, with up to 16 metabolites isolated, many representing small fractions of the administered dose. However, similar metabolic transformations are observed in most species. The primary routes of voriconazole metabolism involve fluoropyrimidine N-oxidation to form UK-121,265 (Fig. 4, pathway A), fluoropyrimidine hydroxylation, and methyl hydroxylation (Fig. 4, pathways B and C). Other metabolites arise from combinations of these pathways to form multiple oxidized metabolites. In addition, N-oxidation of the fluoropyrimidine ring facilitates carbon-carbon bond cleavage resulting in loss of the fluoropyrimidine moiety and giving rise to a major conjugated human metabolite (Fig. 4, pathways D and E). Several of the oxidized metabolites are conjugated with glucuronic acid in all species, resulting in a generally good similarity in the nature of secondary metabolites.

A minor human glucuronide-conjugated metabolite arose from de-fluorination of UK-215,364 (Fig. 4, pathway F) and was not detected in any of the animal species studied. This metabolite represented a minor proportion of the excreted dose (5%), was extremely polar in nature, and was not identified in the circulation. It is the end product of four metabolic steps from voriconazole, and the presumed intermediate precursors (UK-51,060 and UK-215,364) have been identified in animals. Conjugation with glucuronic acid is typically considered to be a detoxifying mechanism (Smith and Williams, 1966) since glucuronides are generally less biologically or chemically reactive than the parent aglycone, exhibiting higher polarity and thereby facilitating excretion.

Release of triazole was a major route of voriconazole metabolism in rat (38% of the dose). This was only observed in one other species, the dog, and it was a minor route of metabolism (5% of the dose). Similar loss of triazole has been observed for fluconazole in mice and dogs (Humphrey et al., 1985). Fluconazole is remarkably metabolically stable, and metabolism accounts for less than 10% of the excreted dose in these species. However, the triazole metabolite accounted for around 3% of the excreted urinary dose of fluconazole in mouse and dog.

Metabolism of voriconazole in the rabbit was unique compared with other species. Only two metabolites were excreted exclusively in the urine, and no unchanged voriconazole was detected. The two metabolites are isomeric products of hydroxylation on the fluoropyrimidine ring.

The major circulating metabolite in rat, dog, and human is the voriconazole N-oxide UK-121,265. Upon administration as a single intravenous dose to rat and dog, UK-121,265 was eliminated rapidly with a half-life of less than 2 h in rat and dog. There was evidence for a sex difference in clearance of the N-oxide in the rat, and one can speculate that it is metabolized by the same cytochrome P450 isoenzymes as voriconazole. It is proposed that further metabolism of UK-121,265 in animals and humans results in metabolites arising from pathways D, E, and F (Fig. 4). Following an intravenous dose of UK-121,265, there was evidence for reduction of the N-oxide back to voriconazole in both rat and dog.

A comparison of dose-normalized AUC values for UK-121,265 in rat and dog following multiple oral voriconazole administration and intravenous dosing of UK-121,265 gives a ratio that represents the

![Fig. 7. Plasma concentration profiles of the N-oxide metabolite UK-121,265 (■) and voriconazole (●) in rat and dog following single intravenous administration (1 mg/kg) of UK-121,265.](image)
fractional metabolism by the N-oxide route (Table 6). Values greater than unity for male rat and female dog are a consequence of the nonlinear elimination of voriconazole. Nevertheless, the high values obtained indicate that a significant proportion of a voriconazole dose is metabolized to the N-oxide in rat and dog and that this process represents a pivotal clearance step. Based on the similar importance of the pathway in humans, it is likely that the same statement can be applied. The N-oxide metabolite is not thought to contribute to the antifungal efficacy of voriconazole, since its activity against a wide range of fungal pathogens is at least 100-fold less than that of voriconazole (data not shown).

In summary, voriconazole shows capacity-limited elimination in all species investigated, and its pharmacokinetics are therefore dependent upon the administered dose. Consequently, it is not possible to accurately predict pharmacokinetics across species, even when nonlinear modeling is employed. Autoinduction of metabolism upon multiple dosing in most animal species (although not in humans) further complicates the pharmacokinetic profile of this compound. In keeping with its relative lipophilicity, voriconazole is well absorbed following oral administration, and it is cleared almost exclusively by metabolism. The major routes of metabolism in humans are all represented in animal species; therefore, appropriate preclinical species have been used for safety testing.

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References


