Pharmacokinetic Evaluation of UK-49,858, a Metabolically Stable Triazole Antifungal Drug, In Animals and Humans

M. J. HUMPHREY, S. JEVONS, AND M. H. TARBIT*
Pfizer Central Research, Sandwich, Kent, CT13 9NJ, United Kingdom

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The pharmacokinetic profile of UK-49,858 (fluconazole), a novel triazole antifungal agent which is being developed for oral and intravenous use, was determined in mice, rats, dogs, and humans. Comparative data following oral and intravenous administration showed that bioavailability was essentially complete in all four species. Peak concentrations in plasma of drug normalized to a 1-mg/kg dose level following oral administration, were relatively high: 0.7, 0.6, 1.1, and 1.4 μg/ml in mice, rats, dogs, and humans, respectively. The volumes of distribution ranged between 1.1 liter/kg in mice and 0.7 liter/kg in humans, which are approximate to the values for total body water. Whole body autoradiography studies in mice following intravenous administration of [14C]UK-49,858 demonstrated that the drug was evenly distributed throughout the tissues, including the central nervous system and the gastrointestinal tract. Plasma protein binding was low (11 to 12%) in all species. Marked species differences were observed in elimination half-lives, with mean values of 4.8, 4.0, 14, and 22 h in mice, rats, dogs, and humans, respectively. The major route of elimination of the drug was renal clearance, with about 70% of the dose being excreted unchanged in the urine in each species. Studies with [14C]UK-49,858 on metabolism and excretion (intravenous and oral) in mice and dogs showed that about 90% of the dose was recovered as unchanged drug in urine and feces, confirming the metabolic stability of the drug. This pharmacokinetic profile is markedly different from that of imidazole antifungal drugs and undoubtedly contributes to the excellent efficacy of UK-49,858 in vivo.

UK-49,858 [2-(2,4,-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol; fluconazole; Fig. 1] is a new systemically acting antifungal agent which has shown activity in several animal models of infection (14; P. F. Troke, R. J. Andrews, K. W. Brammer, M. S. Marriott, and K. Richardson, Antimicrob. Agents Chemother., submitted for publication). Typical oral 50% protective dose values against acute lethal systemic candidiasis in mice range between 0.1 and 0.3 mg/kg, demonstrating that the compound is between 20- and 100-fold more potent than ketoconazole in this model. Results of several studies with imidazole antifungal agents have shown that the pharmacokinetics of these compounds have a significant impact on their comparative efficacy in vivo. The pharmacokinetic profiles of such imidazole antifungal agents as miconazole (3, 12), econazole (13), and clotrimazole (7, 15) were characterized by poor oral bioavailability and low plasma concentrations due to significant first-pass metabolism and a large volume of distribution. The recent introduction of ketoconazole (1) has seen the advent of imidazole antifungal drugs with good systemic bioavailability and relatively high plasma concentrations. However, in common with previous imidazole, ketoconazole is highly bound to plasma proteins (4, 11), is extensively metabolized (9), and exhibits dose-dependent kinetics in both animals (1) and humans (2, 5). This report describes the pharmacokinetic profile of the bistriazole UK-49,858, which is markedly different from that of ketoconazole and related imidazoles and assesses the potential contribution of this profile to the efficacy of the drug in vivo.

MATERIALS AND METHODS

Formulation of the dose. UK-49,858 was dissolved in aqueous Cremophor EL (10% [vol/vol]; BASF, Ludwigshaven, Federal Republic of Germany) at concentrations up to 4 mg/ml for oral and intravenous administrations to rodents and in 0.15 M hydrochloric acid–ethanol (50% [vol/vol]; pH 2.4) at a concentration of 50 mg/ml for oral and intravenous administration to dogs. [14C]UK-49,858 (specific activity, 22.2 μCi/mg) was administered to mice and dogs in excretion studies as described above, with the exception that the oral dose for the dogs was dry mixed (1:4 [wt/wt]) with excipients (lactose–maize starch–sodium stearate: 3:1:0.05 by weight) and delivered in capsules since this was the method of dosing used in long-term toxicity tests. Human volunteers received the unlabeled drug dissolved in 0.1 M citric acid (pH 2.1) at a concentration of 1 mg/ml. Concentrations of drug solutions in all studies were monitored by the method used for analysis of plasma and urine samples and were within 10% of nominal concentrations.

Pharmacokinetic studies. Treatment of both rodent species in the pharmacokinetic experiments was essentially similar. Groups of five female mice (CD1; weight, 100 g; Charles River Breeding Laboratories, Ltd., Manston, United Kingdom) or female rats (CD; weight, 100 g; Charles River) were given oral or intravenous doses of UK-49,858 in solution in each experiment. Dose levels were 1 mg/kg (four experiments) and 40 mg/kg (one experiment) in the mouse studies and 20 mg/kg (one experiment) in the rat studies. Groups of animals were sacrificed by exsanguination under ether anesthesia at various times up to 24 h following oral or intravenous administration of the drug. Animals sacrificed at 24 h were housed in small animal metabolism cages (Biotech Consultants Ltd., Clackmannanshire, United Kingdom) for collection of urine.

The pharmacokinetic study in dogs was carried out with two male littermate beagles (weights, 13 kg) at a dose of 10 mg/kg using a crossover design. Intravenous doses were given as a slow bolus into the saphenous vein; oral doses...
were given by esophageal tube and washed in with water (10 ml). Blood samples were drawn from the cephalic veins by venipuncture at intervals up to 72 h after the dose was administered. Oral and intravenous doses to the same animal were separated by 14 days.

The pharmacokinetic profile in humans was determined in four male volunteers (age range, 18 to 45 years; weight range, 63 to 70 kg). Following an overnight fast each subject received a solution of UK-49,858 at a dose of 1 mg/kg followed by 150 ml water. A standard meal was provided 2 h after dosing. Blood samples were drawn from the antecubital veins at intervals up to 72 h after the dose was administered. Total voided urine was collected at daily intervals up to 72 h.

Blood from all studies was collected in lithium heparin tubes and centrifuged to separate plasma, which was stored at –20°C.

Pharmacokinetic parameters. Pharmacokinetic parameters for the drug were estimated by fitting the plasma concentration data to one-compartment open models by least-squares regression analysis. Values for area under the plasma concentration-time profiles, oral bioavailability, total plasma clearance, renal clearance, volume of distribution, and half-life were calculated by conventional formulas described previously (10). All parameters were normalized for body weight.

Tissue distribution. Tissue distribution of [14C]UK-49,858 was studied in male mice by whole body autoradiography following intravenous administration at a dose of 40 mg/kg. Animals were sacrificed at either 1 or 24 h after dosing. Whole body sections were prepared by the method of Ullberg (18), and were apposed to x-ray film (OSRAY M3; Agfa-Gevaert, Brentford, United Kingdom) for periods of up to 2 weeks.

Plasma protein binding. Protein binding of [14C]UK-49,858 was determined by adding drug to samples of pooled plasma from untreated mice, rats, dogs, and humans to produce initial concentrations of 0.1, 1.0, and 10.0 µg/ml. Duplicate samples were dialyzed against 0.1 M sodium phosphate buffer (pH 7.4) for 3 h at 37°C with a rotating dialyzer (Dianorm M.S.E. Ltd., Crawley, United Kingdom). Drug concentrations in buffer and plasma were determined by liquid scintillation counting.

Disposition studies. The disposition of [14C]UK-49,858 in mice following oral or intravenous administration at a dose level of 10 mg/kg was studied with two groups with three female mice each. Animals were housed in metabolism cages (Biotech Consultants Ltd.) designed for the separate collec-

![FIG. 1. Structure of [14C]UK-49,858. Asterisks denote position of 14C.](image)

![FIG. 2. Concentrations of UK-49,858 in plasma of various animal species following oral administration. Data are normalized to a 1-mg/kg dose level. Values are for pooled samples of plasma from groups of five mice (●) and rats (□) at each time point and mean data from individual dogs (n = 2; ■) and human volunteers (n = 4; △). Actual dose levels were 1, 20, 10, and 1 mg/kg in mice, rats, dogs, and humans, respectively.](image)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Conc (µg/ml) at the following dose levels (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>0.63</td>
</tr>
<tr>
<td>1</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>0.49</td>
</tr>
<tr>
<td>6</td>
<td>0.54</td>
</tr>
<tr>
<td>8</td>
<td>0.47</td>
</tr>
<tr>
<td>24</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Concentration data were obtained by using pooled plasma samples from five animals for each time point. Area under the curve values for 8.5 and 259 ug h/ml for the 1- and 40-mg/kg dose levels, respectively.
tion of urine and feces. Pooled samples of urine and feces were collected daily for 4 days. The disposition of the drug in dogs was determined with two female beagle littermates (weight, 15 and 16 kg) in a crossover experiment at a dose of 7.5 mg/kg. In addition, two female beagle littermates (weight, 12 kg each) were given either an intravenous or an oral dose at the same dose level. Dogs were housed singly in metabolism cages (Modular Systems & Developments Company Ltd., London, United Kingdom), and urine and feces were collected from individual animals daily for 6 days. All samples were stored at -20°C prior to analysis.

**Analysis of samples.** Samples of plasma and urine from pharmacokinetic studies were assayed by a gas chromatographic method specific for the drug (M. H. Tarbit and P. R. Wood, manuscript in preparation). Briefly, samples (0.1 to 1 ml) were made basic with 1 M sodium hydroxide (1 ml) and extracted with ethyl acetate (3 ml). The organic phase was transferred to clean glass tubes and back extracted with 1 M HCl (2 ml). The organic phase was then discarded, and the aqueous phase was again made basic with 5 M NaOH (1 ml) followed by extraction with ethyl acetate (3 ml). The organic layer was transferred to clean glass tubes and evaporated to dryness under nitrogen at 40°C. The samples were reconstituted in ethyl acetate (50 μl) for gas chromatography. Sample volumes (2 μl) were injected onto a column which was deactivated to reduce adsorption. The column contained 10% SE32 silicone gum (Perkin Elmer, Buckinghamshire, United Kingdom) on a benzoylated stationary phase (Chromasorb G. NAW; Chromatography Services Ltd., Wirral, United Kingdom) (17). The column temperature was 270°C. Detection was by electron capture at 350°C. Accuracy of the method, determined by assay of quality control samples, was ±5%.

Concentrations of radioactive compounds in samples of urine were determined by liquid scintillation counting in Instagel (Packard Instruments Ltd., Berkshire, United Kingdom). Samples of feces were homogenized and lyophilized prior to combustion in sample oxidizer. The resultant 14CO2 was trapped with Carbosorb (Packard Instruments Ltd.) and measured by liquid scintillation counting. Chromatographic analysis of samples of neat urine was carried out on silica gel plates (5735; Merck, Darmstadt, Federal Republic of Germany) in solvent systems comprising either butan-1-ol-acetic acid-water (60:15:25, by volume) or chloroform-methanol-0.880 ammonia (80:20:1, by volume). Samples of freeze-dried feces were dialyzed with methanol (recovery, 99% of total radioactivity), and chromatographic analysis of the methanolic extract was carried out as described above.

**RESULTS**

**Pharmacokinetics.** The concentrations of UK-49,858 in plasma, normalized to a 1 mg/kg dose, in mice, rats, dogs, and humans following single oral doses are shown in Fig. 2. Peak concentrations were observed within 4 h of dosing and were similar in all four species, ranging from 0.7 μg/ml in mice to 1.4 μg/ml in humans. Concentrations of UK-49,858 in the plasma of mice following oral administration at 1 and 40 mg/kg are shown in Table 1. The concentrations show good linearity, indicating that systemic exposure to the drug is proportional to the dose over a 40-fold dose range in this species.

Oral bioavailability, calculated by the ratio of area under the curve values in plasma following oral and intravenous administrations, was complete in mice and dogs (Table 2). Although intravenous studies were not conducted in rats or humans, values for percentages of dose excreted unchanged in urine following oral doses (82 and 64%, respectively; Table 2) confirm that oral bioavailability is high in all four species.

Pharmacokinetic parameters of the drug in each species are shown in Table 3. Values for volume of distribution, calculated from plasma clearance and half-life values following intravenous administrations, were 1.1 and 0.7 liter/kg in mice and dogs, respectively, and were similar to values calculated assuming complete bioavailability in oral studies in rats and humans. This volume of distribution is approximate to that for total body water and suggests that the drug

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mouse Oral</th>
<th>Mouse Intra</th>
<th>Rat Oral</th>
<th>Rat Intra</th>
<th>Dog Oral</th>
<th>Dog Intra</th>
<th>Human Oral</th>
<th>Human Intra</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (liter/kg)</td>
<td>0.9</td>
<td>1.1</td>
<td>0.8</td>
<td>—</td>
<td>0.8</td>
<td>0.7</td>
<td>0.7 ± 0.06</td>
<td>—</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>5.1</td>
<td>4.5</td>
<td>4.0</td>
<td>—</td>
<td>15</td>
<td>13</td>
<td>22 ± 3.5</td>
<td>—</td>
</tr>
<tr>
<td>CLplasma (ml/min/kg)</td>
<td>2.0</td>
<td>3.9</td>
<td>2.2</td>
<td>—</td>
<td>0.62</td>
<td>0.65</td>
<td>0.40 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>CLA (ml/min/kg)</td>
<td>1.6</td>
<td>3.1</td>
<td>1.8</td>
<td>—</td>
<td>0.38</td>
<td>0.46</td>
<td>0.27 ± 0.07</td>
<td>—</td>
</tr>
</tbody>
</table>

* Pharmacokinetic parameters were derived from plasma data obtained by using groups of 40 mice and rat and pooled samples from five animals per time point), from 2 dogs, and from 4 normal human subjects. Mean values ± standard deviation are given for humans. Oral data assumes complete bioavailability. Abbreviations: V, volume of distribution; t1/2, half-life; CLplasma, plasma clearance; CLA, renal clearance.

* — No data were obtained in these species via the intravenous route.

* Calculated using data for urinary excretion of drug (Table 5).
FIG. 3. Whole body autoradiograph of a mouse showing distribution of radioactivity at 1 h following intravenous administration of [14C]UK-49,858 at 40 mg/kg. Abbreviations: b, brain; bl, blood; g, gastrointestinal tract; k, kidney; l, liver; s, spleen; u, urinary bladder; t, testes.

is not extensively bound to tissues. A whole body autoradiograph at 1 h after an intravenous administration of [14C]UK-49,858 in a mouse is shown in Fig. 3. The results show that the drug was evenly distributed throughout the tissues, including the central nervous system and the gastrointestinal tract. Only the organs responsible for elimination of the drug, the liver and kidneys, showed accumulation above that in blood. Autoradiographs at 24 h after the dose was administered showed that the drug and its metabolites were essentially eliminated within 24 h of dosing. Plasma protein binding was low in all species, with values (±standard deviation) being 12 ± 0.8, 12 ± 0.7, 11 ± 1.1, and 11 ± 0.4% in mice, rats, dogs, and humans, respectively, and did not vary over the concentration range tested.

A marked species difference was evident in the elimination half-life of UK-49,858 (Table 3), with half-life values ranging from about 4 h in rodents to 22 h in humans. Since the volume of distribution of the drug was similar in all species, the different half-life values reflect species differences in total plasma clearance. Analysis of urine for the percentage of dose excreted unchanged (Table 2) showed that the major route of elimination of drug in all species was by renal clearance.

Disposition. Detailed studies on the disposition of [14C]UK-49,858 were carried out in mice and dogs following oral and intravenous administrations. The percentages of dose excreted in urine and feces in each species are shown in Table 4. The major route of excretion of drug-related material in mice and dogs was via the urine, accounting for about 80% of the dose in both species following intravenous administration. Radioactivity in the feces (Table 4) accounted for 10% in mice and 5% in dogs following intravenous administration, confirming that this was a minor route of excretion. Chromatographic analysis of urine and feces from mice, in two solvent systems (only one is shown; Table 5), showed that the major radioactive component excreted was unchanged drug. [14C]UK-49,858 represented 91.2 and 90.8% of urinary radioactivity (75 and 81% of the dose) and 69 and 86% of fecal radioactivity (13.9 and 8.4% of the dose) following oral and intravenous administration, respectively. Thus about 90% of the dose was excreted as unchanged drug in mice following intravenous administration. The excretion pattern in dogs was similar (Table 5), with unchanged drug in urine representing 88.9 and 90.5% of urinary radioactivity (64 and 71% of the dose) following oral and intravenous administration, respectively. Chromatographic analysis of dog feces was not attempted as excretion of radioactivity was low.

Three minor radioactive metabolites were observed in the urine of both species (Table 5), none of which accounted for more than 4% of the dose. One of these minor metabolites was identified as [14C]1,2,4-triazole, indicating that at least one of the other radioactive metabolites arises as a result of loss of triazole, presumably by N-dealkylation.

**DISCUSSION**

This report describes the pharmacokinetic profile of UK-49,858 in the three animal species and in humans, which were used in efficacy and safety tests of the drug. The results demonstrate that the relatively high plasma concentrations of drug observed in all species are related to the common properties of high oral bioavailability and a volume of distribution of about 1 liter/kg. Both of these pharmacokinetic properties are desirable for an antifungal drug intended for oral treatment of systemic and superficial infections. The volume of distribution of the drug, allied with its low binding to protein, lead to high free concentrations throughout the body, including the brain and the gastrointestinal tract, suggesting that the drug may be effective against infections in a variety of body sites.

The major species difference in the pharmacokinetic profile was the relative rate of drug elimination from plasma. Differences in half-lives between mice, used extensively as a model species for efficacy, and humans, the therapeutic target, were about fivefold. Renal clearance of unchanged drug accounted for about 70% of total clearance in each species, and since volumes of distribution were similar, the differences in half-lives can be attributed to the different glomerular filtration rates in each species. However, the values for renal clearance were about 10 to 15% of the respective values for the glomerular filtration rate in each species. Protein binding was uniformly low, indicating that the drug is extensively reabsorbed in the urinary tract. This combination of high renal filtration and extensive tubular reabsorption suggests that UK-49,858 may be more effective against fungal infections in the urinary tract than imidazole antifungal agents, which are highly protein bound and,

**TABLE 4. Excretion of radioactivity in dogs and mice following administration of [14C]UK-49,858**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)*</th>
<th>Percentages of radioactive dose recovered in:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 to 24 h</td>
<td>24 to 96 h</td>
</tr>
<tr>
<td>Mouse</td>
<td>10 (i.v.)</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>10 (p.o.)</td>
<td>56</td>
<td>27</td>
</tr>
<tr>
<td>Dog</td>
<td>7.5 (i.v.)</td>
<td>42 ± 25</td>
<td>37 ± 23</td>
</tr>
<tr>
<td></td>
<td>7.5 (p.o.)</td>
<td>36 ± 13</td>
<td>36 ± 5</td>
</tr>
</tbody>
</table>

* Intravenous (i.v.) and oral (p.o.) routes of administration are as indicated.

* Values are for pooled samples in mice (three per metabolism cage) and mean ± standard deviation for individual samples in dogs (n = 3).
consequently, are not filtered to any great extent in the kidneys.

Drugs which are predominantly excreted by passive renal clearance generally exhibit predictable kinetics. This property was evident for UK-49,858 which exhibited good dose proportionality in systemic exposure in the mouse over a 40-fold dose range. First-order kinetics and predictable steady-state concentrations also have been a feature of long-term safety evaluation tests with the drug in mice and dogs and pharmacokinetic and toleration studies over a 12-fold dose range in humans (S. Jevons and M. H. Tarbit, International Society for Human and Animal Mycology, IXth International Congress, Atlanta, 1985). Thus the kinetics of UK-49,858 are likely to be predictable over the clinically relevant dose range.

The pharmacokinetic properties of UK-49,858 differ markedly from those reported for other systemically acting imidazole antifungal drugs in clinical use. Thus the high oral bioavailability of the drug in all species is in marked contrast to the poor bioavailability of miconazole, which undergoes significant first-pass metabolism (12) and is limited to topical and intravenous use. Comparison of oral bioavailability of UK-49,858 with that of ketoconazole is difficult. No data have been published on systemic exposure following intravenous administration of ketoconazole in animals or humans, and excretion of unchanged drug in urine following oral administration is less than 5% (11). Consequently, absolute oral bioavailability cannot be determined. However, in contrast to UK-49,858, the oral bioavailability of ketoconazole appears to be variable in laboratory species (9) and dose dependent in animals (1) and humans (2).

Protein binding of UK-49,858 is low (11 to 12%); consequently, the major portion of the drug present in the body is unbound and is available for antifungal activity in tissues. This is in contrast to the imidazole antifungal agents, including ketoconazole, in which plasma protein binding is typically in excess of 98% (4). This 40- to 50-fold difference in unbound fractions of UK-49,858 and ketoconazole in plasma is likely to be a significant contributory factor to the 20- to 100-fold potency advantage of UK-49,858 in vivo over ketoconazole (14; Troke et al., submitted). The significant penetration of UK-49,858 into the brain and cerebrospinal fluid of mice observed in the whole body autoradiography studies is also in contrast to the poor and variable levels in cerebrospinal fluid reported for miconazole (6, 8) and ketoconazole (4). These findings suggest that UK-49,858 may exhibit greater efficacy against fungal infections of the brain than current imidazole drugs.

A further possible contribution to the improved efficacy of UK-49,858, relative to that of ketoconazole, is the greater and more prolonged systemic exposure to the drug, reflecting a smaller volume of distribution and lower clearance. Half-lives for UK-49,858 in plasma were longer in each species than those reported for ketoconazole, which has half-life values of 2.0, 1.1, 2.8, and about 3.0 h in mice (unpublished data), rats (9), dogs (9) and humans (2), respectively. The difference in half-lives between the compounds in humans is most striking, and it is apparent that continuous exposure to significant concentrations of UK-49,858 can be achieved by administration once daily.

The clearest distinction between the bis-triazole UK-49,858 and current systemic imidazole antifungal agents is evident in the routes of elimination. Renal clearance is the major route of excretion of UK-49,858, with metabolism accounting for less than 10% of total drug excreted in mice and dogs. This pattern of excretion reflects the relative polarity and metabolic stability of the drug, unlike other imidazole antifungal drugs, including miconazole and ketoconazole, all of which are extensively metabolized in animals and humans (3, 4, 11, 13, 16).

The results presented here demonstrate that UK-49,858 has novel pharmacokinetic properties, including good oral bioavailability and prolonged systemic exposure, low protein binding, even tissue distribution, and extensive renal clearance. This profile is undoubtedly a major contributory factor to the efficacy of the drug in animal models of fungal infections and may lead to enhanced efficacy in humans.

LITERATURE CITED


