Compartmental pharmacokinetics and tissue distribution of the antifungal triazole ravuconazole following intravenous administration of its di-lysine phosphoester prodrug (BMS-379224) in rabbits

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Objectives: Ravuconazole is a broad-spectrum antifungal triazole in clinical development. We investigated the compartmental plasma pharmacokinetics and tissue distribution of ravuconazole following administration of its novel intravenous (iv) di-lysine phosphoester prodrug, BMS-379224.

Methods: Normal catheterized rabbits received the prodrug at 1.25, 2.5, 5, 10, 20 and 40 mg/kg once daily as 5 min iv bolus for 8 days. Serial plasma levels were collected at days 1 and 7, and tissues were obtained 30 min after the eighth dose. Concentrations of ravuconazole were determined by a validated HPLC method. Plasma concentration data were fitted to a three-compartment pharmacokinetic model. Pharmacokinetic parameters were estimated by weighted non-linear least squares regression analysis using the WinNonlin computer program.

Results: Following single dosing, ravuconazole demonstrated linear plasma pharmacokinetics across the investigated dosage range. Cmax, AUC0–1, Vss, CL and terminal half-life (means ± SEM) ranged from 2.03 to 58.82 mg/L, 5.80 to 234.21 mg·h/L, 5.16 to 6.43 L/kg, 0.25 to 0.18 L/h/kg and 20.55 to 26.34 h, respectively. Plasma data after multiple dosing revealed non-linear disposition at the 20 and 40 mg/kg dosage levels as evidenced by a dose-dependent decrease in CL from 0.104–0.147 to 0.030 and 0.022 L/h/kg; P = 0.1053) and an increase in the dose-normalized AUC0–1 (from 2.40–3.01 up to 11.90 and 14.56 mg·h/L; P = 0.0382). Tissue concentrations 30 min after the last dose were highest in the liver (12.91–562.68 mg/g), adipose tissue (10.57–938.55 mg/g), lung (5.46–219.12 mg/g), kidney (3.95–252.44 mg/g) and brain tissue (2.37–144.85 mg/g).

Conclusions: The pharmacokinetics of ravuconazole fitted best to a three-compartment pharmacokinetic model. The compound revealed non-linear pharmacokinetics at higher dosages, indicating saturable clearance and/or protein binding. Ravuconazole displayed a long elimination half-life and achieved substantial plasma and tissue concentrations including in the brain.

Keywords: mycoses, chemotherapy, drug development

Introduction

Ravuconazole is a novel antifungal triazole that is structurally related to fluconazole but has an improved structure–activity relationship and enhanced potency against opportunistic fungi.1 Ravuconazole has an extended spectrum of antifungal activity without cross-resistance to polyenes and echinocandins. In vitro, ravuconazole has demonstrated potent, broad-spectrum activity against Candida spp., Cryptococcus neoformans and other yeast-like fungi, and potent, potentially fungicidal activity against Aspergillus spp., certain other hyaline moulds, dematiaceous moulds, endemic moulds and dermatophytes.2–8 The compound
displays excellent antifungal efficacy in animal models of superficial candidiasis,\textsuperscript{9,10} disseminated candidiasis,\textsuperscript{10,11} disseminated\textsuperscript{10,12,13} and invasive pulmonary aspergillosis,\textsuperscript{10,14} intracranial\textsuperscript{10} and disseminated cryptococcosis,\textsuperscript{4} and disseminated histoplasmosis,\textsuperscript{7} and has entered clinical development as an oral formulation.\textsuperscript{16–18}

The availability of an intravenous (iv) formulation is critical for treatment of fungal infections in severely ill immunocompromised patients. Ravuconazole di-lysine phosphoester (BMS-379224) confers solubility in aqueous solution and appears to be well-tolerated.\textsuperscript{19–21} In contrast to the cyclodextrins used as the vehicle for the parenteral formulations of itraconazole and voriconazole,\textsuperscript{22} the lysine and phosphate residues are readily cleared and will not accumulate in the state of renal insufficiency. Little is known, however, about the disposition of ravuconazole following administration of this novel iv prodrug. The purpose of this study was therefore to characterize the compartmental plasma pharmacokinetics and tissue distribution of ravuconazole after administration of ravuconazole di-lysine phosphoester over a large dosage range in healthy laboratory animals.

Materials and methods

Experimental design

Study drug. Ravuconazole di-lysine phosphoester (BMS-379224), the iv prodrug of ravuconazole (BMS-207147; Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA),\textsuperscript{19} was dissolved in sterile 5% dextrose (D5W) to produce a 150 mg/mL stock solution that was maintained at +4°C. Prior to use, the drug was freshly diluted with sterile D5W as appropriate. Ravuconazole was administered at ambient temperature in a total volume of 5 mL as a slow iv bolus over 5 min through the indwelling central silastic venous catheter.

Animals. Healthy female New Zealand White rabbits (Hazleton, Herndon, VA, USA) or normal rabbit tissue homogenates. Blank samples of all matrices also were extracted to ensure the absence of interfering peaks.

Analytical assay. Concentrations of ravuconazole were determined using reversed phase HPLC. The mobile phase consisted of acetonitrile/deionized water (58:42 v/v), delivered at 1.2 mL/min. Samples were maintained in the autosampler at room temperature in glass vials. The injection volume was 125 μL. Ravuconazole eluted at ~10 min, using a C-18 YMC ODS-AQ analytical column (150 × 4.6 mm ID, 5 μm particle size; YMC Inc., Wilmington, NC, USA) maintained at room temperature and ultraviolet detection at 284 nm.

Quantification was based on the peak height and the non-weighted concentration response of the external calibration standard BMS-207147 (Bristol-Myers Squibb Pharmaceutical Research Institute). Ten- to 13-point standard curves (range of concentrations, 0.05–2.5 and 0.05–80 mg/L for plasma; 0.05–2.5 mg/L for other body fluids; and 0.05 to up to 80 mg/L for tissues) were linear with $r^2$ values >0.980. Samples containing concentrations exceeding the upper limit of the high-range standard curves (0.05–80 mg/L) were assayed after dilution with mobile phase and determination of over-curve concentration–response linearity. The lower limit of quantification (LLQ) was 0.05 mg/L in plasma and body fluids and 0.25 μg/g in tissues. Accuracies in plasma were within ±12% and <10%, respectively.

Pharmacokinetic data analysis

Pharmacokinetic modelling. Pharmacokinetic parameters for ravuconazole were determined using compartmental analysis. Experimental plasma concentration-versus-time profiles were fitted to a

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A three-compartment open model with iv bolus input and linear first-order elimination from the central compartment using iterative weighted non-linear least squares regression with the WinNonlin computer program (Scientific Consultants, Lexington, KY, USA). Model selection was guided by visual inspection of the observed plasma profiles and Akaike’s information criterion. The model fit the data well with $r^2$ values for the individual fits ranging from 0.992 to 1.000 (mean 0.999). The regression lines through the plot of observed versus estimated concentrations did not differ from the line of identity, and no bias was observed. $C_{\text{max}}$ values were determined as model-estimated concentrations at 6 min after the start of the iv bolus, and $C_{\text{min}}$ values as model-estimated concentrations at 24 h post dosing, respectively. $\text{AUC}_{0-\infty}$ values were calculated from estimated 24 h plasma concentration profiles using the trapezoidal rule and extrapolation to infinity by standard techniques. Dose-linearity after single and after multiple dosing was determined by comparison of the dose-normalized $\text{AUC}_{0-\infty}$ across dosage levels by ANOVA and linear regression analysis. Accumulation was assessed for each dosage level by comparing the mean AUC between doses after multiple dosing as an approximation of AUC between doses at steady state with the mean $\text{AUC}_{0-\infty}$ after single dosing. Distribution and clearance terms were normalized to body weight to allow for comparison across species.

Statistical analysis. All values are presented as means of three animals each ±SEM. Differences between the means of pharmacokinetic parameters across dosage levels were evaluated by Kruskal–Wallis non-parametric ANOVA. A two-tailed $P$ value of <0.05 was considered to be statistically significant.

Results

Single-dose studies

The estimated plasma concentration-versus-time profiles of ravuconazole following single-dose administration of the di-lysine phosphoester prodrug are shown in Figure 1, and corresponding mean compartmental pharmacokinetic parameters are listed in Table 1.

Intravenous bolus administration at dosages of 1.25–40 mg/kg resulted in rising mean peak plasma levels that ranged from 2.03 ± 0.32 to 58.82 ± 3.05 mg/L and approximately dosage-proportional increases in $\text{AUC}_{0-\infty}$ ranging from 5.80 ± 1.08 to 234.21 ± 6.27 mg·h/L. Plasma concentration profiles showed a rapid initial distributive phase, followed by a slower β-phase and a prolonged γ-phase with an estimated mean terminal elimination half-life ranging from 15.83 ± 1.88 to 26.34 ± 7.08 h. Mean plasma levels at the end of the dosing interval of 24 h increased dosage-dependently and ranged from 0.05 ± 0.01 at the lowest to 3.03 ± 0.18 mg/L at the highest dosage. Consistent with dose-independent, linear plasma pharmacokinetics, total plasma clearance (CLt) and dose-normalized $\text{AUC}_{0-\infty}$ were not different across the investigated dosage range ($P = 0.2321$ and 0.1142, respectively). Similarly, the mean apparent volume of distribution at steady state ($V_{ss}$) did not change with the dosage and was between 4.97 ± 0.62 and 6.97 ± 1.96 L/kg.

Multiple-dose studies

The estimated plasma concentration-versus-time profiles of ravuconazole following once daily administration of the di-lysine phosphoester prodrug for six continuous days are shown in Figure 2, and the corresponding mean compartmental pharmacokinetic parameters are listed in Table 2.

Following multiple dosing, there was a trend toward a dose-dependent decrease in CLt at the 20 and 40 mg/kg dosage levels ($P = 0.1053$) together with a significant increase in the dose-normalized $\text{AUC}_{0-\infty}$ ($P = 0.0382$) (Figure 3) and a significant decrease in $V_{ss}$ ($P = 0.0309$). The mean AUC-based accumulation factor in plasma of ravuconazole was between 1.36 and 1.75 at dosages between 1.25 and 10 mg/kg and increased to 3.0 and 3.70 at the 20 and 40 mg/kg dosage levels ($P = 0.0188$) (Figure 3). The mean terminal elimination half-life overall increased to values of up to 50.99 ± 4.95 and 57.89 ± 24.4 h at the two highest dosage levels.

Tissue distribution

Mean tissue concentrations of ravuconazole 30 min following the last of seven consecutive doses were overall highest in the liver (12.91 ± 1.50 to 562.68 ± 33.03 μg/g), followed by adipose tissue (10.57 ± 0.17 to 938.55 ± 33.28 μg/g), marrow (7.34 ± 0.93 to 790.38 ± 39.57 μg/g), and lung (5.80 ± 0.32 to 285.27 ± 18.02 μg/g).

Figure 1. Concentration-versus-time profiles of ravuconazole (RCZ) in plasma after single intravenous bolus administration of di-lysine phosphoester ravuconazole over 5 min. Each point plots the mean level of three rabbits each ±SEM at that time point.
### Table 1. Single-dose compartmental pharmacokinetic parameters of ravuconazole in plasma

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</th>
<th>C&lt;sub&gt;min&lt;/sub&gt; (mg/L)</th>
<th>AUC&lt;sub&gt;0-&lt;infty&gt;&lt;/sub&gt; (mg.h/L)</th>
<th>V&lt;sub&gt;1&lt;/sub&gt; (L/kg)</th>
<th>V&lt;sub&gt;2&lt;/sub&gt; (L/kg)</th>
<th>V&lt;sub&gt;3&lt;/sub&gt; (L/kg)</th>
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<th>CL&lt;sub&gt;3&lt;/sub&gt; (L/h/kg)</th>
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<th>t&lt;sub&gt;1/2b&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2Y&lt;/sub&gt; (h)</th>
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<tbody>
<tr>
<td>1.25</td>
<td>2.03 ± 0.32</td>
<td>0.05 ± 0.01</td>
<td>5.80 ± 1.08</td>
<td>0.57 ± 0.16</td>
<td>1.50 ± 0.37</td>
<td>3.12 ± 1.32</td>
<td>5.16 ± 1.47</td>
<td>3.34 ± 0.34</td>
<td>0.49 ± 0.34</td>
<td>0.25 ± 0.10</td>
<td>0.07 ± 0.01</td>
<td>1.99 ± 0.99</td>
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<td>2.50</td>
<td>3.11 ± 0.92</td>
<td>0.14 ± 0.01</td>
<td>11.14 ± 1.84</td>
<td>0.82 ± 0.36</td>
<td>1.49 ± 0.68</td>
<td>3.80 ± 1.18</td>
<td>6.12 ± 1.61</td>
<td>5.24 ± 1.68</td>
<td>0.64 ± 0.14</td>
<td>0.22 ± 0.04</td>
<td>0.05 ± 0.00</td>
<td>1.55 ± 0.63</td>
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<td>5</td>
<td>3.32 ± 0.27</td>
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<td>1.47 ± 0.07</td>
<td>4.35 ± 1.81</td>
<td>6.97 ± 1.96</td>
<td>14.8 ± 13.28</td>
<td>0.85 ± 0.34</td>
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<td>0.07 ± 0.09</td>
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<td>10</td>
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<td>45.66 ± 3.42</td>
<td>0.60 ± 0.04</td>
<td>1.24 ± 0.31</td>
<td>2.73 ± 0.69</td>
<td>4.76 ± 0.62</td>
<td>7.85 ± 1.27</td>
<td>0.61 ± 0.23</td>
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<td>20</td>
<td>36.63 ± 6.13</td>
<td>1.43 ± 0.13</td>
<td>112.93 ± 20.5</td>
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<td>1.38 ± 0.18</td>
<td>3.31 ± 0.79</td>
<td>5.05 ± 0.45</td>
<td>3.69 ± 0.26</td>
<td>1.11 ± 0.43</td>
<td>0.18 ± 0.04</td>
<td>0.05 ± 0.02</td>
<td>1.38 ± 0.66</td>
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<tr>
<td>40</td>
<td>58.82 ± 3.05</td>
<td>3.03 ± 0.18</td>
<td>234.21 ± 6.27</td>
<td>0.63 ± 0.08</td>
<td>0.93 ± 0.11</td>
<td>4.41 ± 1.90</td>
<td>6.43 ± 1.96</td>
<td>4.69 ± 0.90</td>
<td>0.32 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.05 ± 0.00</td>
<td>0.99 ± 0.39</td>
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C<sub>max</sub>, peak plasma concentration; C<sub>min</sub> (24h), plasma concentration at the end of the recommended dosing interval (24h); AUC<sub>0-<infty></sub>, area under the concentration-time curve from zero to infinity; V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub>, volume of distribution of the first, second and third compartment; V<sub>ss</sub>, apparent volume of distribution at steady state; CL<sub>2</sub> and CL<sub>3</sub>, distributional clearances; CL<sub>t</sub>, total plasma clearance; t<sub>1/2a</sub>, distributional half-life; t<sub>1/2b</sub>, apparent elimination half-life; t<sub>1/2Y</sub>, terminal elimination half-life.

All values represent the means ± SEM of three rabbits each.

*For the comparison among dosage groups by Kruskal-Wallis non-parametric ANOVA.

### Table 2. Multiple-dose compartmental pharmacokinetic parameters of ravuconazole in plasma

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</th>
<th>C&lt;sub&gt;min&lt;/sub&gt; (mg/L)</th>
<th>AUC&lt;sub&gt;0-&lt;infty&gt;&lt;/sub&gt; (mg.h/L)</th>
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<th>V&lt;sub&gt;2&lt;/sub&gt; (L/kg)</th>
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<th>CL&lt;sub&gt;3&lt;/sub&gt; (L/h/kg)</th>
<th>t&lt;sub&gt;1/2a&lt;/sub&gt; (h)</th>
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<th>t&lt;sub&gt;1/2Y&lt;/sub&gt; (h)</th>
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<tr>
<td>1.25</td>
<td>1.90 ± 0.16</td>
<td>0.14 ± 0.02</td>
<td>11.13 ± 2.92</td>
<td>0.53 ± 0.20</td>
<td>0.93 ± 0.11</td>
<td>2.35 ± 0.51</td>
<td>3.83 ± 0.48</td>
<td>6.11 ± 5.21</td>
<td>0.32 ± 0.12</td>
<td>0.10 ± 0.03</td>
<td>0.05 ± 0.04</td>
<td>1.52 ± 0.58</td>
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<tr>
<td>2.50</td>
<td>3.89 ± 0.24</td>
<td>0.29 ± 0.06</td>
<td>22.24 ± 8.50</td>
<td>0.46 ± 0.19</td>
<td>1.25 ± 0.37</td>
<td>2.47 ± 1.36</td>
<td>4.20 ± 1.18</td>
<td>7.57 ± 6.02</td>
<td>0.52 ± 0.12</td>
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<td>5</td>
<td>5.39 ± 0.52</td>
<td>0.50 ± 0.09</td>
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<td>1.28 ± 0.37</td>
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<td>17.37 ± 2.29</td>
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<td>1.07 ± 0.32</td>
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<td>20</td>
<td>22.46 ± 1.14</td>
<td>6.93 ± 1.39</td>
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<td>0.76 ± 0.23</td>
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<td>7.28 ± 9.53</td>
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<td>0.13 ± 0.19</td>
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<td>40</td>
<td>44.34 ± 3.68</td>
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<td>1677.0 ± 349</td>
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<td>0.02 ± 0.00</td>
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<td>1.33 ± 0.74</td>
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C<sub>max</sub>, peak plasma concentration; C<sub>min</sub> (24h), plasma concentration at the end of the recommended dosing interval (24h); AUC<sub>0-<infty></sub>, area under the concentration-time curve from zero to infinity; V<sub>1</sub>, V<sub>2</sub> and V<sub>3</sub>, volume of distribution of the first, second and third compartment; V<sub>ss</sub>, apparent volume of distribution at steady state; CL<sub>2</sub> and CL<sub>3</sub>, distributional clearances; CL<sub>t</sub>, total plasma clearance; t<sub>1/2a</sub>, distributional half-life; t<sub>1/2b</sub>, apparent elimination half-life; t<sub>1/2Y</sub>, terminal elimination half-life.

All values represent the means ± SEM of three rabbits each.

*For the comparison among dosage groups by Kruskal-Wallis non-parametric ANOVA.
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Figure 2. Concentration-versus-time profiles of ravuconazole (RCZ) in plasma after intravenous bolus administration of di-lysine phosphoester ravuconazole over 5 min for six consecutive daily dosages. Each point plots the mean level of three rabbits each ±SEM at that time point.

319.41 ± 23.14 µg/g), kidney (3.95 ± 0.58 to 252.44 ± 41.59 µg/g), lung (5.46 ± 0.27 to 219.12 ± 4.75 µg/g), brain (2.37 ± 0.06 to 144.85 ± 22.61 µg/g), spleen (2.13 ± 0.09 to 130.88 ± 11.12 µg/g), choroid (0.59 ± 0.04 to 76.69 ± 7.38 mg/L) and muscle tissue (0.86 ± 0.02 to 51.26 ± 3.32 µg/g) (Table 3). Concentrations of ravuconazole in CSF, vitreous and aqueous were comparatively lower and exceeded the LLQ of 0.05 mg/L only at dosages exceeding 2.5, 2.5 and 5 mg/kg, respectively. Concentrations in the liver, adipose tissue, marrow, kidney, lung, brain and spleen exceeded concurrent plasma concentrations at the selected sampling time of 30 min post-dosing; whereas concentrations in all other tissues and body fluids were less than or equal to concurrent plasma concentrations.

Toxicity

Compared with values obtained in drug-naive animals, there was a trend toward increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values (P = 0.1020 and 0.1183, respectively) and decreased potassium (P = 0.0522) and alkaline phosphatase (P = 0.0106) values at the 20 and 40 mg/kg dosage levels after treatment for 7 days (Table 4). Abnormal elevations in the mean blood urea nitrogen, serum creatinine, and bilirubin were not observed in samples determined after 8 days of treatment. Throughout the pharmacokinetic study, no apparent infusion-related toxicities or other clinical or behavioural abnormalities were observed and no abnormal weight changes were noted.

Discussion

The results of this study reveal linear plasma pharmacokinetics of ravuconazole following multiple once daily bolus administration of its iv di-lysine phosphoester prodrug to rabbits at dosages between 1.25 and 10 mg/kg/day. With further dose escalation to 20 and 40 mg/kg/day, the disposition became non-linear, indicating saturable clearance from the bloodstream and/or saturable protein binding at higher dosages. Plasma concentration data fitted best to a three-compartment open pharmacokinetic model with an apparent elimination half-life in the order of 20–30 h. Ravuconazole achieved and maintained total plasma concentrations that were multiple times in excess of MIC90s reported for susceptible opportunistic fungi. Tissue concentrations near the completion of the initial distributive phase showed substantial disposition in liver, adipose tissue, bone marrow, lung, kidney, spleen and skeletal muscle. While drug concentrations in non-inflamed CSF, aqueous and vitreous fluid were low, substantially higher concentrations were found in brain tissue and choroid. The compound was well tolerated in rabbits without evidence for clinical or major laboratory toxicities.

Published data on the pharmacokinetics of ravuconazole are limited. After oral administration of 10 mg/kg of ravuconazole to female rats, the mean elimination half-life was 16.9 h; throughout the 72 h wash out, drug concentrations in the lung were four- to 20-fold higher than concurrent plasma concentrations.27 In normal human volunteers, following 14 daily dosages ranging from 50 to 400 mg given as gelatin capsules, ravuconazole exhibited approximately linear pharmacokinetics with mean Cmax values ranging from 1.22 to 6.02 mg/L, mean AUCl0–∞ ranging from 24.68 to 119.12 mg·h/L, and the mean half-life ranging from 103 to 240 h. In comparison with the first dose, there was an 8.5- to 10.8-fold accumulation of ravuconazole on day 14.28 Following iv administration, across species, the di-lysine phosphoester prodrug is readily converted to ravuconazole.19–21 In rats, dogs and monkeys, there were no principal differences in disposition compared with oral ravuconazole. The mean elimination half-life was in the order of 12, 14 and 7.4 h, respectively, and antifungal efficacy of the prodrug was similar to oral ravuconazole in a murine model of disseminated candidiasis.19,20 In a single-dose pharmacokinetic study in healthy male human volunteers, ravuconazole di-lysine phosphoester was administered as 1 h infusion at escalating dosages ranging from 25 to 600 mg. Ravuconazole exhibited linear pharmacokinetics; mean Cmax values ranged from 0.56 to 12.95 mg/L, mean AUCl0–∞ values from 28.60 to 787.6 mg·h/L, and the mean half-life was between 76 and 202 h.21 Overall, with its prolonged elimination half-life and significant accumulation in plasma and tissues, the pharmacokinetics of ravuconazole in the
ravuconazole after multiple once daily dosing for 7 days revealed substantial drug concentrations in lung, liver, spleen, marrow, adipose tissue and skeletal muscle near the completion of the initial distributive phase in plasma. As is characteristic for a lipophilic compound\(^1\) with high (>95% in mice and humans) binding to plasma proteins,\(^1\) drug concentrations were comparatively low in non-inflamed CSF, aqueous and vitreous fluid, but considerably higher in brain tissue and choroid, suggesting the potential therapeutic usefulness of ravuconazole also in these secluded compartments.

Ravuconazole achieved plasma and tissue concentrations that were several fold in excess of MIC\(_{90}\)s of large collections of clinical Candida and Aspergillus isolates.\(^6,7\) In plasma, concentrations above these values (1 mg/L for Aspergillus spp. and 0.25 mg/L for Candida spp.) were maintained in a dose-dependent manner for up to 24 h. Similar to fluconazole,\(^32,33\) the ratio of free drug AUC\(_{0–24}/\text{MIC}\) was the critical pharmacokinetic/pharmacodynamic parameter associated with treatment efficacy in a murine kidney-target model of invasive candidiasis.\(^11\) Whether this parameter may also be predictive of the efficacy of ravuconazole against invasive pulmonary aspergillosis remains to be determined. In vitro pharmacodynamic studies of itraconazole and voriconazole have demonstrated time- and concentration-dependent antifungal dynamics of antifungal triazoles against Aspergillus fumigatus.\(^34\)

In persistently neutropenic rabbits with experimental invasive pulmonary aspergillosis, an AUC\(_{0–24}/\text{MIC}\) ratio of ≥60 and plasma levels exceeding the median MIC of the test isolates for the entire dosing interval were equally associated with effective treatment.\(^14\) Nevertheless, formal pharmacodynamic studies comparing single versus split dosing regimens will be needed for a rigorous assessment of the most predictive pharmacokinetic/pharmacodynamic parameter in invasive pulmonary aspergillosis.

In the absence of severe illness, immunosuppression or any concurrent medication, ravuconazole was well tolerated. Laboratory abnormalities consisted of a trend toward mild dose-dependent increases in hepatic transaminases (ALT and AST) and a dose-dependent decrease in serum potassium. These trends appeared to be limited to the highest multiple dosage levels of 20 and 40 mg/kg. Increases in hepatic transaminases and decreases in serum potassium are known class effects of antifungal azoles.\(^22,35\) However, preliminary safety data in human subjects indicate no evidence of dose-dependent changes in hepatic transaminases or serum potassium.\(^21,28\) and a similarly low level of hepatic and electrolyte disturbances when compared with fluconazole.\(^17\) The sporadic non-dose-dependent changes in serum alkaline phosphatase are of uncertain significance. These effects are usually not a component of triazole-induced hepatic changes. Effects on bone metabolism through interference with mammalian sterol and vitamin D metabolism may alter serum alkaline phosphatase activity.\(^36,37\) However, such changes are usually dosage dependent.

In conclusion, following administration of its iv di-lysine phosphate prodrug, the pharmacokinetics of ravuconazole in healthy rabbits were best described by a three-compartment pharmacokinetic model. The compound displayed non-linear disposition in plasma at escalating dosages, indicating saturable elimination from the bloodstream and/or saturable protein binding. Ravuconazole achieved and maintained potentially therapeutic total plasma concentrations exceeding MICs of susceptible opportunistic fungi and produced high concentrations in tissues that are common sites of invasive fungal infections. The compound was well tolerated without evidence of clinical or major laboratory

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**Figure 3.** (a) Plot of dose normalized AUC versus dosage of ravuconazole after multiple once daily dosing with di-lysine phosphate ester ravuconazole for six consecutive days. Open circles indicate values of individual animals; filled circles indicate corresponding means ± SEM. The slope of the regression line deviates significantly from zero, indicating non-linear disposition over the investigated dosage range. (b) Areas under the concentration-versus-time curve from 0 to infinity (AUC\(_{0–\infty}\)) between dosages (multiple dosing) of ravuconazole following administration of di-lysine phosphate ester ravuconazole. Each bar represents the mean value of three rabbits each ±SEM. Note the onset of significant drug accumulation in plasma over time (\(P = 0.0188\) after multiple once daily dosing with 20 and 40 mg/kg/day.

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\(^{1}\) Cognizant of the limitations of drug concentrations in tissue homogenates\(^29,30\) and the prevalence of different equilibria during the dosing interval, assessment of tissue concentrations of ravuconazole after multiple once daily dosing for 7 days revealed substantial drug concentrations in lung, liver, spleen, marrow, adipose tissue and skeletal muscle near the completion of the initial distributive phase in plasma. As is characteristic for a lipophilic compound\(^1\) with high (>95% in mice and humans) binding to plasma proteins,\(^1\) drug concentrations were comparatively low in non-inflamed CSF, aqueous and vitreous fluid, but considerably higher in brain tissue and choroid, suggesting the potential therapeutic usefulness of ravuconazole also in these secluded compartments.

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Table 4. Effects of ravuconazole on laboratory values after multiple dosing over seven days to healthy rabbits

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<td>Normal values</td>
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For treated rabbits, all values represent the means ± SEM of three animals 0.5 h after the last of seven doses; normal values stem from 24 healthy rabbits naive to any drug therapy.

aBy Kruskal–Wallis non-parametric ANOVA.

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Acknowledgements

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References


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