Pharmacokinetics of L-671,329 in Rhesus Monkeys and DBA/2 Mice

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The time course of plasma drug levels and urinary recovery for two lipopeptide antifungal antibiotics, L-671,329 and cilofungin, were measured in male rhesus monkeys (Macaca mulatta) and in female DBA/2 mice. The antibiotics were administered intravenously at 10 mg/kg of body weight in phosphate-buffered saline–26% polyethylene glycol for the rhesus monkeys and in 5% dimethyl sulfoxide for the mice. Plasma and urine drug concentrations were determined by high-pressure liquid chromatography and/or a microbiological assay versus Aspergillus niger, and pharmacokinetic parameters were determined for both species. In each of the two rhesus crossover tests as well as in the mouse studies, the pharmacokinetics of the two compounds were similar; however, a marked difference was evident between species. The half-lives of L-671,329 and cilofungin in plasma were 39 and 34 min in the mice and averaged 1.8 and 2 h in the rhesus monkeys, respectively. In mice and rhesus monkeys, urinary recovery was <4% for both compounds.

In the search for new therapeutic agents, animal models of disease states have been an indispensable tool for the prediction of efficacy in humans. For any number of reasons, a therapeutic advantage of one agent over another demonstrated in an animal model may not translate analogously to humans. Thus, it behooves an investigator to be critical of the models he relies on and to develop additional experimental procedures to confirm his predictions. It is customary in our laboratory, for example, to explore any species-specific pharmacokinetic properties of a compound of interest. A comparison in rodents versus primates provides some of the additional parameters necessary for projecting success or failure in humans.

In this study, species-specific pharmacokinetics of two recently reported, structurally similar (Fig. 1a and b) lipopeptide antifungal antibiotics, L-671,329 and cilofungin (LY-121019, L-646,991), are presented. These agents have fungicidal, narrow-spectrum activity against Candida and Aspergillus spp. (1, 5, 6), and L-671,329 has also been shown to have activity against Pneumocystis carinii (9, 10). They both have the potential for improved efficacy and tolerance over existing therapy.

MATERIALS AND METHODS

Mice. Female DBA/2 mice weighing 20 ± 2 g were used in these studies. The mice were housed and fed in accordance with National Institutes of Health guidelines (2).

Rhesus monkeys. Two male rhesus monkeys (Macaca mulatta) weighing 4.8 kg were used in these studies. They were also housed and fed in accordance with National Institutes of Health guidelines (2).

Drugs. L-671,329 and cilofungin were prepared in our laboratory by published methods (4, 11). They were diluted in 5% dimethyl sulfoxide to 1.0 mg/ml or phosphate-buffered saline–26% polyethylene glycol (molecular weight, 300) to 10 mg/ml. Samples were kept on ice at 0°C and administered the same day.

HPLC conditions. Conditions for high-pressure liquid chromatography (HPLC) were as follows. The column was a Whatman Partisil 5 ODS-3 column (4.5 mm by 12.5 cm) with a guard cartridge. The mobile-phase buffers were as follows: A, 10 mM potassium phosphate (pH 7.0)–acetonitrile (90:10); B, acetonitrile-water (90:10). The flow rate was 1.2 ml/min. Samples were injected at 50 μl. UV detection of L-671,329 and cilofungin was done at 220 and 255 nm, respectively. Chromatography was isocratic at 45% buffer B for L-671,329 and 42% buffer B for cilofungin. The peak areas were linear from 200 to 0.5 μg/ml (limit of detection based on a peak height signal-to-noise ratio of 3:1).

Microbiological assay. Drug concentrations were determined by zone diameter measurements on Antibiotic Medium 3 (Difco) agar seeded with spores of Aspergillus niger (ATCC 6275; NRRL 334). All samples were assayed in triplicate with 0.25-in. (ca. 0.64-cm) analytical paper discs (Schleicher & Schuell) on petri dishes (15 by 100 mm) containing 5 ml of agar. The sensitivity of the assay for both compounds was 0.08 μg/ml.

Sample preparation. Samples of whole blood or urine, unknowns, and controls spiked with known concentrations were diluted in 3 volumes of 50% methanol–50 mM potassium phosphate buffer (pH 6.5). This treatment was suitable for the microbiological assay. Further treatment for HPLC (blood only) involved dilution of the above-described mixture in acetonitrile (1:1; final dilution, 1:8), 1 h of incubation at 0°C, and centrifugation at 10,000 × g.

Pharmacokinetics. Three mice were used per time point; each mouse was given 200 μg of drug intravenously (i.v.) in 0.2 ml of 5% dimethyl sulfoxide. Blood was collected by heart puncture with heparinized syringes at 1, 5, 15, 30, 60, 90, 120, and 180 min. Blood from three mice was pooled prior to being processed. Urine was collected from a group of five mice over a 3-h period in a metabolism cage, allowing the separation of urine and feces.

Two male rhesus monkeys were anesthetized with ketamine hydrochloride (Ketaset; 100 mg/ml) given intramuscularly at 10 mg/kg of body weight as needed to maintain anesthesia throughout the experiment. To promote urine flow and allow the measurement of glomerular filtration rates, we started an i.v. infusion of 5% mannitol–saline containing 0.5 μCi of 51Cr-EDTA per ml and maintained it at 1.0 ml/min for the duration of the procedure. The monkeys were then each given 1 μCi of 51Cr-EDTA and 10 mg of L-671,329 or cilofungin per kg of body weight in 4.8 ml of phosphate-buffered saline–26% polyethylene glycol by i.v. bolus injection.
bolus injection through the saphenous vein at ≤3.0 ml/min. No adverse reactions were observed in either monkey with either antibiotic. Blood was drawn at 5, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 min or at 5, 30, 50, 70, 90, 110, 130, 150, 170, 190, and 210 min after drug administration from a heparin-locked i.v. catheter placed in the opposite leg vein. Urine was allowed to flow from an indwelling urethral catheter and was collected at 30, 50, 70, 90, 110, 130, 150, 170, and 190 min or at 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 min after drug administration. Glomerular filtration rates were determined in all rhesus monkey experiments by measuring the plasma and urine $^{51}$Cr-EDTA concentrations with a model 1260 Multi Gamma II (LKB/Wallace) gamma counter.

Pharmacokinetic parameters were determined by performing unweighted, nonlinear regression on the time course of plasma measurements with the two-compartment biexponential equation $C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ (8, 13), where $C$ = plasma concentration, $A$ = zero-time intercept for $\alpha$ (distribution phase), $B$ = zero-time intercept for $\beta$ (elimination phase), and $e = 2.7183$, and applying standard equations to calculate area under the plasma concentration-time curve (AUC), volume of distribution at steady state ($V_{as}$), half-life at the elimination phase ($t_{1/2B}$), total clearance (CL), and renal clearance (CLR) (3).

**RESULTS**

The microbiological assay was used for most assays (all monkey samples and mouse urine), as it was the simplest to

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>$V_{as}$ (liters/kg)</th>
<th>CL (ml/min/kg)</th>
<th>$t_{1/2B}$ (min)</th>
<th>AUC$_{0-3}$ (μg · h/ml)</th>
<th>AUC$_{0-\infty}$ (μg · h/ml)</th>
<th>Urinary recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-671,329</td>
<td>0.025</td>
<td>5.1</td>
<td>39</td>
<td>31.5</td>
<td>32.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Cilofungin</td>
<td>0.03</td>
<td>6.5</td>
<td>34</td>
<td>25.2</td>
<td>25.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>
implement. While adequately sensitive, the assay suffers operationally in a number of ways. The compounds diffuse poorly, yielding a zone/concentration slope of about 2 mm per double dilution, and the measurement precision is at best ±0.2 mm or roughly 10% because of the ragged zones produced by mycelial growth. In addition, a marked attenuation by whole plasma necessitates further dilution, resulting in reduced sensitivity and potential volumetric errors. The HPLC assay, while less sensitive, is simpler to perform and is generally reproducible to within 3% at all concentrations tested.

The pharmacokinetic parameters (Tables 1 and 2) established for L-671,329 and cilofungin demonstrated the equivalence of these two lipopeptide compounds. The CLs were similar, even in the two monkeys, i.e., 12.8 (L-671,329) versus 16.8 (cilofungin) ml/min/kg in one monkey and 4.9 (L-671,329) versus 4.8 (cilofungin) ml/min/kg in the other. Other parameters, i.e., \( V_{s, e} \), \( t_1/2_B \), \( AUC_{0-3.5} \), and \( AUC_{0-\infty} \) all showed the equality of these two compounds. Urinary recovery, extrapolated to infinity, was uniformly low (<4%) in both the rhesus monkeys and the mice, indicating nonrenal clearance for these compounds in both species.

In mice, L-671,329 and cilofungin were cleared rapidly in the initial distribution phase and then slowly cleared from the central compartment (Fig. 2). The pharmacokinetic parameters found for the mice also showed that these two compounds are very similar in the way that they are processed by this species.

### DISCUSSION

The kinetics of elimination of cilofungin in rabbits have been shown to be nonlinear by Lee et al. (7) and Walsh et al. (14), and the elimination pathway is predominantly hepatic (7). The low urinary recovery (CL >> CLb) observed in our studies of mice and rhesus monkeys suggests that the disposition of both compounds in these species is analogous to the rabbit pathway for cilofungin. This analogy probably extends to the nonlinear phenomenon as well. This hypothesis, of course, is impossible to demonstrate in single-dose, single-subject experiments.

In the studies cited above, the observed deviation from linearity occurred at doses far in excess of the dose used in our studies of L-671,329 and cilofungin. While this fact does not guarantee that each of the elimination processes in our animals was linear, the fits to the two-compartment model, the derivation of which is based on linear first-order transitions, were quite acceptable. Nevertheless, it is important to note that, in the absence of an established physical model, this mathematical model is only an approximation useful for specific comparisons only.

While a two-animal study clearly cannot represent a general model for primate pharmacokinetics, certain features of the kinetic picture are worth highlighting. Trailing plasma drug levels in the rhesus monkey studies (Fig. 3a and b) demonstrated low-level persistence for both compounds despite what appeared to be a very rapid overall clearance rate. It is noteworthy that the clearance of these antibiotics from the mice was very close to that from one of the rhesus monkeys (no. 14338) and slower than that from the other rhesus monkey (no. 14156). Given the usual relationship between CL and body weight [log CL = (0.662 - log body weight) + 0.648] (12) which results in a 20-g mouse clearing a drug approximately sixfold faster than a 5-kg monkey on a milliliter-per-minute-per-kilogram basis, the clearance of these compounds from the mice was unusually slow.

On the basis of in vitro susceptibilities (1), inhibitory levels of these compounds are achievable in the circulation. The pharmacokinetic parameters in the mice and rhesus monkeys clearly demonstrated the similarity of the two compounds evaluated in this study. Given the unusually favorable elimination kinetics in mice, our in vivo efficacy model, it will be a challenge to propose an efficacious dosage regimen in higher species, e.g., humans. Some degree of scaling will be necessary. More knowledge is needed to identify which pharmacokinetic parameter correlates best with therapeutic efficacy. One aspect that might be considered is the apparently slow fungidal mode of action (6). It is likely that therapeutic success may require that the

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**TABLE 2. Rhesus monkey pharmacokinetic parameters**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Rhesus monkey</th>
<th>( V_{s, e} ) (liters/kg)</th>
<th>CL (ml/min/kg)</th>
<th>CLb (ml/min/kg)</th>
<th>GFR* (ml/min/kg)</th>
<th>( t_1/2_B ) (h)</th>
<th>AUC0-3.5 (ug h/ml)</th>
<th>AUC0-\infty (ug h/ml)</th>
<th>Urinary recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-671,329</td>
<td>14156</td>
<td>0.24</td>
<td>12.8</td>
<td>0.01</td>
<td>2.7</td>
<td>1.3</td>
<td>12.9</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>14338</td>
<td>0.084</td>
<td>4.9</td>
<td>0.01</td>
<td>2.9</td>
<td>0.9</td>
<td>33.8</td>
<td>34.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cilofungin</td>
<td>14156</td>
<td>0.77</td>
<td>16.8</td>
<td>0.6</td>
<td>2.9</td>
<td>1.9</td>
<td>9.3</td>
<td>10</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>14338</td>
<td>0.14</td>
<td>4.8</td>
<td>0.1</td>
<td>3.2</td>
<td>2.1</td>
<td>33.7</td>
<td>34.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* GFR, glomerular filtration rate.

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**FIG. 2. L-671,329 (O) and cilofungin (★) AUCs following a 10-mg/kg i.v. bolus dose in mice. Each point represents the pooled blood of three mice. Best-fit parameters for the biexponential equation \[ C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \] were as follows: for L-671,329, \( A = 46.82, \alpha = 0.179, B = 30.32, \\) and \( \beta = 0.0179; r = 0.9963; \) and \( t_1/2_B = 39 \) min; for cilofungin, \( A = 51.55, \alpha = 0.225, B = 26.74, \\) and \( \beta = 0.0203; r = 0.9999; \) and \( t_1/2_B = 34 \) min.
FIG. 3. L-671,329 (○) and cilofungin (●) AUCs following a 10-mg/kg i.v. bolus dose in rhesus monkeys 14156 (a) and 14338 (b).

pathogen be exposed to MICs of a drug for lengthy periods (perhaps >3 h), implying that peak levels are less critical than AUC or time above the MIC (ΔT). The most apparent difference observed between the species in this study was ΔT. The mice maintained levels above 1 µg/ml (a concentration picked arbitrarily as being near the MIC for 90% of Candida isolates) for >3 h, while the rhesus monkey levels dropped to <1 µg/ml after 60 to 80 min. The t_{1/2b} in the rhesus monkeys, while satisfactorily long, is of little utility if drug levels are below the MIC. If scaled appropriately for ΔT, advantage could be taken of this superior t_{1/2b}, allowing for reduced dosage frequency in primates.

ACKNOWLEDGMENTS
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REFERENCES