Tissue Distribution after Intravenous Dosing of Micafungin, an Antifungal Drug, to Rats

Toshiro Niwa,*a Yoshiko Yokota,a Akira Tokunaga,a Yasuhiro Yamato,b Akira Kagayama,b Tomoichi Fujisawa,c Junko Hatakeyama,c Masaharu Anezaki,d Yuko Ohtsuka,d and Akira Takagi*a


The tissue distribution after an intravenous dose of micafungin (1 mg/kg), a new echinocandin-like lipopeptide antifungal agent, to male rats was investigated. Micafungin in plasma disappeared biexponentially with a terminal half-life of 5.03 h. Micafungin concentrations in liver, kidney, and lung at the first sampling time (5 min) after dosing were 1.15, 1.64, and 2.58-fold higher than the plasma concentration, and the AUC∞∞ were 1.61, 3.42, and 2.89-fold higher than that for plasma. The terminal half-lives for these tissues were 5.14, 4.87, and 5.31 h, respectively, which were comparable to those for plasma. These results suggest that micafungin distributes rapidly and moderately into tissues such as the liver, kidney, and lungs, and that the concentrations in tissues decreased in parallel with the unchanged drug in plasma.

Key words micafungin; antifungal drug; tissue distribution; rat; pharmacokinetics

Micafungin, a new echinocandin-like lipopeptide antifungal agent, has potent in vitro and in vivo activity against a variety of pathogenic Candida species and Aspergillus species by inhibiting the biosynthesis of 1,3-β-d-glucan, a major and specific component of the fungal cell wall, and its minimal inhibitory concentrations at which 90% of the isolates were inhibited (MIC90) against Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei, Aspergillus fumigatus, and Aspergillus flavus are less than 0.125 μg/ml.1—6 Micafungin has a significant therapeutic effect against deep-seated mycoses caused by Candida or Aspergillus, the major pathogenic fungi. The clinical responses in trials that examined the safety and efficacy of micafungin monotherapy (micafungin dosage: 12.5—150 mg) in Japan were 60% in invasive pulmonary aspergillosis, 67% in clonie necrotizing pulmonary aspergillosis, 55% in pulmonary aspergilloma, 100% in candidiasis, and 71% in esophageal candidiasis.6—9

It has been reported that micafungin exhibits linear pharmacokinetics after intravenous dosing to rats, mice, dogs, and rabbits, as well as humans.6—9 The radioactivity after intravenous dosing of 14C-labeled micafungin to male rats is widely distributed immediately and the radioactivity in tissues decreases almost in parallel with the radioactivity in plasma.10 Unchanged micafungin concentrations in rabbit tissues, including the liver, kidney, lungs, and spleen, at near peak plasma concentrations 30 min after the last of multiple doses over eight days are several-fold in excess of the MIC90 against the clinical isolates of Candida spp. and Aspergillus spp.7 However, there are few detailed studies on the tissue distribution kinetics of unchanged drug after an intravenous dose of micafungin in animals and humans.

In the present study, we investigated the distribution kinetics in tissues, such as liver, kidney, and lungs, after an intravenous dose of micafungin to male rats.

MATERIALS AND METHODS

Materials Micafungin and internal standard (FR195743)11 were synthesized and supplied by Fujisawa Pharmaceutical Co., Ltd. All other reagents were of the highest purity commercially available.

Animal Studies Male Sprague-Dawley rats of 7 weeks of age and weighing between 287 and 318 g, obtained from Charles River Japan (Kanagawa, Japan), were used. During the experiments, the rats were housed at a temperature of 23±2°C and relative humidity of 55±5% with a 12-h night/day cycle. Micafungin sodium was dissolved in saline (1 mg/ml), and administered as a single intravenous bolus at a dose of 1 mg/kg. Blood and tissue (liver, kidney, and lung) samples were collected predose and at the following times after the dose: 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h. All blood samples were collected from the abdominal aorta under ether anesthesia and immediately centrifuged at 4°C to obtain the plasma. The plasma and tissue samples were frozen at −20°C until analysis.

Determination of Micafungin in Plasma and Tissues The plasma concentrations of micafungin were determined by high-performance liquid chromatographic (HPLC) methods as described previously.11 The lower limit of quantification of micafungin was 0.05 μg/ml using a 0.1 ml plasma sample. For the determination of tissue concentration of micafungin, liver, kidney, and lung were weighed and homogenized with 4 (v/w) volumes of water. The concentrations of micafungin in the homogenates were measured by HPLC11 with an analytical column TSK gel ODS-80TM (150×4.6 mm I.D., Tohsyo, Tokyo, Japan) equipped with TSK guardgel ODS-80TM (15×3.2 mm I.D., Tohsyo). The column temperature was set at 50°C, and the elution was conducted with 20 mM KH2PO4–acetonitrile (4:3) at a flow rate of 1 ml/min. The fluorescence intensity was determined at an excitation wavelength of 273 nm and an emission wavelength of 464 nm. Calibration curves were linear for micafungin...
concentrations ranging from 0.02 to 12.5 μg/ml of homogenate (from 0.1 to 62.5 μg/g tissue), and the coefficients of variation were less than 7.2%.

Data Analysis Pharmacokinetic parameters were estimated using the WinNonlin Standard computer program (Version 3.1, Pharsight, CA, U.S.A.). The elimination rate constant at terminal phase (λz) was calculated by exponential of the last three measurable plasma concentrations in the elimination phase by linear regression. The half-life (t1/2) was calculated using ln 2/λz. The area under the micafungin concentration–time curve (AUC) was determined by the linear trapezoidal rule until the last measurement point (AUC0–∞) and extrapolated to infinity. This was calculated by dividing the theoretical concentration of the last measurement point by λz (AUC0–∞). Total clearance (CL) was calculated by dividing the nominal dose by AUC0–∞. Volume of distribution at steady state (Vdss) was calculated by multiplying the mean residence time (MRT), which was estimated by dividing the area under the first moment curve (AUMC) by CL. Statistical significance was determined using Student’s paired t-test.

RESULTS AND DISCUSSION

Drug concentrations in plasma, liver, kidney, and lung after an intravenous dose of micafungin are shown Fig. 1, and the pharmacokinetic parameters are summarized in Table 1. Micafungin in plasma disappeared biexponentially with a terminal half-life of 5.03 h. The pharmacokinetic parameters, including AUC0–∞, CL, Vdss, and t1/2, were similar to those previously reported.6) The Vdss in rats, estimated in this study, were also comparable to those in mice.6) In rabbits, linear disposition of micafungin in rats as well as mice exhibited a linear profile in the range of 0.32 to 3.2 mg/kg,6) and the pharmacokinetic parameters in rats, estimated in this study, were comparable to the radioactivity 5 min after intravenous dosing of 14C-labeled micafungin.10) On the other hand, unchanged micafungin doses in plasma, liver, kidney, and lung samples at 24 h after dosing was reported to be 7.9%, 8.9%, 11.7%, and 18.7% of the total radioactivity, respectively,12) and the micafungin concentrations in these tissues 24 h after dosing in this study were similar to the estimated concentrations of unchanged micafungin 24 h after dosing of 14C-labeled micafungin.10)

The ED50s of micafungin, evaluated in mouse models of disseminated candidiasis and aspergillosis and pulmonary aspergillosis, are 0.14–0.38 mg/kg, 0.23–0.36 mg/kg, and 0.26–0.45 mg/kg, respectively,13,14) and the minimum effective plasma concentrations of micafungin in mouse candidiasis and aspergillosis are 0.16–0.26 μg/ml and 0.55–0.80 μg/ml, respectively.15) The pharmacokinetics of micafungin in rats as well as mice exhibits a linear profile in the range of 0.32 to 3.2 mg/kg,6) and the pharmacokinetic parameters in rats, estimated in this study, were also comparable with those in mice.6) In rabbits, linear disposition of micafungin at doses of 0.5 to 2 mg/kg was observed, and micafungin concentrations in plasma and tissues at near peak plasma concentrations 30 min after the last of eight daily doses are several-fold in excess of the MIC90 against the clinical isolates of Candida spp. and Aspergillus spp., suggesting the

![Fig. 1. Plasma and Tissue Concentrations after an Intravenous Dose of Micafungin (1 mg/kg) to Male Rats](image)

Micafungin concentrations in plasma (●), liver (□), kidney (□), and lung (△) were determined as described in the Materials and Methods. Results are expressed as the mean±S.D. of three animals.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C1/min (μg/ml)</th>
<th>AUC0–∞ (μg·h/ml)</th>
<th>CL (ml/min/kg)</th>
<th>Vdss (l/kg)</th>
<th>t1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.31±0.24 (100)</td>
<td>13.4 (100)</td>
<td>1.15</td>
<td>0.447</td>
<td>5.03</td>
</tr>
<tr>
<td>Liver</td>
<td>2.65±0.20 (115)</td>
<td>21.7 (161)</td>
<td>—</td>
<td>—</td>
<td>5.14</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.78±0.25* (164)</td>
<td>45.9 (342)</td>
<td>—</td>
<td>—</td>
<td>4.87</td>
</tr>
<tr>
<td>Lung</td>
<td>5.95±0.60* (258)</td>
<td>38.8 (289)</td>
<td>—</td>
<td>—</td>
<td>5.31</td>
</tr>
</tbody>
</table>

C1,min is mean±S.D. of three animals. Values in parentheses are the percent of the values for plasma. * Significantly different from C1,min for plasma (p<0.05).
achievement of potentially therapeutic drug concentrations in plasma and tissues that are common sites of invasive fungal infections.\(^7\) Micafungin concentrations above the MIC\(_{90}\) against the clinical isolates of \textit{Candida} spp. and \textit{Aspergillus} spp. were maintained in liver, kidney, and lung after administering 1 mg/kg to rats for approximately 24 h (Fig. 1).

In healthy male human volunteers administered 2.5 to 150 mg by 0.5 or 2-h intravenous infusion, micafungin exhibited linear pharmacokinetics, and the CL and \(t_{1/2}\) were 0.190—0.225 ml/min/kg and 11.6—15.2 h, respectively.\(^8,9\) Additionally, the maximum plasma concentrations (\(C_{\text{max}}\)) after 0.5-h intravenous infusion of 50 and 75 mg in humans (approximately 0.8—1.3 mg/kg) were reported to be 5.23 and 7.90 \(\mu\)g/ml, respectively.\(^9\) The CL in rat was 6-fold higher than that in humans, and the \(t_{1/2}\) in rats was 3-fold shorter than that in humans, whereas the \(V_{\text{dss}}\) in rats is slightly higher than that in humans, and the serum protein binding of micafungin in rats is similar to that in humans.\(^6,8,9\)

Unchanged micafungin excreted in the urine, feces, and bile in rats was less than 27.5% of the radioactivity in each sample.\(^12\) Therefore, it is speculated that the difference in \(t_{1/2}\) between rats and humans may be mainly due to differences in the elimination clearance in the liver. Notwithstanding the different modes of drug administration, it is believed that information on rapid and moderate distribution in rats is of potential utility in the selection of antifungal therapeutics.

In conclusion, the results of the present study indicate that unchanged micafungin after an intravenous dose to male rats is distributed immediately, and that micafungin in tissues is eliminated in parallel with the unchanged drug in plasma. These results suggest that micafungin achieves and maintains potentially therapeutic plasma concentrations and distributes into tissues of deeply common invasive infections.

REFERENCES