

## Preliminary Animal Pharmacokinetics of the Parenteral Antifungal Agent MK-0991 (L-743,872)

RICHARD HAJDU,<sup>1\*</sup> RANDALL THOMPSON,<sup>1</sup> JON G. SUNDELOF,<sup>1</sup> BARBARA A. PELAK,<sup>1</sup>  
F. AILEEN BOUFFARD,<sup>2</sup> JAMES F. DROPINSKI,<sup>2</sup> AND HELMUT KROPP<sup>1</sup>

*Antibiotic Discovery and Development<sup>1</sup> and Medicinal Chemistry,<sup>2</sup>  
Merck Research Laboratories, Rahway, New Jersey 07065-0900*

Received 25 April 1997/Returned for modification 9 June 1997/Accepted 16 July 1997

**MK-0991 (L-743,872) is a potent antifungal agent featuring long half-life pharmacokinetics. The pharmacokinetics of MK-0991 administered intravenously to mice, rats, rhesus monkeys, and chimpanzees is presented. Unique to MK-0991 is its consistent cross-species performance. The range of values for the pharmacokinetic parameters were as follows: clearance, 0.26 to 0.51 ml/min/kg; half-life, 5.2 to 7.6 h; and distributive volume, 0.11 to 0.27 liters/kg. The level of protein binding of MK-0991 was determined to be 96% in mouse and human serum. The compound exhibited high affinities for human serum albumin and at least two lipid components. The rationale for the selection of MK-0991 as a drug development candidate was based on its two- to threefold superior pharmacokinetic performance in chimpanzees over the performance of an otherwise equivalent analog, L-733,560. Once-daily dosing for MK-0991 is indicated by a graphical comparison of levels in the circulations of chimpanzees and mice. In a study of the pharmacokinetics of MK-0991 in mouse tissue, the organs were assayed following intraperitoneal administration. The area under the concentration-versus-time curves (AUC) segregated the tissues into three exposure categories relative to plasma. The tissues with greater exposure than that for plasma were liver (16 times), kidney (3 times), and large intestine (2 times). The exposure for small intestine, lung, and spleen were equivalent to that for plasma. Organs with lower levels of exposure were the heart (0.3 times that for plasma), thigh (0.2 times), and brain (0.06 times). Kinetically, drug was cleared more slowly from all tissues than from plasma, indicating that terminal-phase equilibrium had not been achieved by 24 h. Thus, some measure of accumulation is predicted for all tissues. Single daily doses of MK-0991 should provide adequate systemic levels of fungicidal activity as a result of its long half-life pharmacokinetics, wide distribution, and slowly accumulating concentrations in tissue.**

The echinocandins are lipopeptides of semisynthetic and natural product origin (3, 8, 9, 24). These compounds are promising agents for the treatment of systemic fungal disease (1-3, 6, 9) and may also be useful for the management of *Pneumocystis carinii* pneumonia (18, 20-23). MK-0991, previously referred to as L-743,872, is an aza-substituted, diamino analog (4) of the natural product L-688,786 (pneumocandin B<sub>o</sub>) and has been selected as a drug development candidate on the basis of its potent in vivo efficacy and for its favorable pharmacokinetic properties.

Nonionic echinocandins are not water soluble, and solvent-based, parenteral formulations are either not acceptable or dose limiting (12). L-693,989, a water-soluble, aryl-phosphate prodrug of L-688,786, was shown to be as potent as L-688,786 in murine models of *Candida albicans* and *P. carinii* infections, despite being intrinsically nonbioactive (3, 21). Amino analogs of L-688,786 are also water soluble, do not require activation for fungicidal activity, and are as much as 70-fold more active than L-688,786 in the *C. albicans* model (9). However, in comparative tests against this organism, relative potencies in vitro were only within a factor of 4 (5, 9) and fungicidal rates were equivalent (5). Pharmacokinetic analyses in mice revealed that the half-lives ( $t_{1/2}$ s) of amino analogs were as much as eightfold longer than that of L-688,786 (14). The conclusion drawn from these observations was that long  $t_{1/2}$  pharmacokinetics was largely responsible for the improved in vivo efficacy. Evidently,

the slow fungicidal rates exhibited by these agents (5) require lengthy periods of drug pressure to effectively reduce the pathogenic load in this model.

Further development efforts leading to MK-0991 involved pharmacokinetic optimization in nonhuman primates. It was learned early on that the pharmacokinetics of the echinocandins were highly species specific (15, 25). In general, the usual rules for body weight correlation with pharmacokinetic parameters were reversed, i.e., clearance (CL) was slower in rodents than in larger species. Characteristically, primates exhibited a rapid early CL, rendering later levels in blood too low for effective fungicidal activity, despite long terminal  $t_{1/2}$ s in some cases. The original diamino analog, L-733,560, appeared to have overcome this limitation in tests with rhesus monkeys (14); however, as will be shown in this report, this favorable performance was not found with chimpanzees. For preliminary preclinical evaluation, we have decided to rely ultimately on the chimpanzee as the best representative of humans. The wisdom of this has yet to be determined; however, historical comparisons of a variety of agents suggest that this approach is reasonable (11, 16). Dosage scaling was accomplished by using a graphic comparison of levels in the circulations of chimpanzees versus those in the circulations of rodents over a 24-h treatment cycle.

### MATERIALS AND METHODS

**Echinocandin compounds.** Materials were furnished by two departments of Merck Research Laboratories, Rahway, N.J. MK-0991 and other semisynthetic echinocandins were prepared by Medicinal Chemistry, and [<sup>3</sup>H]MK-0991 was synthesized by the Radiolabel Synthesis group of Drug Metabolism. The purity of each compound was in excess of 95% at the time of delivery.

**HPLC analysis of echinocandins in plasma and urine.** A direct-injection, column-switching, reversed-phase high-performance liquid chromatography (HPLC)

\* Corresponding author. Mailing address: Antibiotic Discovery and Development, Merck Research Laboratories, 126 E. Lincoln Ave., P.O. Box 2000 RY80T-100, Rahway, NJ 07065-0900. Phone: (732) 594-4486. Fax: (732) 594-5700. E-mail: richard\_hajdu@merck.com.

method was used to determine echinocandin concentrations in plasma and urine. Samples were injected onto a short column of pellicular  $C_{18}$  (Alltech no. 28551) and washed with the mobile phase (0.1% trifluoroacetic acid or 50 mM triethyl ammonium phosphate [pH 2.85]). This cleanup column was then switched in-line with an analytical  $C_{18}$  column, and the chromatogram was developed with an acetonitrile gradient. Echinocandins were detected by fluorescence detection (224-nm excitation, 302-nm emission). Sensitivity was 0.125  $\mu\text{g/ml}$  in plasma and 0.625  $\mu\text{g/ml}$  in urine. The mean coefficient of variability was 5%.

**Protein binding.** [ $^3\text{H}$ ]MK-0991 was used for these studies and was measured by scintillation counting. Ultrafiltration studies were performed with the Centrifree Micropartitioning Device (Amicon). Drug at a range of concentrations of from 1 to 1,000  $\mu\text{g/ml}$  in 1.0 ml of fresh human serum was loaded into the upper chamber and centrifuged at  $1,500 \times g$  for 8 min. The free fraction ( $f_f$ ) of MK-0991 was calculated as the concentration in the filtered portion divided by the initial concentration. The percentage of bound drug was calculated as  $(1 - f_f) \times 100$ . Ultracentrifugation studies were conducted with a 2.0-ml sample at a single concentration of 10  $\mu\text{g/ml}$ . The samples were centrifuged in a SW55Ti rotor at 55,000 rpm ( $368,000 \times g$ ) for 16 h. The sample was removed in 0.2-ml layers, and each sample was assayed for MK-0991. Since the compound itself sediments slightly, the values were corrected by the corresponding levels in nonserum controls. The free concentration was taken as the mean concentration of layers 4 through 6. All samples were run in duplicate.

**Pharmacokinetics.** Mice (female CD-1 mice; weight, 20 g;  $n = 3$  mice per time point) were injected intravenously (i.v.) with 0.2 ml of MK-0991 via the tail vein or with 0.5 ml intraperitoneally (i.p.) at a dose of 1 mg/kg of body weight. At the time of sampling the mice were euthanized by exsanguination via cardiac puncture under  $\text{CO}_2$  anesthesia. Rats (female SD rats; weight, 300 g;  $n = 4$ ) were injected i.v. with 0.3 ml of MK-0991 (tail) at a dose of 1 mg/kg. Timed blood sampling was performed by obtaining blood from the tail vein via a heparinized 25-gauge infusion set. Terminal (24-h) bleeds were performed as described above for mice. Rhesus monkeys (males; weight, 5 to 6 kg,  $n = 3$ ) were restrained in metabolism chairs and were dosed by injection with 0.40 ml of MK-0991 per kg via the saphenous vein by using a 21-gauge infusion set, followed by injection of a 1.0-ml saline flush. The total infusion time was 1 to 1.5 min. Blood samples were collected using heparinized Vacutainers. Blood samples were obtained at the 24-h time point while the monkeys were in their cages under ketamine anesthesia. Chimpanzees (males;  $n = 2$ ) were kept under ketamine anesthesia during the first 4 h of the procedure. A constant infusion of 5% mannitol in normal saline as well as the 0.5-mg/kg dose were delivered via an indwelling i.v. catheter. Plasma sampling was performed via an additional, indwelling, heparin-locked, i.v. catheter, and urine sampling was accomplished by using an indwelling urinary catheter. Blood samples from beyond 4 h were obtained in the cage as described above for rhesus monkeys.

**Scintillation counting.** Samples (50 to 200  $\mu\text{l}$ ) were incubated in a tissue solubilizer (SOLVABLE; DuPont) and mixed with FORMULA-989 (DuPont) scintillation cocktail, and the radioactivity was counted. For the tissue distribution study, a correction for quenching by internal standard addition was used as follows. Samples were spiked with a known quantity of [ $^3\text{H}$ ]MK-0991, and the radioactivity was recounted. The original counts were adjusted according to the efficiency of recovery of the added counts. Correction was also made for residual blood content in tissue.

**Pharmacokinetic data analysis.** Plasma concentration-time curves were fit to a single-, two-, or three-compartment equation by using the program Kaleidagraph (Synergy Software). A  $1/C^2$  weighting (where  $C$  is concentration) was used, and the selection of the appropriate equation was made by using the  $F$ -statistic (10). Pharmacokinetic parameters, CL, terminal  $t_{1/2}$ , volume in the central compartment ( $V_1$ ), and steady-state volume of distribution ( $V_{ss}$ ), were calculated from the fit equations by using the appropriate formulae (26). For tissues, the area under the concentration-versus-time curve from 0 to 24 h ( $\text{AUC}_{0-24}$ ) was calculated by the trapezoidal method (7).

## RESULTS

**Plasma protein binding.** MK-0991 was extensively bound to serum protein. A precise value for the bound fraction has been difficult to obtain due to the unusual physical properties of this compound. Two methods have been used: ultrafiltration and ultracentrifugation. Results from ultrafiltration studies (data not shown; 0% free fraction at all concentrations) suggested that binding was quantitative; however, in the absence of serum the compound interacted significantly with the ultrafiltration apparatus (77%). This may have masked the presence of a small free fraction. Ultracentrifugation studies indicated a free fraction of 3 to 4% (Fig. 1); however, it was evident that a portion of the material was associated with the less dense lipid layers. Figure 1 illustrates the sedimentation patterns of MK-0991 in mouse and human serum, human serum albumin (HSA), and buffer. In serum, in addition to the large recovery

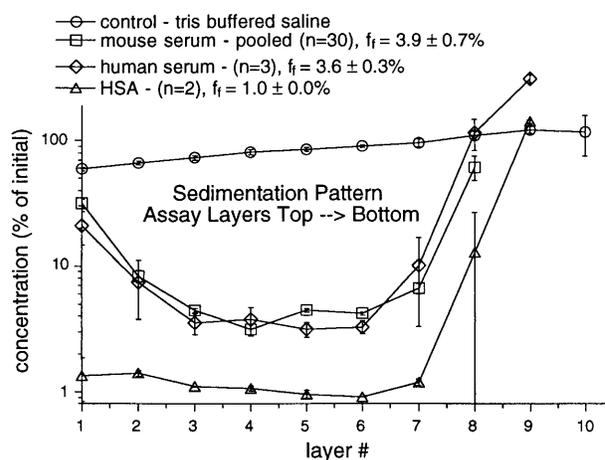


FIG. 1. Determination of serum protein binding of MK-0991 by the ultracentrifugation method. Plotted values are means  $\pm$  standard deviations of (final counts per minute/initial counts per minute)  $\times$  100.

of material in the protein pellet (fractions 9 and higher) much of the drug followed the pattern of lipid distribution. There was evidently some partitioning to the low-density lipoprotein layer (fractions 7 and 8) and to the very low density lipoprotein layer (fractions 1 and 2). It is plausible that some or all of what is measured as free may, in fact, be lipid associated, supporting the notion that binding may be quantitative. At physiological concentrations, binding to human albumin appeared to be tighter than to whole serum (1% free fraction; binding was equivalent in crude fraction V and purified fatty acid-free albumin samples). This contradictory evidence suggests that binding in serum is distributed to albumin and lipoprotein and is probably nearly quantitative. Binding in mouse and human serum was equivalent.

**Animal pharmacokinetics.** The pharmacokinetics of MK-0991 in four animal species is shown in Fig. 2a. The calculated parameters for this compound are summarized in Table 1. For all species, MK-0991 was well tolerated in all subjects and recoveries in urine (data not shown) were consistently in the range of <1 to 3% of the dose, suggesting that, like cilofungin (17), the major route of elimination is hepatic. Distributive volumes and  $V_1$ s for nonhuman primates were approximately twofold lower than those for the rodents.  $t_{1/2}$ s were in the range of 6 to 7 h, and CLs were within a factor of 2 for the nonhuman primates and rodents. Among the echinocandins studied, the findings for MK-0991 were unique in that there was a consistency in pharmacokinetic behavior across species. An example of an echinocandin with unpredictable species specificity is L-733,560, a very close structural analog of MK-0991. L-733,560 had a  $t_{1/2}$  of 12 h in the rhesus monkey (14), but this was not predictive of behavior in the chimpanzee, in which its  $t_{1/2}$  was only 2.7 h (Table 2). For MK-0991 the data for rhesus monkeys was predictive, with  $t_{1/2}$ s in the two species being equivalent within experimental error. After efficacy criteria, comparisons of drugs in chimpanzee pharmacokinetic evaluations formed the basis for product candidate selection, e.g., MK-0991 versus L-733,560 (Fig. 2b and Table 2).

**MK-0991 distribution and tissue pharmacokinetics.** In this study, mice were dosed with 1 mg of [ $^3\text{H}$ ]MK-0991 per kg i.p. to simulate dosing in efficacy models. Compared to i.v. dosing, MK-0991 was 65% bioavailable by this route. Urinary recovery was 2.5% of the dose at 24 h. HPLC measurements confirmed that in most tissues, during the entire sampling period, the

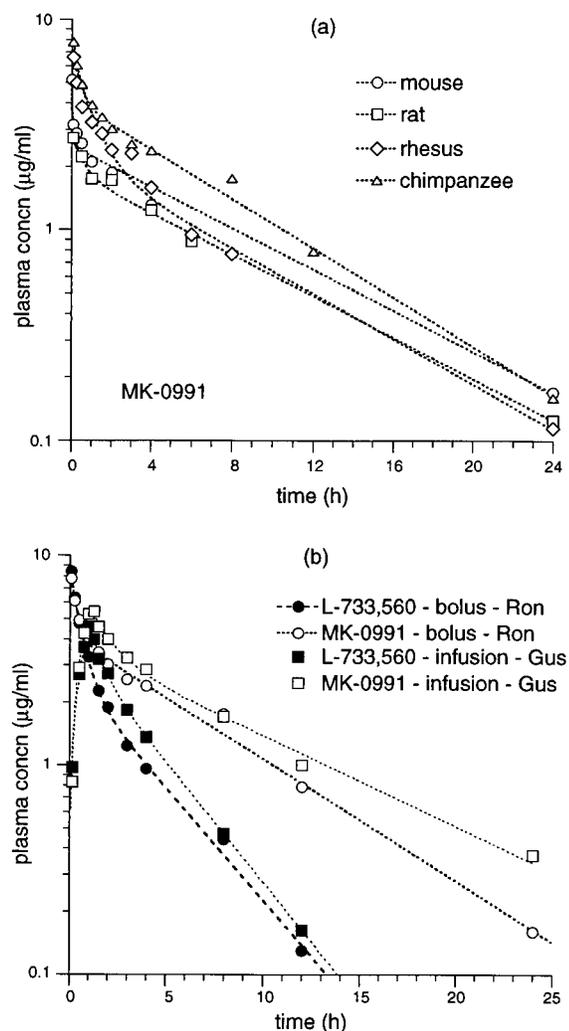


FIG. 2. (a) Comparative pharmacokinetics of MK-0991 in four species receiving i.v. boluses of MK-0991 at 0.5 mg/kg; (b) pharmacokinetics of MK-0991 and L-733,560 in chimpanzees receiving drugs at 0.5 mg/kg. Comparisons of both drugs administered by i.v. bolus to one individual and by 60-min infusion to a second individual are shown.

radiolabel was associated only with the drug. In liver, after 8 h, 5 to 10% of the radiolabel was not drug associated. Figures 3a to d illustrate the time course of the MK-0991 concentrations in various tissues. The profile of the concentration in plasma is included in all four of the plots for reference. Figure 3a shows that MK-0991 rapidly achieved levels in liver and kidney higher than those in plasma and maintained a high tissue/plasma concentration ratio throughout the 24-h period. The liver con-

TABLE 1. Summary of pharmacokinetic parameters of MK-0991<sup>a</sup>

Species (no. of animals)	CL (ml/min/kg)	$t_{1/2}$ (h)	$V_1$ (ml/kg)	$V_{ss}$ (ml/kg)
Mouse (3)	0.44 ± 0.02	7.6 ± 1.0	140 ± 20	250 ± 20
Rat (4)	0.51 ± 0.05	6.5 ± 0.6	210 ± 40	270 ± 40
Rhesus monkey (3)	0.39 ± 0.06	5.6 ± 0.5	88 ± 17	160 ± 30
Chimpanzee (2)	0.24 ± 0.04	6.7 ± 2.1	70 ± 18	120 ± 10

<sup>a</sup> Values are means ± standard deviations. All data best fit to a two-compartment model.

TABLE 2. Pharmacokinetics of L-733,560 and MK-0991 in chimpanzees<sup>a</sup>

Compound and route	CL (ml/min/kg)	$t_{1/2}$ (h)	$V_1$ (ml/kg)	$V_{ss}$ (ml/kg)
L-733,560, i.v. bolus	0.58	2.8	56	110
MK-0991, i.v. bolus	0.26	5.2	57	110
L-733,560, i.v. infusion	0.56	2.6	77	110
MK-0991, i.v. infusion	0.21	8.1	83	120

<sup>a</sup> See legend for Fig. 2b.

tinued to accumulate the compound for up to 8 h, while the levels in the kidney remained fairly constant from 1 to 24 h. Lung and spleen tissues (Fig. 3b) also showed a plateau in concentration over the time course of the study at levels approximately threefold lower than that for the kidney tissue. Muscle tissue (Fig. 3c) contained somewhat lower levels, but it also cleared MK-0991 at a rate slower than the rate at which it was cleared from plasma. The brain (Fig. 3c) had detectable levels of MK-0991 which also remained constant throughout the study. The intestines (Fig. 3d) showed high levels of MK-0991. Since, with time, the appearance of drug in the small intestine led that which appeared in the large intestine, the high concentrations were likely the result of biliary CL. A correlation with the levels in the liver, however, was poor. The concentrations in the small intestine dropped off after 4 h, while the levels in liver continued to climb and remained at a maximum through 16 h. The i.p. route of administration may have complicated the liver and intestine kinetics due to peripheral penetration by the drug. Table 3 presents a summary of the concentrations and AUCs in tissues and plasma, their ratios to plasma, and apparent rate constants.

## DISCUSSION

The antifungal properties of MK-0991 in vitro and in vivo were similar to those of many echinocandin compounds reported previously (1, 2, 4, 5). Measured fungicidal rates were slow (reduction of ~1 log<sub>10</sub> CFU per 5 h), and the minimum fungicidal concentration was as high as 0.5 µg/ml in serum-supplemented media (4). On the basis of these parameters, the pharmacokinetics of MK-0991 in the chimpanzee satisfied our design criteria, which specified a minimum of 10 h of continuous coverage above the 1-µg/ml level. This could be accomplished with a 0.5-mg/kg dose. In addition, the consistent cross-species pharmacokinetic behavior of MK-0991 provided an extra measure of assurance that efficacy in animal models would accurately extrapolate to efficacy in humans.

In the primates the  $V_1$ s of MK-0991 were low and were approximately equal to the fluid volume in plasma. This is likely a consequence of the substantial plasma protein binding; thus, plasma is observed to be a distinct compartment.  $V_{ss}$  was smaller in the primates than in rodents by about a factor of 2. This implies that for a given level in plasma, extravascular levels (peripheral compartment) in primates may be twofold lower than those in mice. Since infections most often reside outside the circulation, this should be accounted for when designing dosing regimens for humans. Conservatively, an equiefficacious time course of MK-0991 in humans might require levels twofold higher than those in mice.

Efficacy projections for MK-0991 in humans were made on the basis of the following reasoning. In the laboratory, routine in vivo efficacy tests are conducted with a twice-daily dosing schedule (6). The two doses are administered 6 h apart. Since at efficacious doses, drug levels in plasma are too low to be

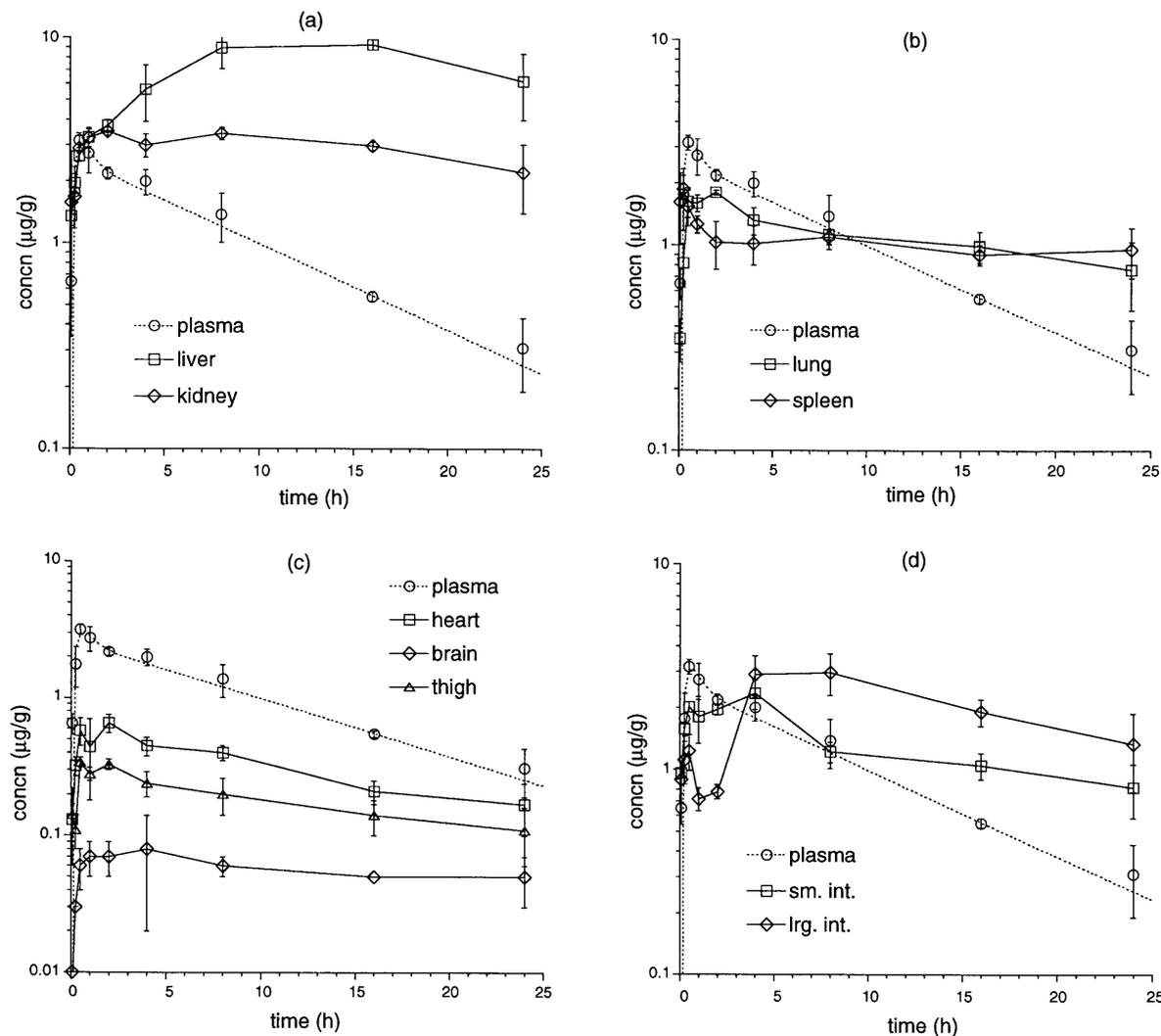


FIG. 3. Concentrations of MK-0991 in tissue versus time in mice after administration of a single i.p. dose of 1 mg/kg. (a) Liver and kidney; (b) lung and spleen; (c) brain and muscle, and (d) small intestine (sm. int.) and large intestine (lrg. int.). The curve for the concentration in plasma is included in each panel for reference. The plotted values are means  $\pm$  standard deviations.

reliably measured, an estimate of the plasma concentration profile under treatment conditions was made by downward extrapolation of pharmacokinetic data. Dose linearity was assumed, and the later dose was added to the first dose, creating a simulation of the 24-h treatment cycle. A plot for the mouse receiving drug at 0.09 mg/kg i.v. twice daily is shown in Fig. 4. This dose was selected as a target level for efficacy on the basis of the following interpretation of results obtained with the in vivo model. For candidiasis (1), the 0.09-mg/kg treatment for 4 days was sufficient to reduce CFU loads in the target organ to below detectable levels in infected animals. This is regarded as a curative regimen, and extrapolation is straightforward. A dose of 0.08 mg/kg i.v. given twice daily was highly effective in a model of disseminated aspergillosis (1). In this survival model, 5 days of this regimen protected 80% of infected animals over a 28-day period. Overlaid in Fig. 4 is the profile of the concentration of drug in the plasma of a chimpanzee into which the drug was infused (0.5 mg/kg i.v. over 60 min). The plot shows a minimum of twofold coverage over the 24-h dosing interval with respect to the projected time course in the rodent model treatment. As mentioned above, it is thought

that twofold coverage may be required to compensate for the smaller distributive volume. This illustrates that if the pharmacokinetics of the drug in humans is adequately represented by the chimpanzee, then a single dosage of 0.5 mg/kg per day would yield a systemic time course of drug concentrations equivalent to or better than one produced by a known efficacious dosing regimen in animals. Some measure of dosage scaling may be necessary for less susceptible organisms.

In the study of the disposition of MK-0991, the fact that  $t_{1/2s}$  in tissue were longer than those in the plasma indicated that during this 24-h observation period, the dynamic equilibrium associated with the terminal elimination phase had not yet been reached. Typically, in a two-compartment system there is a period of time in which the slope of the peripheral concentration is shallower than that for plasma. The interval is the time from peak peripheral level to the start of dynamic equilibrium, at which point the lines become parallel. This interval is short relative to the full elimination time course. In mice treated with MK-0991, however, this condition existed for at least 24 h, suggesting that the full time course of elimination was considerably longer than 1 day. Furthermore, the apparent

TABLE 3. Distribution of [<sup>3</sup>H]MK-0991 in tissue<sup>a</sup>

Organ	$C_{\max}$ ( $\mu\text{g/ml}$ )	$T_{\max}$ (h)	$C_{24}$ ( $\mu\text{g/ml}$ )	$\text{AUC}_{0-24}$ ( $\mu\text{g} \cdot \text{h/g}$ )	Tissue/ plasma ratio		Apparent rate constant ( $\text{h}^{-1}$ )
					$C_{24}$	$\text{AUC}_{0-24}$	
Plasma	3.2	0.5	0.31	25	1.0	1.0	0.098
Liver	9.3	16	6.2	400	20	16	0.023
Kidney	3.5	2	2.2	71	7.1	2.9	0.028
Large intestine	3.0	8	1.3	49	4.3	2.0	0.042
Small intestine	2.3	4	0.82	31	2.6	1.3	0.045
Lung	1.8	2	0.76	26	2.5	1.1	0.025
Spleen	1.9	0.25	0.95	24	3.1	1.0	0.023
Heart	0.66	2	0.17	7.6	0.5	0.3	0.060
Thigh	0.34	0.5	0.11	4.3	0.4	0.2	0.046
Brain	0.08	4	0.05	1.4	0.2	0.1	0.022

<sup>a</sup>  $C_{\max}$  maximum concentration of drug;  $T_{\max}$  time to maximum concentration of drug;  $C_{24}$ , concentration at 24 h; tissue/plasma ratio, ratio of values in tissue to values in plasma. The other abbreviations were defined in the text.

distributive phase (Fig. 2a) was complete within the first few hours, contradicting the notion that the transition to equilibrium was a lengthy process. In a two-compartment system, no combination of rate constants can result in rapid distribution without equilibrium immediately following. It appears, then, that the mathematical model does not fit the physical one. One possible correction would be to introduce a third compartment. While three-compartment pharmacokinetics is sometimes observed with this class, the influence of the additional compartment proposed for MK-0991 is either small or so slow as to delay any observable bending of the log-linear elimination rate until after the first 24 h. Note that longer experiments may have revealed a slower terminal rate; however, higher doses would also have been necessary since, at the dose used, the assay detection limit would have been traversed within the next  $t_{1/2}$ . In analyzing these data with a two-compartment model, the plasma CL is overestimated, since the portion of the dose deposited in the additional compartment is mathematically ascribed to elimination and the potential for multiple-dose accumulation is underestimated. In a regimen in which subsequent doses are administered during a true terminal phase, the steady-state accumulation factor  $R$  for a linear

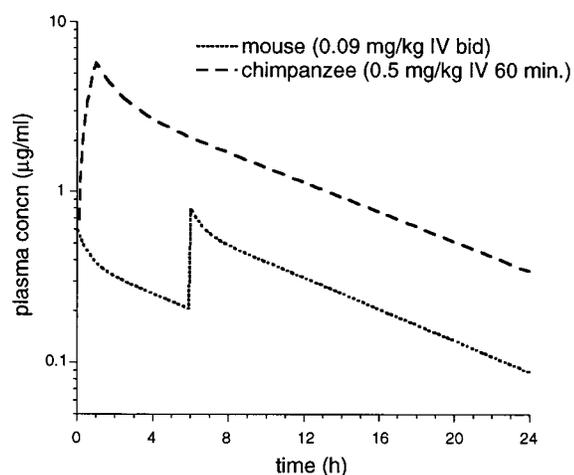


FIG. 4. Graphical comparison of proposed single daily dose of MK-0991 in chimpanzees (representing that in humans) versus the estimated time course of levels in plasma in the mouse model of a curative regimen (see text). bid, twice daily.

system, is calculated by using a simple expression (13):  $R = 1/(1 - e^{-\beta T})$ , where  $\beta$  is the terminal rate constant and  $T$  is the dosing interval. An estimate for MK-0991 in the mouse by using the two-compartment model, a 24-h, interval, and the measured rate ( $\beta = 0.693/t_{1/2}$ ) (Table 1) yields an  $R$  value of 1.13, a modest 13% accumulation. The knowledge that the single-dose terminal phase commences beyond the dosing interval requires the application of a more complex superposition method involving multiple  $R$  factors, the largest of which is associated with the slowest rate constant. Suffice it to say that the slow equilibration rates in tissue likely will govern the terminal rates and the range of rates observed in tissue, 0.02 to 0.06/h (Table 3), yield  $R$  values in the range of 1.3 to 2.6. Thus, some measure of accumulation in tissue beyond that predicted by analysis of the pharmacokinetics in plasma is expected.

The movement in and out of tissue may be of considerable importance on the basis of previously reported work with the echinocandin cilofungin (17, 27). In that pharmacokinetic and pharmacodynamic study with rabbits, nonlinear pharmacokinetics, resulting in disproportionately high circulating levels of drug, was exploited in an effort to demonstrate the requirements for efficacy with this agent. The authors demonstrated that in vivo antifungal activity correlated directly with concentrations in tissue. Furthermore, they report that penetration into tissue was more efficiently accomplished by continuous infusion rather than by intermittent dosing and that time of exposure rather than dose was the more important parameter leading to adequate accumulation in tissue. Efficacy results from other, similar studies support this (19, 28). It follows, then, that long  $t_{1/2}$  pharmacokinetics would be a benefit in establishing efficacious levels in tissue for an agent possessing the mode of action and physical properties of cilofungin. In the cited study earlier (17, 27) the CL of cilofungin from plasma in the most efficacious regimen was 0.6 ml/min/kg and the ratios of the concentration in tissue to the concentration in plasma were in the range of 0.1 to 1.0 for most tissues. On the basis of the work presented in this report, similar pharmacokinetic performance can be expected for MK-0991 with considerably lower daily doses.

#### ACKNOWLEDGMENTS

We acknowledge Kathleen White, Beth Murphy, Bonnie Hegarty-Friscino, and Liza Gantert for expert technical assistance and Hugh Rosen, Milt Hammond, Jim Balkovec, and Julie Stone for review of the manuscript.

#### REFERENCES

1. Abruzzo, G., K., A. M. Flattery, C. J. Gill, L. Kong, J. G. Smith, V. B. Pikounis, J. M. Balkovec, A. F. Bouffard, J. F. Dropinski, H. Rosen, H. Kropp, and K. Bartizal. 1997. Evaluation of the echinocandin MK-0991 (L-743,872): efficacy in mouse models of disseminated aspergillosis, candidiasis, and cryptococcosis. *Antimicrob. Agents Chemother.* **41**:2333-2338.
2. Abruzzo, G. K., A. M. Flattery, C. J. Gill, L. Kong, J. G. Smith, D. Krupa, V. B. Pikounis, H. Kropp, and K. Bartizal. 1995. Evaluation of water-soluble pneumocandin analogs L-733560, L-705589, and L-731373 with mouse models of disseminated aspergillosis, candidiasis, and cryptococcosis. *Antimicrob. Agents Chemother.* **39**:1077-1081.
3. Balkovec, J. M., R. M. Black, M. L. Hammond, J. V. Heck, R. A. Zambias, G. Abruzzo, K. Bartizal, H. Kropp, C. Trainor, R. E. Schwartz, D. C. McFadden, K. H. Nollstadt, L. A. Pittarelli, M. A. Powles, and D. M. Schmatz. 1992. Synthesis, stability, and biological evaluation of water-soluble prodrugs of a new echinocandin lipopeptide. Discovery of a potential agent for the treatment of systemic candidiasis and *Pneumocystis carinii* pneumonia (PCP). *J. Med. Chem.* **35**:194-198.
4. Bartizal, K., C. J. Gill, G. K. Abruzzo, A. M. Flattery, L. Kong, P. M. Scott, J. G. Smith, C. E. Leighton, A. F. Bouffard, J. F. Dropinski, and J. Balkovec. 1997. In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). *Antimicrob. Agents Chemother.* **41**:2326-2332.
5. Bartizal, K., T. Scott, G. K. Abruzzo, C. J. Gill, C. Pacholok, L. Lynch, and H. Kropp. 1995. In vitro evaluation of the pneumocandin antifungal agent

- L-733560, a new water-soluble hybrid of L-705589 and L-731373. *Antimicrob. Agents Chemother.* **39**:1070–1076.
6. **Bartizal, K., G. Abruzzo, C. Trainor, D. Krupa, K. Nollstadt, D. Schmatz, R. Schwartz, M. Hammond, J. Balkovec, and F. Vanmiddlesworth.** 1992. In vitro antifungal activities and in vivo efficacies of 1,3- $\beta$ -D-glucan synthesis inhibitors L-671,329, L-646,991, tetrahydroechinocandin B, and L-687,781, a papulocandin. *Antimicrob. Agents Chemother.* **36**:1648–1657.
  7. **Bent, L. Z., and R. L. Galeazzi.** 1979. Noncompartmental determination of the steady-state volume of distribution. *J. Pharm. Sci.* **68**:1071–1074.
  8. **Bouffard, F. A., J. F. Dropinski, J. M. Balkovec, R. M. Black, M. L. Hammond, K. H. Nollstadt, and S. Dreikorn.** 1996. L-743,872, a novel antifungal lipopeptide: synthesis and structure-activity relationships of new aza-substituted pneumocandins, abstr. F27, p. 104. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  9. **Bouffard, F. A., R. A. Zambias, J. F. Dropinski, J. M. Balkovec, M. L. Hammond, G. K. Abruzzo, K. F. Bartizal, J. A. Marrinan, M. B. Kurtz, D. C. McFadden, K. H. Nollstadt, M. A. Powles, and D. M. Schmatz.** 1994. Synthesis and antifungal activity of novel cationic pneumocandin Bo derivatives. *J. Med. Chem.* **37**:222–225.
  10. **Boxenbaum, H. G., S. Riegelman, and R. M. Elashoff.** 1974. Statistical estimations in pharmacokinetics. *J. Pharmacokinet. Biopharm.* **2**:123–148.
  11. **Brazzell, R. K., Y. H. Park, C. B. Wooldridge, B. McCue, R. Barker, R. Couch, and B. York.** 1990. Interspecies comparison of the pharmacokinetics of aldose reductase inhibitors. *Drug. Metab. Dispos.* **18**:435–440.
  12. **Doebbeling, B. N., B. D. Fine, Jr., M. A. Pfaller, C. T. Sheetz, J. B. Stokes, and R. P. Wenzel.** 1990. Acute tubular necrosis (ATN) and anion-gap acidosis during therapy with cilofungin (LY-121019) in polyethylene glycol (PEG), abstr. 583, p. 183. *In* Program and abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  13. **Gibaldi, M., and D. Perrier.** 1982. *Pharmacokinetics*, 2nd ed., p. 121. Marcel Dekker, Inc., New York, N.Y.
  14. **Hajdu, R., R. Thompson, K. White, B. Stark Murphy, and H. Kropp.** 1993. Comparative pharmacokinetics of three water-soluble analogues of the lipopeptide antifungal compound L-688,786 in mice and rhesus monkeys, abstr. 357, p. 185. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  15. **Hajdu, R., J. G. Sundelof, K. Bartizal, G. Abruzzo, C. Trainor, R. Thompson, and H. Kropp.** 1991. Comparative pharmacokinetics in four animal species of L-688,786 and its water soluble prodrug, L-693,989, abstr. 209, p. 133. *In* Program and abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  16. **Kahan, F. M., H. Kropp, J. G. Sundelof, and J. Birnbaum.** 1983. Thienamycin: development of imipenem-cilastatin. *J. Antimicrob. Chemother.* **12** (Suppl. D):1–35.
  17. **Lee, J. W., P. Kelly, J. Lecciones, D. Coleman, R. Gordee, P. A. Pizzo, and T. J. Walsh.** 1990. Cilofungin (LY121019) shows nonlinear plasma pharmacokinetics and tissue penetration in rabbits. *Antimicrob. Agents Chemother.* **34**:2240–2245.
  18. **Powles, M. A., J. Anderson, P. Liberator, and D. M. Schmatz.** 1996. Efficacy of semisynthetic pneumocandin analog L-743,872 against *Pneumocystis carinii* in murine models, abstr. F42, p. 107. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  19. **Rouse, M. S., B. M. Tallan, J. M. Steckelberg, N. K. Henry, and W. R. Wilson.** 1992. Efficacy of cilofungin therapy administered by continuous intravenous infusion for experimental disseminated candidiasis in rabbits. *Antimicrob. Agents Chemother.* **36**:56–58.
  20. **Schmatz, D. M., M. A. Powles, D. C. McFadden, K. Nollstadt, F. A. Bouffard, J. P. Dropinski, P. Liberator, and J. Andersen.** New semisynthetic pneumocandins with improved efficacies against *Pneumocystis carinii* in the rat. *Antimicrob. Agents Chemother.* **39**:1320–1323.
  21. **Schmatz, D. M., M. A. Powles, D. C. McFadden, L. Pittarelli, J. Balkovec, M. Hammond, R. Zambias, P. Liberator, and J. Anderson.** 1992. Antipneumocystis activity of water-soluble lipopeptide L-693,989 in rats. *Antimicrob. Agents Chemother.* **36**:1964–1970.
  22. **Schmatz, D. M., G. Abruzzo, M. A. Powles, D. C. McFadden, J. M. Balkovec, R. M. Black, K. Nollstadt, and K. Bartizal.** 1992. Pneumocandins from *Zalerion arboricola*. IV. Biological evaluation of natural and semisynthetic pneumocandins for activity against *Pneumocystis carinii* and *Candida* species. *J. Antibiot.* **45**:1886–1891.
  23. **Schmatz, D. M., M. A. Romancheck, L. A. Pittarelli, R. E. Schwartz, R. A. Frontling, K. H. Nollstadt, F. L. Vanmiddlesworth, K. E. Wilson, and M. J. Turner.** 1990. Treatment of *Pneumocystis carinii* pneumonia with 1,3- $\beta$ -glucan synthesis inhibitors. *Proc. Natl. Acad. Sci. USA* **87**:5950–5954.
  24. **Schwartz, R. E., D. F. Sesin, H. Joshua, K. E. Wilson, A. J. Kempf, K. A. Goklen, D. Kuehner, P. Gailliot, C. Gleason, R. White, E. Inamine, G. Bills, P. Salmon, and L. Zitano.** 1992. Pneumocandins from *Zalerion arboricola*. I. Discovery and isolation. *J. Antibiot.* **45**:1853–1866.
  25. **Sundelof, J. G., R. Hajdu, W. J. Cleare, J. Onishi, and H. Kropp.** 1992. Pharmacokinetics of L-671-329 in rhesus monkeys and DBA/2 mice. *Antimicrob. Agents Chemother.* **36**:607–610.
  26. **Wagner, J. G.** 1993. *Pharmacokinetics for the pharmaceutical scientist*, p. 84–85. Technomic Publishing Company, Inc., Lancaster, Pa.
  27. **Walsh, T. J., J. W. Lee, P. Kelly, J. Bacher, J. Lecciones, V. Thomas, C. Lyman, D. Coleman, R. Gordee, and P. A. Pizzo.** Antifungal effects of the nonlinear pharmacokinetics of cilofungin, a 1,3- $\beta$ -glucan synthetase inhibitor, during continuous and intermittent intravenous infusions in treatment of experimental disseminated candidiasis. *Antimicrob. Agents Chemother.* **35**:1321–1328.
  28. **Walsh, T. J., J. W. Lee, E. Roilides, P. Francis, J. Bacher, C. A. Lyman, and P. A. Pizzo.** 1992. Experimental antifungal chemotherapy in granulocytopenic animal models of disseminated candidiasis: approaches to understanding investigational antifungal compounds for patients with neoplastic diseases. *Clin. Infect. Dis.* **14**(Suppl. 1):S139–S147.