

Cilofungin (LY121019) Shows Nonlinear Plasma Pharmacokinetics and Tissue Penetration in Rabbits

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We studied the plasma pharmacokinetics and tissue penetration of cilofungin (LY121019), a new echinocandin antifungal compound, by intermittent and continuous infusion in rabbits. Following a single intravenous dose of 50 mg/kg of body weight, the maximum concentration in plasma was 297 ± 39 $\mu\text{g/ml}$, the area under the curve was 30.1 ± 6.7 $\mu\text{g} \cdot \text{h/ml}$, clearance was 30 ± 10 ml/min/kg , volume of distribution was 0.85 ± 0.23 liters/kg , half-life in distribution phase was 3.7 ± 0.2 min (first 12 min postdose), and half-life in elimination phase was 12.9 ± 0.7 min. When rabbits received cilofungin by continuous infusion (CI) at 10 mg/kg/h over 6 days, sustained concentrations in plasma of 290 ± 56 $\mu\text{g/ml}$ were seen, more than 50-fold higher than predicted if kinetics were linear. Similarly, at 5 mg/kg/h, high levels were also obtained. Such elevated levels in plasma would not have been predicted from the pharmacokinetic characteristics of cilofungin given as a single intravenous dose. Further pharmacokinetic study at several rates of CI suggested that cilofungin elimination follows Michaelis-Menten kinetics. Simultaneous cilofungin levels in plasma and tissue were then determined for rabbits receiving six intravenous, intermittent doses (ID) of cilofungin at 15 mg/kg every 4 min and for rabbits receiving CI as described above. After ID, the mean of the ratios of cilofungin levels in tissue to those in plasma were highest for liver and bile but very low for cerebrum and cerebellum. After CI, ratios were as much as 89 times higher than for ID and significantly greater in the brain, choroid, kidney, and bile ($P < 0.05$). We conclude that following a single dose of cilofungin, the compound is rapidly cleared via first-order kinetics and does not penetrate into the central nervous system, whereas following CI, cilofungin exhibits nonlinear saturable kinetics, is slowly cleared, and significantly penetrates into central nervous system tissues.

Systemic mycoses, especially disseminated candidiasis, have become a common problem with the increasing number of immunocompromised patients and the greater use of potent broad-spectrum antibiotics. Treatment of disseminated candidiasis remains difficult because of a lack of effective yet nontoxic antifungal agents with favorable, defined pharmacokinetic and pharmacologic properties. Currently available drugs are toxic, erratically absorbed, or fungistatic (4, 5, 10). Agents are needed which have high levels of tissue penetration, minimal toxicity, and fungicidal activity against *Candida* species in immunocompromised patients.

Cilofungin (LY121019), a semisynthetic analog of echinocandin B, has been found to have potent fungicidal activity against *Candida* species in vitro, with fungicidal activity comparable with that of amphotericin B against *C. albicans* and *C. tropicalis* (3; J. Lecciones, P. Kelly, J. Lee, R. Schaufele, P. Pizzo, and T. Walsh, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, F 3, p. 16). Cilofungin given intravenously by bolus injections of 50 mg/kg of body weight once daily for 5 days also reduced the recovery of *C. albicans* from the kidneys of mice as effectively as equal doses of amphotericin B (3). Cilofungin was ineffective, however, in a rabbit model of *Candida* endocarditis when doses of 50 mg/kg every 12 h for 7 days were used and was less effective than amphotericin B, 1.0 mg/kg/day, in treatment of *Candida* endophthalmitis in rabbits (6, 7). One possible explanation for the disparity between the in vivo results and in vitro

studies is the rapid elimination of cilofungin in rabbits, whereby an intravenous bolus of 50 mg/kg is cleared from serum with a half-life of less than 10 min, with no drug being detectable after 90 min postdose (7). Moreover, the drug may not penetrate into particular tissue sites, such as the central nervous system (CNS). Administration of cilofungin by continuous infusion should provide more sustained levels in plasma and tissue penetration. However, since little is known about the plasma pharmacokinetics and tissue penetration of cilofungin, we studied these properties during both intermittent doses (ID) and continuous infusion (CI) of cilofungin in rabbits.

MATERIALS AND METHODS

Drug. Cilofungin was provided by Eli Lilly & Co. (Indianapolis, Ind.) as a lyophilized powder. A 50-mg/ml solution was made in 33% polyethylene glycol-300 (Sigma Chemical Co., St. Louis, Mo.) and phosphate-buffered (pH 6.8) physiologic saline. Gentle agitation in an ultrasonic water bath aided in dissolution. Solutions were stored at 4°C and used within 48 h.

Rabbits. Female New Zealand White rabbits weighing between 2.5 and 3.5 kg were used in these studies. Animals were individually housed and provided with food and water ad libitum, according to National Institutes of Health guidelines (1). In order to provide for continued nontraumatic venous access, silastic central venous catheters were surgically placed under sterile technique and general anesthesia, as previously described (9). For CI pharmacokinetic studies,

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each rabbit was equipped with two central venous catheters, one of which was attached to a Parker Micropump (Parker Hannifin Corp., Irvine, Calif.) to deliver cilofungin at preset infusion rates. Pumps were then secured to protective jacks on each rabbit, thereby allowing for free movement.

Single-dose plasma pharmacokinetics. Each of three rabbits was given a single intravenous dose of 50 mg of cilofungin per kg over 6 min. Plasma samples were obtained before the injection and at 0, 2, 4, 6, 8, 10, 12, 16, 20, 30, 40, 50, and 60 min after the completion of the injection.

CI plasma pharmacokinetics. On the basis of the first-order kinetics observed in the single-dose study, cilofungin was administered by CI to yield steady-state concentrations of 5 $\mu\text{g/ml}$ (HiCI) and 2.5 $\mu\text{g/ml}$ (LoCI). Five HiCI rabbits received cilofungin at 10 mg/kg/h and seven LoCI rabbits received cilofungin at 5 mg/kg/h for 6 consecutive days while being treated for experimental disseminated candidiasis by methods previously described (8a). Infusions were interrupted daily for 6 h in order to reload and readjust the micropumps and to allow for maintenance care of each rabbit. Samples were drawn daily at the end of each 18-h infusion period for determination of levels in plasma.

Stepwise increases in CI. Following the CI study, which showed nonlinear kinetics consistent with a saturable excretion route, we further studied cilofungin by stepwise increases in CI rate in order to identify a dosage at which nonlinear clearance would occur. Three rabbits received CI cilofungin at progressively increasing rates of 1, 2, 3.5, 5, and 7 mg/kg/h, with 24-h infusions at each dosage rate. Levels in plasma were determined at 2, 6, 10, and 24 h after the start of each new infusion rate.

Tissue penetration. (i) **ID.** Simultaneous levels of cilofungin in plasma and tissue were determined in three rabbits at sacrifice immediately after the last of six intravenous ID of cilofungin at 15 mg/kg every 4 min. Tissue samples from multiple sites were collected at autopsy. Tissues were carefully dissected to avoid blood contamination, and the bladders were drained prior to dissection to avoid contamination by urine. All plasma and tissue samples were stored at -70°C until time of assay.

(ii) **CI.** Similarly, simultaneous cilofungin levels in plasma and tissue were determined for nine rabbits at the end of each trial of CI at either 5 or 10 mg/kg/h. Five rabbits had levels of cilofungin in tissue determined at near-steady-state concentrations immediately after stopping CI, whereas four rabbits had levels of cilofungin in tissue determined 24 h after discontinuation of CI.

Assay. Levels of cilofungin in plasma were determined by microbiological agar diffusion assay using *Aspergillus montevidensis* (A35137), as previously described (3). Standard plasma samples were made by adding cilofungin to 50% methanol–50% buffer (pH 6) at 1,000 $\mu\text{g/ml}$ and then diluting the solution with 25% methanol–25% buffer (pH 6)–50% control rabbit plasma to obtain final cilofungin concentrations of 0.3, 0.5, 1.0, and 2.0 $\mu\text{g/ml}$. Plasma samples were diluted with 50% methanol–50% buffer (pH 6) so that resulting zone sizes were within the range of the standard curve concentrations. All dilutions were adjusted so that each sample contained the same amount of plasma present in the standard curve samples. *A. montevidensis* was grown on V-8 juice agar medium for 3 days at 30°C . The *Aspergillus* conidia were harvested by streaking a sterile swab repeatedly over the agar plate flooded with 5 to 10 ml of saline. This conidial saline suspension was then adjusted to an optical density of 0.6 at 590 nm on a Bausch & Lomb Spectronic 20 spectrophotometer.

On the day of assay, each 100 ml of melted and cooled (50°C) antibiotic assay medium agar (Difco, Detroit, Mich.) was inoculated with 3 ml of this spore suspension. Three hundred milliliters of this seeded agar was poured onto a glass plate (12 by 12 in.). After allowing the agar to harden, the plate was covered and stored at 4 to 10°C for at least 1 h. Then seven rows of equally spaced 6-mm wells were cut from the agar to form a matrix (7 by 7) of 49 wells. To these wells, 0.05 ml of standard plasma or test plasma samples was added, with three wells dosed per sample. The first 12 wells were required for the four standard samples, whereas the last six wells were reserved for two spiked control rabbit plasma samples of 4 and 15 μg of cilofungin per ml. Thus, 10 test samples could be assayed per plate. The plate was covered and incubated for 24 to 36 h. The diameters of the resultant zones of inhibition were measured to the nearest 0.1 mm with an Image Analyzer (Fryer Co., Carpentersville, Ill.).

The activity of each sample was determined as follows. First, a standard curve was made by plotting the logarithm of the known cilofungin concentration (ordinate) against the unweighted average of the three zone diameters for each standard sample (abscissa). The average of the three zone diameters for each sample was then used to read the concentration of the sample off the curve. There was a good linear relationship between the log of the concentrations and the zone diameters, with a correlation coefficient of 0.9937. The sensitivity of the method was 0.6 $\mu\text{g/ml}$ and the coefficient of variation (including day-to-day variation) at 4 $\mu\text{g/ml}$ was 4%.

Levels of cilofungin in tissue were determined as described above. Each sample was weighed, diluted with 10 ml of 50% methanol–50% buffer (pH 6) per gram of tissue, and then homogenized in a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.). The sample was then centrifuged for 10 min at 2,000 rpm, and the supernatant was assayed as noted above. The standard curve was prepared in 50% methanol–50% buffer (pH 6). Further dilution with buffer was done as necessary. Tissues from untreated rabbits were used as controls. For urine, bile, or vitreous, the samples were simply diluted as required to obtain zones within standard curve range with 50% methanol–50% buffer (pH 6). The sensitivity, linearity, and reproducibility of the assay in tissues were similar to those in the plasma.

Pharmacokinetic calculations. For the single-dose study, the area under the plasma concentration-time curve was calculated by using the linear trapezoidal rule up to the final measured concentration and then extrapolating to infinity (2). The terminal half-life in the postdistributive phase was determined by regression analysis using an unweighted least-linear squares method. The volume of distribution at steady state was determined by using the area under the moment curve. The clearance was calculated by dividing the dose by the area under the plasma concentration-time curve. For all CI studies, the daily levels of drug in plasma were averaged, and since these values were similar after the second day of infusion, all of these values were averaged to obtain a near-steady-state mean concentration in plasma (C_{ss}). For determination of Michaelis-Menten parameters, the C_{ss} at each infusion rate (R_0) was determined by taking the average of the four levels in plasma at 2, 6, 10, and 24 h into each infusion phase for each individual rabbit during the stepwise-increase CI study, then graphing R_0 versus R_0/C_{ss} . The maximum rate of elimination (V_m) and the Michaelis-Menten constant (K_m) values were determined from the y intercept and the negative of the slope, respectively, of the

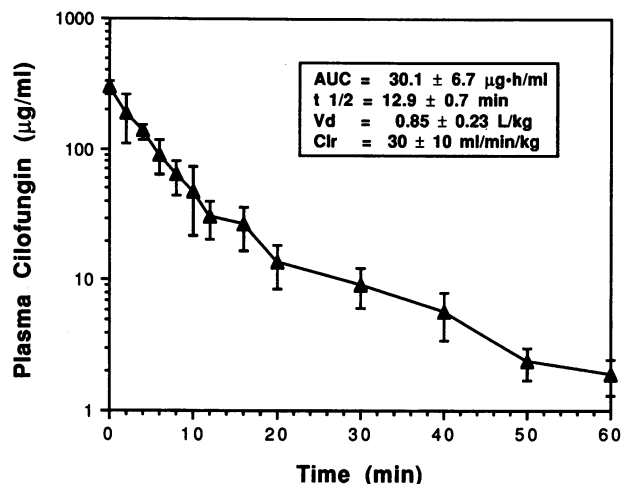


FIG. 1. Plasma cilofungin concentration-versus-time curve for three rabbits after a 50 mg/kg intravenous dose. Each point plots the mean and standard errors of the level in plasma at that time. Mean (\pm standard error of the mean) pharmacokinetic values are shown in the box.

best-fitting line (8). In addition, the overall V_m and K_m were determined by graphing the R_0 versus the mean R_0/C_{ss} for all three rabbits.

To assess the ability of cilofungin to penetrate into tissues, the mean of the ratios of levels in tissue to levels in plasma was calculated by determining the ratios of the concentrations in tissue to concentrations in plasma at sacrifice for each individual rabbit in each group and then calculating the mean of these ratios. Thus, each value is the mean of the ratios and not the ratio of the mean level in tissue over the mean level in plasma. This first method appears more representative of the true degree of tissue penetration, because even if a particular rabbit has low levels in plasma, its level in tissue may be relatively many times higher, and thus its tissue-to-plasma ratio would be high. Differences in the means of each group at each tissue site were evaluated by Student's unpaired t test.

RESULTS

Single-dose plasma pharmacokinetics. The average plasma concentration over time after a single intravenous dose of cilofungin at 50 mg/kg is shown in Fig. 1. A high mean peak level of 297 ± 39 $\mu\text{g/ml}$ was achieved immediately after intravenous bolus, but concentrations fell rapidly with a half-life in distribution phase of 3.7 ± 0.2 min (first 12 min postdose) and a half-life in elimination phase of 12.9 ± 0.7 min. The mean area under the plasma concentration-time curve was thus small at 30.1 ± 6.7 $\mu\text{g} \cdot \text{h/ml}$, whereas the clearance of 30 ± 10 ml/min/kg was correspondingly large. The mean volume of distribution approximated total body water at 0.85 ± 0.23 liters/kg.

CI plasma pharmacokinetics. On the basis of the first-order pharmacokinetic characteristics following single doses, cilofungin was then administered by CI (10 mg/kg/h) to yield a steady-state level in plasma of 5 $\mu\text{g/ml}$. However, cilofungin by CI attained high concentrations in plasma after each 18-h CI period and continued to increase until a near-steady-state level in plasma was achieved at 72 to 144 h at a mean concentration in plasma of 290 ± 56 $\mu\text{g/ml}$ (Fig. 2). The greater than 50-fold-higher near-steady-state level during

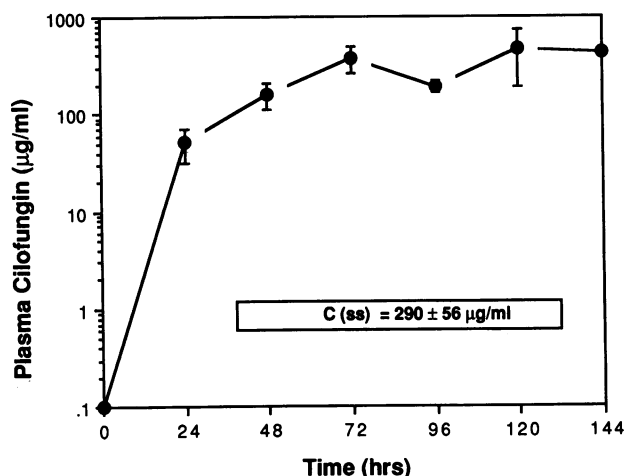


FIG. 2. Plasma cilofungin concentration (\pm standard error of the mean)-versus-time curve for five rabbits during CI of cilofungin at 10 mg/kg/h over 6 days. Interrupted segments of the curve indicate intervals during which infusion was interrupted for readjusting pumps and maintaining rabbits.

HiCI (10 mg/kg/h) indicated that elimination was nonlinear for cilofungin at these rates of CI.

Similarly, at 5 mg/kg/h (LoCI) (Fig. 3), three of seven rabbits accumulated high levels (71 ± 8 $\mu\text{g/ml}$) by the end of the 6-day experiment but did not reach steady state. Four rabbits, however, maintained a constant low-steady-state level (5.0 ± 0.9 $\mu\text{g/ml}$) throughout the duration of infusion. This individual variation suggested that the elimination became markedly nonlinear, possibly because of saturation of elimination pathways in some animals, beginning at about the LoCI rate (5 mg/kg/h).

Stepwise increases in CI. Initially, when mean cilofungin levels in plasma from all three rabbits in this study were reviewed, the levels showed apparently linear increases in steady-state levels for CI rates up to 5 mg/kg/h, although

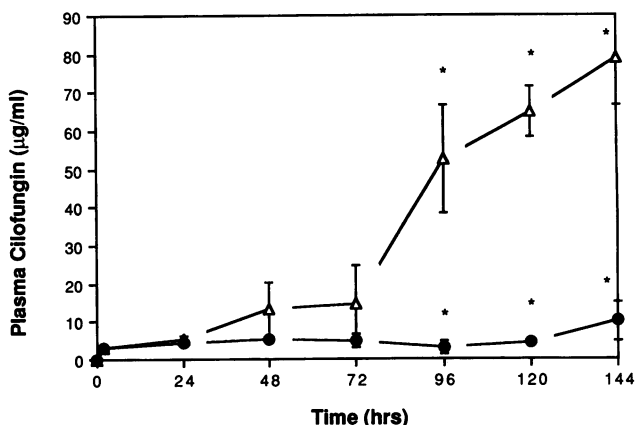


FIG. 3. Plasma cilofungin concentration (\pm standard error of the mean)-versus-time curves for seven rabbits during CI of cilofungin at 5 mg/kg/h over 6 days. Three rabbits (Δ) showed rising mean levels in plasma, whereas the mean levels of the other four rabbits (\bullet) remained constant, with a C_{ss} of 5.0 ± 0.9 $\mu\text{g/ml}$. *, Levels were significantly different ($P < 0.001$, Student's unpaired t test). Interrupted segments of each curve indicate intervals during which infusion was interrupted for readjusting pumps and maintaining rabbits.

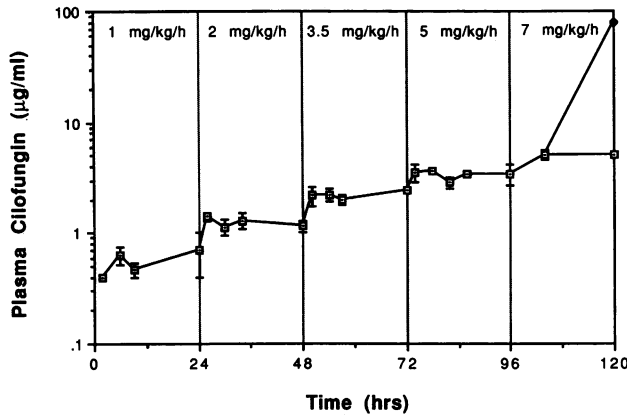


FIG. 4. Plasma cilofungin concentrations (\pm standard error of the mean) during daily stepwise increases in CI of cilofungin between 1 and 7 mg/kg/h in three rabbits. One rabbit appeared to have saturable elimination at 7 mg/kg/h, with rising levels in plasma, whereas the levels in the other two rabbits remained in steady state.

marked nonlinearity occurred in one rabbit at 7 mg/kg/h (Fig. 4). Analysis by graphing the R_0 versus R_0/C_{ss} of each individual rabbit (Fig. 5), however, revealed that the levels approximated Michaelis-Menten pharmacokinetics (mean $r = 0.95 \pm 0.02$, Spearman rank correlation), with an overall K_m of 6.8 $\mu\text{g/ml}$ (range, 4 to 13 $\mu\text{g/ml}$) and overall V_m of 14.6 mg/kg/h (range, 9 to 27 mg/kg/h). There was considerable interrabbit variability in the K_m and V_m , as might be expected among these outbred animals.

Tissue penetration. The mean ratios of cilofungin concentrations in tissue to those in plasma were determined for rabbits dosed intermittently and for rabbits immediately after stopping HiCI and LoCI. Since the ratios were similar for both CI groups, they were combined. The mean ratios for ID rabbits were lower than the mean ratios for CI rabbits (Fig. 6). After CI, ratios of concentration in tissue to concentration in plasma were >0.5 for visceral organs (lung, liver, spleen, and kidney), but following ID, ratios were only <0.1 for all organs except lung and liver. The differences were especially notable in the brain (cerebrum and cerebellum), choroid, kidney, and bile, in which the ratios after CI were significantly higher ($P < 0.05$) by as much as 89-fold

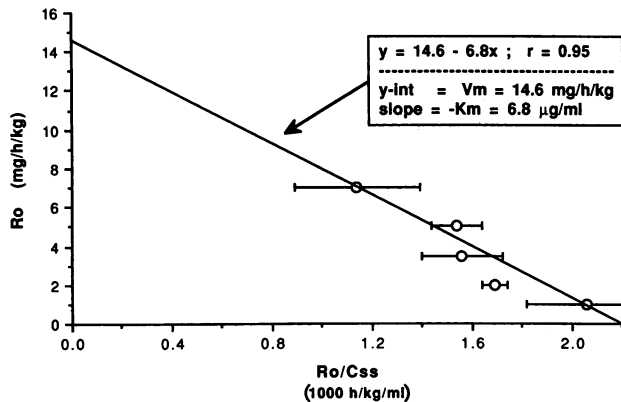


FIG. 5. R_0 versus the mean R_0/C_{ss} (\pm standard error of the mean) for three rabbits given stepwise increases in CI. The best-fitting line is also shown. ($r = 0.95$, Spearman rank correlation), with calculated V_m and K_m .

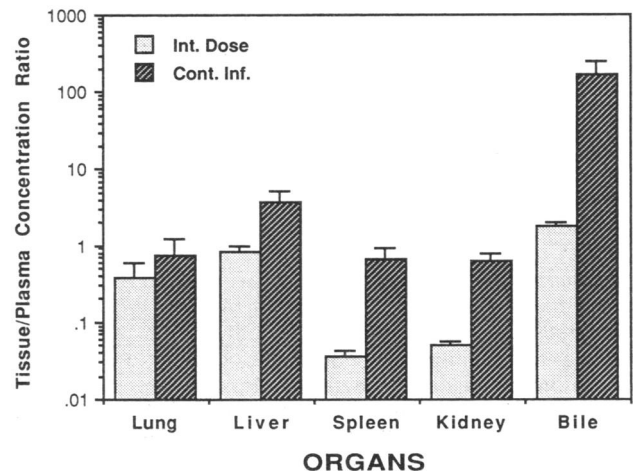
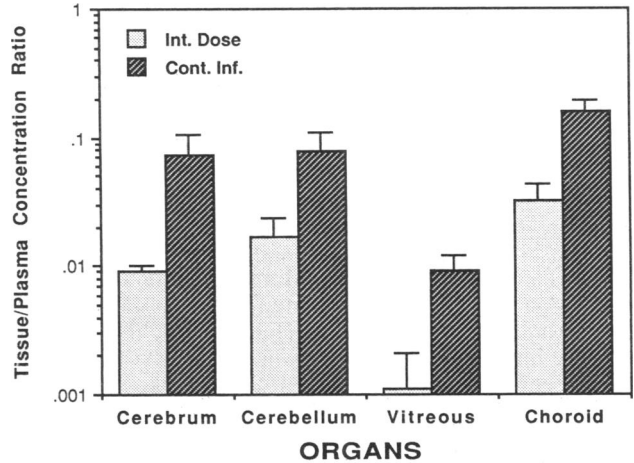


FIG. 6. Means (\pm standard error of the mean) of the ratios of levels in tissue to those in plasma of cilofungin for three ID (15 mg/kg every 4 min times 6 doses) versus five CI (5 or 10 mg/kg/h) rabbits determined immediately after 6-day infusions. Mean ratios for levels in brain and eye are shown above, and those for visceral sites are shown below. Ratios were significantly higher ($P < 0.05$) after CI for brain, choroid, kidney, and bile.

over the ratios following ID. Following ID, ratios of less than 0.02 indicated poor penetration into the brain. Following CI, however, actual levels in brain were $8.7 \pm 1.9 \mu\text{g/g}$, and ratios of concentrations in tissue to those in plasma were significantly sixfold higher than following ID.

In the four rabbits whose cilofungin concentrations in tissue and plasma were determined 24 h after stopping CI, high levels in tissue persisted in the following tissues (in $\mu\text{g/g}$): liver, 222 ± 127 ; choroid, 4.5 ± 1.5 ; and cerebrum, 1.8 ± 0.8 . Persistence of cilofungin in plasma ($1.0 \pm 0.3 \mu\text{g/ml}$) was also found. These findings indicated a slow washout of cilofungin from both tissues and plasma after CI.

DISCUSSION

This study showed that after a single intravenous dose cilofungin achieved high peak concentrations in plasma in rabbits but then was rapidly eliminated following linear, first-order kinetics. During CI, however, a nonlinear, dose-dependent decline in the rate of elimination of this drug was observed, consistent with one or more saturable excretion

pathways. Furthermore, the sustained high levels in plasma resulted in a nonlinear increase in the penetration of cilofungin into multiple tissue sites.

The nonlinearity of cilofungin pharmacokinetics during CI precluded predicting the steady-state concentrations on the basis of single-dose pharmacokinetics. For example, on the basis of a single-dose clearance of 30 ml/min/kg, a constant level in plasma of 5 $\mu\text{g/ml}$ was predicted during HiCI (10 mg/kg/h). Instead, sustained high levels in plasma ($290 \pm 56 \mu\text{g/ml}$) were obtained, indicating a nonlinear decrease in the rate of elimination of this drug. Moreover, saturability of the elimination pathways was suggested by the dose dependency of this nonlinear elimination, as elucidated by the LoCI and stepwise-increase CI studies, in which a rapid rise in cilofungin concentrations in plasma occurred at or above 5 mg/kg/h of CI. Furthermore, the stepwise-increase CI study supported a saturable, receptor-ligand system of elimination, because the drug appeared to follow Michaelis-Menten pharmacokinetics.

The Michaelis-Menten pharmacokinetic parameters determined in the stepwise-increase CI study were consistent with the observed pharmacokinetics of cilofungin in the other studies. According to Michaelis-Menten pharmacokinetics, when a drug is administered at rates approaching V_m (especially above $V_m/2$, the rate when plasma levels = K_m) significant nonlinearity occurs (8). Since it appears that the rabbits had an estimated V_m of 14.6 mg/kg/h, given inter-rabbit variability it is quite possible that the LoCI (5 mg/kg/h) and HiCI (10 mg/kg/h) rates approached V_m for many of these rabbits, leading to the nonlinear, marked rise in levels in plasma of cilofungin observed in most of these rabbits. Conversely, the estimated K_m of 6.8 $\mu\text{g/ml}$ would predict that marked nonlinearity would not occur below this concentration in plasma. Indeed, steady-state was maintained when cilofungin levels remained below 6 $\mu\text{g/ml}$ (Fig. 3 and 4).

The most plausible explanations for the nonlinear, saturable pharmacokinetics of cilofungin involve the principal mode of elimination of cilofungin—biliary excretion. Biliary excretion of unchanged cilofungin in mice accounted for more than 90% of all elimination (R. Gordee, personal communication). The extremely high concentrations of the drug in the bile of our rabbits was also consistent with hepatobiliary excretion. The clearance rate of 30 ml/min/kg could also be explained by rapid hepatic uptake and excretion, since the cardiac output of a 3-kg rabbit is approximately 500 ml/min, and assuming that one-fifth of the cardiac output goes to liver and that the hepatic extraction ratio is 100%, the expected hepatic clearance rate would be 33 ml/min/kg. If this hepatobiliary uptake were dependent on receptors at the hepatocyte or bile canaliculi, these receptors could be saturated by excess drug. Moreover, if such uptake were energy dependent or if the receptors were rapidly consumed during uptake with only slow replacement or if the production of receptors were repressed by constant high levels of cilofungin, then the fall in clearance rate during CI could be explained.

An alternative explanation for the findings of this study could be that cilofungin may have end product metabolites that inhibit the elimination of the parent drug. If so, after intermittent bolus doses the parent drug would be cleared quickly and there may be ample time between doses for metabolites to be cleared to insignificant levels. However, during CI, there may be enough accumulation of metabolites to inhibit parent drug clearance, resulting in rapidly rising levels of cilofungin. Indeed, the delayed buildup of such

metabolites could explain the 72-h delay in starting to accumulate higher drug levels in some of the rabbits receiving LoCI (Fig. 3). Metabolite inhibition could also explain the prolonged washout phase with persistent levels in plasma exceeding 1 $\mu\text{g/ml}$ observed after stopping HiCI, if accumulated metabolites were to continue to inhibit cilofungin elimination. Such metabolite inhibition, however, would be unlikely to cause a stepwise progression in steady-state levels consistent with Michaelis-Menten pharmacokinetics (Fig. 4) but rather would cause a progressive drift upward of levels in plasma. Moreover, it would be difficult to explain the dichotomy of responses seen in Fig. 3 by this hypothesis, unless one were to assume that some rabbits did not produce an inhibitory metabolite (i.e., they lacked the required enzyme pathway).

Penetration of cilofungin into the CNS and other tissues was relatively poor after ID. Mean ratios of concentration in tissue to concentration in plasma were <0.1 for most organs and <0.02 in the cerebrum and cerebellum after 90 mg/kg was given in six divided doses over 24 min. When CI was used, tissue penetration for all organs increased nonlinearly, with ratios of levels in tissue to levels in plasma rising up to 89 times higher than during ID and actual concentrations of cilofungin in brain tissue exceeding 8 $\mu\text{g/g}$. Perhaps these differences in tissue penetration are due in part to total dosage. Preliminary studies, however, in which rabbits dosed with 50 mg/kg twice a day for 6 days showed undetectable levels in tissue 12 h after the last dose, suggested otherwise. The higher levels in tissue could also be partly explained by a mass action effect of the sustained higher concentrations of cilofungin in plasma achieved during CI, but the higher ratios of levels in tissue to levels in plasma were more consistent with the tissues acting as a sink for the compound. Given the lipophilic structure of cilofungin, such an effect may be due to the binding of cilofungin to cell membrane or intracellular sites, allowing for a gradual accumulation of drug in tissue during CI but not during a shorter period of ID. Such tissue binding, if it occurred, could represent a third, slowly equilibrating compartment of a three-compartment model of drug distribution. This model would predict both the accumulation and the persistence of levels in tissue and a prolonged plasma washout period due to the slow leakage of drug back into circulation from this third compartment. Indeed, we observed both the accumulation of high levels in tissue during CI and the persistence of levels in both plasma and tissue as long as 24 h after stopping CI. Moreover, the improvement in CNS penetration with CI was also consistent with this model, since the high lipid content of the CNS may represent an especially large, slowly equilibrating sink for cilofungin, requiring prolonged exposure to high levels in plasma to achieve therapeutically significant concentrations of the drug in these tissues.

Although direct extrapolation from pharmacokinetic data in animals to humans should be done cautiously, this study appears to show that cilofungin, an antifungal compound fungicidal for *Candida* species, can achieve sustained high levels in plasma and tissue if used in an appropriate dosing regimen. Its short half-life and relatively poor CNS tissue penetration suggest that frequent ID or CI may be required to maintain levels in plasma and/or tissue above the MICs for *Candida* species for some life-threatening conditions of disseminated candidiasis. Frequent ID (every 4 or 6 h) may also take advantage of possible nonlinear pharmacokinetics in human beings as well, since such frequent dosing was associated with decreased clearance rates of cilofungin over time (R. Gordee, personal communication). The inability to

maintain fungicidal levels in tissues with 50 mg/kg twice a day may explain the lack of *in vivo* efficacy in previous animal studies (6, 7). For therapeutically difficult sites such as the CNS, this study indicates that ID may fail to clear CNS infections but suggests that CI may improve the chance of achieving enough drug penetration into CNS tissues to eradicate the organisms. Moreover, these improved levels in plasma and tissue are obtainable without much increase in total drug used, since 5 mg/kg/h requires only 20% more total drug than 50 mg/kg twice a day. Indeed, current pharmacodynamic studies conducted in our laboratory have demonstrated a superior effect of CI in treating disseminated candidiasis. Therefore, the sustained high levels in plasma and tissue possible during CI, and especially the improvement in CNS penetration, suggest that CI may be a more effective dosing method for the treatment of some cases of disseminated candidiasis with this drug.

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