

Amphotericin B Pharmacokinetics in Humans

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The pharmacokinetics of amphotericin B were studied in two patients at the conclusion of long-term therapy for disseminated histoplasmosis. The distribution kinetics of this drug were adequately described by a three-compartment mammillary model with a total distribution volume averaging 4 liters/kg. The elimination phase half-life of amphotericin B was approximately 15 days, reflecting slow release of amphotericin B from a peripheral compartment. In accordance with previous reports, renal excretion accounted for only 3% of total amphotericin B elimination. The pharmacokinetic model for one of the patients also was used to compare the simulated amphotericin B serum levels that would be expected if initial therapy followed two recommended regimens.

Dose recommendations for the safe and effective use of amphotericin B have evolved from careful clinical observation of the response of patients who have been treated with this drug (5, 3, 9). The present investigation provides a rational pharmacokinetic basis for analysis of intravenous treatment regimens currently in vogue. This approach is similar to that previously applied to a description of the pharmacokinetics of intrathecally administered amphotericin B (1) and hopefully will serve as a framework for the design of improved treatment regimens with this drug in the future.

MATERIALS AND METHODS

Patients. Two patients were studied at the conclusion of a course of amphotericin B given intravenously. Both were men with disseminated histoplasmosis and adult onset diabetes mellitus not requiring insulin. Patient 217, age 75, received 3.076 g of amphotericin B over 50 days. For the month prior to study he received daily intravenous infusions of 70 mg of amphotericin B. Patient 220, age 45, received 4.110 g of amphotericin B over 136 days. He had received a prior course of 2.025 g, ending 9 months before the present course. For the 2 months before this investigation he received intravenous infusions of 70 mg of amphotericin B every other day.

Amphotericin B assay. Serum and urine specimens were bioassayed by using radial growth inhibition of *Paecilomyces variotii*, as reported previously (5). The lower limit of accurate detection of amphotericin B by this assay is 0.1 µg/ml (5). Urine specimens were kept at 4°C during collection of 24-h samples. Once collected, both urine and serum were stored at -70°C before assay.

Pharmacokinetic analysis. Serum and urine data were analyzed with the SAAM 23 digital computer program developed by Berman and Weiss for multi-

compartmental analysis (4). The program was implemented on a model 6400 Control Data Corp. Computer. Preliminary analyses indicated that systematic errors were apparent when an attempt was made to model the kinetics of amphotericin B distribution with a single two-compartment system. However, only apparently random deviations between the experimental data and the computer-calculated results were evident when a three-compartment system was used. A mammillary model was chosen since it is the three-compartment model most commonly selected to describe drug pharmacokinetics and for theoretical reasons presented in the discussion (Fig. 1).

The assumption was made that, since the patients were studied at the end of their treatment, they were at steady state with respect to their amphotericin B serum and peripheral compartment concentrations. Recovery of amphotericin B excreted in urine was expressed as the rate of renal excretion of amphotericin B (dU/dt) at the midpoint of each collection interval. Where Cl_r is the renal clearance and S the serum concentration of amphotericin B,

$$dU/dt = Cl_r \cdot S$$

Therefore, renal excretion rate data could be used to determine the renal clearance of amphotericin B as well as to estimate serum amphotericin B concentrations below the analytical sensitivity limit of the assay in case 220 (12). Intercompartmental clearances (Q) were determined from the product of the distribution volume of a compartment and the intercompartmental transfer rate constant exiting from that compartment (17).

The computer iteratively adjusted the values of all the parameters of the model to minimize the differences between the theoretical curves describing the serum level and urine excretion rate of amphotericin B and the observed data points. The sum of the squared differences between the data points and the values predicted from the computer fit were 0.0049 for

case 217 and 0.015 for case 220. This corresponds to average deviations of 4.3 and 5.1% for the serum data for cases 217 and 220 and 9.1 and 9.7% for the respective urine excretion rate data.

RESULTS

The results of the analysis of amphotericin B pharmacokinetics in two patients are summarized in Table 1. As can be seen in Fig. 2, the elimination-phase half-life of amphotericin B is approximately 15 days, largely because of the slow return of amphotericin B from the slowly equilibrating peripheral compartment of the pharmacokinetic model (Fig. 1). The volume of this peripheral compartment averages 80% of the total distribution volume of 4 liters/kg, yet its intercompartmental clearance of 9 ml/min is only 10% that of the rapidly equilibrating peripheral compartment. Since the total elimination clearance, averaging 30 ml/min, is greater than the intercompartmental clearance of the slowly equilibrating compartment, estimated amphotericin B concentrations in this compartment persist at considerably higher levels than serum concentrations when therapy is stopped. Even during therapy, most of the amphotericin B at steady state is located in the slowly equi-

brating peripheral compartment, amounting to 67% of total body stores in case 217 and 68% of total body stores in case 220.

Renal excretion appears to be a relatively minor pathway for the elimination of amphotericin B, accounting for only 3.1% of total elimination in case 217 and 3.5% in case 220. Renal clearance of amphotericin B averaged only 3% of creatinine clearance in these two patients. The mode of nonrenal amphotericin B elimination remains to be determined. In the present model, this would include drug decomposition in vivo as well as metabolism and biliary excretion.

Recommendations for starting amphotericin B therapy represent an empirical attempt to provide effective therapy reasonably promptly while minimizing toxicity by administering an initial test dose followed by stepwise incremental doses on subsequent days. Many patients are treated with a 1-mg dose of amphotericin B on day 1, then 5-mg, 10-mg, and 15-mg doses on the next 3 days. The results obtained from the pharmacokinetic analysis of data from case 220 were used to simulate the amphotericin B serum levels that would be anticipated if this regimen were followed in this patient (Fig. 3). Although the minimum inhibitory concentration for *Cryptococcus neoformans* and *Candida albicans* has been reported to range between 0.05 and 1.56 $\mu\text{g/ml}$ (8), in the experience of one of us (J.E.B), at least two-thirds of the isolates have their minimal inhibitory concentrations within the narrower range of 0.2 to 0.5 $\mu\text{g/ml}$. This narrower range was chosen for the purposes of our simulation. It can be seen in Fig. 3 that even on day 4 of therapy serum levels would be maintained above 0.2 $\mu\text{g/ml}$ for less than 10 h. This simulation raises the possibility that this regimen may not provide adequate amphotericin B levels promptly enough to reverse the course of patients who are critically ill.

When the need for effective antifungal therapy is urgent, a more aggressive initial treatment regimen of 0.3 mg/kg on days 1 and 2, followed by gradually increasing doses up to 0.5 to 0.6 mg/kg per day has been recommended (3). The

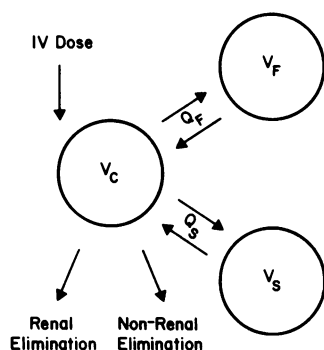


FIG. 1. Pharmacokinetic model of amphotericin B distribution and elimination. After intravenous injection into the central compartment (V_C), amphotericin B distributes into fast- (V_F) and slow- (V_S) equilibrating peripheral compartments. The rate of this distribution is governed by the intercompartmental clearances Q_F and Q_S .

TABLE 1. Summary of pharmacokinetic results

Case no.	Body wt (kg)	Compartment vol. (liter) ^a			Total distribution vol.		Intercompartmental clearances (ml/min) ^b		Elimination half-life (days)	Elimination clearance (ml/min)	Renal clearance (ml/min)	Creatinine clearance (ml/min)
		V_C	V_F	V_S	Liter	Liter/kg	Q_F	Q_S				
217	65.4	26.3	29.2	187.4	242.9	3.71	79.5	8.51	14.0	24.0	0.74	28
220	74.2	35.4	18.9	262.6	316.9	4.27	106.2	9.75	16.5	36.1	1.26	37

^a V_C , Central; V_F , fast equilibrating; V_S , slow equilibrating.

^b Q_F , Fast; Q_S , slow.

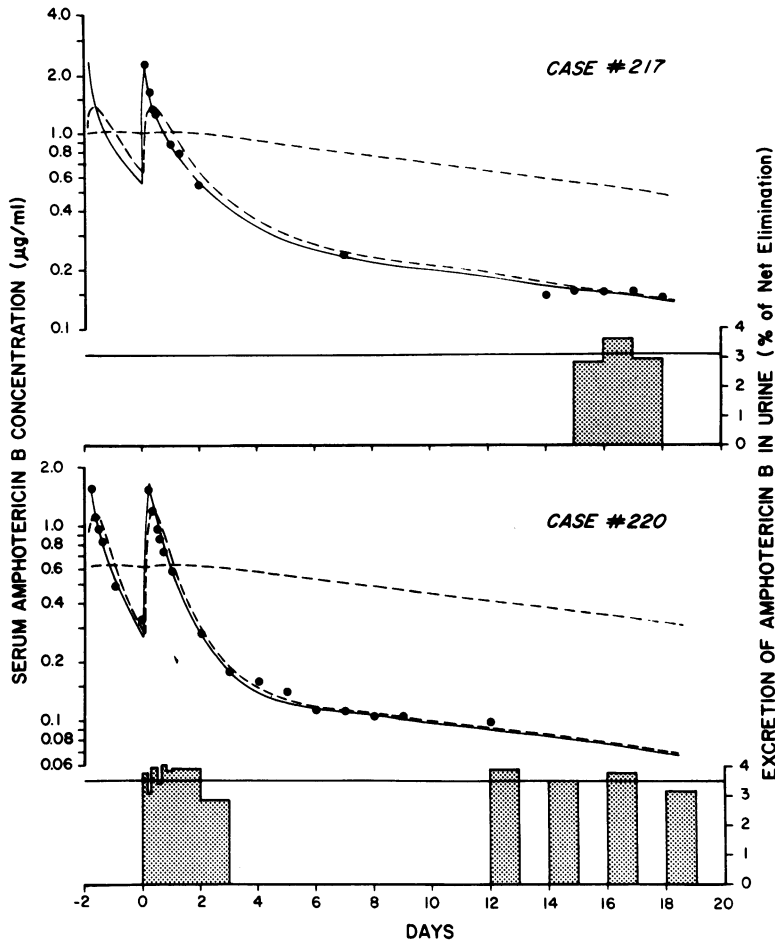


FIG. 2. Pharmacokinetic analysis of plasma concentrations (●) and urinary excretion rate (▣) of amphotericin B at the conclusion of prolonged intravenous therapy of two patients. The last day of therapy is designated day zero. The solid lines are a least-squares fit of the data, based on the model shown in Fig. 1. The dotted lines represent hypothetical amphotericin B concentrations in the fast- and slow-equilibrating peripheral compartments of the model.

initial amphotericin B serum levels that would be expected if case 220 were treated in this fashion are included in Fig. 3 for comparison. On all but treatment day 1, serum levels continuously exceed $0.2 \mu\text{g/ml}$.

Since amphotericin B distribution beyond the intravascular space is probably necessary for maximum antifungal efficacy, serum levels may not reflect therapeutic response under non-steady state conditions. The speed with which a therapeutically effective concentration of amphotericin B is attained throughout its distribution space may be important in this regard, and the pharmacokinetic simulation has been used to compare the two regimens with respect to the percentage of eventual steady state total body stores of amphotericin B reached during

the first 4 days of therapy. On treatment day 4, total body stores of amphotericin B would reach a peak of 19.4 mg if the first regimen were followed and 52.3 mg if the second regimen were followed. This would amount to 8 and 22%, respectively, of the 238.6-mg peak total body stores estimated for this patient under steady state conditions at the conclusion of intravenous infusion on a regimen of 70 mg of amphotericin B every other day. Peak amphotericin B stores estimated for case 217 under steady state conditions were similar, totaling 284.0 mg.

DISCUSSION

For nearly 20 years amphotericin B has been the principal chemotherapeutic agent used to treat systemic mycoses, yet the time course of

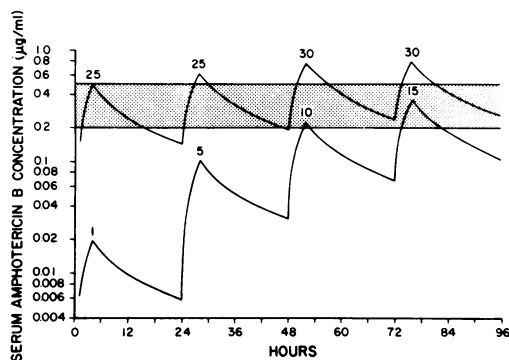


FIG. 3. Computer simulation of the first 4 days of amphotericin B therapy based on the pharmacokinetic results obtained from the study of patient 220. Expected serum levels are compared following daily 4-h intravenous infusion in which doses are administered according to two recommended regimens, as indicated in milligrams by the numbers above each serum concentration peak. The shaded area indicates the usual minimal inhibitory concentrations of *C. neoformans* and *C. albicans*.

serum levels measured in patients after intravenous administration of this drug has eluded formal pharmacokinetic description. A major source of confusion was the fact that after intravenous infusion amphotericin B serum levels fell at an initially rapid rate for 24 h, then more slowly over the next few days (10, 11, 16). Empirically it has been shown that a graph of serum amphotericin B levels versus time could be linearized by plotting the data on log-log coordinates (5). The present investigation demonstrates that this phenomenon results from the multicompartmental nature of amphotericin B distribution and that conventional pharmacokinetic techniques are adequate to describe the distribution and elimination characteristics of this drug.

Previous estimates of a 24- to 48-h elimination half-life of amphotericin B (10, 16) have largely reflected the initially rapid elimination of amphotericin B from the central and rapidly equilibrating peripheral compartments. Since a 24- or 48-h dosing interval is usually selected and the final elimination phase is not reached for almost 6 days, the terminal phase of amphotericin B elimination only becomes apparent when therapy with this drug is stopped. This terminal elimination phase has a half-life of approximately 15 days.

The results of this investigation corroborate earlier reports that less than 10% of an administered amphotericin B dose is excreted unchanged by the kidney (5, 16). Renal amphotericin B clearance averaged only 3% of creatinine

clearance in the two patients, approximating the relative hemodialytic clearances of these two compounds (6). These results are consistent with the view that amphotericin B excretion in urine results from glomerular filtration and is restricted by the greater than 90% protein binding of this drug (6). The data also suggest that impaired renal function would not significantly prolong the elimination half-life of amphotericin B if nonrenal elimination pathways were unaffected by the uremic state. In fact, limited experience has demonstrated that serum amphotericin B concentrations are not unexpectedly increased in patients with impaired renal function (5), or even in anephric patients (6, 10). Certainly the nephrotoxicity of amphotericin B warrants caution in its use, but there is no rational basis for recommending that doses of this drug be automatically reduced in uremic patients.

The long terminal elimination-phase half-life of amphotericin B is of major clinical importance because it implies that a long time will be required to attain pharmacokinetic steady state conditions with repeated doses of this drug. The model indicates that this is not due to slow excretion from the central compartment. Rather, it is the intercompartmental clearance of the slowly equilibrating peripheral compartment that is rate limiting. The large total volume of amphotericin B distribution (4 liters/kg) also contributes to the long elimination half-life of this drug. This apparent volume exceeds total body weight probably because the affinity of cell membranes for amphotericin B exceeds that of serum.

The apparent distribution volume of the central compartment is greater than that of many drugs which have central compartment volumes more nearly approximating plasma volume or extracellular fluid space. Erythrocytes bind amphotericin B avidly, and an erythrocyte-to-saline buffer partition ratio of approximately 30:1 has been found *in vitro* (7). Although serum would be expected to have a higher affinity for amphotericin B than this buffer because of binding to β -lipoproteins (2), it is possible that the central compartment may represent nothing more than total blood volume.

The identity of the peripheral compartments is even more speculative. *In vitro* studies indicate that amphotericin B is approximately 90% bound to serum protein and appears to pass through ultrafiltration membranes while bound to a high-molecular-weight serum constituent that is presumably protein (6). This could explain the three-compartmental nature of amphotericin B distribution since albumin distribution

is also best described by a three-compartment pharmacokinetic model with an intravascular compartment, a rapidly equilibrating extravascular compartment with a small distribution volume, presumably representing the interstitial fluid of tissues with discontinuous capillaries such as liver, spleen, and intestine, and a slowly equilibrating extravascular compartment with a larger distribution volume, presumably representing interstitial fluid of tissues with continuous capillaries such as skeletal muscle and skin (14). A similar pharmacokinetic pattern has been demonstrated for thyroxine which is tightly bound to serum proteins and also appears to distribute into rapidly and slowly equilibrating interstitial fluid compartments (15). These observations also suggest that a mamillary system is more appropriate than a catenary system to model the distribution kinetics of amphotericin B. The most likely explanation for the large distribution volumes of the peripheral model compartments is that amphotericin B binds even more tightly to cell membranes lining these compartments than to interstitial fluid protein, thus increasing their apparent volume in the same way that erythrocyte binding could increase the apparent intravascular distribution volume of this drug.

Correlation of the time course of pharmacological response with non-steady-state pharmacokinetic data requires, at the very least, a consideration of drug concentrations in the biophase, the anatomic site of drug action. When the simulation in Fig. 3 is interpreted with respect to predicted serum levels, the assumption is made that effective amphotericin B concentrations within the central compartment of the model will affect clinical outcome. If this compartment reflects only intravascular volume and effective amphotericin B concentrations are required in the interstitial fluid for a favorable clinical response, interpretation of this simulation becomes more complex. Nonetheless, it seems obvious that serum amphotericin B levels above the minimal inhibitory concentration of the infecting organism are a minimum requirement for clinical efficacy. Although one would like to verify the simulated serum levels by direct experimental measurement of amphotericin B concentration, this is not possible for the first few days of conventional therapy, given the 0.1- $\mu\text{g}/\text{ml}$ lower limit of sensitivity of the method used for amphotericin B assay.

In any case, only a limited interpretation of the results of this pharmacokinetic investigation seems warranted. Because of the uncertain anatomic identity of the model compartments, it is not known in which compartment(s) it is most

crucial to obtain therapeutic concentrations of amphotericin B. In addition, drug elimination is predicted as proceeding entirely from the central compartment of the model, although it is conceivable that amphotericin B also loses biological activity while stored in the peripheral compartments. However, the useful purpose in presenting a pharmacokinetic model of this type is to make the hitherto confusing pharmacokinetics of amphotericin B more understandable. This should facilitate the establishment of an inductive as well as an empirical basis for the design and clinical evaluation of therapeutic regimens with this drug.

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