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Pharmacokinetics of Conventional Formulation versus Fat Emulsion Formulation of Amphotericin B in a Group of Patients with Neutropenia

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The pharmacokinetics of amphotericin B administered in a conventional 5% dextrose (glucose) (5% D) solution and in a 20% fat emulsion formulation (Intralipid; 20% IL) were compared in 16 patients (mean age, 42 years [range, 18 to 70 years]) who had been hospitalized for hematological malignancies and with proven or suspected fungal infections. All of the patients received 50 mg (approximately 1 mg/kg of body weight per day) of amphotericin B daily in random order, either as a 50-ml lipid emulsion (20% IL) (group I) or in 500 ml of 5% D (group II). Five serum samples were taken during the 24 h after drug administration, and the levels of amphotericin B were measured by high-pressure liquid chromatography. Serum amphotericin B concentrations declined rapidly during the first 6 h, and subsequent measurements revealed a slow terminal elimination phase in both groups. The maximum serum amphotericin B concentration was significantly lower when the drug was administered in 20% IL (1.46 ± 0.61 versus 2.83 ± 1.17 μg/ml; $P = 0.02$). The area under the concentration-time curve from 0 to 24 h was also much lower in group I (17.22 ± 11.15 versus 28.98 ± 15.46 μg · h/ml). The half-life of the distribution phase was approximately three times longer in group I (2.92 ± 2.34 h versus 0.64 ± 0.24 h; $P = 0.011$). Conversely, the half-lives of the elimination phase were approximately equal in the two groups (11.44 ± 5.18 versus 15.23 ± 5.25 h). The mean residence times were also similar in both groups (19.41 ± 11.13 versus 19.65 ± 7.86 h). The clearance and the steady-state volume of distribution of amphotericin B in group I were about twice as great as those in group II (62.97 ± 35.51 versus 33.01 ± 14.33 ml/kg/h and 1,043.92 ± 512.10 versus 562.32 ± 152.05 ml/kg [$P = 0.034$], respectively). Finally, the volume of distribution in the central compartment was greater in group I than in group II (618.17 ± 231.80 versus 328.19 ± 151.71 ml/kg; $P = 0.013$), but there were no differences in the volume of distribution in the peripheral compartment (425.75 ± 352.87 versus 234.14 ± 75.92 ml/kg). These results suggest that amphotericin B has a different pharmacokinetic profile when it is administered in 20% IL than when it is administered in the standard 5% D form and that the main difference is due to a clear-cut difference in the steady-state volume of distribution, especially that in the central compartment.

Amphotericin B remains the drug of choice for the treatment of a variety of invasive fungal infections. It is active against a broad range of pathogenic fungi, such as *Candida* spp., *Aspergillus fumigatus*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Sporotrix schenckii* (8).

Amphotericin B is a highly lipophilic drug that is poorly absorbed orally, thus necessitating parenteral administration. It is formulated for intravenous administration as a mixed micellar dispersion with deoxycholate as a surfactant. Amphotericin B should be administered by slow intravenous infusion in 5% dextrose (glucose) (5% D) because normal saline disrupts the colloid and leads to aggregation (8, 11). The infusion of amphotericin B-deoxycholate is often poorly tolerated, and toxic effects are common. Shortening the infusion time results in an increase in the number of infusion-related side effects, and increasing the dosage will lead to a higher rate of chronic side effects (5).

The fungicidal activity of amphotericin B is concentration dependent, as has been shown both in vivo and in animal models (18). The relatively high rate of clinical failures of amphotericin B in granulocytopenic and other immunocompromised patients could be the result of inadequate local concentrations of the active drug in tissue. Because of its dose-related toxicity, the maximal tolerable dosage is 0.5 to 1 mg/kg of body weight per day, which may be suboptimal for clinical success (11).

Encapsulating amphotericin B into liposomes or binding the drug to lipid formulations such as commercially available fat emulsions may overcome this efficacy limitation. By reducing the toxic reactions and preserving the antifungal activity of amphotericin B, these formulations can be administered at higher dosages, resulting in an increased therapeutic index (1–3, 12, 15, 16). It should be remembered that the various formulations differ substantially in their structures and in their pharmacokinetic behaviors, which may have implications for their clinical usefulness.

In the study described here, the pharmacokinetics of amphotericin B administered in conventional 5% D solution and in a 20% fat emulsion formulation were compared in 16 neutropenic patients with proven or suspected fungal infections.

MATERIALS AND METHODS

Study design. A comparative, prospective, open-label randomized study which involves neutropenic patients with hematological disorders was performed in a university general hospital. Patient recruitment began in May 1993 and ended in March 1994. All of the patients received 50 mg (4) of amphotericin B (Fungi-

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TABLE 1. Clinical and demographic data for study patients[a]

Group and sex	Age (yr)	Wt (kg)	Cr (mg/100 ml)	Urea (mg/100 ml)	Diagnosis
Group I					
Male	64	65	0.8	46	AML
Male	46	70	0.7	18	AML
Male	19	78	0.9	89	BMT
Male	46	70	1.1	63	AML
Female	63	51.5	0.8	35	AML
Female	25	54	0.4	16	ALL
Female	25	54.5	0.6	14	BMT
Female	46	66	0.7	44	AML
Group II					
Male	70	70	0.8	28	AML
Male	47	67	0.7	22	AML
Male	58	60	1.0	43	AML
Male	22	65	1.2	22	HDK
Male	21	70	0.7	44	HDK
Male	42	64	0.9	41	AML
Female	56	49	0.8	30	AML
Female	18	65	1.1	98	ALL

[a] Abbreviations: Cr, serum creatinine concentration; urea, serum urea concentration; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; BMT, bone marrow transplantation; HDK, Hodgkin's disease.

zona; Squibb, Barcelona, Spain) daily either as a 50-ml lipid emulsion (Intralipid 20% [20% IL]; KabiPfrimmer, Barcelona, Spain) with a final concentration of 1 mg/ml (group I) or in 500 ml of 5% D (Bieffe Medical, Barcelona, Spain) with a final concentration of 0.1 mg/ml (group II). The composition of 20% IL, as described by the manufacturer, was purified soy oil (20 g), purified egg phospholipids (1.2 g), anhydrous glycerol (2.2 g), and water for injection (up to 100 ml).

Patients. Sixteen patients with a mean age of 42 years (age range, 18 to 70 years), neutropenia (absolute granulocyte count, less than 1,000 cells per mm^3), and proven or suspected fungal infection were enrolled in and completed the study. Eight patients (four males, four females) were assigned to group I; these patients had the following mean values for demographic and clinical parameters: age, 42 years; weight, 63.7 kg; creatinine concentration, 0.75 mg/100 ml; and urea, 40 mg/100 ml. Eight patients (six males, two females) were assigned to group II; these patients had the following mean values for demographic and clinical parameters: age, 42 years; weight, 64.4 kg; creatinine concentration, 0.9 mg/100 ml; and urea, 41 mg/100 ml. Hydration and electrolyte supplementation were given as needed, as determined by the medical staff caring for the patients. Severe adverse reactions during or immediately after the infusion of amphotericin B were treated with 100 mg of meperidine (Dolantina; Bayer, Barcelona, Spain) plus 40 mg of methylprednisolone (Urbason; Hoechst, Barcelona, Spain) by the intravenous route. Individual demographic and clinical data are presented in Table 1.

Drug preparations. Amphotericin B preparations in a commercial fat emulsion were aseptically prepared in a horizontal laminar air flow hood in the Pharmacy Service. Amphotericin B was reconstituted with 10 ml of sterile water for injection (Braun, Barcelona, Spain) to produce a final concentration of 5 mg/ml and was added to 40 ml of 20% IL in a vacuum flask to produce a final drug concentration of 1 mg/ml. Amphotericin B in 5% D was prepared under the same conditions. After reconstitution with 10 ml of sterile water, amphotericin B solution was added to 500 ml of 5% D. Amphotericin B preparations were administered within the first 6 h after preparation.

Although the manufacturer recommends infusion periods of 6 h, a 1-h administration schedule was used to facilitate the management of this type of patient receiving a great number of intravenous medications. In a previous study we had not observed significant differences between the administration of amphotericin B over periods of either 1 or 6 h (6).

Drug pharmacokinetics. Blood samples were obtained at 0.17, 2, 6, 12, and 24 h after the end of the first infusion of amphotericin B, centrifuged in a TDX-Abbott centrifuge at 3,000 rpm to separate the serum, and then frozen at −80°C until they were analyzed.

Determination of amphotericin B concentrations in serum samples. A modification of the method described by Granich et al. (9) was used to measure the amphotericin B concentration in serum samples by high-performance liquid chromatography. The analysis was performed on a Kontron chromatograph equipped with a model 325 solvent delivery system, a model 465 automated sample injector with variable injection volume, and a model 432 UV absorption variable wavelength detector with a 8-μl flow cell. The detector response was monitored with an Acer 1120 SX computer with Kontron PC-integrator software, version 3.00. After deproteinization of serum samples with methanol, the supernatant was injected in duplicate onto a reversed-phase C_{18} column 30 mm in length. The mobile phase was 2.5 mM disodium EDTA–acetonitrile (70:30; vol/vol) at a flow rate of 1.0 ml/min. Amphotericin B was eluted at 1.5 min and was detected at 405 nm. The assay was linear from 0.05 to 25 μg/ml. The sensitivity was 0.05 μg/ml, and the within-day and day-to-day imprecision (coefficient of variation) ranged between 3.61 and 1.33% at 0.5 and 2.5 μg/ml, respectively.

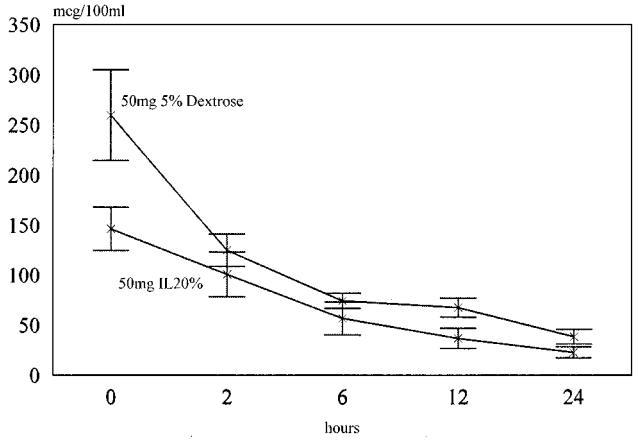

FIG. 1. Mean plasma amphotericin B concentration versus time after administration in lipid emulsion (20% IL) or 5% D. Bars indicate standard errors of the mean.

Pharmacokinetic analysis. Each concentration-versus-time curve of amphotericin B was analyzed by compartmental and noncompartmental methods.

(i) Noncompartmental analysis. The estimation of the pharmacokinetic parameters by the noncompartmental method (clearance [CL], volume of distribution [V_{ss}] and mean residence time [MRT]) is based on the calculation of the area under the curve (AUC) and the area under the moment curve (AUMC). AUC is the area under the concentration-versus-time curve, while AUMC is the area under the curve of a plot of the product of concentration and time versus time.

(ii) Compartmental analysis. The basic pharmacokinetic parameters of the one-compartment model (half-life [$t_{1/2}$]) and the two-compartment model ($t_{1/2}$ of the distribution phase [$t_{1/2\alpha}$], $t_{1/2}$ of the elimination phase [$t_{1/2\beta}$], volume of distribution of the central compartment [V_c], volume of distribution of the peripheral compartment [V_p]) were estimated by using a nonlinear least-squares iterative method. For the estimation process, the package PKCALC (Merrell Dow Pharmaceuticals Inc., Indianapolis, Ind.) was used. The concentrations were weighted statistically, taking weight as the reciprocal of concentration. The *F*-ratio test was used to assess whether individual decay curves were monophasic or biphasic. The serum amphotericin B concentration at 0.17 h was taken as the maximum concentration of drug in serum (C_{max}).

Statistical analysis. Statistical analysis was performed with commercially available software (SPSS/PC$^+$, version 4.0). The distribution of all quantitative variables was examined to detect significant departures from normality by the Shapiro-Wilks test. For descriptive purposes, however, the mean and standard distribution were used independently of the type of distribution. Intergroup comparisons were performed by the Mann-Whitney U test. The critical *P* value retained for significance was 0.05.

RESULTS

The mean serum amphotericin B concentration-versus-time curves after intravenous administration of the lipid emulsion and the conventional formulation are shown in Fig. 1. Serum amphotericin B concentrations declined rapidly during the first 6 h after the administration of both formulations. Subsequent serum amphotericin B concentration measurements also revealed a slow terminal elimination phase in both groups. The *F*-ratio test was significant for all patients, so we considered the individual decay curves as biphasic. Consequently, the results of compartmental analysis are expressed as two-compartment model parameters.

Table 2 provides the pharmacokinetic parameters obtained for both groups of patients. The data for one patient in group

II were not considered for comparative purposes because the values for that individual were more than seven times the standard deviation for the group. The reason for these extreme values is unknown, and they cannot be explained by his clinical status .

The maximum serum amphotericin B concentration for patients given the lipid emulsion was significantly lower than that for patients given the conventional formulation (confidence interval, 1.88 ÷ 1.04 versus 3.71 ÷ 1.95 μg/ml; $\alpha = 0.05$; $P = 0.021$). By contrast, at 24 h there were no statistically significant differences in serum amphotericin B concentrations between the two groups (0.34 ÷ 0.14 versus 0.55 ÷ 0.21 μg/ml; $P = 0.18$). The AUC from 0 to 24 h was lower in group I than in group II, but the difference was not statistically significant (25.10 ÷ 9.34 versus 40.66 ÷ 17.26 μg · h/ml; $P = 0.12$).

The $t_{1/2\alpha}$ after the administration of equivalent doses of amphotericin B was approximately three times longer in group I than in group II (4.58 ÷ 1.26 versus 0.81 ÷ 0.45 h; $P = 0.011$). The $t_{1/2\beta}$, however, was approximately equal in the two groups (18.11 ÷ 10.79 versus 19.19 ÷ 11.27 h; $P = 0.78$). The MRTs were also similar in both groups (27.29 ÷ 11.53 versus 25.59 ÷ 13.71 h; $P = 0.96$).

The CL from serum of amphotericin B administered in 20% IL was approximately twice as great as the CL of the drug administered in 5% D (88.09 ÷ 37.85 versus 43.85 ÷ 22.17 ml/kg/h; $P = 0.11$). The V_{ss} of amphotericin B in 20% IL was about twice as great as that in 5% D (1,406.02 ÷ 681.82 and 677.26 ÷ 447.38 ml/kg, respectively; $P = 0.034$). There were statistically significant differences in V_c (782.07 ÷ 454.27 versus 442.87 ÷ 213.51 ml/kg; $P = 0.013$), but not in V_p (675.27 ÷ 176.23 versus 291.52 ÷ 176.66 ml/kg; $P = 0.40$) between the two groups.

DISCUSSION

Although the pharmacokinetics of amphotericin B administered in 5% D are well defined (7, 8), the development of new delivery systems for this drug has renewed the interest in those types of studies. Depending on the type of amphotericin B formulation, the pharmacokinetic profiles seem to vary considerably (4, 10).

Because of the known fact that the administration of amphotericin B in lipid emulsion is associated with fewer adverse reactions, it seemed worthy to investigate whether the differences in toxicity were or were not related to variations in the pharmacokinetics of amphotericin B. In our experience, the administration of amphotericin B in 20% IL increased V_{ss} by a factor of 1.86. Accordingly, C_{max} and AUC values decreased 52 and 55%, respectively. The CL of the drug increased twofold when amphotericin B was administered in lipid emulsion, while the values of $t_{1/2\beta}$ and MRT remained practically constant, independently of the form of administration (11.44 versus 15.23 h and 19.41 versus 19.65 h, respectively).

It should be remembered with respect to our results that our study involved patients with hematological malignancies with proven or suspected fungal infection and not healthy volunteers. Therefore, our results are the closest to those obtained in clinical practice. On the other hand, the present study included data for only the first 24 h after administration, so we could not detect a third slow compartment and the terminal elimination phase, which has an estimated $t_{1/2}$ of 15 days (7).

It has been postulated that the addition of fat emulsions enhances the CL of amphotericin B from the circulation and results in a faster accumulation in peripheral tissues. The mechanism for the enhanced CL may be related to an active uptake by the reticuloendothelial system. Likewise, the re-

TABLE 2. Pharmacokinetic parameters for two formulations of amphotericin B[a]

Formulation	Noncompartmental					Compartmental			
	C_{max} (μg/ml)	AUC (μg · h/ml)	V_{ss} (ml/kg)	MRT (h)	CL (ml/kg/h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	V_c (ml/kg)	V_p (ml/kg)
IL 20%	1.46 ± 0.61	17.22 ± 11.15	1,043.92 ± 512.10	19.41 ± 11.13	62.97 ± 35.51	2.92 ± 2.34	11.44 ± 5.18	618.17 ± 231.80	425.75 ± 352.87
5% D	2.83 ± 1.17	28.98 ± 15.46	562.32 ± 152.05	19.65 ± 7.86	33.01 ± 14.33	0.64 ± 0.24	15.23 ± 5.25	328.19 ± 151.71	234.14 ± 75.92
P	0.02	NS[b]	0.034	NS	NS	0.011	NS	0.013	NS

[a] Results are expressed as means ± standard deviations.
[b] NS, not significant.

duced toxicity observed could also be explained by the reduced concentrations of the drug in serum (10). We partly agree with such hypotheses. Although it is evident that lower concentrations in serum result in lower levels of toxicity, the explanation for the increase in V_{ss} is not clear, since $t_{1/2\alpha}$ is more than three times higher when amphotericin B is administered in lipid emulsion, which indicates that this formulation results in a slower distribution to peripheral tissues. Moreover, the increase in V_{ss} is mainly due to an increase in V_c since statistically significant differences ($P = 0.013$) in this parameter were found between the two groups. Conversely, V_p remained unchanged ($P = 0.40$).

Comparing the C_{max} values, it is evident that in the lipid form of administration, half of the amphotericin B is rapidly removed from serum. This may give the false impression that only half of the theoretical dose of amphotericin B has been administered or that the bioavailability of the drug in the lipid formulation is 50% compared with the bioavailability of the drug in the standard 5% D preparation. The right explanation could be a different pattern of binding to plasma proteins, with a different degree of protein binding when amphotericin B is administered in 20% IL. Another interesting consideration with regard to this subject is that, assuming a more active distribution to peripheral tissues with the lipid emulsion, one would expect higher $t_{1/2\beta}$ and MRT values as a consequence of the reentry from the peripheral compartment. Surprisingly, neither $t_{1/2\beta}$ nor MRT values were different from those observed when the drug was given in the 5% D preparation.

One must bear in mind the fact that even though the lower incidence of side effects has been related to lower concentrations of amphotericin B in serum, the clinical efficacy does not seem to be compromised by the lower concentrations of the drug in these formulations (2–4, 11–16).

When compared with amphotericin B integrated into liposomes, the pharmacokinetic profile of amphotericin B in lipid emulsion is clearly different. As described by other workers, liposomal amphotericin B shows a reduced V_{ss} (370 ml/kg) and an increased C_{max} and AUC (29.0 μg/ml and 423 μg · h/ml, respectively, when the drug is given at doses of 3 mg/kg of body weight). Elimination parameters are also clearly different, with a reduced CL and an increased $t_{1/2\beta}$ (22.8 ml/kg/h and 23.6 h, respectively) (15). The pharmacokinetic profile of amphotericin B in 20% IL also differs from the profile of the cholesteryl sulfate complex formulation characterized by an increased V (V_{area} = 7,580 ml/kg) and a small increase in AUC (45.6 μg · h/ml), while the CL is lower (21.9 ml/kg/h) (17).

In summary, we can conclude that amphotericin B in lipid emulsion has a pharmacokinetic profile different from those of other amphotericin B formulations. When compared with the standard 5% D form, the main difference is related to an evident increase in V_{ss}, especially in V_c. In view of the limited toxicity and reduced concentrations of drug in serum with this formulation, one may contemplate its use in certain clinical situations in which higher doses of amphotericin B are necessary.

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