Application of amphotericin B in lipid emulsions (AmB/L) reduced membrane toxicity in vitro and decreased amphotericin B-associated toxic side effects in vivo when compared to that of amphotericin B applied in 5% glucose (AmB/G). Therefore, a comparative analysis of the pharmacological parameters of AmB/L and AmB/G was performed. Thirteen patients were analyzed, and nine of these patients received a subsequent treatment with AmB/G and AmB/L. In patients in both treatment groups amphotericin B showed a biphasic elimination from serum, with a prolonged terminal half-life of approximately 27 h. Patients treated with AmB/L showed significantly lower peak concentrations (44.2%; \( P = 0.008 \)) and correspondingly lower area under the drug concentration-time curve (AUC) values (64.3%; \( P = 0.015 \)) compared to the values for the same patients treated with AmB/G at a dose range of 0.6 to 1.5 mg/kg of body weight. The enhanced clearance of AmB/L may be due to a faster initial elimination of amphotericin B-lipid aggregates by the reticuloendothelial system. Lower peak concentrations and AUC values in serum and a correspondingly faster deposition of AmB/L in tissues may at least partly explain the lower toxicity of AmB/L. A comparative pharmacokinetic analysis with data for a single patient treated with AmB/L demonstrated that hemodialysis did not significantly affect the disposition of amphotericin B.

Amphotericin B is a drug with major importance in the treatment of invasive fungal infections. Determinants of an efficient antifungal treatment are early start of therapy and application of high initial doses (1 to 1.5 mg/kg of body weight per day) of amphotericin B (2, 13). Since the bioavailability of amphotericin B in peripheral tissues is rather low, fungicidal concentrations of the drug in tissue may only be provided by early application of high drug doses. This treatment approach is, however, limited by the severe toxicity of amphotericin B. Acute side effects such as fever and chills were observed in up to 60% of patients, while chronic toxicity such as impairment of renal function occurred in up to half of the patients. In search for an improvement of clinical drug tolerability, several investigators described the lower toxicity of amphotericin B when applied in lipid emulsions. In fact, the maximal tolerated dose of amphotericin B in mice could be raised by more than ninefold when the drug was applied in Intralipid (14). This was supported by a number of recent clinical studies reporting that amphotericin B infused in Intralipid induced a significant reduction of nephrotoxicity and acute side effects compared to amphotericin B applied in dextrose (4–6, 16).

The exact mechanism of the Intralipid-mediated reduction of amphotericin B-associated toxicity is still unclear. However, it has been hypothesized that lipid emulsions like Intralipid decrease the amount of oligomeric amphotericin B and thereby reduce the interaction of amphotericin B with cholesterol-containing cell membranes. The remaining monomeric amphotericin B, however, retains its potential to bind to the ergosterol of fungal cell membranes (1, 8, 10, 12).

Binding of amphotericin B to phospholipids may also modulate the pharmacology of the drug and may thereby affect the tolerability of amphotericin B. The present study was designed to analyze the impact of lipid emulsions like Intralipid on the pharmacokinetics of amphotericin B.

**MATERIALS AND METHODS**

**Materials.** Amphotericin B deoxycholate (50 mg/vial) was obtained from Bristol-Myers-Squibb GmbH, Munich, Germany. Intralipid (20%) was purchased from Kabi Pharmacia GmbH, Erlangen, Germany, and Lipofundin (20%) was a product of Braun Melsungen AG, Melsungen, Germany.

---

**TABLE 1. Patient characteristics**

| Patient no. | Age (yr) | Wt (kg) | Diagnosis* |
|-------------|----------|---------|------------|
| 1           | 64       | 66      | AML, presumed aspergillosis |
| 2           | 51       | 80      | ALL, pulmonary aspergillosis |
| 3           | 58       | 60      | Hodgkin’s disease, pulmonary and cerebral aspergillosis |
| 4           | 53       | 85      | CLL, FUO |
| 5           | 76       | 70      | Peritonitis, presumed Candida infection |
| 6           | 49       | 57      | BMT, pneumonia, FUO |
| 7           | 55       | 78      | BMT, presumed Candida pneumonia |
| 8           | 51       | 76      | AML pneumonia, FUO |
| 9           | 56       | 100     | Candida peritonitis |
| 10          | 65       | 65      | Presumed Candida pneumonia |
| 11          | 38       | 88      | Peritonitis, presumed Candida infection |
| 12          | 62       | 75      | Liver transplantation, FUO |
| 13          | 55       | 70      | Presumed pulmonary aspergillosis |

* AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; FUO, fever of unknown origin; BMT, bone marrow transplantation. The term “presumed” and was applied as indicated in Materials and Methods.
**TABLE 2. Comparative pharmacokinetics of AmB/G and AmB/L**

| Patient no. | Dose (mg/kg) | C<sub>max</sub> (μg/ml) | V (liter/kg) | Clearance (ml/min) | t<sub>1/2b</sub> (h) | AUC (μg · h/ml) |
|-------------|--------------|-------------------------|-------------|-------------------|----------------|----------------|
|             | AmB/G        | AmB/L                  | AmB/G       | AmB/L             |                 |                |
| 1           | 0.6          | 2.60                    | 1.02        | 0.54              | 1.10            | 0.54           | 0.95          | 11.8          | 13.3          | 18.9          | 10.5          |
| 2           | 0.6          | 1.4                     | 0.68        | 4.03              | 1.59            | 0.68           | 1.29          | 68.3          | 14.3          | 15.4          | 8.1           |
| 3           | 1.0          | 1.70                    | 1.64        | 2.14              | 1.60            | 0.76           | 0.67          | 32.6          | 27.8          | 21.9          | 25.1          |
| 4           | 1.0          | 1.7                     | 1.08        | 1.34              | 1.52            | 1.57           | 1.58          | 9.87          | 11.1          | 10.6          | 10.5          |
| 5           | 1.0          | 2.07                    | 1.22        | 4.32              | 10.1            | 1.91           | 1.53          | 26.2          | 75.9          | 17.4          | 8.5           |
| 6           | 1.0          | 1.45                    | 0.51        | 2.68              | 3.14            | 0.84           | 4.56          | 37.0          | 7.9           | 19.9          | 3.65          |
| 7           | 1.0          | 2.07                    | 1.23        | 1.12              | 3.45            | 0.59           | 1.02          | 21.9          | 39.2          | 28.3          | 25.0          |
| 8           | 1.0          | 1.60                    | 0.72        | 1.46              | 13.7            | 1.00           | 3.75          | 39.7          | 11.4          | 7.2           | 4.45          |
| 9           | 1.0          | 1.20                    | 0.87        | 1.27              | 2.72            | 1.73           | 5.24          | 14.7          | 9.2           | 29.6          |
| 10          | 1.0          | 1.55                    | 4.07        | 1.71              | 2.74            | 0.85           | 0.88          | 22.6          | 30.7          | 45.3          | 25.7          |
| 11          | 1.5          | 6.7                     | 2.10        | 0.98              | 2.34            | 0.50           | 0.88          | 22.6          | 30.7          | 45.3          | 25.7          |
| 12          | 1.5          | 6.75                    | 2.22        | 0.80              | 1.88            | 0.54           | 0.94          | 17.2          | 23.2          | 46.7          | 26.7          |
| 13          | 2.0          | 2.0                     | 0.92        | 5.24              | 14.7            | 1.00           | 4.13          | 14.7          | 9.2           | 29.6          |
| 8           | 2.0          | 3.08                    | 1.69        | 1.50              | 13.1            | 1.50           | 3.75          | 39.7          | 11.4          | 7.2           | 4.45          |

*Thirteen patients who received either the standard preparation of AmB/G or AmB/L were evaluated. Patients 1 to 7, 11, and 12 received AmB/G on day 1 and then an identical dose of AmB/L on day 2. The concentrations of amphotericin B in serum were determined as described in Materials and Methods. V, volume of distribution; the other abbreviations are defined in the text.*
with nine patients (patients 1 to 7 and 11 and 12) who were first treated with AmB/G and who subsequently received the identical dose of AmB/L (Table 4).

The pharmacokinetic patterns were comparable in both groups. Amphotericin B showed biphasic elimination characteristics, with a short initial and a prolonged terminal half-life. A matched-pairs analysis (Wilcoxon test) revealed a significantly lower $C_{\text{max}}$ (1.30 versus 2.94 µg/ml; $P = 0.008$) for the AmB/L group compared to that for the AmB/G group. Accordingly, AUC values were also significantly lower in the AmB/L group (16.0 versus 24.9 µg · h/ml; $P = 0.015$). In fact, the mean $C_{\text{max}}$ decreased to 44.2% and the mean AUC was reduced to 64.3% when AmB/L was used. Figure 1 demonstrates the comparative pharmacokinetics obtained for patient 1. The volume of distribution was greater in the AmB/L group (1.88 versus 1.34 liters/kg); this difference, however, was statistically not significant. Interestingly, the initial half-life of distribution was significantly longer ($P = 0.03$) in the AmB/L group than in the AmB/G group (0.52 versus 1.0 h). No significant differences between groups were observed with regard to the clearance (1.49 versus 0.88 ml/min) and the elimination half-life ($t_{1/2B}$) of amphotericin B (27.1 versus 27.5 h).

Effect of hemodialysis on the pharmacokinetics of AmB/L. The effect of hemodialysis on the pharmacokinetics of AmB/L was investigated in patient 9. This patient was monitored on 2 subsequent days. On the first day, amphotericin B (1.0 mg/kg) was applied but hemodialysis was not performed. On day 2, a hemodialysis treatment was started, and the identical dose of amphotericin B was applied as a 1-h infusion during hemodialysis. Hemodialysis was carried out for 2.5 h without a negative fluid balance. When the data obtained before and during hemodialysis were compared, it became apparent that hemodialysis did not affect the $C_{\text{max}}$ of amphotericin B (0.8 versus 0.86 µg/ml). The $t_{1/2B}$ was, however, shorter during dialysis (39.3 versus 16.7 h), and accordingly, the clearance of amphotericin B was greater (3.75 versus 4.08 ml/min). As a result, the AUC was slightly lower during dialysis (4.45 versus 4.08 µg · h/ml). The reduced $t_{1/2B}$ cannot be ascribed to hemodialysis since, during the terminal elimination phase, hemodialysis had already been terminated.

Effect of Intralipid on amphotericin B-mediated membrane toxicity. The membrane toxicity of amphotericin B was evaluated by determination of LDH release from human erythrocytes. Amphotericin B was dissolved either in 5% glucose or in a lipid emulsion like Intralipid 20% or Lipofundin 20%. Both lipid emulsions contained identical amounts of phospholipids (12 g/liter). However, the emulsions were different in that 20% Lipofundin contained 100 g of middle-chain triglycerides per liter and 100 g of soybean oil per liter, while 20% Intralipid contained 200 g of soybean oil per liter, but no middle-chain triglycerides.

It was demonstrated (Fig. 2) that increasing amphotericin B concentrations induced a sigmoidal increase in LDH release which reached 100%, i.e., complete lysis of erythrocytes, at an amphotericin B concentration of 40 µg/ml. The release of LDH from erythrocytes was completely prevented by 20% Intralipid at an amphotericin B/Intralipid ratio of 2 mg/1 ml. The identical result was obtained for Lipofundin.

In a subsequent analysis, the impact of the ratio of amphotericin B/Intralipid on membrane toxicity was examined (Fig. 3). This assay was performed at final amphotericin B concentrations of 100 and 200 µg/ml. A decreased ratio of amphotericin B/Intralipid decreased the amount of LDH released, and the combination was more effective at the lower amphotericin B concentration of 100 µg/ml. For both amphotericin B con-

---

### TABLE 3. Effect of dose on pharmacokinetics of amphotericin B

| Dose (mg/kg) | No. of patients | $C_{\text{max}}$ (µg/ml) | $V$ (liters/kg) | Clearance (ml/min) | $t_{1/2B}$ (h) | AUC (µg · h/ml) |
|-------------|----------------|--------------------------|----------------|-------------------|---------------|----------------|
| AmB/G       | AmB/L          | AmB/G        | AmB/L          | AmB/G            | AmB/L         | AmB/G   | AmB/L            | AmB/G          | AmB/L          | AmB/L          | AmB/L          |
| 0.6         | 2              | 2               | 2.00           | 0.85             | 2.29          | 1.35     | 0.61             | 1.12           | 40.1           | 13.8           | 17.2           | 9.3            |
| 1.0         | 6              | 7               | 1.76           | 1.15             | 2.61          | 4.89     | 1.12             | 2.08           | 25.8           | 30.7           | 18.0           | 12.7           |
| 1.5         | 2              | 2               | 6.73           | 2.16             | 0.89          | 2.11     | 0.52             | 0.91           | 19.9           | 27.0           | 46.0           | 26.2           |
| 2.0         | 0              | 2               | 2.0            | 3.47             | 2.82          | 13.9     | 19.4             |                |                |                |                |

$^a$ Amphotericin B pharmacokinetics were evaluated at three different doses in patients receiving AmB/G, while patients treated with AmB/L were additionally evaluated after the administration of a dose of 2.0 mg/kg. Data are presented as means of the respective determinations indicated in Table 2. $V$, volume of distribution; NS, not significant.

$^b$ Number of patients receiving each formulation.

### FIG. 1. Pharmacokinetics of amphotericin B after the administration of amphotericin B at a dose of 1.0 mg/kg to patient 1. Amphotericin B was given as a 1-h infusion. On day 1 the drug was given as AmB/G, and on day 2 the drug was given as AmB/L. The concentrations of amphotericin B in serum were determined at the indicated time points, as described in Materials and Methods.
This evaluation demonstrated that mean treated with the respective regimens on subsequent days (Fig. 1). The possibility of a period effect inducing a bias into the pharmacokinetic parameters cannot be excluded. However, there was no indication for a cumulative rise in amphotericin B concentrations in the sera of patients treated with AmB/G alone on subsequent days (data not shown). Due to the severity of the disease, a prolongation of drug application intervals was generally not regarded as being feasible.

From the data presented above, it was concluded that the reduced toxicity of AmB/L may, at least partly, be accounted for by a reduction of the peak amphotericin B concentration and AUC values.

The question of which mechanism is responsible for the significant decrease in $C_{\text{max}}$ and AUC values then arises. Given the assumption of a reduced interaction of AmB/L with mammalian cell membrane cholesterol (8, 14, 15), a reduced uptake by peripheral tissues and consequently a prolonged elimination from the circulating blood compartment would be expected. In fact, this pharmacological characteristic is observed with liposomal formulations of amphotericin B; however, it is not observed with AmB/L. JANOFF et al. (10) reported that amphotericin B formed nonliposomal, ribbon-like structures when interacting with lipids in an aqueous environment. Comparable structures were also observed with a new amphotericin B-lipid complex (ABLC) (11). Interestingly, ABLC is characterized by significantly lower $C_{\text{max}}$ and AUC values compared to those obtained with amphotericin B deoxycholate. This relates to a rapid elimination of ABLC by organs of the reticuloendothelial system and a correspondingly greater accumulation of ABLC in liver, lungs, and spleen (15). By analogy, it may be suggested that the comparatively low concentrations of AmB/L in serum are, in fact, due to a rapid initial capture of amphotericin B-lipid aggregates by the reticuloendothelial system. In conclusion, AmB/L may be defined as a formulation characterized by low $C_{\text{max}}$ and AUC values in serum and a correspondingly faster deposition in tissue.

DISCUSSION

Application of amphotericin B in lipid emulsions like Intralipid results in a significant reduction of treatment-associated toxicity, while antifungal activity remained unaffected in animal models (12, 14). In an erythrocyte lysis assay, it was demonstrated that Intralipid and Lipofundin effectively prevented amphotericin B-mediated membrane toxicity (Fig. 2). An equally protective effect of Intralipid and Lipofundin was expected since both preparations contain the same amount of phospholipids. The optimal ratio of amphotericin B/Intralipid was established to be $\geq 1$ mg/0.3 ml (Fig. 3) which compares favorably to the clinically recommended ratio of 1 mg/0.5 ml (6).

The following question arises, however: may the reduced clinical toxicity of AmB/L also be attributed to a modulation of the pharmacokinetics of amphotericin B or is it only caused by an alteration of the aggregation status of amphotericin B and consequently results from a modified drug interaction with cell membranes? The present analysis characterizes the pharmacokinetics of AmB/L and compares it to the standard preparation by which AmB/G is applied.

The pharmacology of AmB/G was evaluated at three different doses of amphotericin B, namely, 0.6, 1.0, and 1.5 mg/kg (Table 2), while AmB/L-treated patients were additionally evaluated with a dose of 2.0 mg/kg. The increased dose of 2.0 mg/kg was chosen since the ongoing analysis demonstrated a decrease in peak amphotericin B concentrations in the AmB/L group. For patients treated with AmB/L, a significant correlation between the indicated amphotericin B doses and the respective $C_{\text{max}}$ ($P = 0.04$) and AUC values ($P = 0.04$) was demonstrated, an observation not obtained after AmB/G treatment.

A comparative analysis of the pharmacokinetics of AmB/G and AmB/L was performed with nine patients who were treated with the respective regimens on subsequent days (Fig. 1). This evaluation demonstrated that mean $C_{\text{max}}$ and AUC values were significantly reduced in the AmB/L group, reaching 44.2% ($P = 0.008$) and 64.3% ($P = 0.015$) of the values observed in the AmB/G group, respectively (Table 4). Since the interval of amphotericin B application was only 24 h, the possibility of a period effect inducing a bias into the pharmacokinetic parameters cannot be excluded. However, there was no indication for a cumulative rise in amphotericin B concentrations in the sera of patients treated with AmB/G alone on subsequent days (data not shown).
The differential importance of the concentrations of amphotericin B in serum compared to the concentrations of amphotericin B in tissue remains controversial. However, the Intralipid-mediated decrease in $C_{\text{max}}$ and AUC values combined with the lower toxicity of AmB/L may allow for the use of increased doses of the drug. The application of greater amphotericin B doses is guided by the concept that fast achievement of effective tissue drug concentrations is an important determinant of treatment outcome in invasive fungal infection.

With regard to the clinical application of AmB/L, it was of interest to analyze the effect of hemodialysis on the pharmacokinetics of this formulation. Previous reports demonstrated that the pharmacokinetics of amphotericin B deoxycholate in serum were essentially independent of renal function and were not affected by hemodialysis (7). The comparative pharmacokinetic analysis of a single patient before and during hemodialysis indicates that $C_{\text{max}}$ and AUC values remained essentially unaffected by hemodialysis. Adjustment of the AmB/L dose during hemodialysis therefore does not appear to be necessary.

ACKNOWLEDGMENTS

We thank A. Voigt and A. Bercht for expert secretarial help in the preparation of the manuscript.

REFERENCES

1. Bolard, J., P. Legrand, F. Heitz, and B. Cybulskia. 1991. One-sided action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium. Biochemistry 30:5707–5715.
2. Burch, P. A., J. E. Karp, W. G. Merz, J. E. Kuhlman, and E. K. Fishman. 1987. Favorable outcome of invasive aspergillosis in patients with acute leukemia. J. Clin. Oncol 5:1985–1993.
3. Butler, W. T., and E. Cotlove. 1971. Increased permeability of human erythrocytes induced by amphotericin B. J. Infect. Dis. 125:341–350.
4. Caillot, D., O. Casasnovas, E. Solary, P. Chavanel, B. Bonnotte, G. Reny, F. Entezam, J. Lopez, B. Bonnin, and H. Guy. 1993. Efficacy and tolerance of amphotericin B lipid (Intralipid) emulsion in the treatment of candidemia in neutropenic patients. J. Antimicrob. Chemother. 31:161–169.
5. Caillot, D., G. Reny, E. Solary, O. Casasnovas, P. Chavanel, B. Bonnotte, L. Perello, M. Dumas, F. Entezam, and H. Guy. 1994. A controlled trial of the tolerance of amphotericin B infused in dextrose or in Intralipid in patients with hematological malignancies. J. Antimicrob. Chemother. 33:603–613.
6. Chavanel, P. Y., I. Garry, N. Charlier, D. Caillot, J.-P. Kisterman, M. D’Athis, and H. Portier. 1992. Trial of glucose versus fat emulsion in preparation of amphotericin B for use in HIV infected patients with candidiasis. B. Med. J. 305:921–925.
7. Feldman, H. A., and J. D. Hamilton. 1973. Amphotericin B therapy in an anephric patient. Antimicrob. Agents Chemother. 4:302–306.
8. Gruda, L., and N. Dussault. 1988. Effect of the aggregation state of amphotericin B on its interaction with ergosterol. Biochem. Cell. Biol. 66:177–183.
9. Heinzel, G., R. Wołoszak, and P. Thomann. 1993. Pharmacokinetic and pharmacodynamic data analysis system for the PC. Gustav Fischer, Stuttgart, Germany.
10. Janoff, A. S., L. T. Boni, M. C. Popescu, S. R. Minchev, P. R. Cullis, T. D. Madden, T. Taraschi, S. M. Gruner, E. Shyamsunder, M. W. Tate, R. Mendelsohn, and D. Bonner. 1988. Unusual lipid structures selectively reduce the toxicity of amphotericin B. Proc. Natl. Acad. Sci. USA 85:6122–6126.
11. Janoff, A. S., W. R. Perkins, S. L. Saletan, and C. E. Swenson. 1993. Amphotericin B lipid complex (ABLC™): a molecular rationale for the attenuation of amphotericin B related toxicities. J. Liposome Res. 3:451–471.
12. Joly, V., R. Farinotti, L. Saint-Julien, M. Chéron, C. Carbon, and P. Yeni. 1994. In vitro renal toxicity and in vivo therapeutic efficacy in experimental murine cryptococcosis of amphotericin B (Fungizone) associated with Intralipid. Antimicrob. Agents Chemother. 38:177–183.
13. Khoo, S. H., J. Bond, and D. W. Denning. 1994. Administering amphotericin B—a practical approach. J. Antimicrob. Chemother. 33:203–213.
14. Kirsh, R., R. Goldberg, J. Tarloß, D. Parris, J. Hook, N. Hanna, P. Bugelsky, and G. Poste. 1988. An emulsion formulation of amphotericin B improves the therapeutic index when treating systemic murine candidiasis. J. Infect. Dis. 158:1065–1070.
15. Legrand, P., E. A. Romero, B. E. Cohen, and J. Bolard. 1992. Effects of aggregation and solvent on the toxicity of amphotericin B to human erythrocytes. Antimicrob. Agents Chemother. 36:2518–2522.
16. Moreau, P., N. Milpied, N. Fayette, J.-F. Ramée, and J. L. Harousseau. 1992. Reduced renal toxicity and improved clinical tolerance of amphotericin B mixed with Intralipid compared with conventional amphotericin B in neutropenic patients. J. Antimicrob. Chemother. 30:535–541.