J. Pharm. Pharmacol. 1991, 43: 831-835
Received April 26, 1991



# Tissue Distribution of Amphotericin B Lipid Complex in Laboratory Animals*

STEVEN J. OLSEN, MAVIS R. SWERDEL, BARBARA BLUE, JUNIUS M. CLARK AND DANIEL P. BONNER

*Department of Microbiology, Pharmaceutical Research Institute, Bristol-Myers Squibb Company, Princeton, New Jersey 08543-4000, USA*

**Abstract**—Amphotericin B lipid complex (ABLC), under development for the treatment of serious fungal disease, is not a true liposome but a complex of amphotericin B, dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol with a particle size range of 1·6–6·0 μm. Tissue distribution of ABLC was determined in mice and rats after i.v. or i.p. administration. ABLC resembles typical liposomal preparations with amphotericin B concentrating in the reticuloendothelial system. After a single i.v. treatment with ABLC, amphotericin B was present in high concentrations in liver, lung and spleen of mice and rats while plasma levels were consistently low. Mouse liver contained 48% of the administered dose 1 h after treatment and always contained the largest amount of amphotericin B after ABLC treatment. In mice treated once daily for 7 consecutive days with 10 mg $kg^{-1}$ ABLC, liver amphotericin B concentration reached 377 μg $g^{-1}$. Tissue concentrations of amphotericin B were substantially lower when ABLC was given i.p. instead of i.v. with reticuloendothelial tissues containing 2- to 7-fold more after i.v. treatment. Animals treated with 10 mg $kg^{-1}$ ABLC for 14 consecutive days showed no overt signs of toxicity and had only transient changes in liver and kidney function after treatment.

After more than thirty years of clinical use, the polyene macrolide antibiotic amphotericin B remains the drug of choice for treatment of serious systemic fungal infections (Gallis et al 1990). Patients on chemotherapy or immunosuppressive regimens following organ transplant or individuals with HIV infection present increasing numbers of fungal infections for treatment (Wiebe & DeGregorio 1988; McKinsey et al 1989); however, the narrow therapeutic index for amphotericin B limits the amount of antibiotic which can be used to treat these infections.

Attempts have been made to modify amphotericin B to reduce toxicity and improve solubility without affecting antifungal activity. These programmes have produced soluble ester derivatives but none have been pharmaceutically useful (Bonner et al 1972; Galgiani & VanWyck 1984). Lopez-Berestein et al (1983, 1984) prepared liposomes made from dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG) and amphotericin B, and used this formulation to treat infections in laboratory animals. This preparation used a 7:3 ratio of DMPC:DMPG with amphotericin B at 5 mol percent. In these studies, both normal and neutropenic mice were treated with liposomal amphotericin B for disseminated candidiasis. The liposomal formulation showed an improved therapeutic ratio compared with Fungizone (commercial formulation of amphotericin B and desoxycholate) allowing use of higher doses of amphotericin B without toxic effects. These investigators proceeded into man and have treated 63 patients with systemic fungal infections using liposomal amphotericin B, 46 of whom were evaluated in a clinical study. Twenty-four of the patients were classified as completely responding to therapy and there were no haematologic or pulmonary abnormalities which could be related to the liposomal amphotericin B treatment (Lopez-Berestein et al 1989).

In collaboration with The Liposome Company (Princeton, NJ, USA) we have prepared formulations of amphotericin B with DMPC and DMPG in which the mol fraction of amphotericin B was increased over that used by the Lopez-Berestein group. When amphotericin B content was raised above 25 mol percent unusual lipid structures were formed (Janoff et al 1988). The resulting aggregates were no longer true lipid vesicles but rather an amphotericin B complex. Amphotericin B lipid complex (ABLC) has been shown to be less toxic than Fungizone and to be effective in a variety of infections in laboratory animals ranging from systemic *Candida* sp. infections to cryptococcal meningitis (Clark et al 1991). These studies determine the tissue distribution of ABLC in laboratory animals.

## Materials and Methods

### *Compounds*

Amphotericin B lipid complex (The Liposome Company, Princeton, NJ, USA) and Fungizone Intravenous (Bristol-Myers Squibb, New Brunswick, NJ, USA) were used for all studies. ABLC was diluted in 0·9% NaCl (saline) and Fungizone in 5% dextrose for administration to animals. ABLC is a lipid complex containing a 7:3 molar ratio of DMPC and DMPG with amphotericin B at a concentration of 33 mol percent. It has a particle size distribution ranging from 1·6 to 11·1 μm with 90% of the particles between 1·6 and 6·0 μm (data on file, The Liposome Company). Fungizone is the colloidal dispersion of amphotericin B and desoxycholate used for parenteral administration to patients with serious fungal disease. All doses were based on amphotericin B content of the respective preparation.

### *Animal experiments*

Female Swiss Webster mice and Sprague-Dawley rats from

*This work was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, USA, 1988.

Correspondence: S. J. Olsen, Department of Human Pharmacology, Bristol-Myers Squibb Company, PO Box 4000, Princeton, NJ 08543-4000, USA.

Taconic Farms (Germantown, NY, USA) were dosed i.v. in a lateral tail vein or i.p. with ABLC or Fungizone and bled under anaesthesia from the brachial vessels (mice) or abdominal aorta (rats). Blood was collected into heparinized tubes for plasma separation. Brain, lung, liver, kidney and spleen were removed and homogenized in 4 vol of water. Both plasma and homogenized tissue were extracted in methanol (1 part sample:3 parts methanol) for analysis by HPLC. Extracted tissue was centrifuged at 15 000 *g* for 3 min in a microcentrifuge (Hermle, National Labnet, Woodbridge, NJ, USA) and the supernatant assayed for amphotericin B.

*Analytical methods*

Samples were analysed for amphotericin B by reverse phase HPLC using a 5 $\mu$m $C_{18}$ column (250 × 4 mm i.d., Nicolet Instruments, Madison, WI, USA) with a $C_{18}$ Guard-Pak precolumn module (Millipore/Waters, Milford, MA, USA). The mobile phase was 80% methanol (EM Science, Gibbstown, NJ, USA) and 20% 0·005 M $K_2$EDTA (Sigma, St Louis, MO, USA) run at a flow rate of 2·0 mL $min^{-1}$. The HPLC equipment consisted of an ISS-100 autosampler (Perkin-Elmer, Norwalk, CT, USA) used with two 510 pumps, a 490E detector and a Maxima 820 Workstation (Waters/Millipore, Milford, MA, USA). Amphotericin B was detected at 405 nm using an external standard for assay calibration. The standards were prepared by adding amphotericin B to plasma and extracting with methanol as previously described. Under these conditions minimum detectable levels of amphotericin B were 0·4 $\mu$g $mL^{-1}$ in plasma and 2·0 $\mu$g $g^{-1}$ in tissue.

Plasma from mice treated for 14 days with ABLC or Fungizone was analysed for changes in selected clinical chemistry parameters. A portion of the plasma collected for amphotericin B analysis was removed and tested for blood urea nitrogen (BUN), creatinine, glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) using a VISION clinical chemistry analyser (Abbott Laboratories, North Chicago, IL, USA). Untreated mice of the same age and housed under the same conditions were used for control determinations.

## Results

*Single dose of ABLC or Fungizone to mice*

Mice were dosed i.v. with ABLC at 1 and 10 mg $kg^{-1}$ or with Fungizone at 1 mg $kg^{-1}$. The 1 mg $kg^{-1}$ dose of Fungizone was the maximum non-lethal dose that could be administered to normal mice. Samples were collected 1 h after administration (Table 1). When ABLC was given at 10 mg $kg^{-1}$, high concentrations of amphotericin B were seen in reticuloendothelial system organs; liver (123·2 $\mu$g $g^{-1}$), lung (70·8 $\mu$g $g^{-1}$) and spleen (141·1 $\mu$g $g^{-1}$). Lower amphotericin B levels were seen in kidney (6·5 $\mu$g $g^{-1}$) and plasma (1·7 $\mu$g $mL^{-1}$). All levels were lower after a 1 mg $kg^{-1}$ dose. Administration of Fungizone at 1 mg $kg^{-1}$ resulted in higher amphotericin B concentrations in both kidney and plasma than the equivalent dose of ABLC while concentrations in liver, lung and spleen were one-half to one fifth those in ABLC treated animals. Distribution of amphotericin B was calculated after a single 1 mg $kg^{-1}$ dose of ABLC or Fungizone (Fig. 1). Amphotericin B, as a percent of the administered dose, was highest in liver, lung and spleen for ABLC-treated animals with liver containing 48·2% of the dose 1 h after treatment. Following Fungizone treatment a

Table 1. Concentrations of amphotericin B after a single i.v. dose to mice. Samples were taken 1 h after treatment. ND represents not detectable. Tissue concentration in $\mu$g $g^{-1}$, plasma concentration in $\mu$g $mL^{-1}$. Mean (±s.e.m.) of 2 groups of 3 animals.

| | Concn | | |
|---|---|---|---|
| Tissue | ABLC 10 mg $kg^{-1}$ | ABLC 1 mg $kg^{-1}$ | Fungizone 1 mg $kg^{-1}$ |
| Liver | 123·2 ± 1·8 | 14·8 ± 1·3 | 6·8 ± 1·8 |
| Lung | 70·8 ± 5·2 | 9·9 ± 3·8 | 4·2 ± 0·4 |
| Spleen | 141·1 ± 8·2 | 10·0 ± 1·1 | 2·1 ± 2·1 |
| Kidney | 6·5 ± 0·4 | ND | 3·5 ± 0·6 |
| Plasma | 1·7 ± 0·3 | 0·4 ± 0·4 | 1·7 ± 0·4 |

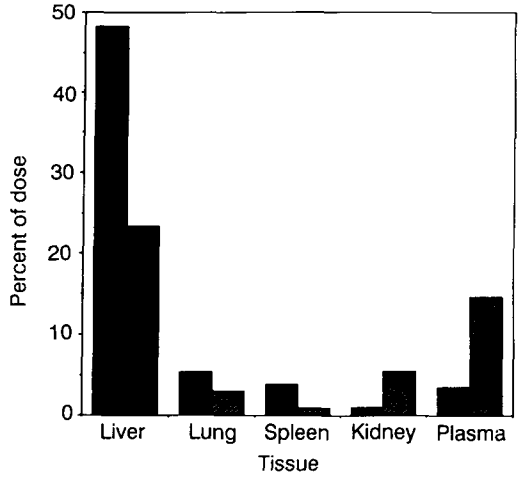


FIG. 1. Distribution of amphotericin B in mouse tissue after i.v. administration of 1 mg $kg^{-1}$ ABLC expressed as a percentage of the total dose. Tissues were taken 1 h after treatment. ■ ABLC; ■ Fungizone.

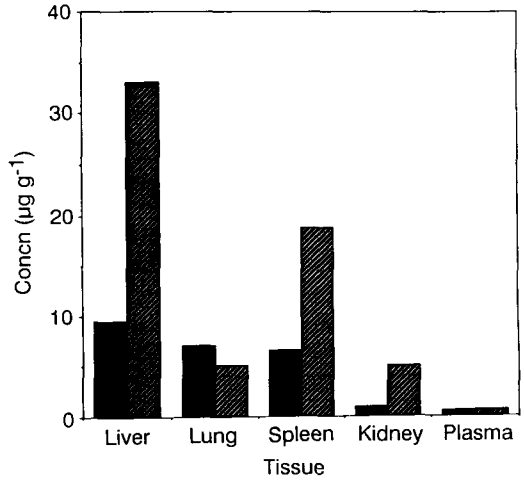


FIG. 2. Concentration of amphotericin B in mouse tissue after 1 and 4 doses of ABLC at 1 mg $kg^{-1}$. Tissues were taken 6 h after treatment. ■ 1 dose; ■ 4 doses.

Table 2. Concentrations of amphotericin B after multiple doses of ABLC to mice. Samples were taken 6 h after the seventh or fourteenth i.v. treatment. Tissue concentration in $\mu g\ g^{-1}$, plasma concentration in $\mu g\ mL^{-1}$. 1 group of 3 animals.

| | Concn | | | |
|---|---|---|---|---|
| | 10 mg $kg^{-1}$ | | 1 mg $kg^{-1}$ | |
| Tissue | 7 days | 14 days | 7 days | 14 days |
| Liver | 377·8 | 175·6 | 57·5 | 41·2 |
| Lung | 43·2 | 34·4 | 8·1 | 7·5 |
| Spleen | 173·2 | 167·1 | 23·8 | 38·7 |
| Kidney | 18·4 | 15·8 | 6·0 | 9·7 |
| Plasma | 1·7 | 2·0 | 1·0 | 0·4 |

higher percent of amphotericin B was recovered in kidney (5·5%) and plasma (14·6%), compared with ABLC.

*Multiple doses of ABLC or Fungizone to mice*

ABLC was given i.v. to mice at 1 mg $kg^{-1}$ once a day for 4 days (Fig. 2). Amphotericin B concentration increased 3- to 5-fold in liver, spleen and kidney after the fourth treatment compared with mice treated once. In the spleen, amphotericin B concentration was 6·6 and 18·8 $\mu g\ g^{-1}$ after 1 and 4 ABLC doses, respectively, whereas in lung and plasma amphotericin B concentrations were similar after 1 and 4 doses. Repetitive dosing with ABLC at 10 and 5 mg $kg^{-1}$ produced low concentrations of amphotericin B in brain tissue of mice. After 1 and 2 doses, amphotericin B was below detectable concentrations but after 3 and 4 daily treatments concentrations ranged from 2 to 7 $\mu g\ g^{-1}$ 6 h after dosing. Amphotericin B was not detectable in brains of animals given 1 mg $kg^{-1}$ ABLC or Fungizone.

Extended treatment schedules with ABLC were used to examine long-term effects on amphotericin B distribution in mice. Tissue concentration after 7 and 14 daily doses of 10 or 1 mg $kg^{-1}$ showed consistent levels over the dosing interval (Table 2). After 7 treatments with 10 mg $kg^{-1}$, amphotericin B concentration was 377·8 $\mu g\ g^{-1}$ in the liver. Plasma levels remained constant over the entire period. A proportional dose response was not seen between groups. Tissue levels were only 2- to 5-fold higher in the 10 mg $kg^{-1}$ compared with the 1 mg $kg^{-1}$ animals. It was interesting to observe that the tissue concentrations did not substantially increase from the 7th to the 14th dose. There were no deaths in any treatment group despite the elevated amphotericin B tissue levels.

After two weeks of treatment with ABLC (10 mg $kg^{-1}$) or Fungizone (1 mg $kg^{-1}$) plasma was analysed for changes in clinical chemistry (Table 3). Transient elevations were evident in SGOT, SGPT and BUN 1 and 6 h after the last dose, and by 24 h these tests had returned to normal values.

*I.v. and i.p. treatment with ABLC*

ABLC was administered i.v. and i.p. to mice at 5 mg $kg^{-1}$ to compare tissue distribution by these two routes. Concentration of amphotericin B was substantially higher in all tissues except plasma after i.v. treatment (Fig. 3). Concentrations in liver, lung and spleen were approximately 3- to 7-fold higher 1 h after i.v. treatment. Liver concentration in i.v. treated animals was 40·7 $\mu g\ g^{-1}$ compared with 5·7 $\mu g\ g^{-1}$ following i.p. dosing. At extended sampling intervals the differences in concentration between the two routes were reduced. The 24 h post-treatment spleen and liver amphotericin B concentrations were 2- to 4-fold higher after i.v. administration. Liver concentration was 60·0 $\mu g\ g^{-1}$ after i.v. treatment compared with 15·4 $\mu g\ g^{-1}$ by the i.p. route. In the spleen, 37·7 $\mu g\ g^{-1}$ was found in i.v. treated animals while i.p. dosing produced 20·8 $\mu g\ g^{-1}$.

Table 3. Effects on selected clinical chemistry values after 14 i.v. doses of ABLC or Fungizone. ABLC was given at 10 mg $kg^{-1}$ and Fungizone at 1 mg $kg^{-1}$, and plasma was taken at 3 time points after the last treatment.

| | | Test[a] | | | |
|---|---|---|---|---|---|
| Treatment | Time (h) | SGOT | SGPT | BUN | Creatinine |
| ABLC | 1 | 114·2 | 58·2 | 42·6 | 0·8 |
| | 6 | 186·0 | 100·2 | 49·4 | 1·0 |
| | 24 | 121·2 | 57·0 | 33·2 | 0·6 |
| Fungizone | 1 | 134·4 | 59·2 | 52·6 | 0·8 |
| | 6 | 126·4 | 57·0 | 57·0 | 0·6 |
| | 24 | 111·1 | 50·2 | 31·2 | 0·6 |
| Control | — | 110·6 | 39·8 | 35·4 | 0·6 |

[a] SGOT, SGPT are IU $L^{-1}$; BUN, creatinine are mg $dL^{-1}$.

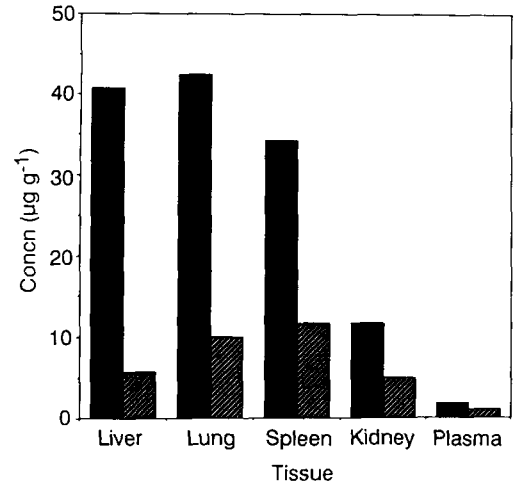


FIG. 3. Concentration of amphotericin B in mouse tissue after i.v. or i.p. treatment with ABLC at 5 mg $kg^{-1}$. Tissues were taken 1 h post-treatment. ■ i.v.; ▩ i.p.

*Single dose of ABLC or Fungizone to rats*

ABLC was administered i.v. at 10 and 1 mg $kg^{-1}$ and

Table 4. Amphotericin B concentration in tissues after a single i.v. dose to rats. Tissues were taken 1 h after treatment. ND represents not detectable. Tissue concentration in $\mu g\ g^{-1}$, plasma concentration in $\mu g\ mL^{-1}$. Mean (±s.e.m.) of 3 animals.

| Tissue | ABLC 10 mg kg$^{-1}$ | Concn ABLC 1 mg kg$^{-1}$ | Fungizone 1 mg kg$^{-1}$ |
|---|---|---|---|
| Liver | 45·9±3·0 | 19·8±3·1 | 12·2±8·7 |
| Lung | 101·7±35·7 | 19·6±7·4 | 6·0±1·1 |
| Spleen | 96·8±40·6 | 11·2±1·1 | 8·7±1·1 |
| Kidney | 6·7±2·3 | 2·0 | 2·0 |
| Plasma | 1·0±0·03 | ND | 0·7±0·8 |

Fungizone was given i.v. at 1 mg kg$^{-1}$ to rats and tissues taken after 1 h for amphotericin B analysis (Table 4). Highest concentrations were again seen in liver, spleen and lung when rats were given ABLC. At equivalent doses, amphotericin B in reticulo-endothelial system tissues was higher in ABLC-treated animals while Fungizone groups produced equivalent or higher levels in kidney and plasma.

## Discussion

ABLC accumulates in the tissues of the reticuloendothelial system as expected for a particulate formulation (Poste 1983); highest concentrations of amphotericin B were found in liver and spleen of both mice and rats.

Concentrations of amphotericin B in plasma remained low following ABLC administration. Even after multiple treatment, ABLC, given at 10 mg kg$^{-1}$ i.v., did not achieve concentrations higher than 2 $\mu g\ mL^{-1}$. Plasma levels of other liposomal amphotericin B preparations show a very different pattern. Amphotericin B, incorporated into small unilamellar vesicles (SUV) and administered to mice at 5 mg kg$^{-1}$, produced peak plasma levels of 5 $\mu g\ mL^{-1}$ within 5 min and remained at 15 $\mu g\ mL^{-1}$ at 24 h (Gondal et al 1989). The vesicles in this preparation were all less than 0·1 $\mu m$ in size compared with ABLC which has a minimum size of 1·6 $\mu m$. Higher levels of amphotericin B were found in serum of patients treated with egg yolk lecithin: cholesterol- and stearylamine-containing liposomes (Sculier et al 1989). The mean liposomal diameter in this preparation was 60 mm for 90–95% of the particles. One patient received eight daily amphotericin B infusions at 4 mg kg$^{-1}$ and showed peak serum levels approaching 60 $\mu g\ mL^{-1}$ by the seventh dose. Smaller amphotericin B-containing vesicles produce higher plasma (serum) concentrations while low plasma levels are apparently characteristic of the larger sized ABLC. This observation was confirmed in data presented by Taylor et al (1982) which described serum levels of amphotericin B after administration of large multilamellar vesicles (MLV)-type liposomes. After a 2 mg kg$^{-1}$ i.v. dose this preparation gave serum levels of 0·13 and 0·14 $\mu g\ mL^{-1}$ at 2 and 24 h, respectively. It appears that plasma levels of amphotericin B are related to the size of the lipid vesicle.

Lipid dispersions made from DMPC and DMPG (7:3) without amphotericin B contained large vesicles in contrast to the compact structures observed with ABLC (Janoff et al 1988). Therefore, it was not possible to administer control liposomes of similar size and composition, and monitor vesicle distribution with and without amphotericin B. The difference in size and structure of ABLC and control vesicles were dramatic enough to prevent direct comparison. This apparently was not the case for all amphotericin B-containing liposomes. Szoka et al (1987) prepared SUV liposomes with and without amphotericin B and monitored tissue distribution using $^{125}I$-labelled lipids. These preparations showed remarkably similar disposition of the radiolabel in tissues whether or not the vesicles contained amphotericin B.

Mice on a two-week i.v. dosing regimen with 10 mg kg$^{-1}$ ABLC suffered no ill effects. Transient changes in SGOT, SGPT and BUN were noted immediately after treatment but returned to normal within 24 h. Similar results were reported by Lopez-Berestein et al (1983) where no gross clinical toxicity was evident in mice after treatment with liposomal amphotericin B during a 42 day observation period. Clinical chemistry studies 21 days after injection showed no abnormalities in BUN, creatinine or SGOT in these animals. Human clinical chemistry data were reported for a SUV formulation of amphotericin B in immunosuppressed patients who had undergone organ or bone marrow transplantations (Tollemar et al 1990). In these individuals the data did not show any toxicity which could not be explained by the underlying disease or post-surgical treatment.

Fungizone is sometimes given i.p. to treat systemic fungal infections in laboratory animals in order to administer sufficient amphotericin B for therapy. Administration of ABLC by this route results in a different distribution pattern where amphotericin B is found in lower concentrations in all tissues except plasma. In view of the altered distribution after i.p. administration and the reduced toxicity of ABLC we recommend that all efficacy studies in laboratory animals employ the i.v. route for therapy.

ABLC has been shown to be well distributed in tissues of laboratory animals. High concentrations were achieved in the tissues of the reticuloendothelial system such as liver, spleen and lung without observable toxic effects. Continued pre-clinical and clinical studies with ABLC are warranted to confirm its therapeutic utility.

## References

Bonner, D. P., Mechlinski, W., Schaffner, C. P. (1972) Polyene macrolide derivatives. III. Biological properties of polyene macrolide ester salts. J. Antibiot. 25: 261–262

Clark, J. M., Whitney, R. R., Olsen, S. J., George, R. J., Swedel, M. R., Kunselman, L., Bonner, D. P. (1991) Amphotericin B lipid complex: therapy of experimental fungal infections in mice. Antimicrob. Agents Chemother. 35: 615–621

Galgiani, J. N., VanWyck, D. B. (1984) Ornithyl amphotericin methyl ester treatment of experimental candidiasis in rats. Ibid. 26: 108–109

Gallis, H. A., Drew, R. H., Pickard, W. W. (1990) Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. 12: 308–329

Gondal, J. A., Swartz, R. P., Rahman, A. (1989) Therapeutic evaluation of free and liposome-encapsulated amphotericin B in the treatment of systemic candidiasis in mice. Antimicrob. Agents Chemother. 33: 1544–1548

Janoff, A. S., Boni, L. T., Popescu, M. C., Minchey, S. R., Cullis, P. R., Madden, T. D., Taraschi, T., Gruner, S. M., Shyamsunder, E., Tate, M. W., Mendelsohn, R., Bonner, D. (1988) Unusual lipid structures selectively reduce the toxicity of amphotericin B. Proc. Natl. Acad. Sci. USA 85: 6122–6126

Lopez-Berestein, G., Mehta, R., Hopfer, R. L., Mills, K., Kasi, L., Mehta, K., Fainstein, V., Luna, M., Hersch, E. M., Juliano, R. L.

(1983) Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposome-encapsulated amphotericin. Br. J. Infect. Dis. 147: 939–945

Lopez-Berestein, G., Hopfer, R. L., Mehta, R., Mehta, K., Hersh, E. M., Juliano, R. L. (1984) Liposome-encapsulated amphotericin B for treatment of disseminated candidiasis in neutropenic mice. J. Infect. Dis. 150: 278–283

Lopez-Berestein, G., Bodey, G. P., Fainstein, V., Keating, M., Frankel, L. S., Zeluff, B., Gentry, L., Mehta, K. (1989) Treatment of systemic fungal infections with liposomal amphotericin B. Arch. Intern. Med. 149: 2533–2536

McKinsey, D. S., Gupta, M. R., Riddler, S. A., Driks, M. R., Smith, D. L., Kurtin, P. J. (1989) Long-term amphotericin B therapy for disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome (AIDS). Ann. Intern. Med. 111: 655–659

Poste, G. (1983) Liposome targeting in vivo: problems and opportunities. Biol. Cell 47: 19–37

Sculier, J. P., Delcroix, C., Brassinne, C., Laduron, C., Hollaert, C., Coune, A. (1989) Pharmacokinetics of amphotericine B in patients receiving repeated intravenous high doses of amphotericin B entrapped in sonicated liposomes. J. Liposome Res. 1: 151–166

Szoka, F. C., Milholland, D., Barza, M. (1987) Effect of lipid composition and liposome size on toxicity and in vitro fungicidal activity of liposome-intercalated amphotericin B. Antimicrob. Agents Chemother. 31: 421–429

Taylor, R. L., Williams, D. M., Craven, P. C., Graybill, J. R., Drutz, D. J., Magee, W. E. (1982) Amphotericin B in liposomes: a novel therapy for histoplasmosis. Am. Rev. Respir. Dis. 125: 610–611

Tollemar, J., Ringden, O., Tyden, G. (1990) Liposomal amphotericin B (AmBisome) treatment in solid organ and bone marrow transplant recipients. Efficacy and safety evaluation. Clin. Transplantation 4: 167–175

Wiebe, V. J., DeGregorio, M. W. (1988) Liposome-encapsulated amphotericin B: a promising new treatment for disseminated fungal infections. Rev. Infect. Dis. 10: 1097–1101