Lipid Formulations of Amphotericin B: Recent Progress and Future Directions

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Three lipid formulations of amphotericin B are now either marketed for clinical use or undergoing further study before they can be approved in various countries worldwide. Amphotericin B lipid complex (ABLC; Abelcet, Liposome Company, Princeton, NJ) is a concentration of ribbonlike structures of a bilayered membrane formed by combining a 7:3 molar ratio of dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol with amphotericin B. Amphotericin B colloidal dispersion (ABCD; Amphocil, Sequus Pharmaceuticals, Menlo Park, CA) is composed of disklike structures of cholesterol sulfate complexed with amphotericin B. AmBisome (Nexstar, San Dimas, CA), the only true liposomal amphotericin B, consists of small unilamellar vesicles made up of a bilayer membrane of hydrogenated soy phosphatidylcholine and distearoylphosphatidylglycerol stabilized with cholesterol in a 2:0.8:1 ratio combined with amphotericin B. All of the preparations appear to be preferentially accumulated in organs of the reticuloendothelial system, as opposed to the kidney. In vivo animal models as well as current clinical experience suggest that use of these formulations results in overall improvement in the therapeutic index. Patients with life-threatening mycosis for whom therapy has failed or who are intolerant to therapy with amphotericin B deoxycholate have been successfully treated with these formulations. However, further study is warranted to help clarify the usefulness of each of the lipid formulations as first-line therapy for documented or suspected invasive fungal infections.

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Invasive fungal infections have been increasingly recognized as a major cause of morbidity and mortality in the immunocompromised host [1, 2]. Patients infected with HIV, recipients of solid organ transplants, and those who are receiving therapy for underlying malignancy are particularly susceptible to these infections [3–7]. The use of high-dose ablative therapy and either bone-marrow or peripheral-blood stem cell transplantation for a variety of malignant and nonmalignant disorders has also contributed to the increasing incidence of invasive fungal infections [8–10].

_Candida albicans_ and _Aspergillus_ species have predominated as the most common fungal pathogens in patients with profound and prolonged neutropenia and in those who have undergone allogeneic bone marrow transplantation [7–12]. Cryptococcal meningitis was recognized as one of the first AIDS-defining illnesses and remains the most common invasive fungal infection in this patient population [3]. However, along with the increasing frequency of fungal infections over the last decade, there has also been an increase in the number of pathogens recognized as causing invasive disease in the immunocompromised host. Non-albicans _Candida_ species, _Torulopsis glabrata_, _Fusarium_ species, and _Trichosporon beigelii_ lead the growing list of newer fungal pathogens [13–14].

Amphotericin B has remained the drug of choice for life-threatening invasive fungal infections over the last 30 years, primarily due to its broad spectrum of activity [15–19]. The usefulness of this polyene has been limited by its narrow therapeutic index. Amphotericin B therapy is associated with a number of adverse events. Although infusion-related side effects such as fever, rigors, and chills are common in patients who receive therapy with amphotericin B, medical management of these symptoms usually suffices to allow continuation of the drug. However, renal insufficiency in association with azotemia, renal tubular acidosis, and impaired urinary concentrating ability resulting in electrolyte imbalance may be severe enough to lead to dose reduction or premature discontinuation of the drug. Moreover, even when full-dose amphotericin B is continued regardless of the development of severe renal insufficiency, therapy for immunocompromised patients with invasive fungal infections often fails. The mortality rates for patients with invasive aspergillosis remain high, and most neutropenic patients die of this infection despite prolonged full-dose therapy with amphotericin B [18, 19].

**Liposomes**

Alec D. Bangham, while investigating the properties of cell membranes at the Institute of Animal Physiology in Cambridge,
England, was the first to demonstrate that, at high concentrations, phospholipids dispersed in water spontaneously form microscopic closed vesicles. These vesicles consisted of water surrounded by bilayered phospholipid membranes. Bangham called these tiny fat bubbles "smectic mesophases." They were later named "liposomes" by his colleague Gerald Weissman [20, 21]. Liposomes are composed of biodegradable phospholipid molecules that are made up of a hydrophilic head attached to a hydrophobic tail. When placed in water, the molecules spontaneously arrange themselves into bilayered membranes, which coalesce to form vesicles. The hydrophilic heads face outward, shielding the hydrophobic tails. Spherical vesicles form to protect the hydrophobic ends (figure 1). Since Bangham's description of liposomes over 30 years ago, numerous methods of creating these molecules have been outlined in several publications. Biochemists, biophysicists, and physiologists have been able to alter the size, charge, permeability, and even number of bilayered membranes in a liposome. Physiologists have used liposomes in the study of cell membrane interactions.

Numerous studies over the last two decades have investigated the practical use of liposomes. Water-soluble substances such as drugs, enzymes, and even genes have been entrapped in the aqueous phase of the bilayer. Fat-soluble substances such as polyene antibiotics can be incorporated into the lipid bilayer.

The pharmacokinetics and pharmacodynamics of drugs incorporated into liposomes may differ greatly from those of the free counterpart. This new drug-delivery system may help to better target certain pharmaceuticals, thus improving their therapeutic index [21]. Studies suggest that drugs incorporated into liposomes or lipid formulations are selectively taken up into the reticuloendothelial system and concentrated in the liver, spleen, lungs, lymph nodes, and, to a lesser extent, bone marrow. Lipid-rich particles are also believed to be ingested by phagocytic cell monocytes, which may help to target sites of infection or inflammation [20, 21].

**Liposomal Amphotericin B**

Amphotericin B, which is highly lipophilic, was first incorporated into liposomes over 10 years ago in an attempt to increase its therapeutic index. In 1981, New et al. reported reduced amphotericin B–related toxicity associated with the use of liposomal-associated drug in an animal model of leishmaniasis [22]. The following year, Graybill et al. and Taylor et al. reported an improved therapeutic index for liposomal-associated amphotericin B in animal models of cryptococcosis and histoplasmosis [23, 24]. These articles suggested that lipid formulations of amphotericin B could significantly reduce the toxicities associated with free drug without compromising efficacy.

Lopez-Berestein et al. extended the in vivo studies with a novel form of liposomal amphotericin B, which they compared to conventional amphotericin B in the treatment of disseminated candidiasis in neutropenic mice. They observed a significant reduction of toxicity with liposomal amphotericin B without loss of efficacy [25] (figure 2). Liposomes created by Lopez-Berestein and Juliano were multilamellar vesicles consisting of a mixture of the two phospholipids dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylylglycerol (DMPG) in a 7:3 molar ratio containing 5%-10% mole ratio of amphotericin to lipid. Preliminary compassionate-use experience with this product in severely immunocompromised cancer patients with progressive fungal infections at the M. D. Anderson Hospital and Tumor Institute (Houston) suggested that invasive disease could be controlled even in patients for whom conventional amphotericin B therapy had previously failed. Moreover, the liposomal product had significantly less nephrotoxicity than conventional amphotericin B even at much higher doses [26, 27].

This experience led to further development of lipid formulations of amphotericin B by the pharmaceutical industry over the last decade. There are three products that currently are undergoing clinical trials in the United States. All three have been licensed for clinical use and are currently available commercially in a number of countries in Western Europe. One lipid formulation of amphotericin B, amphotericin B lipid complex (ABLC; Abelcet Liposome Company, Princeton, NJ) has recently been approved by the U.S. Food and Drug Administra-

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**Figure 1.** Diagram of fat and water-soluble agents encapsulated in a liposome. Reproduced with permission from Hospital Practice (1992;30:53). © 1992, the McGraw-Hill Companies.
tion for use in patients with invasive aspergillosis who are refractory to or intolerant of treatment with conventional amphotericin B.

**Amphotericin B Lipid Complex**

In attempting to explain why the product developed by Berstein and Juliano could be significantly less toxic than conventional amphotericin B while maintaining its efficacy, Janoff and colleagues began a series of experiments to study this form of lipid-associated amphotericin B in their laboratory located in Princeton, New Jersey. They found that the original formulation of lipid-associated amphotericin B contained a variety of liposomal and nonliposomal structures of lipid bilayers. It appeared that the nonliposomal structures in the formation that they called "ribbons" were responsible for the reduction in toxicity [28, 29]. They subsequently performed a series of experiments combining the lipid formulation of DMPC and DMPG in a 7:3 molar ratio with varying concentrations of amphotericin B.

Freeze-etch electron microscopy of formulations made with 0, 5, 25, and 50 mol% of amphotericin B showed significant differences in the structures formed. The formulation made up of 5% mole concentration of amphotericin B, which was similar to the Berstein/Juliano product, consisted of lipid vesicles smaller than the large vesicles that formed in the absence of amphotericin B. It also contained the ribbonlike structures seen in the original product. When the concentration of amphotericin B was increased to 25 mol%, "liposomal" vesicles essentially disappeared and were replaced by tightly packed ribbon structures. At concentrations of >50 mol% amphotericin B, the formulation continued to appear as ribbon structures; however, the amphotericin B and lipid were no longer closely complexed (figures 3 and 4). The ribbon structures formed by complexing DMPC and DMPG with amphotericin B were called "amphotericin B lipid complex" or "ABLC."

In vitro studies suggested that the hemolytic activity of this lipid formulation at low concentrations (<3 mol% of amphotericin B to lipid) was similar to that of conventional amphotericin B, but as the mole ratio of amphotericin B increased and the ribbon structures began to form, there was a marked reduction of overall toxicity. As amphotericin B is increasingly complexed with the lipid, the amount of free amphotericin B in solution is reduced, presumably accounting for the decreased toxicity. The efficacy of ABLC has also been attributed to the ability of lipases from either fungi or inflammatory cells at the site of infection to release the complexed amphotericin B from the lipid formulation. However, the improvement in the therapeutic index may also be related to binding to high density lipoproteins as well as to selective interaction with fungal cell membranes [30].

**Animal models.** ABLC has been compared with conventional amphotericin B in a number of animal models including mice, rats, rabbits, and dogs. Concentrations of amphotericin B in the liver, spleen, and lungs of mice and rats appear to be much higher after a single dose of ABLC than after a single dose of amphotericin B deoxycholate. The level of amphotericin B found in the kidney tissue of mice appeared similar to the level of amphotericin B after injection of ABLC at the 1 mg/kg dose. However, plasma levels of amphotericin B were consistently lower after injection of the lipid complex ABLC than were levels obtained after injection of amphotericin B deoxycholate. When increasing the dose of the lipid complex, the levels of drug in the liver, spleen, and lung tissue rise dramatically; however, this rise is associated with little change in the levels of drug in the kidney and essentially no rise in plasma levels [29, 31, 32]. Studies in mice showed that after a single iv dose the LD₅₀ for the lipid complex of amphotericin B was 40 mg/kg whereas that for conventional amphotericin B was 3 mg/kg. Multiple-dose studies of ABLC continued to show a significant reduction in toxicity even at 10 times the standard.
Figure 3. Freeze-etch micrographs of various DMPC:DMPG/amphotericin B dispersions quenched from 20°C. All systems contained DMPC:DMPG in a molar ratio of 7:3 and an amphotericin B molar percentage (with respect to the bulk lipid) of 0, (a), 5% (b), 25% (c), and 50% (d) (original magnification, ×25,000). Reprinted with permission from [29].

dose of conventional amphotericin B in mice [31] and four times the standard dose in rabbits [33].

The efficacy of ABLC has also been evaluated in a number of animal models of fungal infection and was found to be comparable to that of conventional amphotericin B. Moreover, in a number of cases the lipid formulation was effective when conventional amphotericin B was ineffective at controlling the fungal infection at the maximum tolerated dose [31, 34, 35]. ABLC (10 mg/kg) also demonstrated rapid fungicidal activity in the treatment of experimental cryptococcal meningitis [36]. The ability to deliver much higher doses of amphotericin B in the form of a lipid complex without reaching the maximum tolerated dose was thought to account for the improved therapeutic index [29].

**Human studies.** The pharmacokinetics of ABLC in humans resembles those in animals. Circulating blood levels of amphotericin B were much lower in male volunteers after single-dose infusion of ABLC than after infusion of conventional amphotericin B deoxycholate [37]. The lipid complex is believed to be rapidly taken up by the reticuloendothelial system and concentrated in the liver, spleen, lungs, and other tissues of the body. Tissue levels of amphotericin B were measured at autopsy of a heart transplant patient after 3 days of treatment with ABLC. Relatively high concentrations of amphotericin B were detected in the spleen (290 μg/g), liver (196 μg/g), and lungs (222 μg/g). Lower concentrations were detected in the kidney (6.9 μg/g), lymph nodes (7.6 μg/g), brain (1.6 μg/g), and heart (4.9 μg/g) (J. A. Boyle, unpublished data).

ABLC has been compared with conventional amphotericin B in a number of small phase II and phase III clinical trials of patients with leishmaniasis, coccidioidomycosis, and cryptococcosis. A dose-escalation trial of ABLC vs. conventional
amphotericin B in patients with leishmaniasis showed that the lipid formulation was effective and that it was less nephrotoxic than the standard drug [38]. Preliminary results of two other trials of patients with coccidiodomycosis or cryptococcosis suggest that the lipid complex form of amphotericin B was also effective but that it was less nephrotoxic than conventional amphotericin B [39, 40]. A randomized controlled trial comparing ABLC with conventional amphotericin B in the treatment of candidiasis has been completed and found no significant difference in efficacy between the two treatments. This study randomized 231 patients to receive ABLC (5 mg/[kg · d]) or amphotericin B (0.6–1 mg/[kg · d]) in a 2:1 randomization for the treatment of hematogenous and invasive candidiasis. Response rates (63% for ABLC vs. 68% for amphotericin B) were not statistically different ($P > .5$). Nephrotoxicity was significantly lower in the group of patients who were treated with ABLC ($P = .007$). The frequency of other adverse reactions was similar for both drugs [41].

Approximately 1,000 patients have been treated with ABLC according to an open-label emergency-use protocol. To be eligible to receive this drug, previous treatment with amphotericin B (total dose at least 15 mg/kg or 1,000 mg) or other antifungals, including fluconazole and itraconazole, had to have failed to control documented or presumed invasive fungal infection. The patients could also receive the drug if they were considered to be intolerant of or unable to receive amphotericin B because of renal insufficiency.

The initial patient population of 228 cases was analyzed in detail and included failures of amphotericin B (doses of $\geq$1,000 mg over $\geq$10 days; $n = 116$); failures of other antifungal agents, including fluconazole and itraconazole ($n = 18$); amphotericin B–induced nephrotoxicity (serum creatinine level, $\geq$2.5 mg/dL in adults or $\geq$1.5 mg/dL in children; $n = 73$); and preexisting renal dysfunction (serum creatinine level, $\geq$3.0 mg/dL; $n = 8$).

ABLC was administered for a median duration of 25 days at a mean maximum dose of 5 mg/[kg · d]. Clinical response rates, as assessed by the patient’s primary physician, were 78% (52/67) for candidiasis and 60% (43/72) for aspergillosis. Use of ABLC was associated with a decrease in the serum creatinine level during therapy. The mean serum creatinine level declined significantly ($P = .0001$) from baseline in the entire population and in the subgroup whose initial serum creatinine level was $>2.5$ mg/dL ($n = 62$). ABLC was effective in patients with invasive mycoses who were unresponsive to or intolerant of previous antifungal therapy and was infrequently associated with dose-limiting nephrotoxicity [42].

Therapy with amphotericin B deoxycholate is frequently started empirically long before invasive aspergillosis is documented, making it difficult to compare the lipid formulation to its parent drug in a prospective randomized trial. Data from 151 patients with definite or probable invasive aspergillosis who had been treated with ABLC according to emergency-use protocols were therefore compared to data from medical charts of 122 control patients with aspergillosis who had been treated with conventional amphotericin B. Patients treated with ABLC showed higher overall response rates (40% vs. 23%; $P = .002$) than patients who were treated with amphotericin B. Moreover, patients whose serum creatinine level was $\geq$2.5 mg/dL at the initiation of treatment with ABLC had a significant decrease in their serum creatinine levels between weeks 2 and 5 ($P < .004$) despite continued therapy with 5 mg/[kg · d] of this drug. Although trials with historical controls have major limitations, ABLC appeared to be less nephrotoxic and at least as effective for treating invasive aspergillosis, even in cases where therapy with at least 500 mg of amphotericin B deoxycholate had failed to control infection [43].

**Figure 4.** The putative structure of amphotericin B lipid complex (ABLC). Amphotericin B and lipid are arranged in a 1:1 interdigitated complex. Reprinted with permission from [29].

**Figure 5.** Scanning electron micrograph of amphotericin B colloidal dispersion (ABCD, Amphocil). Reprinted with permission from M. Gurwith (Sequs Pharmaceuticals, Inc., Menlo Park, California).
Amphotericin B Colloidal Dispersion

Amphotericin B colloidal dispersion (ABCD; Amphocil, Sequus Pharmaceuticals, Menlo Park, CA) is a complex of amphotericin B and cholesteryl sulfate in a 1:1 ratio. This lipid formulation of amphotericin B forms disklike structures ~115 nm in diameter when combined with the cholesteryl sulfate, leaving no free drug (figures 5 and 6) [44]. Hanson and Stevens documented in vitro activity of ABCD against 41 isolates of 15 pathogenic species of fungi [45]. MICs and mean fungicidal concentrations of ABCD appeared similar to those of the deoxycholate suspension of amphotericin B. There were, however, some differences: the lipid formulation of amphotericin B was more active against some species of fungi, and the deoxycholate suspension was more active against other species.

Animal models. The pharmacokinetics of ABCD have been extensively studied in animal models. Plasma levels of amphotericin B after single iv bolus injection in the rat are significantly less at 1 hour when ABCD is compared with amphotericin B deoxycholate. The half-life of the lipid formulation, however, is much longer and its volume of distribution much greater. Concentrations of amphotericin B in liver tissue are 2–3 times higher after injection of ABCD than after injection of amphotericin B deoxycholate. At the same time, concentrations of amphotericin B in the kidney are significantly reduced even after 5 mg/kg of the colloidal dispersion is injected [46]. Similar findings of lower plasma concentrations, increased liver deposition, and decreased kidney concentration of amphotericin B have been reported in the dog model [47]. Studies comparing ABCD with amphotericin B deoxycholate in mice, rats, and dogs have shown that this lipid formulation is significantly less toxic than equivalent doses of amphotericin B deoxycholate [48]. Up to 5 mg/(kg·d) of ABCD could be given to dogs before this drug produced side effects that were similar to those produced by 0.6 mg/(kg·d) of amphotericin B deoxycholate [47].

Animal models of systemic coccidioidomycosis, cryptococcosis, and aspergillosis have been used to study the relative efficacy of ABCD compared with amphotericin B deoxycholate [49–53]. Although the doses of amphotericin B deoxycholate (1.3 mg/kg) required to clear the organs of fungi in a mouse model of coccidioidomycosis were lower than those required for ABCD (5 mg/kg), the lipid formulation had a significantly better therapeutic index, as amphotericin B deoxycholate was found to be 5–8 times more toxic than ABCD [49]. Unlike the murine model of coccidioidomycosis, a murine model of cryptococcosis showed that ABCD had equal efficacy when it was compared with amphotericin B on a mg/kg basis. ABCD was again found to be less toxic, suggesting an improvement in the therapeutic index for the lipid formulation [50].

Rabbit models of invasive aspergillosis suggest that amphotericin B deoxycholate is superior to ABCD in tissue clearance of fungi at equal doses of 1 mg/kg [51, 52]. However, increased duration of survival was observed in persistently granulocytopenic rabbits with pulmonary aspergillosis when 5 mg/(kg·d) of ABCD were compared with 1 mg/(kg·d) of amphotericin B deoxycholate [52]. The investigators attributed the improved therapeutic index to an enhanced rate of tissue clearance, decreased pulmonary injury, and reduced nephrotoxicity in the rabbits treated with 5 mg/(kg·d) of ABCD. These findings were further confirmed in a study of the evolution of pulmonary infiltrates in experimental pulmonary aspergillosis. With use of ultrafast CT scanning and an image-analysis algorithm, a dose-dependent clearance of pulmonary infiltrates in rabbits treated with ABCD was documented [53]. Higher doses of ABCD (5 and 10 mg/[kg·d]) exerted a rapid clearing effect on the development of pulmonary lesions in comparison with the response rates of amphotericin B at 1 mg/[kg·d].

Human studies. Hostetler et al. studied relatively low doses of ABCD (up to 1 mg/[kg·d] or 2 mg/kg three times weekly up to 10 weeks) for a group of patients with coccidioidomycosis whose therapy had failed or who were intolerant to prior antifungal therapy. Although fever and rigors were common (75% and 83% of patients, respectively), adverse events rarely limited therapy. Most patients (71%) had at least a minor response to ABCD therapy, and a few (13%) had a major response with use of the clinical scoring system adapted for coccidioidomycosis [54]. A preliminary report by Bowden and Cays of a phase 1 clinical trial of ABCD suggested that doses of up to 7 mg/(kg·d) may be well tolerated and effective in the setting of opportunistic fungal infection [55].

The results of an open-labeled compassionate-use trial of 168 patients with documented or presumed invasive mycosis who were treated with ABCD were recently reported. Patients were eligible for enrollment in the study if their fungal infection had failed to adequately respond to a minimum of 7 days of treatment with conventional amphotericin B deoxycholate or if they had renal insufficiency that was preexisting or secondary to acute drug-related nephrotoxicity (usually due to therapy with conventional amphotericin B deoxycholate).
The initial dose of ABCD was variable (0.5, 1, 2, 3, or 4 mg/(kg·d)) and was dependent on the individual investigator’s judgement. A daily dose of 3 mg/(kg·d) was recommended for candidiasis and other yeastlike fungi, whereas 4 mg/(kg·d) was recommended for infections due to *Aspergillus* species or other filamentous fungi. The dose of ABCD was increased to as high as 6 mg/(kg·d) in some patients. ABCD was administered for a median duration of 12.5 days, with a mean cumulative dosage of 3,962 mg.

In 48 (49%) of 97 cases in which the patients were clinically evaluable, the fungal infection was believed to have partially cleared or resolved after treatment with ABCD. Nineteen (58%) of 33 patients with candidiasis and 11 (34%) of 32 with aspergillosis responded to therapy with ABCD. All 168 patients in the trial were included in the safety analysis. Despite a mean cumulative dose of 4 g of ABCD and daily doses as high as 6 mg/kg, the mean change in the serum creatinine level from baseline until completion of therapy with ABCD was −0.02 mg/dL. This preliminary report suggests that the colloidal dispersion of amphotericin B, like the lipid complex, is active and less nephrotoxic than the parent drug [56].

**AmBisome**

AmBisome (Nexstar, San Dimas, CA) is the only true “liposomal” preparation of the three lipid formulations of amphotericin B undergoing clinical trials. It differs from ABLC and ABCD in that it is made up of small unilamellar lipid vesicles that are uniform and spherical in size and that average 60–70 nm. The lipid bilayer is made up of hydrogenated soy phosphatidylcholine and distearoylphosphatidylglycerol stabilized by cholesterol and combined with lipophilic amphotericin B in a 2:0.8:1:0.4 molar ratio (figure 7) [57]. The in vitro antifungal activity of AmBisome was found to be comparable to that of amphotericin B when a number of clinical isolates from a large cancer center were tested [58].

A recent report, however, suggested that the small unilamellar liposomal form of amphotericin B may be as much as 4–8 times less active than the deoxycholate form against several isolates of *C. albicans* [59]. It is unclear what the significance of in vitro susceptibility testing is for lipid formulations of amphotericin B. Unlike in vitro antibacterial drug susceptibility testing, in vitro antifungal drug susceptibility tests have been hard to duplicate from laboratory to laboratory. Moreover, lipid formulations of amphotericin B were specifically designed to complex or bind the antifungal for targeted drug delivery. There may be a need for the drug to be taken up by the reticuloendothelial system or to be delivered to points of infection to realize a therapeutic advantage.

**Animal models.** The pharmacokinetics, toxicity, and efficacy of AmBisome have been studied in the mouse, rat, and rabbit [60, 61]. Peak plasma levels that can be obtained with the liposomal form of amphotericin B were similar in mice, rats, and rabbits. Liposomal amphotericin B is similar to the other lipid formulations of amphotericin B as it appears to be preferentially concentrated in the liver and spleen of animals; however, the rate of uptake by the reticuloendothelial system appears to be much slower than that by ABLC or ABCD. It is hypothesized that the larger lipid complexes and dispersions may be more readily phagocytized by the macrophages of the reticuloendothelial system than the smaller unilamellar vesicles. The negative charge on the AmBisome particle may also delay uptake by the reticuloendothelial system. These mechanisms may account for the fact that the liposomal form of amphotericin B has much higher peak plasma levels and a prolonged circulation time compared with its larger counterparts.

AmBisome has been found to be less nephrotoxic than conventional amphotericin B in the mouse, rat, and rabbit models. There does, however, appear to be a slight rise in the levels of liver transaminases with repeated infusions of liposomal amphotericin B. The LD₁₀ after a single injection of liposomal amphotericin B was >175 mg/kg in mice and >50 mg/kg in rats. This was 30–60 times greater than the LD₁₀ after a single injection of conventional amphotericin B [60].

The efficacy of AmBisome has been studied in murine models of systemic candidiasis, cryptococcosis, and blastomycosis. Gondal et al. showed that mice infected with *C. albicans* who received any of the doses of liposomal amphotericin B studied
Table 1. Pharmacokinetics of lipid formulations of amphotericin B.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Brand name</th>
<th>Lipid configuration</th>
<th>Size (nm)</th>
<th>Lipids</th>
<th>Mean C_{max} in μg/mL (dose)</th>
</tr>
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<tbody>
<tr>
<td>Amphotericin B</td>
<td>Fungizone (Bristol-Myers Squibb, Princeton, NJ)</td>
<td>NA</td>
<td>NA</td>
<td>Deoxycholate</td>
<td>0.98 (0.25 mg/kg)</td>
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<td>2.9 (0.68 mg/kg)</td>
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<tr>
<td>L-AMB</td>
<td>AmBisome</td>
<td>Small unilamellar vesicle (liposome)</td>
<td>80</td>
<td>Hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol</td>
<td>7.3 (1.0 mg/kg)</td>
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<td>17.2 (2.5 mg/kg)</td>
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<td>57.6 (5.0 mg/kg)</td>
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<tr>
<td>ABCD</td>
<td>Amphocil</td>
<td>Disklike</td>
<td>120–140</td>
<td>Cholesteryl sulfate</td>
<td>Increased</td>
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<td>0.84 (0.5 mg/kg)</td>
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<td>2.19 (1.0 mg/kg)</td>
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<td>2.53 (1.5 mg/kg)</td>
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<tr>
<td>ABLC</td>
<td>Abelcet</td>
<td>Ribbonlike</td>
<td>1,600–11,000</td>
<td>Dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol</td>
<td>Decreased</td>
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<td>0.27 (0.5 mg/kg)</td>
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<td>1.1 (2.5 mg/kg)</td>
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NOTE. "Decreased," "increased," and "similar" are in reference to values for amphotericin B. ABCD = amphotericin B colloidal dispersion; ABLC = amphotericin B lipid complex; AUC = area under the curve; C = pharmacokinetics in children; C_{max} = maximum drug concentration; Cl = clearance; L-AMB = liposomal amphotericin B; NA = not applicable; Vd_{ss} = volume of distribution.

* Model dependent.

(except for 1 mg/kg) lived longer as long as they were treated early [62]. If treatment was delayed until 3 days after inoculation, the drug dose to achieve optimal benefit appeared to be 5 mg/kg. The maximum dose of conventional amphotericin B given to mice by single injection was 2 mg/kg, and 1.5 mg/(kg·d) was the maximum tolerated dose with multiple injections. Although Pahls and Schaffner recently found that AmBisome was significantly less toxic than conventional amphotericin B in their mouse model of candidiasis, they suggested it was 4–8 times less active in clearing the infection [59]. The dosing regimen in this model, however, may not have been optimal to assess maximum antifungal effect.

Francis et al. reported the results of a comparison of amphotericin B deoxycholate with the small unilamellar lipidic form of amphotericin B in persistently neutropenic rabbits with pulmonary aspergillosis [63]. Rabbits were evaluated for duration of survival, lung tissue infection, and hemorrhagic pulmonary lesions by rapid CT scanning. Treatment with the lipidic form of amphotericin B was studied at 1, 5, and 10 mg/kg, whereas therapy with amphotericin B deoxycholate was studied at 1 mg/kg. Although rabbits who received any dose of AmBisome lived longer than those who received amphotericin B, the rate of reduction of pulmonary hemorrhage was greatest at a dose of 5 mg/(kg·d) while the dose of 10 mg/(kg·d) was more nephrotoxic. Thus, 5 mg/(kg·d) was proposed as an optimal dose of AmBisome.

**Human studies.** Peak and trough plasma levels of amphotericin B were measured in 49 patients who received doses of AmBisome of 2, 3, or 4 mg/kg [64]. Mean peak concentrations of up to 46 μg/mL with a corresponding mean trough level of 4 μg/mL were detected in the seven patients who received 4 mg/kg of the liposomal form of amphotericin B. Tissue concentrations of amphotericin B were determined at autopsy in three patients who had received therapy with AmBisome [65]. The highest concentrations of drug were found in the liver and spleen.

The pharmacokinetics of AmBisome in humans appear quite similar to those determined in the animal model. Although the peak plasma level and circulation time are greater for the small unilamellar lipidic form of amphotericin B than for the lipid complex and colloidal dispersion forms, the lipidic form is preferentially concentrated in the liver and spleen. The toxicity profile of AmBisome also appears to parallel that of the other lipid formulations of amphotericin B. Increases in serum creatinine level have occurred with use of liposomal amphotericin B; however, liposomal amphotericin B also appears to be less nephrotoxic than the deoxycholate form of the drug. Renal function improved in a number of patients when their regimen was changed from conventional amphotericin B to AmBisome [66].

The serum pharmacokinetics of liposomal amphotericin B were recently described in a prospective study of 24 persistently febrile neutropenic patients who received this small unilamellar vesicle formulation of amphotericin B as empirical antifungal therapy. Liposomal amphotericin B administered as a 1-hour infusion dose to this population was well tolerated and seldom associated with infusion-related toxicity. Liposomal amphotericin B in serum samples was measured as amphotericin B by high-pressure liquid chromatography. Using model-independent methods, the investigators found that the mean (±SD) area under the serum concentration vs. time curve (AUC_{0→∞}), which was determined on the last day of infusion for 3 dose cohorts (1, 2.5, and 5 mg/[kg·d]), reflected a nonlinear dose
relationship that was consistent with reticuloendothelial uptake and redistribution [67] (table 1).

Phase II experience with the use of AmBisome in immunocompromised hosts with documented or presumed invasive fungal infections has been reported from a number of centers in Europe [65, 72, 73]. Patients were eligible to receive liposomal amphotericin B if they failed to respond to amphotericin B deoxycholate or were intolerant to this therapy. They could also receive the lipid formulation if they had significant underlying renal insufficiency before antifungal therapy was started. The most common reasons for immunodeficiency in these hosts were underlying malignancy, solid organ or bone marrow transplantation, and AIDS. Documented invasive fungal infections were most commonly due to Candida or Aspergillus species. Clinical responses were seen in a number of patients with documented invasive fungal infections even after therapy with amphotericin B deoxycholate had failed. Patients who were neutropenic at the time of therapy appeared to respond, although recovery of neutrophil counts, remission from underlying malignancy, and continued therapy with the liposomal form of amphotericin B appeared to be necessary for resolution of the fungal infection.

A phase II/III trial of AmBisome as prophylaxis against invasive fungal infections in bone marrow transplant patients was conducted in Sweden. Liposomal amphotericin B at 1 mg/(kg·d) was compared to placebo in a double-blind, randomized study. There were fewer fungal infections in allogeneic transplant patients who received liposomal amphotericin B than in those who received placebo, and no fungal infections were documented in autologous transplant patients in either arm of the study. Although the study drug was well tolerated, the overall duration of survival for patients who were treated with placebo was similar to that for patients who were treated prophylactically with liposomal amphotericin B [74]. A number of reports of the compassionate use of AmBisome in immunocompromised children with invasive fungal infections have suggested that this liposomal form of amphotericin B is safe and effective when used in pediatric patients [75–77]. Further studies designed to better define the safety, tolerance, and efficacy of AmBisome are currently under way in the United States.

### Lipid Emulsion Plus Amphotericin B Deoxycholate

Anecdotal reports of the use of amphotericin B mixed with fat emulsions used for parenteral nutrition are increasing in both Europe and the United States. Kirsh et al. reported reduced toxicity without loss of antifungal activity when they treated systemic murine candidiasis with a lipid-emulsion formulation of amphotericin B [78]. Clinical experience with this approach in neutropenic hosts and HIV-infected patients with candidiasis has recently been published [79, 80]. French investigators have reported the results of a prospective randomized trial comparing the use of standard amphotericin B mixed in 5% dextrose vs. amphotericin B mixed with 20% intralipid in a group of patients requiring empirical treatment for persistent fever and neutropenia. Patients treated with the amphotericin B/fat emulsion mixture appeared to have a significant reduction in infusion-related toxicity and renal dysfunction. However, the study was too small to show any difference in antifungal efficacy [81].

Unfortunately, amphotericin B does not appear to mix well with the fat emulsion. When analyzing the combination of 0.6 mg/mL of amphotericin B in 10% or 20% fat emulsion, Trissel
found that the amount and size of undissolved particles were substantially increased in comparison with amphotericin B in 5% dextrose [82]. These particles could be removed by an inline filter; however, this would markedly reduce the antifungal efficacy of this compound. The amphotericin B/fat emulsion mixture does not take into account current models of lipid drug delivery, suggesting that a significant improvement in therapeutic index may not have been seen with use of this mixture.

Methods of preparing this drug have not been standardized, and drug admixture in the pharmacy has not been approved by the U. S. Food and Drug Administration. Lipid emulsions used for parenteral nutrition are known to cause hepatic dysfunction, platelet dysfunction, and increased risk of infection. Mixing lipid emulsions with amphotericin B should be considered investigational, and the use of this compound should be limited to clinical research protocols approved by an institutional review board.

### Future Directions for Research

Lipid formulations of amphotericin B have been undergoing clinical investigation for over a decade. Many questions, however, remain to be elucidated. For example, little is known about the comparative efficacy and pharmacodynamics of these compounds. More work is clearly required in clarifying their mechanism of action, the basis of the improved therapeutic index, and the selective cytotoxicity. Drug interactions as well as the possibility of any long-term toxic effects need to be studied, particularly in patients who receive many months of therapy for chronic invasive mycosis. An understanding of the interaction of lipid formulations of amphotericin B with immunomodulators such as cytokines and interleukins is also necessary, when the use of such combinations is considered. Further definition of the clinical usefulness of the lipid formulations of amphotericin B in specific target populations (e.g., newborn infants, children, diabetics, organ transplant recipients, surgical intensive care patients, HIV-infected patients, and neutropenic patients) and for specific fungal infections is needed. Only through the use of thoughtfully designed, carefully conducted, well-controlled clinical trials will we increase our understanding of the appropriate use of these compounds (table 2).

### Table 2. Ongoing clinical trials of lipid formulations of amphotericin B:

- Empirical therapy for fever and neutropenia
- First-line therapy for documented aspergillosis
- First-line therapy for candidiasis in neutropenic patients
- Dose-escalation trials in patients with other documented fungal infection

### Summary

The lipid formulations of amphotericin B represent an important contribution to the management of invasive fungal infections, particularly in immunocompromised patients who require higher doses of amphotericin B but who often do not tolerate them well. Three products (ABLC, ABCD, and AmBisome) are commercially available and have been licensed for clinical use in a number of countries in Western Europe. ABLC has recently been approved for clinical use in the United States for patients with aspergillosis who are refractory to or intolerant of conventional treatment with amphotericin B deoxycholate.

The lipid formulations of amphotericin B should be considered in the management of invasive fungal infections in patients with dose-limiting renal insufficiency, in patients who are intolerant of amphotericin B, and in patients with specific fungal infections that are progressive despite treatment with amphotericin B deoxycholate (e.g., invasive aspergillosis). Future studies are needed to define their role for first-line therapy, especially in the setting of cost containment. The pharmacoeconomics of the lipid formulations of amphotericin B warrant careful analysis in specific clinical settings; the potential of an improved therapeutic index should be weighed against the increased cost of these drugs in comparison with that of conventional amphotericin B deoxycholate [83].

### References


