Amphotericin B: Insight into the advances in preclinical and clinical development


Abstract

Amphotericin B (AmB) is a recognized antifungal drug derived from a natural source that has been used for more than five decades to treat various fungal as well as protozoal infections. In the past two years, the drug has returned to the forefront of medical discussions as the preferred treatment for COVID-19-associated mucormycosis (CAM), popularly known as black fungus infections. AmB acts by binding to ergosterol present in the fungal cells resulting in the formation of pore ion channels and thus making the fungal cell membrane dysfunctional. It also produces free radicals inside fungal cells and thus impairs vital cellular pathways of the fungus. Low fungal resistance and broad-spectrum activity against an array of fungal species support the continued use of AmB for decades. However, nephrotoxicity produced by conventional formulations of AmB is a major limitation of their use and is the reason for the search for safer advanced lipid-based formulations and nano-formulations. The price of the medication with newer AmB formulations and the intravenous route of administration are the major obstacles to their widespread clinical use. Due to its significant toxicity, AmB is mainly used to treat life-threatening fungal infections and leishmaniasis only. This review provides an insight into the preclinical and clinical advancement of various AmB formulations, including its nano-formulations, promising for the therapy of susceptible infections in humans and animals.

Keywords amphotericin B, antifungal drug, COVID-19-associated mucormycosis, liposomes, nano-formulations

Introduction

Amphotericin B (AmB; synonym: amphozone) belongs to the polyene antifungal antibiotic class which possesses broad activity against yeasts, moulds, and the protozoan Leishmania species [1]. AmB is naturally produced by the soil actinomycete Streptomyces nodosus, which was initially obtained from Venezuela's Orinoco River region. It is included in the WHO Model List of Essential Medicines. AmB has shown remarkable effectiveness in human medicine for the treatment of clinical fungal infections in patients for more than five decades [2]. The first version of AmB, deoxycholate AmB (D-AmB), was produced in the year 1950 to treat mycotic infections. Due to its wider range of antifungal activity, it was promptly approved by the FDA for clinical use in the year 1958, even though its structure was not elucidated at that time [3]. Amphotericin B exhibits a fungicidal action that is concentration-dependent.
and has a post-antifungal effect, meaning that it continues to have an antifungal effect even after the concentrations of AmB decrease below its ‘cidal’ concentration. The fungicidal activity of AmB depends on the dose used, and at low doses, it may exhibit fungistatic action [4]. Although AmB has been used in clinical practice for about more than sixty years, drug resistance has been not reported commonly as compared to other antifungal drugs [5].

AmB, in its conventional form, has serious side effects that may preclude it from being utilized even when a major systemic fungal infection is present. Chronic usage of AmB at the dose rate of 35 mg/day or more is associated with nephrotoxicity as its most common side effect. Through interaction with hepatic cytochrome P450, it also affects the liver’s metabolic capacity [6]. However, the current lipid formulations of the AmB have less nephrotoxicity than the old formula which contained deoxycholate. The lipid formulations release a low free AmB content in the serum and were first created in the year 1990.

**Structure of Amphotericin B**

Amphotericin B (AmB) derived its name so, as it has amphotropic characteristics. The presence of the amino group on mycosamine and the arboxyl group on the major ring provide it with the amphotropic property. It is a 38-membered macrocyclic ring (C_{47}H_{73}NO_{17}) with an unsubstituted heptaene (double bonds) chain which conjugated and produced through lactonization. An amphipathic property is also assured by a polyhydroxylated chain containing 7 free hydroxyl (-OH) groups. A mycosamine residue (lactone) forms a side chain with a free amino group at one end of the molecule [7]. Grossly, the drug molecule of AmB can be seen as an amalgamation of four parts viz. polar head, hydrophilic polyno gene chain, hydrophobic polyene chain, and hydrophilic tail (Figures 1 and 2).

![Figure 1. Structural makeup of amphotericin B](http://www.drugfuture.com/chemdata/amphotericin-b.html)

**Physico-chemical properties**

AmB in powder form has a yellow to orange color and aggregation nature (prisms or needle like) with practically no odour. It is soluble in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and propylene glycol and slightly soluble in methyl alcohol. It is insoluble in water near neutral pH (6-7) but becomes sparingly soluble in water if pH is altered to < 2 or > 11. Adding sodium desoxycholate to water increases solubility for AmB [8, 9]. AmB is kept in the dark for storage since direct sunrays deactivate it.

**Spectrum of Activity**

AmB has broad spectrum antifungal activity against many fungal pathogens viz. *Candida albicans, Coccidioides immitis, Blastomyces dermatitidis, Cryptococcus neoformans, Histoplasma capsulatum,*
Aspergillus fumigates and other pathogens including Mycobacterium leprae (bacteria), and Leishmania brasiliensis (protozoan parasite) [4, 8]. However, the systemic toxicity of AmB limits its use. Therefore, clinical indications for the use of AmB are mainly limited to life threatening invasive infections caused by species of Candida, Blastomyces, Coccidioides, Cryptococcus, Histoplasma, Aspergillus, and other fungus involved in pulmonary mycoses.

Mechanism of action of AmB as an antifungal drug

Classically, the primary antifungal mechanism of amphotericin B (AmB) is owing to its ability to bind directly to ergosterol (a sterol found in the cell membrane of fungus). AmB can create pores in lipid bilayers of fungal cells, induce ergosterol sequestration, and interact with phospholipid moieties in the plasma membrane, and these events contribute to its fungicidal activity. Creating pores in lipid bilayers causes the permeability of tiny ions and electrolytes, which disrupts the intracellular ionic equilibrium [10] (Figures 3 and 4). Both forms of AmB i.e. monomeric and self-aggregated forms can bind with ergosterol, however, it is only self-aggregated AmB forms whose binding causes pores in cholesterol-containing membranes. Thus, formulations, that assure the release of AmB in monomer forms, show an improved therapeutic index [11].

To understand mechanisms of action for liposomal AmB, fluorescently tagged liposomes and gold-labeled liposomes, loaded with amphotericin B or unloaded (empty), were utilized to illustrate in vitro and in vivo binding of the liposome to the cell wall of the pathogenic fungus. Without AmB, liposomes cling to the surface of the fungal cell, but both the fungal cell and the ’empty’ liposomes are unharmed, whereas, binding of amphotericin B-loaded liposomes induces the killing of fungal cells [12-13]. Following the administration of liposomal AmB, free, protein-bound, and liposome-associated amphotericin B comes into the blood circulation. Out of which, the liposomal form adheres to the fungal cell wall in a preferred manner. Then, the active molecules of AmB are released and transferred to the fungal cell membrane which exerts its fungicidal activity [1]. It is possible that binding induces liposomal breakdown and the AmB release, which subsequently acts on membrane ergosterol in the fungal cell. The process is likely aided by the fact that AmB has a higher binding affinity for ergosterol than cholesterol, which is the liposome’s main lipid component. Temperature seems to play a role in the dissociation of AmB from the liposome and shift to the fungus, with the most efficient transfer occurring at body temperature [14].
In vivo, macrophages play a crucial role in the accumulation of both fungal cells and lipid formulated AmB drugs at the sites of fungal infection or in the mononuclear phagocyte system, as they swallow up both lipid–AmB and fungal cells. Thus, macrophages act as reservoirs of AmB and smooth the progress of its transfer to fungal cells within these phagocytic cells [15].

**Different Formulations of AmB**

Currently, there are four major types of amphotericin B formulation available commercially for parenteral use viz. Conventional amphotericin B (D-AmB), Liposomal amphotericin B (L-AmB),
Amphotericin B lipid complex (ABLC), and Amphotericin B colloidal dispersion (ABCD). Some popular trade names of AmB are Fungizone® (conventional deoxycholate form), AmBisome®/Fungisome® (liposomal form), Abelcet® (lipid complex form) and Amphotec® / Amphotec® (colloidal dispersion form).

A popular lozenge form of amphotericin B viz. Fungilin® has been available for decades to treat denture related candidiasis or oral thrush; however, there is the chance of relapse of the signs after drug withdrawal [16-17]. Two commercially available isotonic nanoemulsions viz. Intralipid® and Clinoleic® were used to solubilize AmB without the use of toxic surfactants and were found promising to be used as nanoemulsion aerosols of AmB via nebulization [18]. An inhalation powder formulation of amphotericin B (ABIP, Nektar Therapeutics) was approved by the FDA in February 2006 with orphan drug status for the management of immunocompromised individuals who are at risk for aspergillosis, to prevent pulmonary fungal infections. Preclinical data showed that a better survival rate was observed when inhalable AmB was used in immunocompromised rabbits challenged with Aspergillus fumigatus spores via the pulmonary route. Despite its low bioavailability, the development of oral formulations with improved pharmacokinetic features has been attempted and some of them are in the clinical development phase. Oral AmpB formulations (liquid and capsule), that were safe and tolerable to Beagle dogs following the administration of single and multiple doses, have been developed [19].

Topical formulations of AmB have also been developed. Liposomal AmB (0.5% w/v), an ophthalmic drop has been demonstrated to be an effective substitute for Fungizone® (0.15% w/v D-AmB) which was a cornea irritant drug [20]. A transdermal patch formulation of AmB has been attempted to increase its solubility, bioavailability, and patient compliance [21]. Dissolvable microneedle arrays or microneedle patches for better transdermal and intradermal delivery of AmB have also been formulated to treat cutaneous leishmaniasis and cutaneous or intracorneal fungal infections [22-24].

**Conventional amphotericin B (D-AmB)**

Deoxycholate or conventional form (D-AmB) is the first formula of AmB discovered in the 1950s, produced from the reaction of one part of AmB with two parts of sodium deoxycholate (1:2) and used to treat systemic fungal infections [1]. Fungizone (Bristol-Myers Squibb), an intravenous sodium D-AmB solution, was first introduced to the market in the year 1958 [25]. The clinical efficacy of D-AmB is always accompanied by more severe side effects i.e. dose-related nephrotoxicity. This toxicity restricts the tolerable daily dose of D-AmB typically to 0.7–1.0 mg/kg, making it less efficient for treating systemic mycoses, particularly in immunosuppressed individuals [26].

**Liposomal amphotericin B (L-AmB)**

L-AmB is a more advanced version of AmB that is intended to mitigate the negative consequences or toxicity of D-AmB. It was first launched in the European countries, in the year 1989. FDA approved intravenous infusion formulation of L-AmB for the treatment of visceral leishmaniasis or kala-azar since August 1997 [9]. Ambisome®, a popular AmB liposomal formulation was developed and produced by Gilead Sciences, Inc. The molecular ratio of amphotericin B, cholesterol, distearoyl phosphatidyl glycerol, and hydrogenated soy phosphatidylcholine in the liposomal bilayer membrane is 0.4: 2: 1: 0.8. The detrimental effect of nephrotoxicity is consequently diminished when amphotericin B is present in liposomal form, but the potency of antifungal activity still remains equivalent to deoxycholate form of AmB. The aqueous centre of each spherical vesicle that makes up the L-AmB structure is surrounded by a lipid bilayer. This small, 60-70 nm-diameter unilamellar liposome structure is recognized as a specific form of colloidal system that extends the half-life of AmB in serum. The normal L-AmB dose is 3-6 mg/kg/day, and due to its negative charge, small size, and lack of absorption by mononuclear phagocytic cells, it can maintain a high concentration in plasma [1]. L-AmB has proven to be successful against cases of systemic fungal infections viz.
candidiasis, disseminated histoplasmosis, life-threatening mucormycosis (like in COVID-19 patients), cryptococcal meningitis (in patients with HIV or having febrile neutropenia), and invasive form of aspergillosis. Although, the liposomal structure had a major role in reducing the nephrotoxic effects but did not totally alleviate nephrotoxicity and it was still required to monitor renal function tests after 9 days of initiation of therapy [27]. Further, the use of L-AmB is restricted due to its expensive cost [28].

**Amphotericin B lipid complex (ABLC)**

Formulation of ABLC uses two phospholipids viz. dimyristoyl phosphatidylcholine and dimyristoyl phosphatidyl glycerol in an equivalent molar ratio (1:1) and forms ribbon-like sheets with AmB. It is of large and variable size and has a diameter of 1–10 μm. Initially, it was approved for second-line treatment of aspergillosis and later on US FDA extended its indications to include fungal infections refractory to conventional amphotericin B therapy [29]. The large size of ABLC allows it to be easily engulfed by macrophages and stores it in macrophage-rich organs like the spleen and liver, as well as facilitates ABLC concentration clearance from plasma. Treatment with ABLC, on the other hand, appeared to have a lower risk of kidney injury and a higher concentration in the lungs than other types of AmB, although it did have a higher risk of hepatic damage [9]. ABLC is usually administered at a daily dose of 5 mg/kg body weight.

**Amphotericin B colloidal dispersion (ABCD)**

It is formed by making a complexion between AmB and cholesteryl sulfate in equivalent molar concentrations. It is a stable complex that forms uniform disk-shaped particles (~ 120 nm diameter x 4 nm thick). It has a variety of actions that are comparable to D-AmB, but it varies in that ABCD is promptly removed from circulation by macrophage consumption. It exhibits dose-limiting, infusion-related toxicities [26]. A dose-escalation phase-I study revealed the maximum tolerable dose of ABCD as 7.5 mg/kg/day [30].

**Comparative efficacies**

All three lipid formulations minimize the dose-limiting type of nephrotoxicity caused by conventional amphotericin B, while retaining the antifungal activity, and thus improving the therapeutic index. The order of *in vitro* activity of AmB and its various lipid formulations against *Aspergillus* spp. has been reported in the following order: D-AmB = ABCD > L-AmB > ~ABLC [31]. However, D-AmB (1 mg/kg/day) and L-AmB (5 mg/kg/day) exhibited greater antifungal efficacy than ABCD and ABLC (5 mg/kg/day each) in experimentally-infected rabbits with hematogenous Candida albicans originated meningoencephalitis [32].

**Pharmacokinetic properties**

Despite its lengthy history of use, much about the pharmacokinetics of amphotericin B remains unclear, and large variations in pharmacokinetic data are observed for various kinds of formulation. For example, as per data retrieved from package inserts from the manufacturer, the half-lives of D-AmB, L-AmB, ABLC, and ABCD are 24, 5-10, 173.4 and 28.2 h, respectively [26, 33]. The maximum plasma drug concentrations achieved in humans for L-AmB, ABLC, and ABCD after the dose of 5 mg/kg, were 83, 1.7 and 3.1 μg/mL, respectively [29].

Because it is poorly absorbed through the GI system showing bioavailability <1 % (range: 0.3 - 0.9 %), it is administered intravenously. Off-label routes like topical, intrathecal, or intracameral uses are also reported. Commercial lipid formulations of Amphotericin B are not bioavailable following oral route administration. Amphotericin B strongly binds to lipoproteins, human serum albumin (HSA), and α-1-acid glycoprotein (AAG) due to its amphoteric nature and such bindings may exceed 95%. Due to the poor solubility of AmB in plasma, a concentration-dependent binding
is observed. Therefore, the amount of bound drug improves, as the AmB concentration increases [34]. It is hypothesized that a large portion of the drug escapes the circulatory space and binds to the membranes containing cholesterol. The liver, kidney, spleen, and lungs have the largest amount of AmB, with negligible accumulation in muscles or adipose tissues. The reticuloendothelial system (RES) is important for the accumulation of the L-AmB. Hepatic and splenic accumulation of L-AmB is attributed to the large number of macrophages present within the liver and spleen. Macrophages are known to readily phagocytose liposomes; this feature is beneficial in treating fungi inhabiting within macrophages, such as Cryptococcus neoformans [1]. Body fluids like inflammatory pleura, peritoneum, synovium, and aqueous humor have AmB concentrations equal to nearly two-thirds of serum concentration. In humans, amphotericin B quickly crosses the placenta. Poor penetration is observed when AmB passes through via healthy or inflamed meninges, vitreous humour, and normal amniotic fluid. This unequal distribution may be the reason why some infections are resistant to treatment.

**Nano-formulations of AmB**

Nanotechnology-based approaches have been successfully attempted to develop amphotericin B nano-structures like lipid, metallic, and polymeric nanoparticles, as an effective strategy to reduce toxicity and improve antimicrobial potency [35]. AmB is one of the first bio-pharmaceuticals, commercially marketed as nano-sized formulations [11]. Nanoparticles serve as AmB’s colloidal carriers and are more adept at selectively delivering drugs to the target cells. They demonstrate sustained drug release, enabling longer-lasting drug-pathogen interaction to treat them [36]. Solid nanoparticles have a high density (weight per volume) and thus offer prolonged drug release due to progressive depot diffusion. Drugs can be delivered in greater dosages over a shorter period of time due to this mechanism. The size of commercial amphotericin B by scanning and transmission electron microscopy is 1–2 µm. After nanonization of the drug, particle size was ranged between 10-20 nm, which promotes preferential engulfing of the drug by the macrophages, resulting in drug delivery to specific targets; such strategy was found effective in visceral leishmaniasis, as Leishmania dwells inside the macrophage-phagocytic system [37]. A number of nano-formulations have been developed for Amphotericin B and important ones are presented in Table 1.

**Table 1. Development of nano-formulations of AmB**

<table>
<thead>
<tr>
<th>Type (Name)</th>
<th>Formulation</th>
<th>Efficacy /Activity (Reference)</th>
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</thead>
<tbody>
<tr>
<td>Polymers based nanoparticulate delivery system</td>
<td>Chitosan and Dextran sulfate were used together as a positive and negative charged polymer, respectively, with zinc sulfate to complex Amphotericin B (AmB) nanoparticles</td>
<td><em>In vivo</em> study revealed a reduction in nephrotoxicity [38].</td>
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<tr>
<td>Polymeric nanoparticles (AmB-NPs)</td>
<td>PLGA [poly(lactide-co-glycolide)] nanoparticles entrapped AmB</td>
<td>AmB-NP exhibited reduced <em>in vitro</em> hemolysis and <em>in vivo</em> nephrotoxicity in rats [39]. Bioavailability of the AmB-NP after oral route was more than 8 times as compared to Fungizone®.</td>
</tr>
<tr>
<td>Lipid nanoparticles (AmB-PEG-LNPs)</td>
<td>AmB-entrapping PEG (polyethylene glycol)-LNPs</td>
<td>Reduced cytotoxicity as compared to Fungizone® and AmBisome®. Lower hematotoxicity than that of Fungizone® but at par to that of AmBisome®. Superior <em>in vivo</em> (mice model) and <em>in vitro</em> antifungal activity [40].</td>
</tr>
<tr>
<td>Method</td>
<td>Nanophase Formulation</td>
<td>Outcome</td>
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<tr>
<td>Polymeric nanoparticles (AmB-NPs)</td>
<td>PLGA [poly(lactide-co-glycolide)] nanoparticles entrapped / encapsulating AmB</td>
<td>In mice models of invasive pulmonary disease as well as disseminated aspergillosis, oral formulation proved very effective [41].</td>
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<tr>
<td>Polymersomes formulation (PAMBO)</td>
<td>(PEG)-PLA Copolymers based Polymersomes loaded with AmB</td>
<td>In a fungal mouse model, PAMBO was less harmful and more effective than Fungizone. The survival rate of immunosuppressed mice infected</td>
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<td></td>
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<td>with Candida albicans exhibited a noticeably positive change [42].</td>
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<tr>
<td>AmB attached to functionalized carbon nanotubes (f-CNT–AmB)</td>
<td>AmB bound carbon nanotubes</td>
<td>Greater in vitro and in vivo antileishmanial activity than conventional AmB in hamster and no significant cytotoxic effects in rat</td>
</tr>
<tr>
<td>AmB-loaded PLGA nanoparticles and AmB nanosuspension</td>
<td>Poly (D,L-lactide-co-glycolide) (PLGA) NPs and nanosuspensions with AmB</td>
<td>In vivo efficacy was equivalent or more effective to both ambisome and fungizone in the acute infection of Aspergillus fumigatus in</td>
</tr>
<tr>
<td>Biocompatible polymeric nanoparticles (NANO-D-AMB)</td>
<td>AmB trapped in PLGA and incorporated with DMSA (dimercaptosuccinic acid)</td>
<td>increased antifungal efficacy against Paracoccidioides brasiliensis in BALB/c mice [45].</td>
</tr>
<tr>
<td>Nanoemulsions (NE)</td>
<td>AmB-bound cholesterol-stabilized NE</td>
<td>Higher values for selectivity index were found for AmB-loaded NE than conventional AmB for in vitro antileishmanial activity [46].</td>
</tr>
<tr>
<td>Solid lipid nanoparticles (SLNs)</td>
<td>Topical AmB solid lipid nanoparticles</td>
<td>High skin AmB deposition and distribution with little skin irritation along with potent action against Trichophyton rubrum was noted</td>
</tr>
<tr>
<td>Nanoemulsion (NE)</td>
<td>Topical nanoemulsion formulation of AmB</td>
<td>Based on its ability to retain in the skin, it could have a beneficial local antifungal impact or be used to treat dermal</td>
</tr>
<tr>
<td>AmB nano-assemblies (AmB-NAs)</td>
<td>Synthesis of AmB nanoparticles using extracts of Aloe vera leaf</td>
<td>More efficient in eliminating Candida spp. and elicited fewer drug side effects in the Balb/C mice [49].</td>
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<tr>
<td>Chitosan-coated nanoparticles</td>
<td>Chitosan-coated PCL [poly (ε-caprolactone)] nanoparticles containing AmB</td>
<td>Reduced cytotoxicity and improved gastro-intestinal stability was noted, promising for oral delivery [50].</td>
</tr>
<tr>
<td>Sulfonated Chitosan nanoparticles (NPs)</td>
<td>AmB loaded sulfonated chitosan NPs (AmB-SCNPs)</td>
<td>Resulted in decline of Candida glabrata infection in comparison with the AmB only and AmB-non-sulphonated nanoparticles[51].</td>
</tr>
<tr>
<td>Chitosan-coated NPs</td>
<td>Chitosan-coated PLGA NPs containing AmB</td>
<td>Antifungal activity was retained while decreasing AmB cytotoxicity to RBC [52].</td>
</tr>
<tr>
<td>Nano-structured lipid carriers (NLC)</td>
<td>Chitosan-coated nano-structured lipid carriers for AmB (ChiAmB-NLC)</td>
<td>At pH 5.8 to 6.8, it showed mucoadhesive characteristics that might delay gastrointestinal transit and be used to increase systemic</td>
</tr>
<tr>
<td>Biodegradable and biocompatible Chitosan NPs (AmB-CH-TPP and AmB-CH-Dex)</td>
<td>AmB-loaded chitosan NPs i.e. TriPolyPhosphate sodium (TPP) and Dextran sulphate (Dex)</td>
<td>AmB-CH-TPP NPs demonstrated antileishmanial activity by IV route. Not suitable for topical treatment of cutaneous leishmaniasis as</td>
</tr>
</tbody>
</table>

Amphotericin B in veterinary medicine

As in human medicine, amphotericin B is being used to treat serious fungal illnesses and leishmaniasis in animals but less frequently, mainly due to high cost. Due to cost factors, it is used mainly in small animals and horses. The AmB therapy is done on alternate days or thrice weekly, which may continue for many weeks making the therapy costly. For infusion purposes in small animals, AmB is administered over 2–6 hours after dilution with a 5 % dextrose solution (10 mg of amphotericin B per 100 mL fluid) to reduce nephrotoxicity. It was observed that slow infusion of AmB with the large volume of fluid resulted in less nephrotoxic than rapid intravenous bolus in dogs [60]. It is not recommended to mix in an electrolyte-containing solution, such as lactated Ringer's solution, since doing so could result in the drug precipitating. Abelcet® (ABLC) has been used in small animals to treat cryptococcal meningitis, disseminated coccidioidomycosis, blastomycosis, histoplasmosis, and pythiosis [61]. Cats are more sensitive to the nephrotoxic effect of AmB than dogs, hence lesser dosages are recommended in them. To diminish the side effects of AmB like fever, nausea, and vomiting, drugs like diphenhydramine (0.5 mg/kg, IV) or aspirin (10 mg/kg, PO), or hydrocortisone sodium succinate (0.5 mg/kg, IV) are administered before administration of AmB. It has also been used subcutaneously as dilute dispersion in the successful treatment of canine and feline cryptococcosis [62]. In horses, AmB is used mostly as a topical application to treat fungal ailments of the eye, limb, and upper airway treatment, with infrequent usage as a systemic antifungal. Intravenous regional limb perfusion (IRLP) administration of AmB has resulted in successful treatment of pythiosis in horses [63]. Successful treatment of equine cutaneous pythiosis using topical amphotericin B is also reported [64]. Ophthalmic use of AmB has been indicated as topical (50 mg drug into 10 ml sterile water), subconjunctival (0.8 – 2.0 mg), intravitreal (5 μg), and intracameral (25 μg in 0.05 ml sterile water) applications to treat various ocular fungal illness [65]. In the horse, amphotericin B topical and intralesional therapy has been reported as an effective treatment for nasal conidiofomycosis [66, 67]. There have been few reports of using AmB in birds [68] and wild species like tortoise [69]; but no reports on clinical use of AmB in food-producing animals are available, and no approved AmB formulations exist for such animals.

Amphotericin B in COVID-19-associated mucormycosis (CAM)

Mucormycosis, popularly known as black fungus, a fungal infection primarily affecting immunocompromised individuals, is caused by fungi of the mucorales class. Species of fungi responsible for
mucormycosis include *Mucor, Rhizopus, Rhizomucor*, and *Syncephalastrum* species. It has been noted in the survived COVID-19 patients with a history of long hospitalization and steroid use, as a post-covid complication. Mucormycosis is typically considered a rare disease, but the numbers of black fungus cases were highly increased in COVID times, referred to as COVID-19-associated mucormycosis (CAM). The two major forms of CAM were rhino-orbital-cerebral (ROC) and pulmonary forms with ROC one is more common. Predisposing factors to CAM infections include low oxygen levels, hyperglycemia (diabetic cases), an acidic environment, elevated iron concentrations, and impaired phagocytic activity in COVID-19 patients. Most of the CAM cases were reported from India followed by Egypt, Iran, Turkey, and the United States of America [70]. In India, CAM has been reported in COVID-19 infections caused by the B.1.617.2 (δ) variant, the primary offender for the second COVID wave that has rapidly spread throughout the nation [71].

Amphotericin B, posaconazole, and is a vuconazole are the three drugs found effective in the treatment of black fungus. Among these, the liposomal form of amphotericin (L-AmB) is the most preferred drug and used to treat CAM at the daily dose rate of 5-10 mg /kg [72]. L-AmB is recommended by the European Confederation of Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM) as a drug of choice for the treatment of CAM at a daily dose rate of 5 mg/kg body weight, given in 200 ml 5% dextrose over 2–3 h, for the period of 3–6 weeks [73]. When a CAM infection is diagnosed, a starting dose of 1 mg L-AmB is suggested to be administered over 10-15 minutes in the veins and then given as a once-daily dose based on body weight for the next 14 days [74]. The high cost of the liposomal form of AmB with long dosage regimens (3-6 weeks, sometimes > 6 weeks) remains a constraint in treating CAM. Even with the use of antifungal drugs (including AmB) in the CAM, the mortality rate remains high (> 30 %). Clinical practices of nebulized parenteral liposomal amphotericin B (off-label use: 12.5 mg dissolved in 3 ml sterile water) are reported to reduce the incidences of COVID-19-associated pulmonary aspergillosis (CAPA) [75].

**Amphotericin B associated toxicity**

The median lethal dose (LD50) of AmB following the IV route is reported as 1.2-4, 1.6, and 6.0 mg/Kg, respectively in mice, rats and dogs, whereas oral LD50 was reported high i.e. more than 5 and 8 g/Kg in rats and mice, respectively [8]. The high oral LD50 value of the drug relates to its low oral bioavailability. The most common toxicity reported for the use of amphotericin B is nephrotoxicity (including hypokalemia). Other important common toxicities associated with the use of AmB are infusion related reactions, hematological alterations (anemia), and hepatotoxicity.

**Nephrotoxicity**

After delivery, conventional amphotericin B dissociates rapidly and binds strongly to plasma lipoproteins. The AmB-lipoprotein complex is largely responsible for harmful renal accretion. The incidence rates reported for AmB associated nephrotoxicity were 46.2, 58.6 and 60.8 % [76-78]. L-AmB formulations are consistently found to be less nephrotoxic than conventional or deoxycholate AmB formulations [79]. Results of many clinical trials have been in concurrence with these preclinical findings in laboratory animals, with L-AmB consistently being less nephrotoxic than amphotericin B deoxycholate. Both nephrotoxicity and infusion related reactions were noted less severe for liposomal AmB than the ABLC formulation in a double-blind study on neutropenic patients but exist in both groups [80]. The low mammalian toxicity of L-AmB may be attributed to its greater binding with cholesterol within the liposome bilayer, which renders it more stable and less available to interact with cellular membrane cholesterol [81]. In the studies on hypercholesterolemic rabbits, ABLC was found to be less nephrotoxic and less accumulative in renal tissues as compared to conventional AmB (D-AmB) [82-83]. The patho-physiology of AmB induced nephrotoxicity includes the formation of pores in the renal tubular membrane leading to tubular damage (both oxidative and
ionophoric), significant vasoconstriction that reduces renal blood flow, and lowers glomerular filtration rate that finally results in ischemic injury. Acute renal failures are the most serious complications of amphotericin B therapy [84-85]. Elevated creatinine level is an important marker for AmB associated nephrotoxicity [86]. Several strategies suggested to minimize nephrotoxicity of AmB include lowering infusion speed; salt pre-loading and post-loading; taking care of the hydration state of the patient; adjusting doses based on continuous monitoring of blood urea nitrogen and creatinine; and use of diuretics (like mannitol and furosemide), and liposomal systems for drug delivery [81, 84]. In a study, the heat-treated form of AmB resulting in a ‘super-aggregated form of AmB’ was found less cytotoxic to renal cells without upsetting its antifungal activity [87]. L-AmB induced renal tubular damage may lead to potassium loss (hypokalemia). The high hypokalemia incidence (51.3 %) was reported in L-AmB treated patients [88]. To avoid severe electrolyte imbalances, monitoring serum potassium levels and early potassium supplementation are essential during L-AmB therapy [89-90].

**Other important toxicities**

Infusion-related toxicity is a well-known D-AmB side effect, causing acute fevers and chills. These effects are observed more frequently with the infusion of ABLC and ABCD formulations due to their fast clearance into immune cells. These adverse events may be caused by a pro-inflammatory cytokine response mediated by TLR2 and CD14 cells. Such complement mediated reactions are thought to be due to liposomes rather than the drug and are not dose-dependent [91]. An idiosyncratic response comprising chest pain, abdominal pain, and dyspnea has been observed with the use of L-AmB infusion. Such a reaction subsides if an antihistaminic drug is used and the infusion is stopped [92].

The AmB associated hepatotoxicity is characterized by abnormal liver function tests including elevated liver transaminase enzymes and hyperbilirubinemia. Amphotericin B deoxycholate hepatotoxicity has been reported in 10 % to 18.75 % of patients [93-94] whereas; comparable incidences of hepatotoxicity for liposomal AmB and conventional AmB have been reported as 17.8 and 20.3 %, respectively [95]. Long-term AmB treatment causes normochromic, normocytic anemia due to suppression of erythropoietin (EPO). By deactivating HIF-1, a crucial transcription factor and regulator of EPO production, AmB prevents the EPO gene transcription [96-97]. AmB may cause cardiotoxicity and reversible dilated cardiomyopathy is a rare disorder associated with the use of AmB [98-99].

**Conclusion**

Despite severe drug-induced toxicities, amphotericin B remained the indisputable ‘gold standard’ for the clinical management of invasive fungal infections, for more than six decades. Various lipid-based formulations of amphotericin B have been developed to enhance drug tolerability with a more favorable safety profile while maintaining its antifungal activity. The pharmacokinetic and safety features of different formulations of AmB differ vastly. The development and registration of new formulations with improved safety and biopharmaceutical properties are costly and time-consuming activities but are warranted to reduce toxicity and increase therapeutic tolerance. Pharmaceutical attempts are being made to produce cost-effective oral formulations for better compliance with the therapy needed in longer duration treatments. New prospects for amphotericin B are being fostered by promising nano-formulations but outsized clinical investigations need to confirm the preclinical results.
References


