

AMPHOTERICIN B, ITS LIPID FORMULATIONS AND CONJUGATES WITH POLYMERS

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SUMMARY

The minireview is focused on novel findings concerning mechanism of action, lipid formulations, polymer conjugates, and structural modifications of amphotericin B.

Key words: amphotericin B, fungi, systemic mycoses, liposome, lipid formulation, toxicity, prodrugs

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INTRODUCTION

Incidence and prevalence of severe fungal infections have increased over the last decades mainly in the connection with the expanding

number of immunocompromised hosts. Amphotericin B (AmB) (Fig. 1), a chiral polyene antibiotic produced by the actinomycete *Streptomyces nodosus*, is a broad-spectrum antifungal drug used in the treatment of the life-threatening fungal infections.

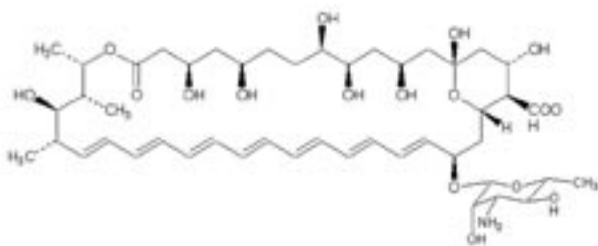


Fig. 1. Structure of amphotericin B

Mechanism of action is connected with its strong affinity for membrane sterols (higher for ergosterol present in fungal membranes than for cholesterol in mammalian membranes) that results in the formation of AmB-sterol aggregates and subsequently molecular channels across cell membranes. The disruption of osmotic integrity of the membrane leads to the leakage of intracellular ions and molecules, and consequently to fungal cell death. AmB is virtually insoluble in water, and that is why it is not absorbed after oral administration. Conventional AmB for intravenous administration is deoxycholate salt of AmB. Unfortunately, AmB is relatively toxic drug and frequent occurrence of adverse events is a limiting factor for clinical use. AmB shows both acute and chronic toxicity - fever, chills, nausea, vomiting, headache, electrolytic imbalance but also nephrotoxicity, thrombocytopenia etc. Amphotericin B-associated nephropathy is the major adverse effect and it involves both glomerular and tubular alterations (1, 2).

RESULTS AND DISCUSSION

Conventional AmB, Modifications of its Physical State and Lipid Formulations of AmB

Although AmB has been used in therapy more than 40 years, its physic-chemical properties, mechanism of action, and pharmacokinetics as well as pharmacodynamics have not been fully understood yet. Recently, important findings have been revealed in this area. Dimers stabilized by van der Waals interactions, linear or cylindrical aggregates of AmB were described in solutions or in artificial lipid membranes by spectroscopic measurements (3). In the absence of sterols, AmB aggregates remained at the surface of artificial lipid bilayers and induced only gel-to-subgel transformation of them whereas ergosterol present in membranes enables the embedding of AmB aggregates in phospholipid bilayers and consequently the formation of AmB pores (4). AmB caused an increase of the orientation order of lipid acyl chains in cholesterol-containing membranes but disorders of them in the presence of ergosterol (5). Based on molecular dynamics simulations, interactions between AmB and ergosterol are more specific and the channels are larger (and probably more stable) than those between AmB and cholesterol (6). However, the interactions of AmB with various sterols depend of the AmB/sterol molar ratio, and the greatest affinity has been found not to ergosterol but to 7-dehydrocholesterol (7).

AmB-related nephrotoxicity is dose-dependent and duration-dependent. Other risk factors are male sex, increased body weight, chronic renal disease, and exposure to other nephrotoxic drugs, e.g. cyclosporin (8). Infusion-related adverse effects of AmB are associated with the expression of several genes enco-

ding pro-inflammatory cytokines and chemokines (9). Other toxic effects of AmB, such as hepatotoxicity (10) or pancreatic toxicity (11), have been observed relatively rarely. AmB exhibited a spermicidal activity in both *in vitro* and *in vivo* experiments (12).

In the last decade, three lipid formulations of AmB have been introduced into practice to improve its properties and to reduce its toxicity: AmB lipid complex (ABLC), AmB colloidal dispersion (ABCD), and liposomal AmB (L-AmB). The pharmacokinetics and tissue distribution of these formulations are different from those of conventional AmB, and from each other as well, whereas their efficacy and spectrum of activity are similar to conventional AmB. Lipid formulations significantly reduce risk of mortality and nephrotoxicity but the reduction of acute infusion-related reactions is not significant (13-15). Because of their high cost, lipid formulations of AmB are indicated after the failure of conventional AmB treatment or in the case of nephrotoxic reactions (15). A comparative analysis of ABLC, ABCD, and L-AmB was recently published (16).

Heating of AmB-deoxycholate to 70 °C for 20 min induces a superaggregation of AmB which is connected with a decrease of toxicity (probably by a lower induction of cytokine and chemokine production), whereas antifungal activity is similar and antileishmanial activity is higher in the comparison with conventional AmB (17, 18). In the connection with treatment of leishmaniasis, a nanosuspension of AmB has been developed which shows high adhesion to gastrointestinal mucosa and enables an uptake of AmB via the gastrointestinal tract (19). Although its antifungal activity has not been evaluated yet, this nanosuspension is an interesting example suggestive of possibility that an oral form of AmB for antifungal treatment could be developed. New amphiphilic copolymers for delivery of AmB also appear to be promising (20). However, some other, relatively simple and inexpensive ways can contribute to a reduction of AmB toxicity: massive hydration and sodium supplementation (21), solubilization of AmB by cheaper synthetic bilayer fragments instead of treatment with AmB lipid formulations (22), or continual infusion of AmB-deoxycholate instead of traditional administration of the same dose during 4-6 h (23).

Conjugates of AmB with Polymers

Polymer conjugation is a method for alteration of pharmacokinetics and reduction of drug toxicity. Poly(ethylene glycol) (PEG), one of the best biocompatible polymers, is suitable for drug delivery (24, 25). A preliminary study with water-soluble AmB-methoxypoly(ethylene glycol) conjugate showed that it has *in vitro* similar spectrum and acute toxicity but higher antifungal activity than conventional AmB in terms of $\mu\text{mol.l}^{-1}$ (26, 27). Biodegradable disubstituted PEG-AmB derivatives with the linker between the drug and PEG are water-soluble, similarly active *in vitro* as conventional AmB but 6 times less toxic (28). Arabinogalactan is a highly-branched natural polysaccharide highly soluble in water, biodegradable, and non-toxic. Its conjugates with AmB possess an improved antifungal activity against *Candida albicans* and reduced toxicity in the comparison with conventional AmB (29).

Structural Modifications of AmB

N-Methyl-*N*-D-fructosyl amphotericin B methyl ester (MFAME), a semisynthetic derivative of AmB, showed a good water solubi-

lity, lower toxicity to mammalian cells and comparable *in vitro* antifungal effect with AmB against *Candida albicans*. However, the incorporation of MFAME into liposomes did not further improve its toxicity (30).

CONCLUSION

This review paper with 30 references includes new findings concerning amphotericin B, an important antifungal drug for the treatment of systemic fungal diseases. Several strategies have been used to increase of the therapeutic index of AmB, e.g. modifications of AmB molecule or modification of its physical state ("heated" AmB), incorporation of AmB into lipid formulations, and conjugation of AmB with polymers.

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