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A review on liposomal amphotericin b in antifungal therapy

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Abstract

To reduce the in-vivo toxicity of the broad-spectrum antifungal drug amphotericin B, various lipid formulations of amphotericin B, ranging from lipid complexes to small unilamellar liposomes, have been developed and subsequently commercialized. These structurally diverse formulations differ in their serum pharmacokinetics as well as their tissue localization, tissue retention, and toxicity. This difference can affect the choice of formulation for a given infection, the time of initiation of treatment, and the dosing regimen. Although preclinical studies have shown similarities in the in-vitro and in-vivo antifungal activity of the formulations with comparable dosing, their acute, and chronic toxicity. Profiles are not the same, and this has a significant impact on their therapeutic indices, especially in high-risk, immunosuppressed patients. With the recent introduction of new antifungal drugs to treat the increasing numbers of infected patients, the amphotericin B lipid formulations are now being studied to evaluate their potential in combinational drug regimens. With proven efficacy demonstrated during the past decade, it is expected that amphotericin B lipid formulations will remain an important part of antifungal drug therapy.

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Introduction

Opportunistic fungal infections, mainly caused by candida and Asperigillus spp., may be life-threatening in severely immunocompromised patients, such as organ and bone marrow transplant recipients. Amphotericin B has been the drug of choice [1]. Amphotericin B is a macrocyclic polyene antibiotic derived from *Streptomyces nodosus* and is administered complexed with deoxycholate. However, the therapeutic use of amphotericin B has been limited by its acute toxicity, including headache, chills, fever, nausea, vomiting, diarrhoea, anorexia, malaise, muscle pain, phlebitis, hypocalcemia, anemia, bronchospasm, arrhythmias, and, above all,

nephrotoxicity. Especially in transplant recipients treated with the immunosuppressive drug cyclosporin, therapy with amphotericin causes a synergistic nephrotoxicity. To reduce toxicity, amphotericin B has been encapsulated in liposomes, which allows higher doses. In experimental animal studies as well as clinical trials, liposomal amphotericin B shown to be effective against invasive fungi. The incorporation of amphotericin B into liposomes, this alters the pharmacokinetic properties of the drug, which leads to changes to in tissue distribution, antifungal activity [2]. And, most of all, tolerability. The first preparation amphotericin B were prepared at investigational centres shortly before therapy, because they could not be any amount of time. However, more than ten years ago, lyophilized formulation consisting of liposomal amphotericin B incorporated into small unilamellar liposomes (mean diameter 45-80 nm) composed of hydrogenated soy phosphatidyl choline, cholesterol and distearoyl phosphatidylglycerol combined in a molar ratio of 2:1:0.8 was introduced. At Huddinge hospital, we were the first to use and report the experience of liposomal amphotericin B (AmBisome). We

have now experienced 12 years of use of AmBisome in transplant recipients [3].

Liposomes

The term liposomes mean lipid body. In 1960's liposomes were first made by A.D. Bangham. The size of liposomes ranges from 25-500nm. Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous of compartments. For administration of nutrients, liposomes are used as a vehicle in drug delivery system. Liposomes, these are one amongst the various drug delivery system used to target the drug to particular tissue [4, 5].

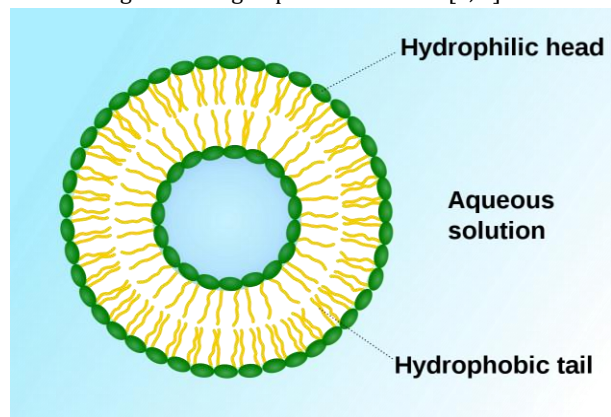


Fig 01: liposomes Structure and Components

Advantages

- Liposomes are suitable for hydrophilic and hydrophobic drugs.
- Liposomes are biocompatible, non-toxic.
- It reduces exposure of sensitive tissues to toxic drugs.
- It protects the encapsulated drug from the external environmental conditions.
- Reduced toxicity and increased stability- As therapeutic activity of chemotherapeutic agents can be improved through liposome encapsulation. This reduces deleterious effects that are observed at concentration similar to or lower than those required for maximum therapeutic activity.

Disadvantages

- It has short half-life.
- Production cost is high.
- There is a chance of leakage of encapsulated drug.

Types

Liposomes are classified on the basis of

[A] Based On Structural Parameters

1. Unilamellar vesicle

- Small unilamellar vesicle (SUV): Sizes ranges from 20-40 nm.
- Medium unilamellar vesicle (MUV): Size ranges from 40-80 nm.

- Large unilamellar vesicle (LUV): Sizes ranges from 100 nm- 1,000 nm.
2. Oligolomellar vesicle
- These are made up of 2-10 bilayers of lipids surrounding a large internal volume.
3. Mutilamellar vesicles
- They have several bilayers. They can compartmentalize the aqueous in an infinite numbers of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayers of LUV/MLV Enclosing a large number of SUV etc.

[B] Based On Method of Liposomes

Four basic stages which involve in the preparation of liposomes.

- Drying down lipids from organic solvent.
- Dispersing the lipid in aqueous media.
- Purifying the resultant liposome.
- Analysing the final product.

Amphotericin B [6]

Amphotericin B is an antifungal medication that fight infections caused by fungus. Amphotericin B has a broad antifungal spectrum which includes most fungi that causes human diseases. Amphotericin B was licensed in 1959. It was initially designed for the treatment of local mycotic infection and later approved for the treatment of progressive and potentially fatal fungal infections.

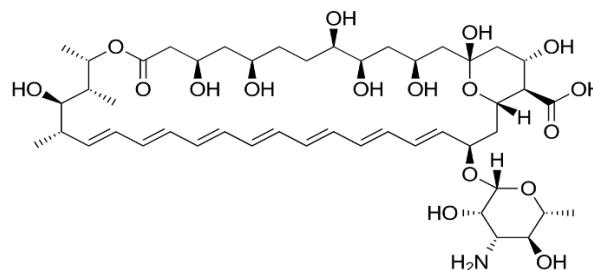


Fig 02: Structure of Amphotericin B

Basic chemistry

1. Chemical formula - C₄₇H₇₃NO₁₇.
2. Molecular weight - 924.084.
3. Solubility - Amphotericin B is insoluble in water, in dehydrated alcohol, in ether, in benzene and in toluene.
4. Physical properties - Amphotericin B appears as deep yellow.
5. It is odourless; it should be stored below 8 ° C.
6. It is stored in tight containers and protected from light.

Adverse effects

The most common side effects of amphotericin B includes:

- Loss of potassium.
- Loss of magnesium.
- Anaphylaxis.
- Fevers.
- Nephrotoxicity.

Therapeutic uses

- In addition to its antifungal action, amphotericin B is used in the treatment of visceral leishmaniasis (L-AMB formulation), caused by the parasitic leishmania.

Liposomal Amphotericin B (L- Amb)

Incorporation of amphotericin B in to liposome significantly altered its toxicity, tissue distribution, and efficacy. Compared with intravenously administered amphotericin B-desoxycholate, liposome amphotericin B showed reduced acute toxicity anti maximal tolerable dose 9 times greater than amphotericin B-desoxycholate. Liposome-amphotericin B also produced higher tissue and lower serum concentrations than amphotericin B-desoxycholate, and was significantly more effective in prolonging survival of mice infected with *Histoplasma Capsulatum*. Liposome is a class of drug delivery system that has opened several new possibilities in improving target to fungal treatment increasing the affinity between amphotericin b and ergosterol and reduction of body damage. In L-AMB, amphotericin b is interacted in to liposomes consisting of hydrogenated soy, phosphatidylcholine and cholesterol. L-AMB is less toxic than ABELCET and AMBD. L-AMB is usually used in febrile or neutropenic due to fungal aspergilliosis infections, candida infections, cryptococcus infections refractory to AMBD. In addition, L-AMB includes comparatively less adverse effects to ABLC. However, liver function should be closely monitored during the medication because the drug concentration in the liver is higher than then spleen and kidney, which could induce higher liver damage compared to common formulations. L-AMB is a unique lipid formulation of amphotericin b that has been used for nearly 20 years to treat a broad range of fungal infections. While the antifungal activity of amphotericin b is retained following its incorporation in to a liposome bilayer, its toxicity significantly reduced. The drug exposure – effects relationships for L-AMB differ significantly from DAMB and remain poorly understood [7].

Preparation of liposomal Amphotericin B by the SCF – CO₂ Method

Liposomes containing AMB were prepared by SCF – CO₂ Method that was reported in a Korean patent. The experimental apparatus, as shown as figure, was made up of the following components: CO₂ syringe pump, circulatory and cooling lines for maintaining the CO₂ pump head, and CO₂, which flowed out of a storage (-7 ° C); and, reaction vessel (72cm³) containing a magnetic stirrer, pressure indicator, and temperature indicator [8].

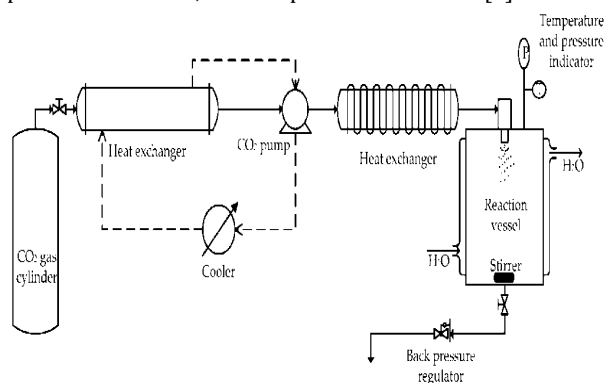


Fig 03: Preparation of liposomal Amphotericin B by the SCF – CO₂ Method

Solution of MeOH and CHCl₃ at 65 ° C. Two hundred milligrams of vitamin C was sonicated to dissolve in 2.0 ml of DMA; next, 50.0 mg of AmB was added to the DMA – vitamin C solution at 65 ° C. The mixture was then transferred to the DSPG solution. The AmB –DSPG lipophilic complex was formed by heating at 65 ° C for several minutes. Further, 213 mg of HSPC was dissolved in 1.0 ml of an equivolume solution of MeOH and CHCl₃ at 65 ° C to yield a clear solution. Fifty – two milligrams of cholesterol was dissolved in a 1.0 ml of equivolume solution of MeOH and CHCl₃ at 65 ° C. The cholesterol and HSPC solution were then mixed with the AmB –DSPG complex solution. The resulting solution and 90 mg (9%W/V) of anhydrous lactose was sealed in the reaction vessel. The temperature of the vessel varied between 35-65 ° C and the pressure varied between 10-30 MPa. Supercritical CO₂ was introduced into the vessel until the desired pressure was reached. After approximately 30 minutes. With stirring at equilibrium, additional supercritical CO₂ continued to flow into the vessel for about 30 min. To wash out any remaining solvent, the vessel was then depressurized to atmospheric pressure; the AmB – Phospholipid mixture was coated onto the surface of the lactose particles, forming a thin film. The resulting thin film was then hydrated with 10 ml of milli-Q water at a temperature of 65 ° C to form a liposomal AmB suspension. Liposomes that were obtained using this process were termed SCF-CO₂ liposomes.

Preparation of Liposomal Amb by the Conventional Method

The liposomal AmB was also prepared by the conventional thin film hydration method for comparison with the liposomes that were prepared by the SCF-CO₂ method. AmB was dissolved in an organic solvent and mixed with a solution of phospholipid dissolved in organic solvents in a manner that was similar to that in the SCF-CO₂ method. The mixture was then transferred to a round-bottom flask and connected to an EYELA rotary evaporator (N-1110V-W; EYELA, Shanghai, China) and water bath (SB-1200; EYELA), with the temperature being maintained at 45 ° C with proper mixing. The Organic solvents were then removed under reduced pressure to obtain a thin film on the wall of the vessel; the resulting was hydrated with 9% lactose aqueous solution at a specific temperature of 65 ° C. After hydration, multilamellar liposomal AmB was obtained, and the resulting liposomes were sonicated were (Beckman XL-80 ultracentrifuge, Branson, MO, USA) for 30 minutes, for vesicle size reduction [9].

Mechanism of Action

Amphotericin B is a polyene antifungal that exerts its activity by binding to ergo sterol in fungal cell membranes, developing holes in the membranes and allowing cell components to leak out, causing cell death.

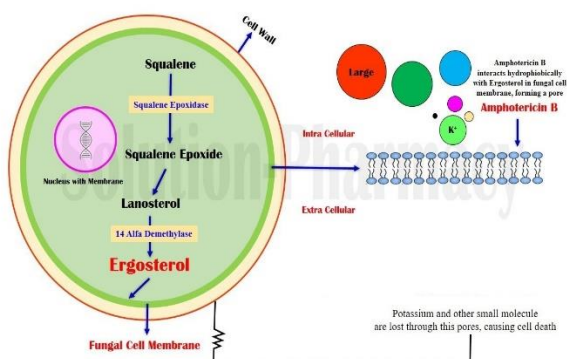


Fig 04: Diagrammatic Representation of Mechanism action for Amphotericin B

Molecular Pharmacology of Liposomal Amphotericin B

Since their first description in 1965, liposomes have been extensively investigated for using drug delivery. They are spherical vesicles characterised by an aqueous core surrounded by a lipid bilayer. The composition of the liposome has a significant impact on the resultant pharmacokinetic properties. Liposomes can be engineered to maximize antifungal activity and minimize drug related toxicity. The liposome specifically used in LAmB was designed to enable parental administration, facilitate the stability of amphotericin B within the liposome, yet enable the active compound to engage with the fungus when encountered within various tissue sites [10].

The unilamellar lipid structure of LAmB has three major components. The first is hydrogenated soy phosphatidylcholine, which comprises the majority of the lipid bilayer. It has the advantage of a gel to liquid-crystal phase transition point of $> 37^{\circ}\text{C}$, meaning it is not readily hydrolysed at body temperature. Secondly, distearoylphosphatidyl glycerol was selected and has a net negative charge. Under the slightly acidic conditions used to prepare liposomes, the amino group of amphotericin B, with its net positive charge, forms an ionic complex with the distearoylphosphatidyl glycerol thus promoting the retention of amphotericin B within the liposomal bilayer. The third component cholesterol, was the liposomal bilayer. Currently available lipid formulations of amphotericin B are not orally bioavailable, although early efforts to develop lipid formulations suitable for oral administration are promising. Other lipid formulation of amphotericin B in clinical use includes amphotericin lipid complex (ABLC) (abelcet, sigma Tau, Gaithersburg, MD) and amphotericin B colloidal dispersion (ABCD) (amphocil/amphotec, three rivers pharmaceuticals, Cranberry Township, pharmacokinetic characteristics. ABLC is composed of flattened, ribbon-like multilamellar structures with particles 1600-11000nm in size, resulting in a greater volume of distribution, perhaps from sequestration in the liver and spleen. Plasma concentrations of amphotericin B following ABLC are lower compared with LAmB [11, 12]. ABCD is a complex of amphotericin B and cholesteryl sulphate that forms thin disc shaped structures that are approximately 120 nm in

diameter, which are rapidly removed from the circulation by the reticulo endothelial system. Given the significant differences between the formulations of amphotericin B conclusions from one compound cannot be necessarily extrapolated to another [13].

Efficacy of Liposomal drug delivery

Liposomal drug delivery systems are very effective in the delivery of vaccines and genes due to their adjuvant property and targeting ability which elicit the immune response of the body through antibody formation and corrected gene inputs. Along with this, the mechanism of vaccine and gene delivery is also explored. There have been several recent investigations into vaccine delivery by liposomes approved by the USFDA and many are in the development stage [14]. In gene delivery, various factors like liposomal preparation, size and various types of liposomes such as cationic and anionic and responsible for the efficiency of transfection of the gene. First generation liposomes in gene delivery suffered from several limitations such as poor encapsulation efficiency, poor release, and lower in vivo targetability. Among second-generation liposomes, cationic liposomes have found better efficiency and good targeting ability for DNA delivery as compared with conventional liposomes. In the area of liposomal-based vaccine and gene delivery, transfection efficiency, toxicity, cellular, and gene delivery need to be studied in future to make it more efficient in this regard [15, 16].

Conclusion

Liposomal amphotericin B is a safe and efficacious antifungal drug in the treatment of severe invasive fungal infections and fever of unknown origin. Nephrotoxicity is usually not a limiting factor when using liposomal amphotericin B, if it is administered in approved dosage.

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