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Topical Amphotericin B formulas: Promising new application

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ABSTRACT

Amphotericin B (AmB) is an old drug used over more than 50 years in clinical medicine for treating various fungal infections in the human body. Opportunistic systemic infection is the most common type of fungal infection mainly treated by AmB. Damage in the fungal plasma membrane by pore forming is the acceptable mechanism of action of AmB. Nephrotoxicity as an important adverse effect of AmB may restrict its use even in the presence of serious systemic fungal infection. New lipid formulas are preferred types to reduce the side effects of old deoxycholate AmB (D-AmB) form. Intravenous administration is the main route of AmB usage for clinical treatment of various systemic fungal infections in the human body. Topical application of AmB is a new approach which is still under primary evaluation. Various pharmaceutical forms of topical preparation of AmB were discussed in this review.

Conclusion: Topical application of AmB provides a promising branch of treatment to reduce the adverse effects of intravenous usage.

Keywords: amphotericin B, topical, systemic fungal infection.

INTRODUCTION

Amphotericin B (AmB) is one of polyene group that has a wide antimicrobial activity against most types of yeasts, molds and a protozoan *Leishmania* spp. [1-2]. It produces naturally by *Streptomyces nodosus*, which is one of the soil Actinomyces [3]. General characters of AmB include its yellowish color and aggregation nature with a low solubility in water or in most organic solvents, but can increase solubility at pH below 2 or at more than 11 [4]. Over more than 50 years, AmB still prefer to use with a high efficiency in clinical medicine to treatment various fungal infections in the human body [5-6]. Deoxycholate AmB (D-AmB) is the first form of AmB developed in 1950 to use as a treatment for systemic fungal infections [1]. Thus, it quickly approved by FDA for clinical apply in 1958 although its structure was unknown due to its broad spectrum antifungal activity [6]. In 1958, an intravenous formula of sodium D-AmB solution was presented in the markets under the name Fungizone-Squibb [7]. Low fungal resistance and broad spectrum antifungal activities are the most valuable pharmaceutical characters encourages continuous usage of AmB [8]. Although wide clinical use of AmB for more than five decades, resistance of fungi has been a rare until now in compared to other antifungal agents [7, 9, 10, 11]. As with any drug, AmB has adverse effects that may prevent it used even in the presence of serious systemic fungal infection. Nepherotoxicity is the major side effects yield from chronic used of more than 35 mg/day of AmB [12]. It also influences on the liver metabolic capacity through interaction with hepatic cytochrome P450 [13]. However, the old formula of AmB that contain deoxycholate have

more nephrotoxicity effects than new lipid formula developed in 1990, which release low free AmB concentration in serum [4].

Mechanism of action of AMB

There is no clear mechanism of action is confirmed to explain the antifungal effect of AmB although it used for more than 50 decades. It activity to bind with the ergosterol of the fungal plasma membrane is the more acceptable one, which causing dysfunction of fungal cell through forming of ion pore channel [1, 7, 9, 14, 15]. Pore formation will cause inhibition of fungal glycolysis and rapid efflux of K^+, and Mg^+ ions inside fungal cells leading to increase the acidity of these cells and cell death [3]. However, this mechanism can support by the higher affinity of AmB to bind with ergosterol than with mammalian cholesterol and its bigger molecule size that cause more membrane damage through conducting high amount of ion [16]. Two main domains in the chemical structure of AmB molecule play a role in pore forming in fungal plasma membrane, including hydrophobic (hydrocarbon chain) which is the direct responsible for pore development and hydrophilic (polyhydroxyl chain) that facing the interior of the pore [3].

Oxidative stress is another mechanism of AmB action against fungi through production of free radicals inside the fungal cells [7, 17- 18]. The oxidation effect of AmB will lead also to form superoxide anion and oxygen depletion in which effect on the fungal cell pathways [19]. Moreover, AmB has the ability to induce proinflammatory immune response due to its immunomodulatory properties [7]. This will give the infected individual, especially those with immunocompromised state, another protective process against fungal infection.

AmB pharmaceutical forms

The adverse effects of D-AmB, especially nephrotoxicity, infusion reaction and dose limitation, are always under the view to limit by development new formulas with the same antifungal activity [3]. Three lipid formulas, including liposomal amphotericin B (L-AmBand), Amphotericin B lipid complex (ABLC) and Amphotericin B colloidal dispersion (ABCD) are becoming more reliable to use with less side effects [1, 3, 20- 23]. Although all of these formulas contain AmB, they have different therapeutic properties such as reticuloendothelial clearance, size, visceral diffusion and shape [22]. This various properties will also associate with other characters effect on the antifungal activity of each formula such as type of infection, time of therapy starting, required dose, toxicity level, tissue location and retention, and pharmacokinetic properties [24]. Thus, usages of new AmB formula provide various choices to treatment different fungal infection even in patients with renal impairment and conventional AmB failure [21]. Suitable choice is mainly depended on the low infusion-related toxicity, especially for L-AmB, and possibly for ABLC [3].

Treatment with AmB still considered the first chose against systemic fungal infection such as Cryptococcosis (Cryptococcal meningitis), Aspergillosis, invasive Candidiasis and other lethal opportunistic mycosis diseases as with zygomycosis and fusariosis [22, 24]. Liposomal AmB is more preferred formula of AmB to treat brain fungal infection due to its high penetration through brain membrane and low toxicity compared with other formulas [24]. However, various AmB formulas have different antifungal activity as proved by clinical utilization depending on the site of infection and the immune state of infected individuals [4].

The usual dose of AmB should be 3-5 mg/kg/day and the effect of this dose may differ from one formula to another [5]. The AmB is parenterally administrated because of its low oral bioavailability (0.2–0.9%) [23]. However, the lipid formulas are quite more expensive than old one [21].

Type of AmB formulas

1- Deoxycholate Amphotericin B (D-AmB)

Deoxycholate Amphotericin B (D-AmB) is the first discovered formula of AmB in 1950 results from mixing sodium deoxycholate with AmB and used for treatment of systemic fungal infections [1]. The mixture consists of AmB to deoxycholate ratio of 1:2 [4]. The antifungal efficiency of D-AmB is always concomitant with more serious dose-related nephrotoxicity side effects [12]. This toxic effect limited the maximal tolerated dose of D-AmB to 0.7–1.0 mg/kg/day, which is less effective to treatment systemic fungal infection, especially in immunocompromised persons [3].

2- Liposomal amphotericin B (L-AmB)

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Liposomal amphotericin B (L-AmB) is more development formula of AmB which is designed to reduce the side effects of D-AmB [1]. It presented to the European market in 1989 and approved by FDA as first drug to treat visceral Leishmaniasis in August 1997 [4]. The L-AmB structure composes of spherical vesicles that distinguished by lipid bilayer surround aqueous core [1]. This small unilamellar liposome structure which has 60-70 nm diameters is also regarded as a particular sort of colloidal system that increase serum half-time of AmB [4]. The usual dose of L-AmB is 3-6 mg/kg/day and can be remained at high concentration in plasma by the effect of its negative charge, small size as well as it avoids ingestion by the mononuclear phagocytic cell [3]. The commercial name of L-AmB is Ambisome® [4].

The L-AmB has proved to be effective against a wide range of systemic fungal infection caused by opportunistic fungi such as Candidiasis, cryptococcal meningitis in HIV and febrile neutropenia patients, disseminated histoplasmosis, life threatening mucormycosis and invasive aspergillosis [1-2]. The pharmacokinetic of L-AmB started when the liposomal vesicle becomes in contact with the fungal element in infection site, then release AmB from holding vesicle to attach with the ergosterol of fungal plasma membrane and destroy it [1]. The liposomal structure also has an important role to reduce the nephrotoxicity effects of AmB when it used alone [2], but it still required monitoring after 9 days of the management beginning [25]. However, utilizing of L-AmB is also restricted by its expensive cost [2].

3- Amphotericin B lipid complex (ABLC)

The commercial name of Amphotericin B lipid complex is Abelcet®. It consists of two phospholipids with AmB in 1:1 molar ratio with a diameter of 2-5 µm of ribbon-like shape [4]. The large size of ABLC makes it easily ingested by macrophages and deposit in organs rich with this cell such as spleen and liver and also facility the clearance of ABLC concentration from plasma [3]. However, treatment with ABLC appeared low risks of kidney damage and more concentration in lung than other types of AmB, but showed risks of hepatic disorders [4]. The usual dose of ABLC is 5 mg/kg/day [3].

4- Amphotericin B colloidal dispersion (ABCD)

Amphotericin B colloidal dispersion (ABCD), which has a commercial name as Amphotec®, is characterized by its content of equal molar concentrations of cholesterol sulfate [4]. The diverse effects of ABCD usage are quietly similar to that of D-AmB, but it differs by quickly removing from the plasma by macrophage ingestion [3].

Amphotericin B usage

Invasive systemic fungal infections have recently consider the major cause of morbidity and mortality in immunocompromised individuals who have an immunodeficiency condition such as those with AIDS, transplant recipients or patients receiving immunosuppressive chemotherapy for tumors treatment [4]. The *in vitro* and *in vivo* usage of AmB proved its broad spectrum activity against various fungi. It found an *in vitro* action of AmB to inhibit the growth of 89% of 448 clinical isolates molds at ≤1 µg/ml [26]. The minimum inhibitory concentration (MIC) of AmB is usually required less value to inhibit the mold growth than for minimum fungicidal concentration (MFC) as noted with some mold such as *Trichoderma longibrachiatum* (MIC; 0.87, MFC; 5 µg/ml) and *Rhizopus arrhizus* (MIC; 0.36, MFC; 2.2 µg/ml) [27]. Synergism with other antifungal agents could also increase AmB activity against pathogenic fungi as the combination with fucytosine against melanized fungi of Chaetothyriales order that cause primary cerebral infections [28]. However, yeast growth required less concentration of AmB as MIC (0.25 to 1.0 µg/ml) to inhibit compared to molds [27].

The AmB can use to treat different types of systemic mycoses that mainly caused by opportunistic fungi such as *Aspergillus* spp., *Candida* spp., and zygomycetes and those by primary infectious fungi such as *Histoplasma capsulatum*, *Blastomyces* spp., *Coccidioides immitis*, *Cryptococcus* spp. and *Paracoccidioides* spp. [4, 22, 24, 29]. Intravenous injection of 3.39 mg/kg/day of ABLC in 23 patients with paracoccidioidomycosis revealed 100% cure rate [29]. About 100% survival rate of mice infected with two strains of *Aspergillus fumigatus* (wild and azole resistance strains) was found after treated with 16 mg/kg of L-AmB for 14 days [30]. Combining with other immune materials such as IFN-γ can also increase antifungal activity of AmB against *A. fumigatus*, *Saccharomyces cerevisiae*, but not against

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C. albicans [31]. A pharmacokinetic/pharmacodynamic (PK/PD) model is *in vitro* designed to simulate releasing of AmB from plasma. After tested against three clinical isolates of *Aspergillus* spp., it revealed that *A. fumigatus* was completely inhibited at C_{max} of ≥2.4 mg/liter, partial inhibition of *A. flavus* with growth delay of 1 to 50 hrs at C_{max} of 0.6 to 4.8 mg/liter, and delay of *A. terreus* growth over 8 h for all C_{max}s. [32]. Leishmaniasis is another infection can also treat by AmB in which 85% of cutaneous leishmaniasis and 77% of old world mucosal leishmaniasis due to *Leishmania infantum* was healing after treated with AmB [33].

Nanotechnology approach introduces a promising field to increase the antifungal activity of AmB. Other goals of this new technique are increasing deposition rate of AmB in the spleen and liver, but not in kidney or lung and also to decrease its adverse effects on the human body [4]. Nanoemulsions containing AmB and cholesterol had been shown higher cure rate against Leishmaniasis with limited toxicity toward macrophages [34].

Usage of AmB in animal model

In vitro test of any new drug is always considered the first step to evaluating its therapeutic activity, followed by choosing a suitable animal model to determine the curative nature of such new drug [35]. Infections in the animal model, as an alternative choice for human, provide the answer to many questions about the mechanism of pathogenesis and host defense against infection [36]. For dermatophytes infection, animal model introduces many benefits to understand dermatophytes pathogenesis, evaluation of new drug activity and increasing our knowledge of immune response mechanisms [37]. Otherwise, a variation between human and animals in the immune response, in the causative fungal agents, and differences in skin structure make a challenge in the establishment of dermatophytic infections in animal models [36]. However, other difficulties in using of animal model to evaluate new drugs for dermatophytoses may include low response of rodents to anthropophilic dermatophytic infections, inflammation results from preparation of infection site by shaving, and chosen of suitable animal models [37].

The dog is chosen to be a model to evaluate the curative ability of polyaggregated Amphotericin B (FPA) against infected by *Leishmania infantum*. After 6 months of intravenous injection of FPA (5 mg/Kg), three times every two weeks, there was no significant enhancement in clinical or parasitological characters [38]. Experimentally infected of mice with *Leishmania major* is used to evaluate the efficiency of a therapeutic combination of AmB and chitosan platelets against such type of parasite infection. Histological and immunohistochemical examination of the treated skin lesion revealed a significant reduction of inflammatory granuloma and parasite load compared with D-AmB alone [39]. Another combination of AmB 3% and oleic acid 5% in emulgel formula also showed the same efficiency in the treatment of cutaneous leishmaniasis in a mouse model after usage twice a day for twelve days [40].

Topical usage of AmB for the human

Intravenous administration is a common route of AmB to clinical use in the treatment of various systemic fungal infections in the human body [4, 29]. Topical application of AmB is a new approach which is still under primary evaluation (table 1). Compresses soaked in a solution of 5 mg ABLC was successfully used every 2 days until 5 weeks as topical postoperative treatment of patient with rhinomaxillary mucormycosis caused by *Lichtheimia ramose* [41]. Bronchial instillation is another type of tropical used of AmB. A patient with pulmonary chromomycosis caused by *Scedosporium prolificans* that developed after lung transplantation failed to respond to systemic itraconazole. An improvement of bronchial obstruction was noted after 3 instillations by AmB that continuous as once every 3 months for 2 years [42].

Gel is one of the promising topical forms of AmB to use against various skin fungal infections. Fungisome™ gel which contains liposomal amphotericin B (0.1% w/w) had applied to treat a patient with cutaneous sporotrichosis failed to respond to other antifungal agents. Complete healing of lesion observed after 8 weeks from treated with topical AmB [43]. A gel of AmB and γ- cyclodextrin complex was *in vitro* tested against 11 different fungal species, while it *in vitro* and *in vivo* tested against Leishmaniasis and its causative agent. An antifungal efficiency was observed with 48%, 28%,

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and 69% higher compared with AmB Neo-Sensitabs ® disks, AmB dissolved in dimethyl sulfoxide and clotrimazole cream, respectively. The complex also revealed high *in vitro* leishmanicidal efficiency and *in vivo* activity against an experimental model of cutaneous Leishmaniasis [44].

Structure of ultradeformable liposomes containing AmB (AmBUDL) with 107 ± 8 nm diameters is prepared to test antifungal activity of AmB and its characters on mammalian skin cells. It's revealed a significant antifungal activity against *C. albicans* and non-albicans Candida with less cytotoxic effects on mammal cells and 40 times higher accumulation rate on the human skin than AmBisome. The AmBUDL also displayed 100% of *L. brasiliensis* promastigot and 75% of amastigote at 1.25 µg/ml [45]. Moreover, vaginal suppositories of 50 mg AmB were showed a successful treatment of 70% of ten women with non-albicans Candida vaginitis after giving nightly for 14 days. The medicine is also revealed less local side effects and well tolerated [46].

Preparation of topical eye drops of AmB is progressively developed. Liposomal AmB (AmBisome®) as 0.5% (w/v) eye drop is suggested to be an alternative chose to cornea irritant Fungizone ® containing 0.15% (w/v) D-AmB. The stability of L-AmB new drop has been quite good for 6 months at room temperature or at +2-8 °C [47]. Topical AmB as eye drop may fail to cure fungal infections in the eyes of some cases. Thus, a combination of AmB with other antifungal agent could increase its therapeutic activity. After AmB failed to give a positive curative result, topical combination of voriconazole and AmB eye drop for 5 weeks revealed a successful curative activity of women with keratitis caused by *Scedosporium apiospermum* [48].

Treatment with topical AmB may not always give satisfying results than ordinary drug in the treatment of fungal infection. Therapeutic efficiency of topical L-AmB solution in the treatment of 110 patients with cutaneous leishmaniasis for 8 weeks was evaluated. There was no significant different from that of intralesional glucantime [49]. Whereas, usage of systemic administration of L-AmB for treatment of necrotizing skin and soft-tissue mucormycosis in an infant with bilineal leukemia was completed successful after used of topical D-AmB [50].

Incorporation of AmB in nanoparticles is a new approach of AmB application as tropical drug for treatment of fungal infection. Nanoparticles encapsulated AmB (AmB-np) exhibited a significant *in vitro* and *in vivo* inhibitory activity against fungi. Growth and biofilm metabolic activity of *Candida* spp. is reduced to 72.4-91.1% and 80%-90%, respectively after 4 hours of *in vitro* tested of AmB-np. By using a murine full-thickness burn model, topical AmB-np showed a quicker efficiency to treat wound of mice infected with *Candida* spp. during three days [51]. Theoretical design of nanoemulsion formula of AmB based on pseudo-ternary phase diagram is also developed to recommend usage of AmB as topical treatment of skin infected with Candidiasis and Aspergillosis and to reduce its side effects [52]. A stable formula of AmB in microtube nanonmaterial consists from 12-hydroxystearic acid (1%) had shown a similar antifungal efficiency than with D-AmB against skin pathogenic fungi [53]. Solid lipid nanoparticles (SLNs) are another carrier design of vehicle containing AmB to increase its topical antifungal activity. This formula exhibited a high drug skin permeation and more inhibitory action against *Trichophyton rubrum* [54].

Conclusions

Amphotericin B (AmB) is the more effective drugs used against different types of systemic fungal infections. Although it used over more than 50 years, it's still the first choice for treating serious fungal infections in the human body. The mechanism of AmB action is mainly explained by pore forming in the fungal plasma membrane leading to cell death. New lipid formulas are preferred to use to reduce the side effects of old deoxycholate AmB (D-AmB) form, especially nephrotoxicity. Intravenous administration is the main route of AmB usage for clinical treatment of various systemic fungal infections in the human body. Topical application of AmB is a new approach which is still under primary evaluation. The primary experiments provide promising results about the efficiency of topical formulas of AmB against fungi and to reduce the adverse effects of intravenous usage.

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Table 1: AmB formulas for topical usage

Year	Type of disease	Type of fungi	AmB formula and Dose	Duration of treatment	Ref. No.
2005	non-albicans Candida vaginitis	non-albicans Candida	vaginal suppositories of 50 mg AmB	nightly for 14 days	46
2007	keratitis	Local eye infection with different fungi	Liposomal AmB (AmBisome®) as 0.5% (w/v) eye drop	Test pharmaceutical characters only that show stability for 6 months	47
2011	cutaneous leishmaniasis	*L. tropica* and *L. major*	topical L-AmB solution	3–7 drops twice daily for 8 weeks	49
2012	rhinomaxillary mucormycosis	*Lichtheimia ramose*	ABLC solution (5 mg/ml)	2 days until 5 weeks	41
2012	Horse with tumoral mass located at the left flank	*Pythium insidiosum*	solution of 50 mg of AmB in 10 mL of sterile water	75 days	55
2013	*In vitro*	skin pathogenic fungi	AmB in microtube nonmaterial		53
2013	keratitis	*Scedosporium prolificans*	Combination of voriconazole and AmB	5 weeks	48

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			eye drop (1%)		
2014	cutaneous Leishmaniasis	*Leishmania* spp.	gel of AmB and γ-cyclodextrin complex		44
2014	Candidiasis in mice wound	*Candida* spp.	Nanoparticles encapsulated AmB (AmB-np)	3 days	51
2014	Mucormycosis in an infant with bilineal leukemia	*Rhizopus* spp.	topical D- AmB	Successful outcome	50
2015	cutaneous sporotrichosis	*Sporothrix* spp.	Fungisome™ gel (liposomal amphotericin B 0.1% w/w	8 weeks	43
2016	mammalian skin cells	*C. albicans*, non-albicans Candida, *Leishmania brasiliensis*	ultradeformable liposomes containing AmB (AmBUDL)	40 times higher accumulation rate on the human skin than AmBisome	45
2016	*In vitro* model against dermatophytes	*Trichophyton rubrum*	Solid lipid nanoparticles (SLNs) containing AmB	formula exhibited a high drug skin permeation and more inhibitory action	54
2018	pulmonary chromomycosis	*Scedosporium prolificans*	0.5 mg/ml aqueous solution of AmB instillation	instillations continuous as once every 3 months for 2 years	42

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