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Unmet clinical needs in the treatment of systemic fungal infections: The role of amphotericin B and drug targeting

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ABSTRACT

Recently an increase in both the prevalence and incidence of invasive fungal infections have been reported. The number of fungal species that can cause systemic mycoses are higher and current antifungal therapies are still far from ideal. The emergence of antifungal resistances has a major clinical impact when using azoles and echinocandins leading to possible treatment failure and ultimately putting the patient's life at risk. Amphotericin B can play a key role in treating severe invasive mycoses as the incidence of antifungal resistance is very low combined with a high efficacy against a wide range of fungi. However, the use of this drug is limited due to its high toxicity and the infusion-related side effects often necessitating patient hospitalisation. New medicines based on lipid-based systems have been commercialised in the last decade, these treatments are able to reduce the toxicity of the drug but intravenous administration is still required. An oral or topically self-administered amphotericin B formulation can overcome these challenges, however such a product is not yet available. Several drug delivery systems such as cochleates, nanoparticulate and self-emulsifying systems are under development in order to enhance the solubility of the drug in aqueous media and promote oral absorption and cutaneous permeation across the skin. In this review, the type of drug delivery system and the effect of particle size on efficacy, toxicity and biodistribution will be discussed.

1. Introduction

Over the last decade, the treatment of systemic fungal infections has been significantly improved; however, the current antifungal therapies are still far from ideal due to the limited arsenal of clinically available drugs, the development of resistances and also the challenges of adequate and early diagnosis (Roemer and Krysan, 2014 Zhai and Lin, 2011). Recently an increase in both the prevalence and incidence of invasive fungal infections have been reported. Moreover, the number of fungal species that cause systemic mycoses is higher. Several factors have triggered this serious public health such as: (i) the increase of immunosuppressed patients due to HIV, cancer therapies and organ trasplants; (ii) the increase in the use of broad-spectrum antibiotics for a long time leading to resistances and (iii) the increase of catheter-related bloodstream infections (Florez, 1998; Yang et al., 2016).

The most common species that cause fungal systemic infections are: *Candida albicans (C. albicans), Aspergillus fumigatus* and *Cryptococcus neomorfans,* as well as other opportunist fungi such as *Histoplasma capsulatum, Coccidioides immitis* and *Fusarium.* In addition, new pathogenic fungi resistant to the current antifungal therapy have appeared, such as *Acremonium, Scedosporium, Paecilomyces* and *Trichoderma* (Yang et al., 2016). Among hospital-acquired infections, *Candida* accounts for 50% of the mycosis followed by *Aspergillus* (6,8%), *Cryptococcus* (4,5%), molds (4,3%), zygomycetes (1,4%) and other fungi (33%) (Fig. 1) (Peman, 2008; Roemer and Krysan, 2014).

The number of agents available to treat invasive fungal infections has increased by 30% since the turn of the millennium (Dodds et al., 2006). Nevertheless, the number of therapeutic drugs available for the treatment of invasive fungal infections is quite limited when compared to those available to treat bacterial infections (Roemer and Krysan, 2014). Amphotericin B is one of the oldest antifungal drugs that in spite of its high toxicity, still

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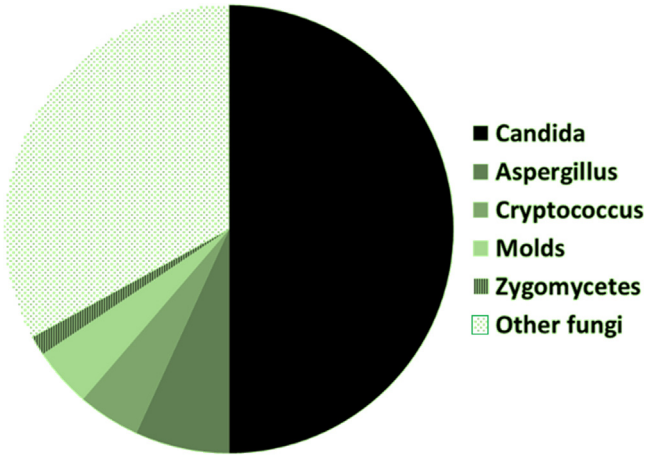

Fig. 1. Prevalence of systemic fungal infections.

remains in clinical practice as one of the first-line treatments because of its broad spectrum of activity and low incidence of clinical resistances which is of great advantage over the azoles. Several formulations have been developed and commercialised in the last decade in order to overcome challenges associated with poor aqueous solubility and toxicity. This review focuses on the role of amphotericin B in clinical practice to treat invasive mycoses and the effect of drug delivery system and particle size on efficacy, toxicity and biodistribution of the drug. New technological approaches under development to improve the clinical outcome of the amphotericin B therapy will be also discussed.

2. Current antifungal therapies for invasive mycoses

The main therapeutic agents to treat invasive fungal infections are described in Fig. 2 (Lorenzo, 2008; Peman, 2008). Briefly, the antifungal drugs can be classified based on their pharmacological targets in the following categories:

- Cellular wall: echinocandins, such as caspofungin, anidulafungin and mycafungin, which are semisynthetic drugs with the ability to inhibit the 1,3-β-glucan synthase required to form the cellular wall.
- Plasma membrane: polyenes, such as nystatin and amphotericin B (produced by *Streptomyces nodosus*) which are able to bind to the ergosterol of the plasma membranes and form pores that destabilise the cell leading to apoptosis.
- Ergosterol synthesis: azoles, such as miconazole, ketoconazole, itraconazole, fluconazole, posaconazole, voriconazole, isavuconazole and ravuconazole, which are able to block out the ergosterol synthesis, essential to form the plasma membrane, by inhibiting the 14-demethylase.
- Nucleic acids synthesis: 5-fluorocytosine which is able to enter the cell through permeases and block out the DNA or RNA synthesis.

Table 2 summarises the most commonly used drugs for the treatment of systemic fungal infections. Only the azoles and the 5-fluorocytosine are orally administered. The rest of the medicines require intravenous administration, which increases infusion-related side effects, necessitating patised drugs for the treatment of systemic fungal infections. Only the azoles and the 5-fluorocytosine are orally administered. The rest of the medicines require intravenous administration, which increases infusion-related side effects, necessitating patient hospitalisation. Similar drugs are utilised in immunocompromised patients which suffer from systemic fungal infections in higher prevalence. Overall, one of the major drawbacks of the drugs before mentioned is the appearance of resistance, the adverse effects and the interactions with other concomitant treatments. The appearance of resistances has a major clinical impact for azoles and echinocandins leading to treatment failure and putting at risk patient's life (Perlin, 2007; Sanguinetti et al., 2015). For these reasons, the role of amphotericin B to treat severe invasive mycoses is key as the incidence of resistance is very low and the efficacy is high against a wide range of fungi. This is crucial taking into account that not always is

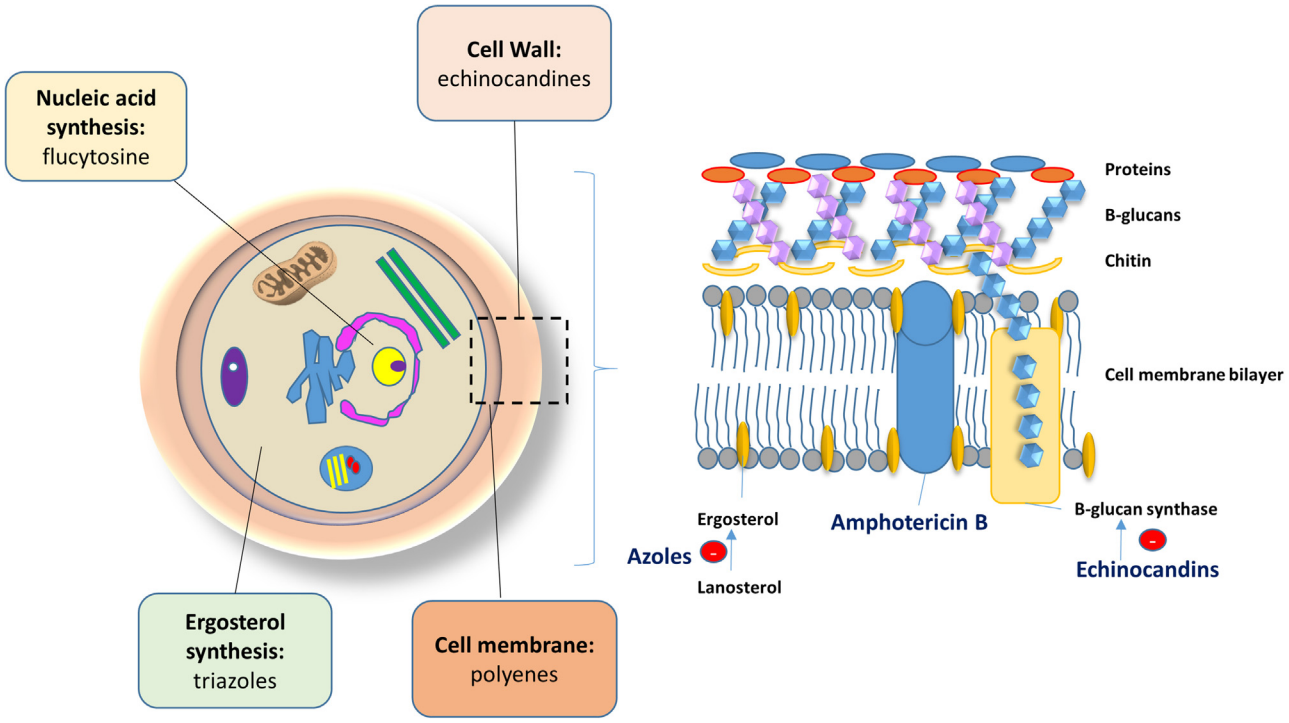

Fig. 2. Antifungal therapies for invasive mycoses and pharmacological targets.

possible to determine the pathogen that causes the disease and an empiric treatment has to be instaured as soon as possible. However, the use of amphotericin B is limited due to its poor aqueous solubility and high toxicity being required patient hospitalisation in order to monitor drug plasma levels during the treatment course.

3. Amphotericin B

3.1. Physicochemical properties

Amphotericin B has been the "gold-standard" of the antifungal therapy since its commercialisation in the 1950s. It is a potent antifungal drug and it can be considered as the most broad spectrum antifungal available in the market (Tables 1–3) (Helmerhorst et al., 1999; Romer and Krysan, 2014). It is a macrolide produced by *Streptomyces nodosus* (Florez, 1998; Lorenzo, 2008). Its chemical structure gives amphotericin B two main physicochemical properties: amphipilic behavior, due to the apolar and polar sides of the lactone ring; and amphoteric behavior, due to the ionizable carboxyl and amine groups. It has a low solubility in aqueous enviroments at physiological pHs and in many organic solvents. Aqueous solubility can be increased at highly acid or highly basic pH, due to salt formation; but these conditions trigger amphotericin B degradation and the salt form possesses a reduced antimycotic activity, so its use is not recommended in clinical practice (Torrado et al., 2008).

Also, a key factor is the aggregation state of the drug itself. Amphotericin B can be found in three different aggregations states: monomeric, dimeric and poly-aggregated (Figs. 3 and 4). These states can be easily studied using spectrophotometry. The monomeric form exhibits a clear yellowish colour and has a peak at 406–409 nm, while the dimeric form is a clear orangish solution with a peak at 328–340 nm. At higher concentrations of dimeric amphotericin B in the medium, the clear solution turns into a translucent due to drug aggregation. The poly-aggregated form is an opaque suspension characterised by small intensity peaks at: 406–420 nm, 383–385 and 360–363 nm (Torrado et al., 2008). As observed in Fig. 4, needle-like crystals aggregate like a rosette in aqueous media.

Table 1
In vitro activity of amphotericin B against different pathogens. Key: 1; IC_{50}, dose that inhibits 50% of growth.

Species	IC_{50} (μM)
C. pseudotropicalis	7.2 ± 1.9
C. albicnas	2.2 ± 0.8
C. neoformans	1 ± 0.1
C. krusei	>70
C. parapsilosis	6 ± 1

Table 2
Most commonly used drugs for the treatment of systemic fungal infections. Key: IV, intravenous, po, oral, BID, twice a day, TID, three times a day, QID, four times a day. Amphotericin B adverse effects are less common with lipid formulations than with the conventional formulation (Fungizone®). Isavuconazole is a prodrug (372 mg of isavuconazonium is equivalent to 200 mg of isavuconazole) (Revankar and Sobel, 2017; Allen, 2010).

Class	Drug	Indicated use	Route	Dose	Most frequent adverse effects
Polyenes	Amphotericin B	Most fungal infections except for *Pseudallescheria* sp.	IV	Fungizone®: 0.5–1.0 mg/kg/day Lipid formulations (AmBisome®, Abelcet®, Amphocil®): 3–5 mg/kg/day	-Infusion-related side effects: thrombophlebitis, rash, hypokalemia, anaphylactic reactions. -GI upset: vomits, diarrhoea -Anemia -Nephrotoxicity
Echinocandins	Anidulafungin	Candidiasis	IV	Loading dose: 200 mg (day 1) Maintenance: 100 mg/day	-Infusion-related side effects: hypokalemia, -GI upset: diarrhoea -Hepatitis
	Caspofungin	Aspergillosis Candidiasis	IV	Loading dose: 70 mg (day 1) Maintenance: 50 mg/day	-Infusion-related side effects: phlebitis, rash -GI upset -Headache
	Micafungin	Candidiasis	IV	100 mg/day	-Infusion-related side effects: phlebitis, rash -GI upset: nausea -Headache -Hepatitis
Azoles	Fluconazole	Candidiasis Cryptococcal meningitis Coccidioidal meningitis	IV/po	100–800 mg/day (higher loading dose may be required)	-GI upset -Hepatitis -QT prolongation
	Isavuconazole	Aspergillosis Mucormycosis	IV/po	Loading dose: 372 mg/8 h Maintenance: 372 mg/day	-GI upset: nausea, vomiting -Hepatitis
	Itraconazole	Dermatomycosis Histoplasmosis Blastomycosis, Coccidioidomycosis Sporotrichosis	po	100 mg/day to 200 mg/BID	-Infusion-related side effects: phlebitis, rash, edema, hypokalemia, hypertension -GI upset: nausea -Headache, dizziness -Hepatitis -QT prolongation
	Posaconazole	Prophylaxis for invasive aspergillosis and candidiasis	po	200 mg/TID	-Infusion-related side effects: rash -GI upset -Hepatitis -QT prolongation
	Voriconazole	Invasive aspergillosis Fusariosis Scedosporiosis	IV/po	Loading dose: 6 mg/kg IV Maintenance: 200 mg po/12 h or 3- 6 mg/kg IV/12 h	-Infusion-related side effects: rash, edema -GI upset -Hepatitis -QT prolongation -Transient visual disturbances
Nucleic acids	Flucytosine	Candidiasis Cryptococcosis	po	12.5–37.5 mg/kg/QID	-Bone marrow toxicity (pancytopenia) -Hepatic & renal toxicity -GI upset: nausea, vomiting, colitis -Neuropathy

Table 3
Amphotericin B spectrum of action compared to other systemic antifungal drugs (adapted from: (Murray at al., 2006)). Key: antifungal activity is classified in: inactive or not recommended (0), occasional activity (+), middle activity but with descriptions of resistances (++), reliable activity with occasional resistance (+++), very active with, rare or not described resistance (++++), NA, non-available.

Microorganism	Amphotericin B	Flucytosine	Ketoconazole	Itraconazole	Fluconazole	Voriconazole	Caspofungin
Candida	++++	++++	+++	++++	++++	++++	++++
C. albicans	+++	++++	++	++	++	+++	++++
C. glabrata	++++	++++	+++	++++	++++	++++	+++
C. parapsilosis	+++	++++	+++	+++	++++	++++	++++
C. tropicalis	++	+	+	++	0	++++	++++
C. krusei							
Cryptococcus neoformans	++++	+++	+	++	+++	++++	0
Genre Aspergillus	++++	0	0	++++	0	++++	+++
Genre Fusarium	+++	0	0	+	0	+++	0
Zygomycetes	++++	0	0	0	0	0	+
Blastomyces dermatitidis	++++	0	++	++++	+	++++	++
Coccidioides immitis	++++	0	++	++++	++++	++++	++
Histoplasma capsulatum	++++	0	++	++++	++	++++	++
Penicillium marneffei	++++	0	++	++++	++	++++	NA
Sporothrix schenckii	++++	0	++	++++	++	NA	NA
Dematiaceous micelial fungi	+++	+	++	+ ++	+	+++	0

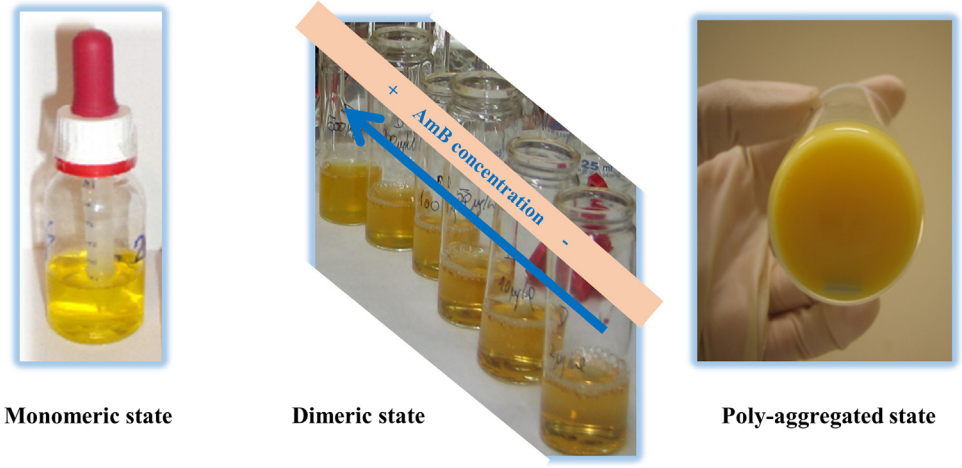

Fig. 3. Amphotericin B aggregation states in aqueous media.

Due to its poor aqueous solubility at physiological pH and poor permeability (Ching et al., 1983; Torrado et al., 2013), its topical delivery is not optimal as well as its oral bioavailability which has been reported to be very low (0.2–0.9%). For this reason, currently, all commercialised formulations are intended for intravenous administration (Florez, 1998).

3.2. Pharmacological properties

Amphotericin B binds to sterols in the cell-membrane showing a high affinity for ergosterol, which is located only in fungal cells and some parasites such as *Leishmania*. It can also bind with less selectivity to the cholesterol of mammalian cells leading to

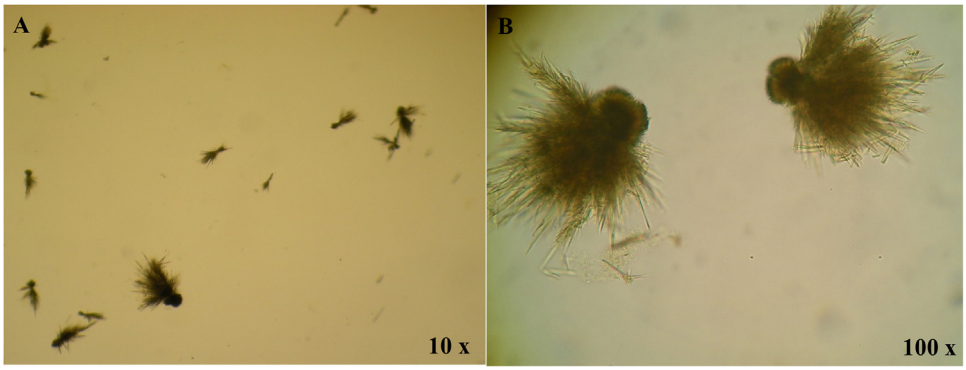

Fig. 4. Images of Amphotericin B aggregates using optical and electron microscopy: A) Amphotericin B in DMSO/H_2O (1:3) and; B) Amphotericin B in DMSO/H_2O (1:1).

toxicity. Amphotericin B can intercalate within the plasma cell membrane and form pores which leads to alteration of Na^+, $K^+$and H^+ permeability and loss of carbohydrates and proteins, ultimately, being lethal to the cell. Depending on the strain of fungus and the drug concentration, amphotericin B can behave as a fungicide or fungistatic agent. Table 3 (Murray et al., 2006) shows a comparison between amphotericin B and other systemic antifungal drugs which highlights the broad-spectrum activity of this drug over other agents.

3.3. Toxicological profile

Amphotericin B can also bind to the cholesterol of mammalian cells causing toxicity, especially in kidney cells which are rich in cholesterol. For this reason, the incidence of nephrotoxicity is very high in patients treated with amphotericin B which is one of the major limitations in clinical practice.

Currently, there are several formulations of amphotericin B in the market including micellar dispersions, lipid complexes, liposomes and colloidal dispersions with different efficacy/safety profiles which will be discussed in the next section.

4. Marketed parenteral formulations

4.1. Convencional-deoxycholate amphotericin B (Fungizone®)

Fungizone® is a micellar dispersion of amphotericin B and sodium deoxycholate (1:2 molar ratio) (Serrano et al., 2013a). This was the first marketed amphotericin B formulation and it was considered the "first-line treatment" for more than three decades because its broad-spectrum activity and low incidence of clinical resistances. However, Fungizone® produces severe adverse effects and was relegated to a second-line treatment when lipid-based medicines (AmBisome®, Abelcet® and Amphocil®) were marketed in the 1990s.

Fungizone® is marketed as a lyophilized yellow powder which is reconstituted in dextrose 5% before intravenous infusion. Intravenous infusion should be given over a period of time of approximately 2 to 6 h depending on the dose with the aim of reducing the infusion-related side effects of the drug. The optimal dose is unknown; thus, the dosage must be individualised and adjusted according to the patients clinical status. Total daily dosage may range up to 1.0 mg/kg per day or up to 1.5 mg/kg when given on alternate days. It is usually used in severe infections caused by *Aspergillus, Blastomyces, Candida, Coccidioides, Cryptoplasma, Histoplasma, Absidia, Mucor, Rhizopus, Canidiobolus, Basidiobolus* and *Sporothrix,* American mucocutaneous leishmaniasis (second-line treatment); and also in immunosuppresed patients in which common antimicrobial therapy has failed.

Fungizone® produces many adverse effects. It is highly nephrotoxic, leading to decrease in renal blood flow and glomerular filtration and alteration of electrolyte reabsorption in proximal and distal tubules. Nephrotoxicity appears in almost every conventional amphotericin B-treated patient, but it's usually a reversible injury that disappears when treatment is over (Florez, 1998). It can also produce azotemia (abnormally high blood nitrogen compounds levels), an increase in serum creatinine, hypocalcemia, hyposthenuria, renal tubular acidosis and nephrocalcinosis. Pain at the injection site is very common, causing phlebitis or thrombophlebitis, which can be prevented by adding 100 U heparine after the intravenous infusion (Florez, 1998). Fever and shaking chills are also frequent.

Fungizone® has a plasma half-life of 24 h, while the elimination half-life consists in 15 days. Amphotericin B in plasma binds highly to plasma proteins (90%). The drug is mainly found in pleural, peritoneal and sinovial liquids and aqueous humor. It is mainly excreted via feces and blood concentrations are not influenced by hepatic or renal failure (Florez, 1998).

In Fungizone®, amphotericin B is in dimeric form due to the interaction with the sodium deoxycholate forming small micelles of about 35 nm in size which can be easily excreted by the kidney increasing its nephrotoxicity (Dupont, 2002). Several authors have described the preparation of simil Fungizone® at lab scale using a simple manufacturing process consisting on: (i) the solubilisation of sodium deoxycholate and phosphate salts in aqueous media and (ii) a pH shift to 12 followed by the addition of the drug. At this pH, the solubility of the drug is much higher which facilitates the interaction with the deoxychoalte and avoids the amphotericin B aggregation once the pH is shifted back to 7.4 resulting in a transparent orangish formulation (Fig. 5) (Serrano et al., 2013b).

To overcome the high toxicity of Fungizone®, pharmaceutical industry has developed amphotericin B lipidic formulations, such as lipidic complexes, micelles or liposomes (Florez, 1998; Lorenzo, 2008; Ruiz-Camps and Cuenca-Estrella, 2009). Currently, amphotericin B lipidic formulations are the first-line treatment in developed countries, but due to its high cost compared to Fungizone®, the last one is still used in developing countries. These formulations were designed to get a lower serum concentration of amphotericin B while higher concentrations in targeted tissues. Ultimately, this can decrease the risk of nephrotoxicity associated to the convencional medicine (Torrado et al., 2008).

Amphotericin B has a great tendency to form self-aggregates in aqueous media due to its poor aqueous solubility (Torrado et al., 2008). The aggregation state plays a key role in the activity, the toxicity of the molecule and also its pharmacokinetic profile. The larger the aggregates, the lower the toxicity and the greater the half-life in the body after intravenous administration (Serrano et al., 2013b). One pharmaceutical approach that has been employed in developing countries to reduce the toxicity of the Fungizone® is to heat the formulation either 20 min at 70 °C (Belkherroubi-Sari et al., 2013) or 1 h at 70 °C (Torrado et al., 2008), in order to promote aggregation before intravenous administration. Larger aggregates are formed, thus glomerular filtration is reduced and consequently, nephrotoxicity is minimised (Belkherroubi-Sari et al., 2013; Torrado et al., 2008). However, this is not a standarised process and if the aggregates formed are too large, they could lead to vein blockages. For this reason, scientists are developing novel approaches to produce drug aggregates with a controlled particle size. The manufacturing process is similar to the one utilised in the preparation of simil Fungizone® (Fig. 5) with the difference that the pH is not changed before adding the drug. Therefore, the drug is not completely solubilised and aggregates of amphotericin B interacts with sodium deoxycholate. Particle size can be controlled based on centrifugation speed cycles and time. Studies have shown that poly-aggregated amphotericin B is less toxic than Fungizone® and at the same time it is an affordable formulation due to the ease of preparation and low-cost excipients (Serrano et al., 2013b).

4.2. Amphotericin B lipid complex (Abelcet®)

Abelcet® with a ribbon-like structure, consists of amphotericin B complexed with two phospholipids in a 1:1 drug-to-lipid molar ratio with a particle size of about 1.6–11 μm. The two phospholipids, L-dimyristoylphosphatidylcholine and L-dimyristoylphosphatidylglycerol, are present in a 7:3 molar ratio (Lorenzo, 2008; Moreno-Perez et al., 2016; Peman, 2008).

Amphotericin B lipid complex is used to treat severe systemic mycosis in patients with renal insufficiency or when convencional amphotericin B is contraindicated, visceral leishmaniasis and also

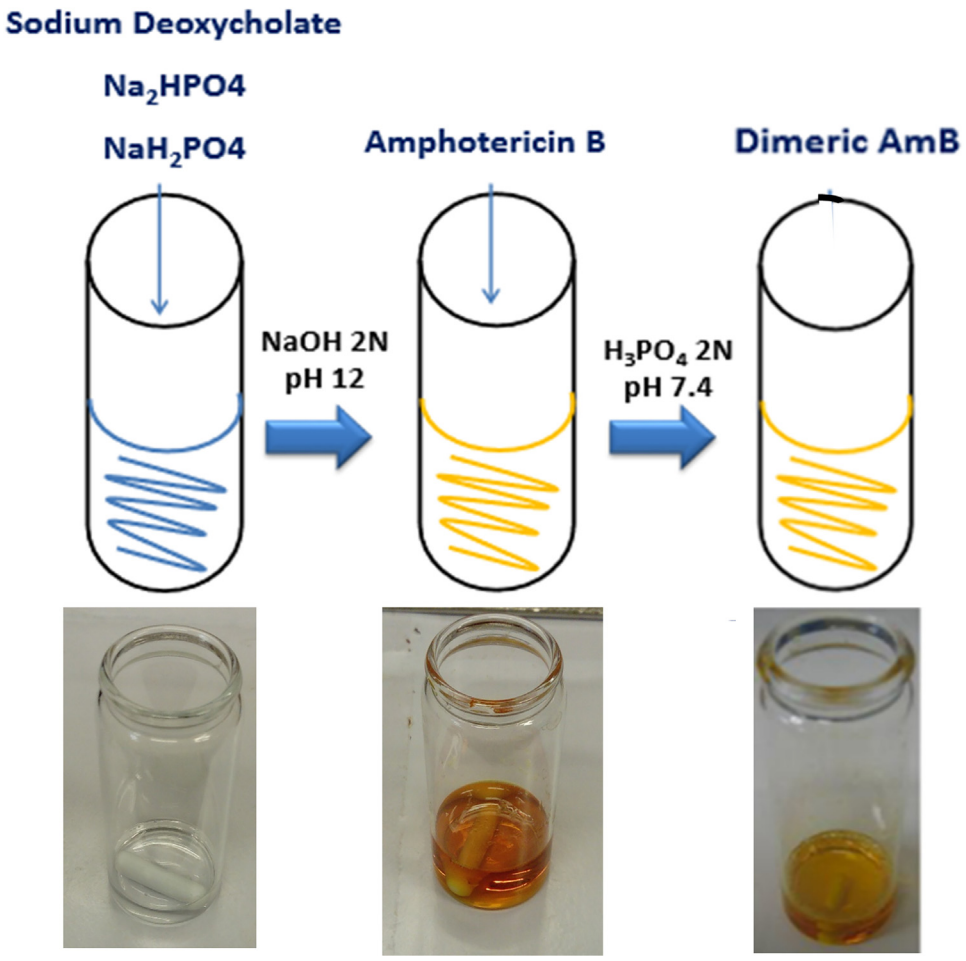

Fig. 5. Preparation of simil Fungizone at lab scale.

to prevent visceral leishmaniasis in VIH-infected patients (Moreno-Perez et al., 2016).

Abelcet® is better tolerated compared to Fungizone® which allows the administration of higher doses (0.6–5 mg/kg/day) (Moreno-Perez et al., 2016). Even though, Abelcet® perfusion requires patient hospitalisation and medical supervision because can also produce adverse reactions, destroying the renal function, leading to an increase in creatinine, azotemia and hypocalcemia. Its intravenous administration can produce anaphylactoid acute reactions. For this reason, it is common the administration of antihistamines, corticosteroids and paracetamol as pre-treatment medication before the injection (Moreno-Perez et al., 2016).

4.3. Colloidal dispersion (Amphocil®/Amphotec®)

Amphocil®, with a disc-like structure, consists of amphotericin B and cholesteryl sodium sulfate in a 1:1 molar ratio with a particle size of 110–140 nm (Dupont, 2002). Also, Amphocil® includes tromethamine, disodium edetate, hydrochloric acid and monohydrate lactose as excipients. This formulation is presented as a lyofilized powder to intravenous perfusion with two different doses: 50.000 UI or 100.000 UI of amphotericin B in each vial.

It is used in invasive aspergillosis (due to its higher accumulation in the lungs) especially in those patients with renal insufficiency who cannot tolerate convencional amphotericin B or this cannot be administered in a high dose.

Amphocil® is less toxic than Fungizone® but, as well as Abelcet®, Amphocil® may produce anaphylactoid acute reactions related to perfusion that can be treated using antihistamines and adrenal corticosteroids (Moreno-Perez et al., 2016).

4.4. Liposomal amphotericin B (Ambisome®)

Ambisome® is composed of small unilamellar vesicles with a particle size of about 70 nm containing 50 mg of amphotericin B encapsulated in the bilayer of liposomes consisting of approximately 213 mg hydrogenated soy phosphatidylcholine, 52 mg cholesterol, 84 mg distearoylphosphatidylglycerol (as the sodium salt), 0.64 mg alpha-tocopherol together with 900 mg sucrose and 27 mg sodium succinate hexahydrate (Adler-Moore et al., 2016; Azanza et al., 2015; Lorenzo, 2008; Peman, 2008; Siedner et al., 2016).

Amphotericin B liposomes are used in the treatment of *Candida, Aspergillus and Cryptococcus* systemic infections, visceral leishmaniasis, prophylaxis of transplant patients and febril neutropenic patients unresponsive to broad spectrum antibiotic agents (Siedner et al., 2016). Currently, it is the first-line treatment in *Candida* spp. infections. Immunocompromised-patients, such as HIV, are commonly treated with a 1–1.5 mg/kg/day dose for 21 days. Due to the risk of recurrences, they may also take a maintenance or re-induction therapy.

Ambisome® is a less toxic formulation than Abelcet®, and by far than Fungizone® (Azanza et al., 2015; Florez, 1998). In fact,

Ambisome® is the only amphotericin B formulation that does not need pre-medication before its administration. Also, Ambisome® has shown a decrease in the incidence of nephrotoxicity to one half compared to the conventional Fungizone® (Azanza et al., 2015).

5. Influence of the drug delivery system on biodistribution and efficacy/safety profile

The particle size and type of drug delivery system lead to markedly differences in the pharmacokinetic profile of the amphotericin B formulations (Fig. 6). Formulations with a larger particle size such as Abelcet® and Amphocil® are characterised by a fast decline of amphotericin levels from plasma (lower AUC and larger volume of distribution and half-life). A higher accumulation in other organs from the reticulo-endothelial system such as liver and spleen is also characteristic. Accumulated amphotericin B is released from tissues to bloodstream and subsequently, excreted in the biologically active form through the urine and the bile (Serrano et al., 2013; Torrado et al., 2008 Torrado et al., 2008). However, the pharmacokinetic profile of Ambisome® is marked by a very high plasma concentration and reduced volume of distribution. This is because of the liposome size which is low enough for not being recognised by the reticulo-endothelial system (leading to prolonged circulation in plasma) but high enough to avoid glomerular filtration and drug renal excretion (opposite to Fungizone®), minimising the interaction between the drug and the tubular cells and then decreasing the renal toxicity (Florez, 1998). For this reason, the administration of higher doses is possible, which can be even 10 times bigger (5–10 mg/kg/day), enhancing efficacy and reducing patient hospitalisation. However, one of the major limitations of Ambisome® is its high cost due to the complex manufacturing process.

Table 4 compares the four marketed amphotericin B formulations. It shows that commonly the four formulations above mentioned have the same efficacy profile but with remarkedly different toxicity profile. All lipidic formulations showed a lower renal toxicity, nevertheless, only the liposomal formulation is able to decrease infusion-related side effects associated with the intravenous administration.

6. Novel technological approaches

In literature, there are many amphotericin B formulations for intravenous administration with the aim of improving the efficacy/toxicity balance (Table 5). Microemulsions, nanoparticle systems and micelles are the most common drug delivery systems under research. Most of them have shown a reduced toxicity (lower haemolytic and renal toxicity). However, very few have also succeeded in improving the efficacy over the lipidic commercialised formulations. Amongst all the drug delivery systems, polymeric nanoparticles (from PLGA and polybutylcyanoacrylate and polysorbate 80) have shown the greatest improvement characterised by lower toxicity and retained *in vitro* antifungal activity. (Van de Ven et al., 2012; Xu et al., 2011)

Nowadays, there are also many amphotericin B formulations under development especially for oral and topical use. The major advantage of the oral delivery of amphotericin B is probably the lower nephrotoxicity and the fact that hospitalisation would not be required reducing indirect health costs and promoting treatment access worldwide. The most promising novel formulations under development currently in clinical trials are the following:

- Amphotericin B cochleates which are in Phase II clinical trials (Perlin, 2004). Amphotericin B is encapsulated within cochleates which are stable phospholipid-cation precipitates that roll up into a spiral without aqueous space. They are more stable than liposomes as they suffer less from oxidation. The drug releases slowly as long as the cochleates unroll or dissociate. They have shown less toxicity than Fungizone® and less immunogenicity. Also, they are stable as a lyophilized powder for long periods of time at room temperature, which is an advantage in developing countries as cold-chain supply is not required (Perlin, 2004).
- Nanoparticulate systems in which the amphotericin B is encapsulated within *N*-palmitoyl-*N*-methyl *N*,*N*-dimethyl-*N*,*N*,

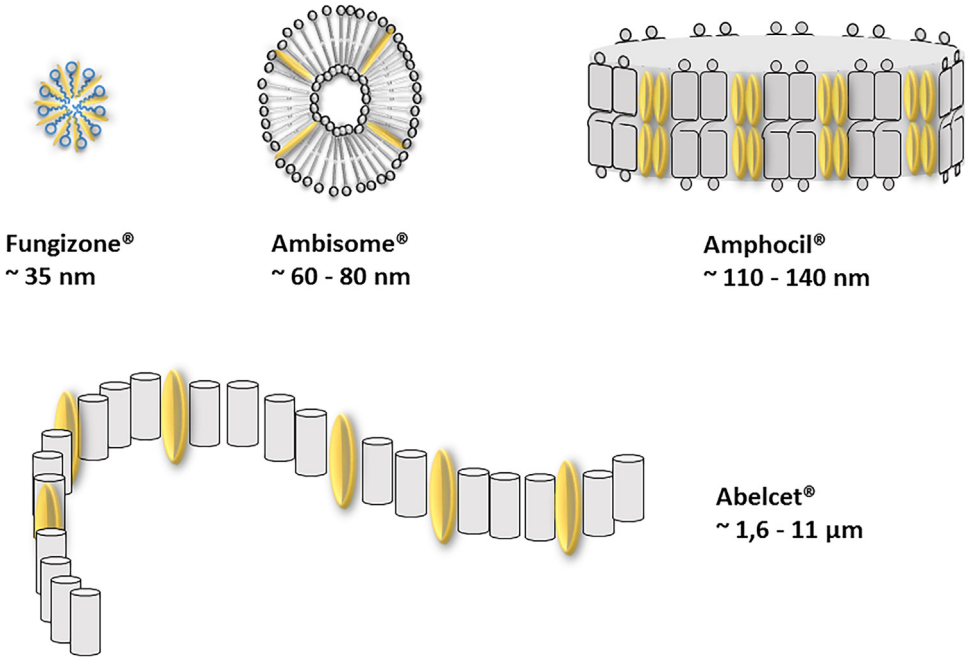

Fig. 6. Drug delivery systems of amphotericin B formulations. Adapted from: (Serrano et al., 2013a).

Table 4
Efficacy and toxicity of different amphotericin B marketed formulations. Key: ABCD, Amphocil®; ABLC, Abelcet®, L-AmB, AmBisome®, D-AmB, Fungizone®.

Formulations	Pathogen	Efficacy	Lower toxicity		Ref.
			Renal	Infusion-related	
D-AmB vs L-AmB	*Cryptococcus* spp.	Similar	L-AmB	L-AmB	Leenders et al. (1997)
D-AmB vs L-AmB	*Histoplama capsulatum*	Higher L-AmB	L-AmB	L-AmB	Johnson et al. (2002)
D-AmB vs L-AmB	Empiric treatment Neutropenic fever	Similar	L-AmB	L-AmB	Prentice et al. (1997)
D-AmB vs ABLC	*Candida* spp.	Similar	ABLC	Similar	Anaissie et al. (1995)
D-AmB vs ABCD	*Aspergillus* spp.	Similar	ABCD	D-AmB	Bowden et al. (2002)
D-AmB vs ABCD	Empiric treatment Neutropenic fever	Similar	ABCD	D-AmB	White et al. (1998)
ABLC vs L-AmB	Leukemia and fungal infections	Similar	L-AmB	L-AmB	Fleming et al. (2001)
ABLC vs L-AmB	Empiric treatment Neutropenic fever	Similar	L-AmB	L-AmB	Wingard et al. (2000)
D-AmB vs L-AmB	Visceral leishmaniasis	Similar	L-AmB	L-AmB	Sundar et al. (2004)
D-AmB vs ABLC	Visceral leishmaniasis	Similar	ABLC	ABLC	Sundar et al. (2004)

Table 5
Novel Amphotericin B formulations for intravenous administration. Key: AmB, amphotericin B; LD_{50}, dose that kills 50% of animals; Brij, polyoxyethylated alkyl ether; Peceol, glycerol monooleate; Mys40, polyetylenglycol 40 stearate; Solutol HS 15, polyethylenglycol 15-hydroxystearate; PLGA, poly(lactic-co-glycolic) acid; Angiopep-2, peptide with high capacity to cross the blood-brain barrier; PEG-PE, 1,2-dystearoil-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy(polyethylenglycol)-2000; PVA, polyvinyl alcohol.

Formulation (particle size)	Composition	Toxicity	Efficacy	Ref.
Microemulsion	Polysorbate 80 Isopropyl myristate Lecitine	Lower nephrotoxicity than Fungizone®	*In vivo*: higher efficacy than Fungizone® against *C. albicans*	Brime et al. (2003)
Microemulsion (45 nm)	Brij 96 Isopropyl myristate Lecitine	Higher LD_{50} than Fungizone®	–	Brime et al. (2002)
Microemulsion (84 nm)	Peceol Mys40 Solutol HS15	Lower haemolytic toxicity and higher LD_{50} than Fungizone®	*In vitro*: same as Fungizone® against *C. albicans*	Darole et al. (2008)
Microspheres (4 μm)	PEG-albumin	Lower haemolytic toxicitiy than Fungizone®	*In vitro*: same as Fungizone® against *C. albicans*	Angra et al. (2009)
Nanoparticles	PLGA Mercaptosuccinic acid	Lower hepatic and renal toxicity than Fungizone®	*In vivo*: same as Fungizone® against. Paracoccidioidomycoses	Amaral et al. (2009)
Nanoparticles (86–153 nm)	PLGA	Lower haemolytic toxicity than free-AmB but higher than AmBisome®	*In vitro*: higher than Fungizone® and same as AmBisome® against. *A. fumigatus*	Van de Ven et al. (2012)
Nanoparticles (69 nm)	Polybutylcyanoacrylate Polysorbate 80	Lower haemolytic and renal toxicity than AmBisome®	*In vivo*: higher than AmBisome® against meningitis by *Cryptococcus*	Xu et al. (2011)
Nanocapsules	Chitosan	Lower haemolytic and macrophage toxicity than Fungizone® and AmBisome®	Higher in vivo activity against. *L. donovani*	Asthana et al. (2013)
Nanospheres (96 nm)	PLGA Sorbytan monostearate Polysorbate 80	Lower haemolytic toxicity than free-AmB	*In vitro*: better than free-AmB against *C. albicans*	Gharib et al. (2011)
Nanospheres (282 nm)	Poly(ε-caprolactone) Poloxamer	Lower haemolytic toxicity and higher LD_{50} than Fungizone®	*In vivo*: lower than Fungizone® against *C. albicans*	Espuelas et al. (2003)
Nanosuspension (118 nm + 440 nm)	PVA	Lower haemolytic toxicity than free-AmB but higher than AmBisome®	*In vitro*: higher than Fungizone® and AmBisome® against. *A. fumigatus*	Amaral et al. (2009)
Mixed micelles (358 nm)	Poloxamer	Lower haemolytic toxicity and higher LD_{50} than Fungizone®	*In vivo*: lower than Fungizone® against *C. albicans*	Espuelas et al. (2003)
Polymeric micelles (24.7 nm)	Angiopep-2 conjugated to PEG-PE	Lower haemolytic toxicity and cytotoxicity in brain endothelial cells than Fungizone®	*In vivo*: higher than Amphocil® against meningitis *Cryptococcus*	Shao et al. (2010, 2012)
Polymeric micelles	Polyoxyethylene block copolymer conjugated to poly-aspartic	Lower haemolytic toxicity than free-AmB	*In vivo*: same as Fungizone® against *C. albicans*	Adams et al. (2003)
In situ forming gel	Carboxymetylcelulose Dextran	No toxicity in the administration zone (peritoneum)	11 days of sustained release and 3 weeks of *in vitro* activity against *C.albicans*	Hudson et al. (2012)

N-trimethyl-6-*O*-glycol chitosan nanoparticles (GCPQ) (Serrano et al., 2015). Currently, it is in clinical trials Phase I. This drug delivery system enhances the oral bioavailability of the drug until 24%. The nanoparticles of about 200 nm protect the drug from gastric degradation, reduce nephrotoxicity and enhance lymphatic absorption while selectively target the drug to lung, liver and spleen similar to Abelcet® or Amphocil®.

– Self-emulsifying drug delivery systems (SEDDS), which are currently in Phase I, are able to enhance the oral amphotericin B bioavailability too. They are several SEDDS systems composed of a mixture of amphotericin B with Peceol® and distearoylphosphatidylethanolamine (DSPE)-$(PEG)_{2000}$ or Gelucire® 44/14 and vitamin E-TPGS which remains highly effective even after the exposure to tropical temperatures (Wasan et al., 2015; Risovic et al., 2007).

Topical administration also has some advantages over the oral or intravenous route as it allows to treat localised infections on the skin or mucoses while the toxicity is very limited. Due to the poor permeability of the drug and consequently, the low pass to the

bloodstream, the use of this route of administration to treat systemic mycoses is negligible. Regarding the topical use of amphotericin B, lipid based systems have shown the best outcomes. In India, Fungisome® gel is already commercialised. This liposomal formulation is consisting on multilamellar vesicles able to entrap the drug in 0.1% w/w (Alhijjaj et al., 2016). Cyclodextrin-derived formulations have also shown promising results by protecting amphotericin B from degradation and increasing the availability of dissolved drug molecules at the barrier surface (Torrado et al., 2013). Amphotericin B-gamma cyclodextrin complexes have been developed exhibiting a good therapeutic index not only to treat fungal cutaneous infections but also in cutaneous leishmaniasis (Ruiz et al., 2014). Because cyclodextrins are GRAS (Generally Recognised As Safe) excipients, ophtalmic formulations are under development in order to treat fungal keratitis which are mainly caused by *Candida* species (Serrano et al., 2012).

7. Concluding remarks

Over the last decade, the treatment of systemic fungal infections has been significantly improved. However, the current antifungal therapies are still far from ideal. The emergence of antifungal resistances has a major clinical impact when using azoles and echinocandins leading to possible treatment failure and ultimately putting the patient's life at risk. Amphotericin B can play a key role in treating severe invasive mycoses as the incidence of antifungal resistance is very low combined with a high efficacy against a wide range of fungi However, its use is limited due to its high toxicity necessitating patient hospitalisation. Fungizone® (micelar dispersion) was considered the "first-line treatment" for more than three decades but due to its high toxicity, it was relegated to a second-line treatment when lipid-based medicines (AmBisome®, Abelcet® and Amphocil®) were marketed in the 1990s. Novel strategies are under development to produce affordable formulations for developing countries such as controlled particle size aggregates with limited toxicity after intravenous administration. Oral delivery systems are also in clinical trials such as cochleates, nanoparticulate systems and SEDDS making treatment access worlwide possible specially in developing countries where patient hospitalisation is not always easy.

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