

Oral Amphotericin B: The journey from bench to market

Dolores R. Serrano^{1,2} and Aikaterini Lalatsa^{3,*}

¹Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040-Madrid, Spain.

²Instituto Universitario de Farmacia Industrial (IUPI), School of Pharmacy, University Complutense, Avenida Complutense, 28040 Madrid, Spain.

³School of Pharmacy and Biomedical Sciences, University of Portsmouth, St. Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK.

*Corresponding author

Dr. Aikaterini Lalatsa

School of Pharmacy and Biomedical Sciences

University of Portsmouth, St. Michael's Building

White Swan Road, Portsmouth PO1 2DT, UK.

Email: katerina.lalatsa@port.ac.uk

Tel: 023 9284 3929

Abstract

Since the 1950s, amphotericin B (AmB) has been used in clinical practice to treat systemic fungal infections and leishmaniasis, a neglected parasitic disease that can be fatal if left untreated. Fungizone[®] (a micellar dispersion) was the “gold standard” for more than three decades but due to the safer profile of novel lipid-based medicines (AmBisome[®], Abelcet[®] and Amphocil[®]), it is now used as second-line in the developed world. Lipid-based medicines possess a more favourable safety profile (mainly lower nephrotoxicity and infusion-related side effects) allowing the administration of larger doses and therefore similar efficacy with fewer administrations. However, all formulations require parenteral administration because AmB has low oral bioavailability (0.2-0.9%) due to the precipitation in aqueous media. In the last decade, strong partnerships between academia and industry has led to the development of innovative drug delivery systems able to deliver and target orally AmB in effective concentration while reducing its nephrotoxicity and infusion-related side effects. Currently, three major platform technologies (cochleates, chitosan nanoparticles and SEDDS) that are undergoing clinical trials that are discussed in this review. The pharmacokinetic and pharmacodynamic profile against visceral leishmaniasis, systemic candidiasis and aspergillosis of novel delivery systems will also be discussed.

Keywords: Amphotericin B, Ambisome[®], oral delivery, cochleates, self-emulsifying drug delivery systems (SEDDS), chitosan nanoparticles.

Introduction

Since the 1950s, amphotericin B (AmB) is used in clinical practice to treat initially systemic fungal infections and several years later also leishmaniasis, a neglected parasitic disease that can be fatal when left untreated [1, 2]. A micellar dispersion of AmB and sodium deoxycholate (Fungizone[®]) was considered the “gold standard” for more than three decades due to its broad-spectrum activity and low incidence of clinical resistance in spite of causing severe adverse effects such as nephrotoxicity, anaemia and infusion-related side effects [3]. However, Fungizone[®] was relegated to a second-line treatment in developed countries when lipid-based medicines (AmBisome[®], Abelcet[®] and Amphocil[®]) were marketed in the 1990s. Lipidic formulations exhibit similar efficacies but more favourable safety profiles (mainly lower nephrotoxicity) which allows the intravenous administration of higher doses (5-10 mg/kg compared to the 0.5-1 mg/kg recommended dose for Fungizone[®]) [4, 5]. Infusion-related side effects and the need for patient hospitalization to monitor for AmB side effects remain still an issue for lipidic formulations and limits the access to AmB treatment in developing countries. An oral self-administered AmB formulation can overcome these challenges, however, this has not yet been materialized.

Why has an oral AmB medicine not been marketed till now?

In clinical practice, AmB is administered orally only when a local action is desired, for example to prevent nosocomial infections in the oesophageal and gastrointestinal tract [6]. The main reason of requiring IV administration is the low fraction of AmB that is orally absorbed (0.2-0.9%) due to the precipitation of crystalline drug in aqueous media (Figure 1a) [6, 7]. The zwitterionic and amphiphilic character of AmB with an asymmetrical distribution of ionisable carboxyl and primary amine groups and hydrophobic and hydrophilic groups respectively leads to poor aqueous solubility (< 1 mg L⁻¹ at physiological pH) [7, 8]. AmB violates Lipinski's rule of five possessing a molecular weight of 924 Da, 12 H-bond donors and 18 H-bond acceptors [3, 9]. Thus, to achieve clinical effective AmB concentrations in targeted organs after the oral administration, chemical modification of the drug or reformulation using appropriate excipients is required. The latter will require excipients able to increase AmB's aqueous solubility [10, 11] as well as ensure stability of the drug throughout all the harsh environment of the gastrointestinal tract (GIT) such as the low pH of the stomach and

facilitate the drug absorption through the enterocytes (reaching the portal blood) or M-cells (till the lymphatic route).

Pharmacokinetics of oral AmB

AmB was originally orally studied for its pharmacokinetics in humans in 1957 even at very high doses of 2-10 g daily (Table 1) [12-14]. Two decades later [6], lower doses of AmB were orally administered (30-40 mg/day) for infections of the GIT. Interestingly both high doses and lower doses resulted in similar plasma concentrations (Table 1). Researchers speculated that the absorption process may be saturated (reaching a maximum plateau) after oral administration and thus, multiple administrations of lower doses could results in higher plasma levels than a single high dose of AmB.

Advancements in drug delivery technologies and the emergence of nano-enabled formulations have enhanced research efforts of academic and industrial researchers in the last two decades. Various delivery strategies including polymeric and solid lipid nanoparticles [15-17], micellar dispersions [18], cochleates [19], nanosuspensions [20], carbonanotubes [21] and lipid-based nanomedicines [22-24] have been reported with varying degrees of success in achieving effective plasma and tissue after oral administration and moving further in human clinical trials (Table 2-6).

Single dose pharmacokinetics studies in animals have illustrated that tissue-plasma ratio is a key factor to take into account after oral administration of AmB (Table 2). A low plasma concentration does not necessarily indicate that the formulation is ineffective. Due to its physicochemical properties, AmB accumulates in tissues for longer periods of time leading to long half-life after intravenous administration [25]. Tissue accumulation is similarly observed after oral administration. For this reason, determination of AmB levels in organs of interest such as liver and spleen for visceral leishmaniasis and lungs, kidneys, brain and liver for fungal infections is critical in the evaluation of an AmB oral candidate formulation. Bearing in mind the *in vitro* effective concentrations of AmB against fungal and parasitic strains (reported IC₅₀ between 0.1 to 2 µg/ml [26-28]), levels reached in tissues of interest equal or higher to the *in vitro* IC₅₀ after oral administration could be effective.

After evaluating the published data on single oral administration of AmB in rodents, several conclusions can be drawn (Table 2). Saturated absorption impacts on the overall

oral bioavailability and AmB follows a non-linear pharmacokinetic profile. No significant higher levels were achieved when a dose of 50 mg/kg of Peceol-AmB was administered compared to 5 mg/kg [18, 24, 29]. P-glycoprotein ATPase activity may be involved in the saturation process. However, Osei-Twum et al. have shown that AmB was not a substrate of P-glycoprotein mediated efflux [30]. Lipid-based formulations appear to have a later T_{max} (between 2-12.5 hours) and the T_{max} increases with increasing dose. Lymphatic absorption can contribute to the later T_{max}. Doses below 5 mg kg⁻¹ fail to produce plasma levels above 0.1 µg mL⁻¹ which are required *in vitro* to elicit an effect. The situation is more complicated for polymeric nanoparticles. Polylactic-co-glycolic acid (PLGA, 50:50) nanoparticles have illustrated a sustained response, while chitosan based nanoparticles illustrated an early onset T_{max} and greater plasma concentrations (Table 2). Single dose pharmacokinetic levels in both plasma and tissues are critical for designing a multiple dose regime that can prove to be more efficacious exploiting the accumulation of AmB in the body and its high tissue distribution. The gastrointestinal toxicity of AmB, which manifests as mild nausea, vomiting, diarrhea and loss of appetite, remains a major limitation for oral strategies taking into account the higher oral AmB dose administered needed for ensuring oral bioavailability and tissue accumulation. A high oral dose combined with non-linear and variable pharmacokinetics might result in acute nephrotoxicity and this needs to be taken into account in multiple dose regimes.

Multiple doses have been reported for lipid-based and polymeric formulations with doses ranging between 2.5-20 mg kg⁻¹ twice daily. Similarly, a dose above 5 mg kg⁻¹ elicited adequate plasma concentrations and an equivalent or higher AmB concentration in liver compared to kidney. However, in visceral leishmaniasis (VL) infected animals, a lower accumulation in liver has been reported compared to non-infected animals after intravenous administration of Ambisome (2 mg kg⁻¹) or lipid-based nanoemulsions orally (10-20 mg kg⁻¹ twice daily for 5 days). Researchers have speculated that the liver and spleen macrophages of VL infected mice lose their phagocytic activity [24]. Macrophages infected with *L. donovani in vitro* illustrate a lower phagocytic activity compared to uninfected cells [31].

The most successful formulations able to deliver greater amounts of drug in tissues in rodent model were cochleates [19], lipid-based systems [22, 29] and polymeric nanoparticles [15]. From all the formulations, AmB-GCPQ (N-palmitoyl-N-methyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan) nanoparticles showed oral

bioavailability not only in rodents but also in dogs and possesses one major differential characteristic compared the other formulations: preferential drug accumulation in liver, spleen and lung leading to highest spleen/kidney and high liver/kidney and lung/kidney ratios. Cochleates were the only formulation to show almost four fold accumulation in the lungs compared to the liver and kidneys [19].

Pharmacodynamics of oral AmB

AmB activity after oral administration has been mostly tested against VL, systemic candidiasis and aspergillosis (Tables 4-6) but also research against other parasitic diseases such as trypanosomiasis is recently published [32].

The size of particulate formulations is a key characteristic for ensuring adequate targeting of AmB strategies in murine VL models to reach reticuloendothelial (RES) organs (Table 4). Poor activity levels were achieved after the oral administration of a nanosuspension consisting of polysorbate 80, pluronic F68 and sodium cholate with a particle size greater than 500 nm [20]. However, greater parasitaemia reduction in liver and spleen was obtained with: i) AmB attached to functionalized carbon nanotubes (long axial, width approximately 50 nm) [21], ii) lipid-based emulsions such as iCo-10 (approximately 200 nm) composed of 60/40 (v/v) mono- and diglycerides (Peceol/Gelucire 44/14) with vitamin E-TPGS (D- α -tocopheryl polyethylene glycol succinate) [33, 34] and iii) polymeric AmB-GCPQ nanoparticles with similar particle size (approximately 200 nm) [15]. Therapeutic regimes of 2.5 mg/kg twice daily were insufficient to eradicate the parasitemia while doses equal or above 5 mg/kg once or twice daily for at least 5 consecutive days were able to deliver enough AmB to elicit its pharmacological effect. *Leishmania* is an intracellular parasite accumulated in the RES organs (liver, spleen, bone marrow) and targeting these organs will lead to a better therapeutic outcome. The oral route is ideal for liver targeting as the drug reaches the liver via the portal blood circulation after absorption, while lymphatic uptake can enable bone marrow targeting.

A complication in comparing the efficacy of oral AmB formulations in aspergillosis is the use of different infection models (Table 5), which use different number of colonies to induce the infection that impacts in treating animals of varying degrees of neutropenia with tested formulations and does not allow direct comparison of the strategies and doses used. Compared to parasitic infections like VL, fungal aspergillosis caused by *A. fumigatus* is more severe and higher doses ($> 15\text{-}20\text{ mg kg}^{-1}$) and longer duration of therapy (>10

days) are necessary to prolong the survival rate and inhibit the growth of *Aspergillus* in infected tissues. Also, the infection is more disseminated, thus, higher levels of AmB are required not only in liver and spleen but also in plasma, lungs, kidneys and brain. Oral AmB strategies for aspergillosis involved lipid based systems [35, 36], polymeric nanoparticles [15, 17] and cochleates [37], with the latter being the most successful formulation in preclinical studies. The same formulations were also tested in a systemic candidiasis animal model and treatment for longer periods of time was more efficacious at eradicating the *C. albicans* from the infected tissues after oral AmB administration (Table 6) and are the ones closer to translating into an oral AmB formulation.

A step closer to oral AmB

Engineering oral AmB drug delivery systems requires: i) an increased AmB dissolution in the gastrointestinal tract, (ii) enhancement of lymphatic absorption, (iii) reduction in drug degradation in the acidic pH of the stomach, (iv) enhancement of the gastrointestinal transit and (v) finally targeting of the formulation in organs of interest and reducing off-target side-effects (e.g. nephrotoxicity). The three most promising delivery strategies that have entered clinical trials are described in more detail below:

1. CAMB- Amphotericin B cochleates (Aquarius Biotechnologies- Matinas Biopharma) [38]. Clinical trials phase I have been completed and currently, the company is recruiting participants to initiate a Phase II clinical trial [39]. This platform technology is based on the encapsulation of AmB within cochleates which are stable cationic phospholipid precipitates composed of commonly used excipients such as phosphatidylserine and calcium. They have a multilayer structure consisting of a large, continuous, solid lipid bilayer sheet rolled up in a spiral without aqueous space (Figure 1b) [40]. The structure of the cochleates provides protection from the acidic gastric pH because the entire cochleate structure is composed of a series of solid layers and even though the outer layers can be exposed to harsh environmental conditions, the interior of the cochleate remains intact. AmB cochleates have shown comparable efficacy *in vivo* to parenteral liposomal AmBisome® in three disease states (VL, candidiasis and aspergillosis) [37, 40-42]. However, the scaling-up process can be complex as one of the major limitations is that the cochleate structure is not always uniform resulting in either aggregates or stacked sheets and cochleates or large size needles-like structures [43].

2. NM0147 METAmphizon (Nanomerics) [44]. AmB nanoparticles are obtained using Molecular Envelope Technology (MET) and engineered from a biocompatible polymer. AmB is encapsulated within N-palmitoyl-N-methyl N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan (GCPQ) nanoparticles of about 200 nm in size [15]. The particles have a core-shell structure with the ionic units forming the particle shells and the hydrophobic groups forming the particle core (Figure 1c). The hydrophobic tail of the polymer has the same number of carbons as the hydrophobic domain of AmB, which enhances the hydrophobic interaction and the formation of a nanocomplex [15]. The oral bioavailability of AmB-GCPQ nanoparticles is 24% and similar to AmB cochleates, AmB encapsulated in GCPQ nanoparticles also has shown a high efficacy against candidiasis, aspergillosis and VL compared to parenteral administered AmBisome® [15]. Currently, METAmphizon is in clinical trials Phase I and preclinical studies have shown ability to: protect AmB from gastric degradation, reduce nephrotoxicity and enhance lymphatic absorption while target to lung, liver and spleen. The lower accumulation in the kidneys is favourable as the nephrotoxicity is reduced but at the same time, it can be a limitation in those cases where the microorganisms are localised in the kidneys.

3. iCo-Amphotericin B (iCo Therapeutics Inc) [45]. iCo Therapeutics has developed self-emulsifying drug delivery systems (SEDDS) to enhance oral AmB bioavailability which are currently in Phase I. There are two main formulations in their pipeline: iCo-009 and iCo-010. The iCo-009 system is composed of AmB mixed with Peceol® (glyceryl monooleate) and distearoylphosphatidylethanolamine (DSPE)-(PEG)₂₀₀₀ and is characterised by excellent homogeneity and no crystalline features [35]. Accumulation of AmB in liver, spleen, kidney and lung tissues was reached after the oral administration of the formulation without inducing gastrointestinal toxicity leading to high efficacy against candidiasis, aspergillosis and leishmaniasis [22]. The iCo-010 system is a novel oral AmB formulation consisting of Peceol®, Gelucire® 44/14 (lauroyl macrogol-32-glycerides) and vitamin E-TPGS that remains highly effective against murine systemic candidiasis following exposure to tropical temperatures (43 °C). This is advantageous bearing in mind that neglected diseases occur mainly in tropical regions and AmB is a thermally unstable drug [46]. However, one of the limitations of this system is the low level of AmB solubilized in the aqueous fraction (<20%) during lipolysis studies which is indicative of the amount of the drug that is available for absorption [47].

Oral administration can enable liver targeting as AmB will be transported to the liver, an important site of replication of the leishmania parasite, via the portal circulation. Oral administration of particulate formulations (<300nm) can target M cells and result in lymphatic uptake and has been shown to play a key role in the oral bioavailability of both AmB-GCPQ nanoparticles and SEDDS [15, 48]. Khan et al. have suggested that a particle size of 100-500 nm is optimal for lymphatic uptake via the GI lymphatic system [49, 50]. Apart from particle size, the particle composition is critical. Chitosan polymers have been shown to adsorb great amounts of bile salts [51] during their transit through the GIT which can facilitate enterohepatic recirculation and enable their incorporation into chylomicrons and entrance in the lymphatic vessels, a process similar to what has been described for lipid-based SEDDS [52]. Bearing in mind that the *Leishmania* parasites reside in lymph nodes and they accumulate in RES tissues, the lymphatic uptake of AmB facilitates accumulation of the drug in tissues of interest enabling efficacious treatment of this parasitic disease [53].

Translating oral nanomedicines of AmB: cost-effectiveness

Even though the number of doses needed will be higher in the oral regime compared to a single IV administration of the drug to obtain the same level of efficacy, the overall cost of treatment may be lower taking into account both direct and indirect costs. Parenteral administration requires patient hospitalization and drug monitoring due to the acute and sometimes fatal infusion-related adverse effects such as anaphylactic reactions and nephrotoxicity which prolongs hospitalization. Parenteral formulations need to be sterile, while oral formulations such as the SEDDS or polymeric nanoparticles are cheaper to produce under GMP conditions and are easily scalable compared to liposomes (AmBisome®). Oral AmB permit self-administration and can be distributed in rural areas and results in fewer adverse effects. Moreover, oral AmB formulations have been shown to be stable at tropical conditions not requiring a cold transport chain and cold storage reducing further the final cost of the oral formulations.

Clinical translation of oral AmB has been only evaluated in VL and not in other clinical manifestations of leishmaniasis such as mucocutaneous (MCL) and diffuse cutaneous (DCL) forms alone or in combination with topical treatments.

Future perspectives and concluding remarks

Several years before an oral AmB would be unthinkable. Higher doses and longer duration of treatment than those used parenterally are required to eradicate the microorganisms from affected tissues. Further toxicological studies in humans upon longer administration periods are required to assess the risk-benefit ratio. However, in the last decade, strong partnerships between academia and industry has led to the development of innovative drug delivery systems able to deliver oral AmB in enough concentration in targeted tissues to elicit its pharmacological effect while reducing its nephrotoxicity and infusion-related side effects. Currently, the road to market for oral AmB medicines is paved by three major platform technologies (cochleates, chitosan nanoparticles and SEDDS) undergoing clinical trials. Apart from the toxicity, other major limitations of these systems that have to be carefully taken into consideration are the scaling-up of the fabrication as well as the polymer synthesis and GMP production in order to get overall cost-effective formulations allowing the access of oral AmB treatment worldwide especially in developing countries.

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Figure captions:

Figure 1. AmB electron micrographs: A) TEM of Crystals of AmB in dextrose 5% (w/v) (reprinted with permission from [15]. Copyright (2015) American Chemical Society); B) Electron micrograph freeze-fracture of cochleates [54]; c) TEM of AmB- GCPQ nanoparticles in deionised water (reprinted with permission from [15]. Copyright (2015) American Chemical Society).

Table 1: Oral pharmacokinetics studies in humans with AmB. Key: - data non available.

Formulation	Number of patients	Treatment (duration)	Regime	Sampling	Cmax plasma (µg/ml)	Ref.
AmB	5	2 g / day	Single dose	-	0.040	[12]
	5	4 g / day (1 day)	Multiple		0.080	
	5	10 g / day (3 days)	Multiple		0.210	
AmB	15	4.2 g / day (2 days)	Multiple	-	0.180	[14]
Solubilised AmB	9	5.8 g / day (10 days)	Multiple	-	0.054	[13]
Tablet AmB 10 mg (Fungilin®)	3	30 mg / day (2 days)	Multiple	3 h after dose in the morning	0.068	[6]
	2	40 mg / day (2 days)	Multiple	3 h after dose in the morning	0.068	

Table 2: Single dose oral pharmacokinetics animal studies with AmB. Key: -, data no available; ND, no detectable, below limit of quantification; D-AmB, Sodium deoxycholate – Amphotericin B; Albelcet, AmB-lipid complex suspension; Intralipid-AmB, AmB incorporated into 10% intralipid; Peceol-AmB, AmB incorporated in 100% Peceol (Self-emulsifying drug delivery system formulation: SEDDS); PLGA: polylactic-co-glycolic Acid (50:50), DSPE/PEG2000 (iCo-009), distearoylphosphatidylethanolamine-poly-(ethylene glycol)2000; iCo 010, 60/40 (v/v) mono- and diglycerides with vitamin E-TPGS (D- α -tocopheryl polyethylene glycol succinate); AmB-cochleates* data are extrapolated from the graph pub. N°: US 2003/0228355 A1 patent; SLN, solid lipid nanoparticles; GCPQ- N-palmitoyl-N-methyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan.

Formulation	Animal model (n° animals)	Therapy	Collection of the samples	T max plasma (h)	Cmax plasma ($\mu\text{g/mL}$)	AmB concentration in tissues ($\mu\text{g AmB/ g tissue}$)						Ref.
						Liver	Spleen	Kidney	Lung	Brain	Heart	
D-AmB	Rat (n=10)	1.6 – 16.7 mg/kg	-	5 - 9	0.040 – 0.100	-	-	-	-	-	-	[55]
D-AmB (Fungizone®)	Rat (n=6)	5 mg/kg	24 h post-administration	ND	ND	ND	ND	ND	ND	-	ND	[18]
Albelcet®	Rat (n=6)	50 mg/kg		8	0.0398	ND	ND	0.009	0.731	-	ND	
Intralipid-AmB	Rat (n=6)	50 mg/kg		10	0.0485	ND	ND	ND	0.558	-	0.0459	
Peceol-AmB	Rat (n=6)	5 mg/kg		2	0.719	0.0134	ND	ND	43	-	ND	
Peceol-AmB	Rat (n=6)	5 mg/kg		2	1.187	0.0061	ND	ND	ND	-	ND	
Peceol-AmB	Rat (n=6)	50 mg/kg		4	1.469	0.0109	0.0765	ND	ND	-	0.0128	
AmB PLGA nanoparticles	Rat (n=3)	10 mg/kg	-	24	0.176	-	-	-	-	-	-	[56]
D-AmB (Fungizone®)	Rat (n=3)	10 mg/kg	-	6	0.0602	-	-	-	-	-	-	
Peceol/DSPE/PEG2000 - AmB	Rat (n=6)	4.5 mg/kg	72 h post-administration	6.3	0.071	0.017	0.091	0.079	0.117	ND	ND	[23]
	Rat (n=8)	10 mg/kg		12.5	0.096	0.033	0.039	0.283	0.055	ND	ND	
AmbiOnp (SLN)	Rat (n=4)	200 mg/kg	-	9	0.124	-	-	-	-	-	-	[16]
AmB dissolved in DMSO	Rat (n=4)	200 mg/kg		12	0.142	-	-	-	-	-	-	
AmB-Cochleates*	Mice C57BL16 (n=3)	10 mg/kg	1 h post-administration	-	-	0.04	ND	0.04	0.04	0.02	0.04	[19]
	Mice C57BL16 (n=3)	10 mg/kg	6 h post-administration	-	-	0.72	0.6	0.96	0.96	0.48	0.1	
	Mice C57BL16 (n=3)	10 mg/kg	24 h post-administration	-	-	0.4	0.36	0.16	0.22	ND	ND	
AmB-GCPQ nanoparticles	Mice CD-1 (n=4)	5 mg/kg	-	4	0.308	0.985	1.364	1.119	1.608	-	-	[15]
	Beagles (n=3)	4 mg/kg	-	8	0.065	-	-	-	-	-	-	

Table 3. Multiple dose oral pharmacokinetics animal studies with AmB. Key: -, data no available; ND, no detectable, below limit of quantification; Peceol (Self-emulsifying drug delivery system formulation: SEDDS); DSPE/PEG2000 (iCo-009), distearoylphosphatidylethanolamine-poly-(ethylene glycol) 2000; iCo 010, 60/40 (v/v) mono- and diglycerides with vitamin E-TPGS (D- α -tocopheryl polyethylene glycol succinate); VL-mice, visceral leishmaniasis infected mice; BID, twice a day; GCPQ- N-palmitoyl-N-methyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan.

Formulation	Animal model (n° animals)	Therapy (duration)	Collection of the samples	Conc. plasma ($\mu\text{g/mL}$)	AmB concentration in tissues ($\mu\text{g AmB/g tissue}$)						Ref.
					Liver	Spleen	Kidney	Lung	Brain	Heart	
AmB-Cochleates	Mice C57BL16 (n=3)	10 mg/kg/day (10 day)	Organs collected 24 h post-administration	ND	2.4	1.5	2.3	9.5	0.1	0.3	[19]
Peceol/DSPE/PEG₂₀₀₀ - AmB	Mice BALB/c (n=8)	2.5 mg/kg BID (5 days)	Organs collected 12 h post-administration	0.166	0.714	0.329	0.112	0.589	0.082	0.160	[22]
	Mice BALB/c (n=8)	5 mg/kg BID (5 days)		0.168	0.957	0.751	0.323	1.449	0.112	0.289	
	Mice BALB/c (n=8)	10 mg/kg BID (5 days)		0.390	3.151	1.920	0.928	4.076	0.160	0.626	
	Mice BALB/c (n=6)	20 mg/kg BID (5 days)		0.519	4.728	4.194	1.589	5.048	0.184	0.621	
Peceol/DSPE/PEG₂₀₀₀ - AmB	Mice BALB/c (n=5)	10 mg/kg BIB (5 days)	Organs collected 60 h post-administration	-	0.328	0.152	-	-	-	-	[24]
	VL- Mice BALB/c (n=6)	10 mg/kg BID (5 days)		-	0.125	0.233	-	-	-	-	
	Mice BALB/c (n=5)	20 mg/kg BID (5 days)		-	0.536	0.477	-	-	-	-	
	VL- Mice BALB/c (n=6)	20 mg/kg BID (5 days)		-	0.171	0.428	-	-	-	-	
iCo-010	Mice BALB/c (n=6)	2.5 mg/kg BID (5 days)	Organs collected 12 h post-administration	0.172	0.446	0.342	0.495	0.408	0.059	0.084	[29]
	Mice BALB/c (n=6)	5 mg/kg BID (5 days)		0.232	0.836	0.916	0.813	1.168	0.112	0.156	
	Mice BALB/c (n=6)	10 mg/kg BID (5 days)		0.418	2.543	1.407	2.268	2.014	0.157	0.338	
	Mice BALB/c (n=7)	20 mg/kg BID (5 days)		0.538	3.494	1.939	3.685	3.179	0.169	0.366	
AmB-GCPQ nanoparticles	Mice CD-1 (n=3)	5 mg/kg/day (5 days)	Organs collected 24 h post-administration	0.229	0.892	1.716	1.405	1.548	0.139	-	[15]
	Mice CD-1 (n=3)	5 mg/kg BID (5 days)	Organs collected 12 h post-administration	0.462	6.281	7.884	3.346	7.456	0.280	-	

Table 4. Oral efficacy studies with AmB against visceral leishmaniasis. Efficacy compared with non-treated controls. Key: -, data no available; ZP, zeta potential, p.i, post infection; f-CNTs, functionalized carbonnanotubes; iCo 010, 60/40 (v/v) mono- and diglycerides (Peceol/Gelucire 44/14) with vitamin E-TPGS (D- α -tocopheryl polyethylene glycol succinate); BID, twice a day; QD, four times a day; GCPQ- N-palmitoyl-N-methyl-N, N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan, p.i, post infection.

Formulation composition	Formulations characteristics	Animal model	Infection model	N° of animals	Therapy	Parasitemia load reduction (%)		Reference
						Liver	Spleen	
Nanosuspension 0.4% AmB (w/w), 0.5% Tween 80 (w/w), 0.25% Pluronic F68 (w/w), 0.05% sodium cholate (w/w)	Size: 528 nm ZP: - 38 mV Stability: > 21 days at 20 °	BALB/c mice	IV infection with 1 x 10 ⁷ <i>L. donovani</i> amastigotes. Treatment: 7 days p.i. Sacrifice: 14 days p.i.	-	5 mg/kg (4-5 days)	28.6	-	[20]
Lipid-based formulation	-	BALB/c mice	IV infection with 5 x 10 ⁷ <i>L. donovani</i> promastigotes. Treatment: 7 days p.i. Sacrifice: 14 days p.i.	4	2.5 mg/kg BID (5 days)	64.7	-	[33]
				4	5 mg/kg BID (5 days)	96.4	-	
				5	10 mg/kg BID (5 days)	99.5	-	
				5	20 mg/kg BID (5 days)	99.8	-	
iCo – 010	Size \approx 200 nm Stability: AmB > 75% over 60 days a 30 and 43 °C	BALB/c mice	IV infection with 5 x 10 ⁷ <i>L. donovani</i> promastigotes. Treatment: 7 days p.i. Sacrifice: 14 days p.i.	4	2.5 mg/kg BID (5 days)	83	-	[34]
				4	5 mg/kg BID (5 days)	98	-	
				4	10 mg/kg BID (5 days)	99	-	
				4	20 mg/kg QD (5 days)	96	-	
AmB attached to f-CNTs	-	Golden hamster	Intracardiac infection with 1 x 10 ⁸ <i>L. donovani</i> promastigotes. Treatment: 30 days p.i. Sacrifice: 37 days p.i.	8	5 mg/kg/day (5 days)	-	90.2	[21]
				7	10 mg/kg/day (5 days)	-	96.5	
				8	15 mg/kg/day (5 days)	-	98.2	
AmB-GCPQ nanoparticles	Size: 216 nm ZP: + 3 mV Stability: AmB > 95% at 4°C	BALB/c mice	Intracardiac infection with 1 x 10 ⁷ <i>L. infantum</i> promastigotes Treatment. 24 days p.i. Sacrifice: 31 days p.i.	8	5 mg/kg/day (5 days)	-	-	[15]
				8	5 mg/kg/day (10 days)	98.9	92.1	

Table 5. Oral efficacy studies with AmB against aspergillosis. Efficacy compared with non-treated controls. Key: -, data no available; CFU, colony forming units; p.i, post-infection; NSD, no significant differences (p>0.05) between treatment and control groups; DSPE/PE, distearoylphosphatidylethanolamine – polyethylenglycol, PLGA: polylactic-co-glycolic Acid; IP, intraperitoneally; SC, subcutaneously; BID, twice a day; GCPQ- N-palmitoyl-N-methyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan, p.i, post infection; * values obtained 8 days p.i.

Formulation	Animal model	Infection model	N° of animals	Therapy (duration)	% of survival		Aspergillus CFU reduction (%)						Reference
					After 4 days p.i.	After 14 days p.i.	Liver	Spleen	Brain	Lungs	Heart	Kidneys	
AmB-cochleates	BALB/c mice	Neutropenia with cyclophosphamide 200 mg/kg day -3 p.i. IV infection with 10 ⁶ spores of <i>A. fumigatus</i> . Treatment immediately after infection.	10	20 mg/kg/day (14 days)	70	60	-	-	-	-	-	-	[37]
			10	40 mg/kg/day (14 days)	90	70	-	-	-	-	-	-	
AmB incorporated into Peceol	Sprague-Dawley Rat	IV injection with 2.1-2.5 x 10 ⁷ CFU of <i>A. fumigatus</i> . Treatment: 48 h p.i.	7	50 mg/kg (4 days)	-	-	NSD	98	95	NSD	NSD	NSD	[36]
Peceol/DSPE/PEG ₂₀₀₀ – AmB	Sprague-Dawley Rat	IV injection with 2.9-3.45 x 10 ⁷ CFU of <i>A. fumigatus</i> . Treatment: 48 h p.i. Sacrifice: day 3	7	10 mg/kg BID (2 days)	-	-	NSD	90.1	79.2	NSD	NSD	87.9	[35]
AmB – PLGA nanoparticles	CD-1 mice	Neutropenia with cyclophosphamide 200 mg/kg IP+ cortisone 250 mg/kg SC: day -2 and +3 p.i. Infection by nebulization of 8.1 x 10 ⁸ CFU of <i>A. fumigatus</i> spores/ml 1h. Treatment: 5 h p.i. Sacrifice: 101 h p.i.	6	5 mg/kg	-	-	-	-	-	95.0	-	-	[17]
			6	5 mg/kg/day (4 days)	-	-	-	-	-	99.7	-	-	
		5	2 mg/kg	-	-	-	-	-	-	-	-	78.1	
		5	5 mg/kg	-	-	-	-	-	-	-	-	98.0	
AmB-GCPQ nanoparticles	OF-1 mice	Neutropenia with cyclophosphamide 200 mg/kg IP and fluorouracil 150 mg/kg IV at day -1 p.i. IV infection with 1 x 10 ⁴ CFU of <i>A. fumigatus</i> . Treatment: 24 h p.i. Sacrifice: 14 days.	10	5 mg/kg (10 days)	100	25	-	-	-	60*	-	40*	[15]
			15	7.5 mg/kg (10 days)	100	25	-	-	-	>95*	-	>95*	
			15	15 mg/kg (10 days)	100	20	-	-	-	>95*	-	>95*	

Table 6. Oral efficacy studies with AmB against candidiasis. Efficacy compared with non-treated controls. Key: NSD, no significant differences ($p>0.05$) between treatment and control groups; -, data no available; DSPE/PEG2000 (iCo-009), distearoylphosphatidylethanolamine-poly-(ethylene glycol) 2000; BID, twice a day. GCPQ- N-palmitoyl-N-methyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan, p.i, post infection.

Formulation	Animal model	Infection model	N° of animals	Therapy	% of survival	Candidiasis CFU reduction (%)				Ref.
					After 15 days p.i.	Lungs	Kidneys	Spleen	Liver	
Peceol/DSPE/PEG ₂₀₀₀ – AmB	Sprague-Dawley Rat	IV injection with $1-1.35 \times 10^6$ CFU of <i>C. Albicans</i> . Treatment: 48 h p.i. Sacrifice: day 3	5	5 mg/kg BID (2 days)	-	NSD	75	-	-	[35]
			7	10 mg/kg BID (2 days)	-	NSD	95	-	-	
AmB - cochelates	BALB/c mice	IV injection with 10^6 blastoconidia of <i>C. Albicans</i> . Treatment: 24 h p.i. Sacrifice: day 17 p.i.	10	0.5 mg/kg/day (15 days)	100	NSD	NDS	-	-	[57]
			10	1 mg/kg/day (15 days)	100	NSD	NDS	-	-	
			10	2.5 mg/kg/day (15 days)	100	100	99.97	-	-	
			10	5 mg/kg/day (15 days)	100	100	99.98	-	-	
			10	10 mg/kg/day (15 days)	100	100	99.97	-	-	
			10	20 mg/kg/day (15 days)	100	100	99.97	-	-	
AmB-GCPQ nanoparticles	BALB/c mice	IV injection with 1×10^6 CFU of <i>C. albicans</i> . Tretament: 24 h p.i. Sacrifice: 10 days p.i.	5	5 mg/kg/day (9 days)	100	-	NSD	100	100	[15]

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