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REVIEW ARTICLE



# Liposomal formulations in the pharmacological treatment of leishmaniasis: a review

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**ABSTRACT**

Conventional chemotherapy for leishmaniasis includes considerably toxic drugs and reports of drug-resistance are not uncommon. Liposomal encapsulated drugs appear as an option for the treatment of leishmaniasis, providing greater efficacy for the active and reducing its side effects by promoting superior tissue absorption, favouring drug penetration into the macrophages, and retarding its clearance from the site of action. In this paper, a review on the advances achieved with liposome-based anti-leishmaniasis drug delivery systems is presented. Formulations prepared with either conventional or modified (sugar-coated, cationic, niosomes, peptides- and antibodies-bounded) liposomes for the delivery of pentavalent antimonials, amphotericin B, pentamidine, paromomycyn, and miltefosine were covered. This literature review depicts a scenario of no effective therapeutic agents for the treatment of this neglected disease, where liposomal formulations appear to improve the effectiveness of the available antileishmania agents.

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## Leishmaniasis: biology and general aspects of the disease

Leishmaniasis is a group of endemic diseases caused by the *Leishmania* intramacrophagic parasite and mainly affects the poorest populations. According to the World Health Organization and the Special Program for Research and Training in Tropical Diseases, leishmaniasis is a tropical disease that is among the six most important parasitic diseases – from the point of view of public health – along with malaria, schistosomiasis, filariasis, Chagas disease, and trypanosomiasis (Desjeux and Alvar 2003, Okwor and Uzonna 2016).

The transmission of this disease is increasing at high rates in several areas of the world, as a consequence of situations that increase the likelihood of exposure to vector insects (phlebotomine), such as the establishment of new settlements in endemic, wild areas of high risk and areas where zoonotic transmission may occur; the deterioration of social and economic conditions in the poorer suburbs of cities; and increased migration of populations from rural to urban areas (Romero and Boelaert 2010).

Leishmaniasis is endemic in several regions of the world, including deserts and rainforests in tropical and subtropical regions of Africa, America and Asia, and suburban and rural areas of southern Europe (Davies *et al*. 2003, Rotureau 2006, Okwor and Uzonna 2016). It is estimated that 350 million people worldwide are at risk of becoming infected; about 12 million people are infected, and the annual occurrence is about 1.5–2 million cases of cutaneous form and 500 000 cases of the visceral form of the disease (McGwire *et al*. 2014).

Leishmaniasis causes considerable morbidity and mortality, and has traditionally been classified into three main forms (Table 1), based on their clinical manifestations (Handman 2001, McGwire *et al*. 2014). The most deadly form is visceral leishmaniasis (VL), which, if left untreated, leads to death. Several species of *Leishmania* cause cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), which, if not fatal, are responsible for considerable morbidity in a large number of people in endemic foci (Peters *et al*. 1983, McGwire *et al*. 2014).

*Leishmania,* as a flagellated promastigote, lives in the digestive tract of the phlebotomine vector, where it undergoes a series of distinct physiological and morphological transitions before differentiating into metacyclic promastigotes, a highly infective form of the parasite (Figure 1), which is introduced into the skin of the mammalian host during the blood sucking process. The promastigotes are resistant to lysis by the complement system and rapidly invade the macrophages recruited at the site of the mosquito bite. After internalization in the macrophage phagolysosome compartment, the parasites differentiate into small, non-mobile amastigotes. The amastigotes perpetuate the disease in the mammalian host as a result of its proliferation and continuous release due to lysis of infected macrophage or selective exocytic events that allow the invasion of other macrophages (Rittig and Bogdan 2000). This complex life cycle includes

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Table 1. Three major classes of leishmaniasis.

| Type of leishmaniasis | Main features | Leishmania species |
|---|---|---|
| Cutaneous leishmaniasis (CL) | Most common form of the disease. Localized injuries that heal themselves | *L. major, L. tropica, L. aethiopica* (in the Old World) and *L. mexicana, L venezuelensis, L. amazonensis, L. braziliensis, L. panamensis, L. guyanensis* e *L. peruviana* (in the New World) |
| Mucocutaneous leishmaniasis (LMC) | Injuries that cause extensive destruction of the oral and nasopharyngeal cavities | *L. aethiopica* (Old world), and *L. braziliensis, L. guyanensis, L. mexicana, L. amazonensis e L. panamensis* (New World) |
| Visceral leishmaniasis (VL) | The most devastating form of leishmaniasis. It affects spleen, liver (mainly) and bone marrow; characterized by progressive anaemia, pancytopenia, and hyperglobulinaemia | *L. donovani complex* (Old World) *and, L. infantum, (L. chagasi)* (New World) |

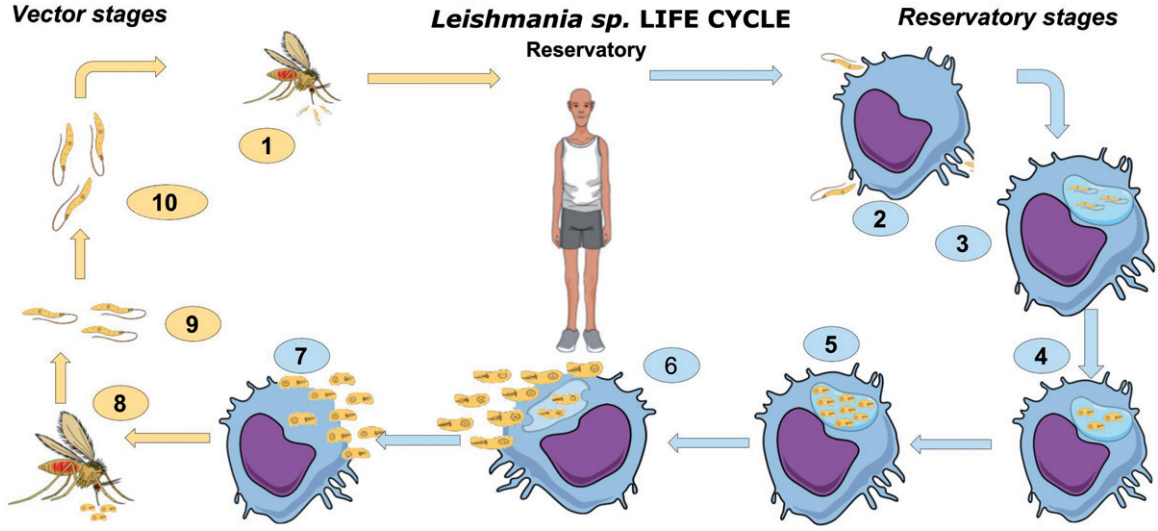


Figure 1. *Leishmania* life cycle. (1) Phlebotominae sandfly bites and takes a blood meal of the reservatory, injecting the parasitics promastigote forms. (2) Promastigotes are adhered by macrophages. (3) Promastigotes are phagocytized by macrophages. (4) Promastigotes are transformed into amastigotes inside the parasitophorous vacuole. (5) Amastigotes proliferation inside the parasitophorous vacuole. (6) Macrophage lysis and amastigote liberation. (7) Amastigote re-invasion in cells of various tissues. (8) Phlebotominae sandfly bites and takes a blood meal of the reservatory, ingesting cells infected with amastigote forms. (9) Amastigotes are transformed into procyclic promastigote forms in midgut. (10) Division and transformation into procyclic promastigote forms in midgut and migrate to proboscis.

several facets that could be exploited for drug design, optimization and development (Hammarton *et al.* 2003).

Tropical diseases, such as leishmaniasis, are globally dispersed and have a major socio-economic impact, affecting mainly poor people in developing countries. Therefore, commercial interest in the development of new pharmaceutical compounds for these diseases is limited because their treatment must be accessible to ensure access of the affected poor population. In addition, advances in understanding the biology of *Leishmania* have not been satisfactorily translated into effective chemotherapeutic compounds (Trouiller *et al.* 2002, Renslo and McKerrow 2006, Cruz *et al.* 2009, Handler *et al.* 2015).

Since our aim in this review is to present research carried out with drugs entrapped in liposomes as alternative therapies for the treatment of leishmaniasis, it is important to first describe, the conventional antileishmania agents employed thus far.

## Leishmaniasis treatment: conventional chemotherapy

The main therapeutic agents against leishmaniasis are pentavalent antimonials, amphotericin B (AmB), pentamidine, paromomycin, and miltefosine. Besides those, other chemotherapy agents *against* infectious *diseases* (e.g. primaquine and doxurrubicin) have been tried, as well as some alternative drugs such as oryzalin, trifluralin, furazolidone, desferrioxamine, bisnaphtalimidopropyl polyamide derivatives, and buparvacone (Pund and Joshi 2017). Nevertheless, none of them have proven to be ideal for the treatment of leishmaniasis due to high toxicity and serious adverse reactions, e.g. gastrointestinal disorders and cardiac arrhythmias, long duration of treatment, or by inadequate mode of administration leading to treatment withdrawal. In addition, even the drugs most frequently used, such as meglumine antimoniate (AME), and AmB do not completely eliminate the parasites, generating resistant parasites (Sundar *et al.* 2011, van Griensven and Boelaert 2011).

### Pentavalent antimonials

The first choice drugs for leishmaniasis treatment are pentavalent antimonials ($Sb^{V}$). They were first used by Brazilian physician Gaspar Vianna in 1912, in its trivalent form (trivalent antimony – $Sb^{III}$). After that, the so-called tartar emetic (a mixture of potassium tartrate and antimony), achieved

some success (Ram and Nath 1996). However, this formulation was difficult to administer and also presented toxicity, causing cough, chest pain and depression. In 1937, the treatment with sodium stibogluconate (Pentostan®), a drug derived from sotonic acid wherein the antimony is in the form ($Sb^V$) was described to reduce some side effects and toxicity of tartar emetic. Nowadays, the pentavalent antimonial used in *South American* and European countries is AME (Berman 1988, Handler *et al.* 2015), for which a liposomal formulation is presently in clinical trials (Bozzuto and Molinari 2015).

The antimonials have shown variable efficacy against CL, MCL, and VL, requiring intravenous or intramuscular injection (Murray *et al.* 2005). Due to side effects, such as elevated cardiotoxicity, pancreatitis and nephrotoxicity, patients with VL should be hospitalized and monitored (Zaghloul and Al-Jasser 2004, Shahian and Alborzi 2009).

The antimonials have a broad mechanism of action. The pentavalent antimony ($Sb^V$) enters the host cells (macrophages), crosses the phagolysosomal membrane and is converted to trivalent antimony ($Sb^{III}$). It is believed that the anti-leishmania mechanism of $Sb^{III}$ is related to its interaction with biomolecules containing sulfhydryl groups such as peptides, thiols, and enzymes (Frézard *et al.* 2009). Thus, $Sb^{III}$ acts against the amastigote form, where it compromises the redox potential of intracellular thiols, inducing their efflux and, consequently, inhibiting trypanothione reductase (Wyllie *et al.* 2004). The reduction of $Sb^V$ may be non-enzymatic – under acidic conditions such as those found in the phagolysosome – by glutathione, glycylcysteine and trypanothione, or enzymatically by thiol-dependent reductase (Denton *et al.* 2004) and AME reductase. $Sb^V$ can also kill parasites by indirect mechanisms, such as increased levels of cytokines (Pathak and Yi 2001). Antimony agents also act at the DNA level, inducing DNA damage *in vivo* (Lima *et al.* 2009) and inhibition of DNA topoisomerase I (Cheesman 2000).

### Amphotericin B

Amphotericin B deoxycholate or Fungizone® is a systemic antifungal agent that has been used in the treatment of leishmaniasis since the 1960s (Saha *et al.* 1986). Due to increasing resistance to antimonials, AmB is used as an alternative medicine for leishmaniasis (Calderón Gómez *et al.* 2015). It is highly toxic, which requires careful and slow intravenous administration, causing stiffness, chills and fever associated with myocarditis and nephrotoxicity (Berman 2009). AmB binds to ergosterol, the predominant sterol in *Leishmania*, but it also recognizes cholesterol in human cells, forming complexes that open pores in the membranes, altering ion balance and causing cell death (Roberts *et al.* 2003). Although expensive, AmB was the first antileishmania liposomal product launched, as discussed below.

### Pentamidine

Pentamidine is an aromatic diamidine, which has anti-trypanosomatid, antifungal, antibacterial, antiviral, and antitumor activity (Berman 1988). In the treatment of leishmaniasis, important adverse effects were reported such as diabetes mellitus, severe hypoglycaemia, hypotension, myocarditis, and renal toxicity, which can cause death (Singh *et al.* 2012). Although the drug is currently poorly used due also to the appearance of cases of resistance (Bray *et al.* 2003), and high toxicity associated with low efficacy (Jain and Jain 2013), a recent report claimed its appropriateness, at a single (7 mg/kg) doses regimen, to increase patients' adherence to treatment (Gadelha *et al.* 2015). In that phase II pilot study, 20 patients infected with *L. guyanensis* were treated and, despite the mild side effects observed (pain at the site of injection, indurated area followed by abscess formation at the site of injection) the authors claimed the bolus treatment to be as efficient as repeated intramuscular injections (4 mg/kg) every two days. The selective inhibitory action of pentamidine in a broad range of protozoan, including *Leishmania* would include DNA-binding and inhibition of replication, heme binding, inhibition of peptidase activity and disruption of calcium homeostasis (Sands *et al.* 1985), but it seems to play a special role in mitochondria where it accumulates, causing collapse of mitochondrial membrane potential (Bray *et al.* 2003).

### Paromomycin

Paromomycin is an aminoglycoside antibiotic, extracted from *Streptomyces rimosus*, licensed in Europe for the parenteral treatment of bacterial infections (Davidson *et al.* 2009). It has a large spectrum of parasitic activity, not seen in any other aminoglycoside antibiotic (Murray *et al.* 2005). Paromomycin is used as an alternative treatment of leishmaniasis: in the topical form (CL) or intravenously for VL (Jain and Jain 2013). However, parenteral formulations can cause serious adverse reactions, including nephrotoxicity and ototoxicity and, more rarely, hepatotoxicity (Fong *et al.* 1994, Jhingran *et al.* 2009). Its mechanism of action is the inhibition of protein synthesis, through binding to ribosomal proteins inducing the misreading of mRNA. In this way, it interferes in the complex of peptides formation and causes disruption of the polysomes in nonfunctional monosomes. Paromomycin also affects mitochondrial membrane potential, leads to airway dysfunction, and changes membrane fluidity and lipid metabolism (Jhingran *et al.* 2009).

### Miltefosine

Miltefosine (hexadecylphosphocholine) was originally developed for the treatment of cancer (Unger *et al.* 1989). The drug has been used in India for the treatment of patients with VL refractory to conventional antimonial treatment, with very promising results (Sindermann *et al.* 2004) and currently stands out as the best alternative for the treatment of VL, since the drug can be administered orally, unlike others (Sundar and Olliaro 2007, McGwire *et al.* 2014). However, its use is prevented for pregnant women, since their most serious side effects are the induction of teratogenesis, in addition to the a high rate of treatment failure (Sundar

*et al.* 2011). The mechanism of drug action may be direct against *Leishmania*, affecting its lipid metabolism (Croft and Olliaro 2011) leading to apoptosis of the parasite (Paris *et al.* 2004). Miltefosine has also been shown to act in the host cell, stimulating the production of nitric oxide synthetase 2 that catalyses the generation of nitric oxide, which kills the parasite inside the macrophage (Wadhone *et al.* 2009).

Besides the drugs mentioned above, other compounds with some efficacy against leishmaniasis are rifampicin, tamoxifen, and allopurinol among others (Kandil 1973, Croft and Coombs 2003, Croft *et al.* 2006, Singh *et al.* 2012).

In relation to selective leishmanicidal activity, pentavalent antimony may act against different *Leishmania* species, while pentamidine has limited activity against specific *Leishmania* species (Alvar *et al.* 2006). Paromomycin can be used topically to treat localized leishmaniasis caused by *L. braziliensis*, but not by other *Leishmania* species (Minodier and Parola 2007).

Drugs targeting intracellular pathogens should overcome the host cell membrane barriers and release and maintain the drug intracellular, at a therapeutic level and for a desired period of time. But the development of multidrug resistance is increasing, which makes the treatment of intracellular diseases even more challenging (Chakravarty and Sundar 2010). For instance in the case of *L. mexicana and L. donovani* resistant to AmB and pentamidine, which presents alterations in the levels of saturated fatty acids that affect membrane fluidity, making it difficult to keep the drugs within the parasite (Mbongo *et al.* 1998, Al-Mohammed *et al.* 2005). Therefore, there is a need for the development of advanced treatment methods to better control intracellular infections. Another study showed a quick development of resistance to miltefosine in *Leishmania* promastigotes, seen by a decreased miltefosine accumulation in the cell. The lower miltefosine accumulation could be achieved by two independent mechanisms, an increase in drug efflux, mediated by the overexpression of the ABC transporter P-glycoprotein and a decrease in drug uptake, which is easily achieved by the inactivation of proteins known to be responsible for the miltefosine uptake, the miltefosine transporter LdMT (Pérez-Victoria *et al.* 2006).

An alternative approach for the treatment of leishmaniasis is the use of sustained release systems, such as liposomes. Such systems provide greater efficacy and safety once the drugs are adsorbed or encapsulated in carriers, reducing the dose and adverse reactions of conventional formulations.

## *Liposomes in drug delivery*

As drug delivery vehicles, liposomes are phospholipid micro or nanocapsules organized in bilayers and concentrically enclosing one or more aqueous spaces. When some lipids are suspended and agitated in excess of aqueous solution, they spontaneously give rise to a population of vesicles that can range in diameter from tens of nanometres to tens of microns; and in the number of lipid bilayers (Figure 2(A,B)) (Samad *et al.* 2007).

During its formation, the liposome encapsulates part of the aqueous medium in which it is dispersed encompassing the hydrophilic substances present in the medium. The bilayer, in turn, is able to accommodate hydrophobic molecules and behaves as a semipermeable membrane, relative to the material encapsulated in the aqueous core of the vesicles. In addition, substances with an amphipathic nature can be partitioned in the lamellar interface (Figure 2(C)). Due to this versatility in carrying water-soluble compounds and lipids, liposomes are considered drug-delivery carriers of wide application (Bawarski *et al.* 2008), being able to deliver drugs, proteins, enzymes, and genetic materials to living cells

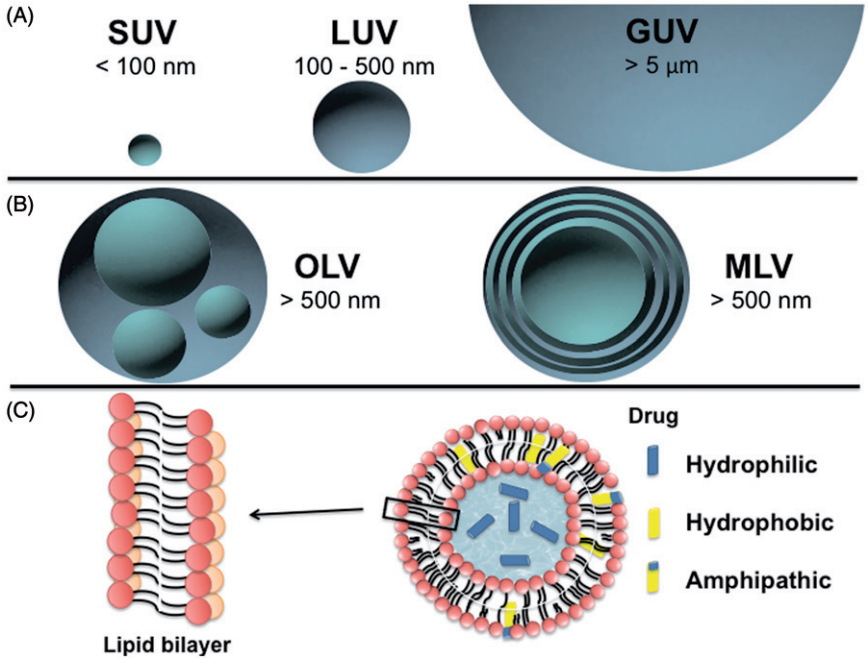


Figure 2. Liposomes in drug-delivery. (A) Unilamellar vesicles of increasing size: SUV, LUV and GUV. (B) Oligo (OLV) and multivesicular (MLV) vesicles. (C) Representation of the lipid bilayer and incorporation of hydrophilic, hydrophobic and amphipathic compounds inside the liposomes.

(Torchilin 2012). The ability of liposomes to trap hydrophilic and hydrophobic drugs with modulation of their activity, as well as their versatility and ability to modify their surface is the main factor responsible for their popularity in drug delivery research.

The most common classification describes the liposomes in relation to the number of concentric lipid bilayers they have (e.g. uni, oligo, or multilamellar vesicles), which can be small, large or giant (SUV, LUV, or GUV, respectively), as depicted in Figure 2 (Torchilin 2012).

The role of liposomes as carriers is to release the drugs encapsulated therein into specific targets (Grant and Bansinath 2001). Liposomes have been used in immunoassays and as sustained release systems for a diversity of drugs including antivirals, antifungals, antineoplastic, and antileishmaniasis drugs (Rongen *et al.* 1997, Allen and Cullis 2013, Bozzuto and Molinari 2015). The drug encapsulated in liposomes appears as an alternative treatment, since the active principle is protected from elimination and/or rapid degradation. Liposomes may be internalized and slow drug release into the target cell, potentiating its action and reducing undesirable side effects, such as systemic toxicity induced by high plasma levels.

In general, several properties influence the *in vivo* performance of liposomes, including lipid composition, phase transition temperature of their constituent phospholipids, method of preparation, lamellarity, size and surface charge (Allen and Cullis 2013).

## Liposomes in the improvement of anti-leishmania therapy

After intravenous injection, liposomes can be rapidly removed by the phagocytic cells of the spleen and liver (also referred to as passive targeting) and, at least in part, are located in the lysosomes of these cells. Such observation has stimulated scientists to explore liposomes application to the treatment of bacterial and parasitic intracellular infections (Bakker-Woudenberg 1995). In fact, one of the first applications of liposomes was its targeting for macrophages, in the treatment of *Leishmania* infection (reviewed by Owais and Gupta 2005).

Liposomes are better studied for treatment of leishmaniasis than for any other parasitic disease, mainly because *Leishmania* colonizes macrophages, which are also responsible for liposome clearance *in vivo*. Thus, the use of conventional liposomes for the targeting of anti-leishmaniasis drugs with the possible reduction in their toxicity profile was early envisaged. The efficacy of liposomal formulations in the treatment of leishmaniasis is related to their clearance by phagocytes resident in the liver, spleen, and bone marrow, what is very formulation specific (Frézard *et al.* 2000, Pund and Joshi 2017).

In the following sections, we discuss studies addressed with conventional and modified liposomes (sugar-coated, cationic liposomes, niosomes, liposomes modified with peptides for macrophage activation, immunoliposomes) in the context of leishmaniasis, from drug delivery to clinical studies.

### Conventional liposomes

For the therapy of leishmaniasis, as mentioned earlier, antimonials are the drugs of choice, despite their recognized toxic effects. Accordingly, they are also the most studied antileishmania agents, in drug delivery (Pund and Joshi 2017). Due to that, Table 2 provides a detailed survey of literature, involving the use of antimonials encapsulated in liposomes for the treatment of leishmaniasis. As shown in Table 2, the first reports in rodent and dog models occurred in the 1980s (Alving *et al.* 1978a, 1978b, 1980, 1984, Alving and Steck 1979, Weldon *et al.* 1983, Chapman *et al.* 1984, Berman *et al.* 1986), when different authors verified that liposomal formulations were up to 700 times more effective in the treatment of VL, when compared to treatment with non-encapsulated antimonials, using lyophilized SUVs composed of distearoyl-glycero-phosphocholine, cholesterol, dodecylphosphocholine (5:4:1 mol%), reconstituted in phosphate buffer, pH 7.2. In addition, electron microscopy studies revealed significant disintegration of *Leishmania* in hamster Kupfer cells after treatment with such antimonial liposomes, possibly resulting from phagolysosomal fusion (Alving *et al.* 1978a). After those, the efficacy of various antimonial drugs such as AME or sodium stibogluconate when encapsulated in liposomes has been compared to that of the free drug, showing to be superior always in the liposome formulations (Frézard *et al.* 2000, Frézard and Demicheli 2010, Schettini *et al.* 2006, Kalat *et al.* 2014, New *et al.* 1983, Sinha *et al.* 2015). In 2013, Momeni *et al.* (2013) prepared liposomal formulations for three antileishmanial drugs: glucantime, miltefosine, and paromomycin, using different lipids and a modified emulsion/freeze dry process. The encapsulation efficiency (%EE) of the liposomes prepared by this method was up to 90%, but the liposomes had bimodal size distribution, which caused the %EE to decrease 50% during the sterilization and filtration steps. These authors also showed that the effect of the surface charge is significant on the preparation process, with smaller sized liposomes and higher %EE in formulations with negatively charged lipids (zeta potential = –60 mV). In our research group, the leishmanicidal effect of a liposomal formulation (composed of egg PC, Chol, PS, and α-tocopherol) for the sustained release of AME has been investigated. This formulation was prepared, physicochemically characterized and *in vitro* evaluated in dog macrophages infected with *L. infantum*. The liposomal formulation promoted a significant decrease (>50%) in parasite load of infected canine macrophages in comparison to the free AME treatment (Gaona *et al.* unpublished results).

Considering the problem of resistance associated with antimonials, efforts were also made to evaluate the efficacy of second-line agents, such as miltefosine (Papagiannaros *et al.* 2005) and mainly, AmB (Bray *et al.* 2003, Hammarton *et al.* 2003, Minodier and Parola 2007, Nylén and Gautam 2010) in liposomal form, for the treatment of all types of leishmaniasis. Papagiannaros *et al.* (2005) demonstrated advantages of using liposomal miltefosine over free miltefosine, such as the improvement in the susceptibility of miltefosine-resistant *Leishmania* promastigotes.

Liposomal amphotericin B (L-AmB) was 350–750 fold more active than AME, and 2–5 times more active than

Table 2. Liposome-encapsulated antimonial formulations for the leishmaniasis treatment.

| Year | Treatment(s) | Dose(s) | Type of liposome | Composition and proportions (mol%) | Results | Reference |
|---|---|---|---|---|---|---|
| 1978 | Experimental infection ***L. donovani*** in Hamsters<br>**Administration route:** Intravenous and intracardiac | 0.3–1.3 mg Sb/kg<br>1.3–2.5 mg Sb/kg<br>2.5–5.2 mg Sb/kg<br>10–30 mg Sb/kg<br>40–80 mg Sb/kg<br>For 4 days | Positively and negatively charged MLV's<br>pH: 7.3 | DMPC/CHOL/DCP (2:1.5:0.22 and 2:1.5:0.88)<br>SM/CHOL/DCP<br>SM/CHOL/SA<br>Egg-PC/CHOL/DCP<br>Egg-PC/CHOL/SA<br>DSPC/CHOL/DCP<br>DSPC/CHOL/SA (2:1.5:0.22)<br>DMPC/CHOL/SA (2:1.5:0.22; 2:1.5:0.44; 2:1.5:0.88) | Liposomes containing Egg PC (positively charged) are less effective than negatively charged liposomes.<br>Liposomes containing PS (negatively charged) were the least effective.<br>Liposomes containing highly saturated long chain phospholipids (DPPC), CHOL and a negative charge were the most effective.<br>The concentration range of 1.3–2.5 mg Sb/kg of encapsulated drug resulted in a concentration of 823 mg Sb/kg of free drug, 332–640 times greater in the prevention of leishmaniasis mortality.<br>Empty liposomes showed toxicity in the mice. | (Alving *et al.* 1978b) |
| 1978 | **Experimental infection:** *L. donovani*<br>***In vivo:*** Hamsters<br>**Administration route:** Intracardiac | 1 mg Sb/kg for 4 days | Negatively charged MLVs (2 μm) | DPPC/CHOL/DCP (2:1.5:0.22) | The dose required for 50% suppression of infection was approximately 350 times greater for the free drug compared to the encapsulated drug.<br>The increase in the antileishmania effect of the liposome-encapsulated drug may be greater in a chronic disease than in an acute infection.<br>At 17 days post infection the animals treated with the encapsulated drug had 61% parasite suppression, and the free drug had only 18% suppression. | (Alving *et al.* 1978a) |
| 1980 | **Experimental infection**: *L. donovani*<br>***In vivo:*** Hamsters<br>**Administration route:** Intracardiac, parenteral, intravenous, intramuscular | 12–25 mg Sb/kg | MLVs | DPPC/CHOL/DCP (2:1.5:0.22) | The encapsulated drug was 276 times more effective than unencapsulated antimoniate.<br>The suspensions with the drug encapsulated and unencapsulated together were 8.5 times more effective than the drug alone.<br>20/21 animals were free of parasites 5 weeks after infection.<br>Empty liposomes did not show leishmanicidal activity. | (Alving *et al.* 1980) |
| 1983 | **Experimental infection**: *L. donovani*<br>***In vivo***: Hamsters<br>**Administration route**: Intracardiac | 5 μL/g (L-AME) | MLVs<br>pH 7.2 | As in Alving *et al.* (1978a) | Liposome and parasite interactions were found with Kupffer cells.<br>Liposomes having saturated phospholipids as the main phospholipid, such as those made with DPPC or SM, are much more resistant to enzymatic attack by phospholipases C or A (found in the macrophage) when compared to liposomes made with egg lecithin.<br>Liposomes with high amounts of saturated phospholipids were more effective against leishmaniasis, compared to liposomes made with unsaturated phospholipids.<br>A change in the structure of the liposomes is shown, with the polymorphic cheek appearance in the degradation process, at the onset of drug release. | (Weldon *et al.* 1983) |
| 1984 | **Experimental infection:** *L. donovani*<br>***In vivo***: Hamsters<br>**Administration route:** Intracardiac | 11–42 mg Sb/kg | MLVs<br>pH 7.3 | As in Alving *et al.* (1978a) | The encapsulated drug was 100–120 times more effective than the free drug.<br>Death suppression in type II infection was 100%.<br>The suppression of death in type III infection was 80%. | (Alving *et al.* 1984) |

(*continued*)

Table 2. Continued

| Year | Treatment(s) | Dose(s) | Type of liposome | Composition and proportions (mol%) | Results | Reference |
|---|---|---|---|---|---|---|
| 1984 | **Experimental infection:** *L. donovani*<br>***In vivo*:** Dogs<br>**Administration route:**<br>Intravenous | 0.5 mL/kg<br>1.94 mg Sb/kg<br>(1 day)<br>0.61 mg/kg<br>(1–4 days)<br>0.061 mg<br>(1 day)<br>0.046 mg/kg<br>(10 days)<br>0.0152 mg/kg | MLVs<br>pH 7.3 | As in Alving *et al.* (1978a) | The highest percentage of inhibition was presented at the concentration of 0.61 mg L-AME/kg, at the treatment of four consecutive days, with 97.4%, close to the percentage of free drug 99.2% higher at the concentration of 104 mg Sb/kg for 10 days.<br>The SD 50 dose of L-AME was 0.029 mg Sb/kg and this same dose in the free drug was at 24 mg Sb/kg.<br>The 0.046 mg L-AME/kg/day dose had a low parasite suppression. This indicates that this dosage level is close to the minimum dose of drugs that may be effective when delivered directly to the parasite. | (Chapman *et al.* 1984) |
| 1997 | ***In vivo:*** Dogs<br>**Administration route:**<br>Intramuscular<br>Subcutaneous | 25–85 mg Sb/mL | MLVs<br>prepared with high-Speed dispersion<br>(250 nm) | Soy-PC/CHOL/DCP<br>(2:1:0.2) | The pharmacokinetics of L-AME have three phases: (1) absorption; (2) decrease of drug serum levels; (3) elimination phase.<br>The subcutaneous form has a slow absorption and little half-life.<br>The intramuscular form exhibits a rapid absorption, high concentrations of the drug in the plasma and arrives fast to the blood system. | (Valladares *et al.* 1997) |
| 2000 | **Experimental infection:**<br>*L. chagasi*<br>***In vivo*:** Hamsters<br>**Administration route:**<br>Intraperitoneal | 60 mg Sb/kg | MLVs, DRVs, SUVs<br>FDELs<br>pH: 7.2 | DCPC/CHOL/<br>DCP<br>(5:4:1)<br>(1:0.58 lipid/meglumine ratio) | DRV method was the most efficient for the encapsulation of meglumine antimoniate into liposomes.<br>All animals treated with L-AME showed less than 70 amastigotes per 1000 nuclei of the host cell in the liver or spleen, and some animals (4 of 9) appeared to be parasite free.<br>It is suggested that treatment with four doses of 2 mg Sb/kg per week with L-AME may be satisfactory, since each dose is 70-fold lower than the cumulative dose in one week with conventional treatment. | (Frézard *et al.* 2000) |
| 2001 | **Experimental infection**<br>*L. infantum*<br>***In vivo*:** Dogs<br>**Administration route:**<br>Subcutaneous and intravenous | 9.8 μg/kg de Sb | – | PC (94–98%) in sodium cholate emulsion | The concentration of Sb in plasma after intravenous administration of L-AME has two phases:<br>1. Rapid distribution in tissues and decrease of plasma Sb concentrations<br>2. Slower phase of drug decrease<br>The plasma concentration of the drug after administration of L-AME is always high and is maintained over a long period of time compared to the levels achieved by the free drug. | (Valladares *et al.* 2001) |
| 2003 | **Experimental infection:**<br>*L. chagasi*<br>***In vivo:*** Dogs<br>**Administration route:**<br>Intravenous | 3.8 mg Sb/kg | FDELs<br>pH 7.2 | DSPC/CHOL/DCP<br>(5:4:1) | Treatment with L-AME showed high levels of Sb in the liver and spleen for a long period of time.<br>The critical organ for L-AME treatment was the bone marrow, with low levels of Sb.<br>A single dose of L-AME was not sufficient for total elimination of bone marrow parasites. | (Schettini *et al.* 2003) |
| 2004 | **Experimental infection:**<br>*L. chagasi*<br>***In vitro*:** macrophages | 342.17–0.47 μM Sb | Negatively charged SUV's<br>radio-labeled with 30 μCi of [$^3$H] DPPC | Egg-PC/PS/CHOL<br>(5:1:4; 4:4:2) | The formulation showed no toxicity in the peritoneal macrophages.<br>The amount of free drug used in the treatment was 16 times greater than that of the encapsulated drug.<br>The $IC_{50}$ for liposomal antimony was 14.11 μM achieving the complete elimination of the parasite load in the macrophages.<br>The targeting of liposomes to infected macrophages through interaction with the SRs receptor was improved. | (Tempone *et al.* 2004) |

(*continued*)

Table 2. Continued

| Year | Treatment(s) | Dose(s) | Type of liposome | Composition and proportions (mol%) | Results | Reference |
|---|---|---|---|---|---|---|
| 2005 | **Experimental infection:** *L. chagasi* ***In vivo***: Dogs **Administration route:** Intravenous | 6.5 mg Sb/kg 4 days administration | FDELs (1.2 μm) pH 7.2 | DSPC/CHOL/DCP (5:4:1) | The treatment significantly reduced the number of parasite positive dogs with less than 1 amastigote per 1000 guest cells, but did not achieve total elimination of the parasites. Low levels of AME have been achieved in the bone marrow due to the larger size of the liposomes used (1.2 μm) and it is believed that this organ is more accessible to smaller sized liposomes. | (Schettini *et al.* 2005) |
| 2006 | **Natural infection:** *L. chagasi* ***In vivo***: Dogs **Administration route:** Intravenous | 5.7 mg Sb/kg 100 mg Sb/kg 4.2 mg Sb/kg | FDELs mixed 3: 1 with sucrose solution (400 nm) pH 7.4 | DSPC/CHOL/DCP (5:4:1) | Liposomes larger than 1 μm present problems for their intravenous application. The use of cryoprotectant sugars controls membrane fusion during freeze-drying by obtaining smaller size liposomes with high encapsulation efficiencies. The kinetics of drug release were biphasic, with a first phase of rapid drug release, followed by a second extended release phase. The amount and rate of drug release during the first phase was found to be significantly higher for the formulation prepared with sucrose as compared to that prepared without sugar. Thus, the presence of sucrose causes increased release of antimony from liposomes and a high retention in the tissues. In addition to better targeting to the organs. Even with an antimonial dose 23 times lower, the liposomal formation resulted in antimonial levels – two times – 63 and 68 times higher in the bone marrow, liver and spleen, respectively, when compared with other drugs. | (Schettini *et al.* 2006) |
| 2008 | **Natural infection:** *L. chagasi* ***In vivo***: Dogs **Administration route:** Intravenous | 4.2 mg Sb/kg 6.5 mg Sb/kg | FDELs mixed 3: 1 with sucrose solution pH 7.2 | DSPC/CHOL/DCP (5:4:1) | The cumulative dose by L-AME was 20-fold lower compared to the cumulative dose achieved in conventional treatment. The symptoms of the disease after treatment decreased significantly. Adverse signs were found after the first 15 minutes of treatment. More than a 95.7% reduction in parasite load was achieved at the nodes of the liver, spleen, and cervical lymph nodes after treatment with liposome-encapsulated drug, compared to treatment with either empty liposomes or saline solution. | (Ribeiro *et al.* 2008) |
| 2008 | **Experimental infection:** *L. chagasi* ***In vivo***: Hamsters **Administration route:** Intraperitoneal | 0.75–75 mg Sb/kg | Negatively charged SUV's (20 nm) | Egg-PC/PS/CHOL (5:1:4) | Reduction of 100% of amastigotes in the liver at the dose of 0.75 mg/kg. No cytotoxicity was observed. Efficacy of 16-fold higher liposomal formulation compared to free drug. With neutral charge liposomes an antiparasitic effect of the lipid formulation was found. | (Tempone and Andrade Jr. 2008) |
| 2011 | **Experimental infection:** *L. major* ***In vitro***: macrophages | 10.4 mg/mL Sb | Fluorescent SUVs marked with DIL-L or DIL-PS-L pH 7.4 | PC/CHOL/D-L-α tocopherol (10:4:0.1) PC/PS/CHOL/ D-L-α tocopherol (10:4:0.1) | L-AME was five times more effective, with reduced toxicity in macrophages. The concentration required to kill 100% of the intracellular amastigotes was 40-fold lower in the liposomal antimoniate formulations containing PS compared to the free drug. The IC 50 in the PC/CHOL/D-L-α tocopherol liposomes was 10.5 μM. PS containing liposomes had 100% killing leishmanicidal activity of all amastigotes at a concentration of 25 μM. | (Borborema *et al.* 2011) |

(*continued*)

Table 2. Continued

| Year | Treatment(s) | Dose(s) | Type of liposome | Composition and proportions (mol%) | Results | Reference |
|---|---|---|---|---|---|---|
| 2013 | **Experimental infection:** *L. major* ***In vivo:*** BALB/c **Administration route:** Subcutaneous | 25–200 µg/mL Sb | SUVs Freeze-drying double emulsion mixed with sucrose solution (101 ± 23 nm) | PC/PE/CHOL/PG | The lesion size decreased significantly compared to the control groups. The amastigotes count in the skin lesions decreased in all groups treated with the liposomal formulation. The efficiency of liposome encapsulation was up to 90%; However, they inherited a bimodal size distribution causing their encapsulation efficiency to be reduced to 50% during sterilization filtration. | (Momeni *et al.* 2013) |
| 2014 | **Experimental infection:** *L. infantum* **Administration route:** Intraperitoneal | 30 mg Sb/kg | FDELs mixed 3: 1 with sucrose solution (400 nm) pH 7.2 | DSPC/CHOL/DCP (5:4:1) | Treatment with AML decreased 41% of the parasite load on the liver and spleen together with less organ damage. Oral treatment with WEP associated with a single dose of liposomal MA reduced parasite burden in both the liver and spleen at the same level observed with liposomal antimonial treatment (40 and 30%, respectively). | (Ferreira *et al.* 2014) |
| 2 | **Experimental infection:** | AME and Sb | LUV's | PC/CHOL/PS (5:4:1) | Evaluation of the $IC_{50}$ values demonstrated that the promastigotes were not susceptible to free AME or the liposome-encapsulated drugs. Instead, promastigotes were susceptible to Sb salt solution ($IC_{50}$=67.61 µg/mL). Analysis of the anti-leishmanial activity revealed that AME-FEL treatment inhibited the parasite with an $IC_{50}$ value of 0.95 µg/mL, whereas the $IC_{50}$ value for free AME was 60.28 µg/mL. Free and encapsulated Sb samples were more active in the amastigotes than AME. Sb-FEL showed an $IC_{50}$ value of 0.21 µg/mL, whereas the $IC_{50}$ value for free Sb was 9.05 µg/mL. | (Borborema *et al.* 2016) |
| 0 | *L. infantum* | 81 mg/mL | FEL-liposomes | | | |
| 1 | **Administration route:** | 25 mg/mL | Lipid concentration: | | | |
| 6 | Subcutaneous ***In vitro:*** Macrophages | | 111 mg/mL | | | |

unencapsulated AmB, when tested against experimental VL. In addition, when tested *in L. donovani* infected squirrel monkeys, L-AmB affected 99% of the amastigotes in liver with a concomitant improvement in the AmB therapeutic index (Bodhe *et al.* 1999). L-AMB is quite stable, exhibits certain macrophage targeting, and good potency against human VL, accordingly to its specific liposome composition and sterol (cholesterol vs. ergosterol) binding thermodynamics (Bodhe *et al.* 1999, Amato *et al.* 2004, Sundar and Chakravarty 2010, Chattopadhyay and Jafurulla 2011, Stone *et al.* 2016, Zielinska *et al.* 2016).

Other lipid-based amphothericin B products such as AmB colloidal dispersion (Amphocil®) and the AmB lipid complex Amphotec® have been shown to be effective against VL, but the L-AmB formulation was superior *in vivo* to any of them (Yardley and Croft 2000).

Recently, a new ergosterol-rich L-AmB formulation (Kalsome™10) has been proposed for its leishmanicidal efficacy, tolerability and immunomodulatory activity; it was found to induce no hepato- or nephrotoxicity in murine models, leading to significant reduction in parasite burden and almost complete clearance of parasites from liver and spleen. Additionally, the treatment almost completely inhibited the secretion of (disease promoting) cytokines IL-10 and TGFβ, and significantly elevated the levels of cytokines IFNγ and IL-12, related to the control of the disease (Asad *et al.* 2015).

## *Sugar-coated liposomes encapsulating classic leishmanicidal agents and natural actives*

Sugar-coated liposomes were designed to target more specifically macrophages by directing leishmanicidal agents to the cell surface, which contains receptors that recognize terminal glycoside residues of galactose, mannose, and fucose (Owais and Gupta 2005). Considering this, urea-stibamine (the first compound based on pentavalent antimony) was encapsulated in conventional and sugar-coated liposomes for the treatment of experimental leishmaniasis in hamsters, the effectiveness and toxicity of urea-stibamine being analysed and compared with those elicited using different sugar-coated liposomes (Banerjee *et al.* 1996, Bray *et al.* 2003). The liposomal form with mannose was shown to be more efficient for the transport of urea-stibamine to the macrophage. Subsequently, the advantage of mannose-coated liposome delivery systems was confirmed for classical non-antimonial drugs, such as pentamidine isethionate and its analogues, evaluated *in vitro* for leishmanicidal activity (Nandi *et al.* 1993, Bray *et al.* 2003).

Also hamycin, a polyene antibiotic, was prepared in mannose-coated liposomes and tested *in vitro* against experimental leishmaniasis in a hamster model. The formulation was found to be more potent than regular hamycin in reducing the burden of spleen parasites. Moreover, drug toxicity was reduced in the liposomal formulation, as determined by the methemoglobin levels and enzymatic markers of the liver function, mainly with liposomes prepared with higher esterol contents (Banerjee *et al.* 1994).

Satisfactory responses were also found when the natural compounds piperine and andrografolide (extracted from Indian medicinal plants) were encapsulated in mannose-coated liposomes and tested against experimental leishmaniasis, in hamsters (Raay *et al.* 1999). In all cases, mannose-coated liposomal systems were more effective in reducing the parasitic load in the spleen, and the overall toxicity when compared to conventional liposomes and the free drugs.

Different sugar-coated liposomes or the incorporation of plant glycosides or synthetic glycosides was designed for delivery systems, and their macrophage specificity was tested *in vitro*. For instance, plant glycosides contained in liposomes for the treatment of leishmaniasis, in which the glycoside partitioned into the lipid matrix being responsible for the anti-leishmanicidal activity, whereas the sugar portion on the liposomal surface acted as a ligand for specific receptors on the macrophage surface (Dutta *et al.* 1994, Kole *et al.* 1994). The authors evaluated the potential of several plants, such as asiaticosides glycosides, acaciaside, amarogentine, and bacopasaponine for this purpose. As expected, liposome systems showed a lower toxicity potential, in comparison to the free drug (Medda *et al.* 1999, Basu and Lala 2004).

## Cationic and surfactant-containing liposomes to direct the delivery of leishmanicidal drugs

While the presence of negatively charged lipids in the liposomes were found to favour the %EE (Momeni *et al.* 2013), insertion of synthetic, positively-charged, lipids (cationic liposomes) have gained great attention, mainly because of their ability to improve target cell delivery. In addition, there have been reports of better drug uptake by macrophages, increasing the release to the liver and spleen, when entrapped in positively charged liposomes, as compared to neutral or negatively charged liposomes (Banerjee *et al.* 1998, Qi *et al.* 2016). This observation led researchers to explore the potential of cationic liposomes in the treatment of *Leishmania* infection. In fact, using L-AME prepared with a fraction of phosphatidylserine (PS), Tempone *et al.* (2004) and Tempone and Andrade Jr. (2008) demonstrated a high *in vivo* efficacy, reducing by 133 times the dose of total antimony administered in infected hamsters. In the same way, Borborema *et al.* (2011) developed L-AME formulations containing PS and determined their leishmanicidal activity and macrophage uptake, showing that the mean inhibitory concentration with L-AME was 10-fold lower that of the free drug. In another study, these authors found *in vitro* activity for that L-AME formulation and other Sb-containing liposomes against intracellular *L. infantum* amastigotes; these liposomes were 63-fold and 39-fold more effective than equivalent free drugs (Borborema *et al.* 2016). Interestingly, Dey *et al.* (2000) reported that a single dose of positively charged liposomes containing phosphatidylcholine (PC) and stearylamine (SA) significantly reduced hepatic parasite load in a model of experimental VL. Pal and coworkers studied the effect of lipid composition using cationic liposomes and monosodium antimonium gluconate (SAG) in a VL model. The association of SAG with PC-SA liposomes significantly improved the leishmanicidal activity, resulting in significant reduction (98%) of the parasite load in the spleen, compared to empty liposomes of PC-SA (60%) and free SAG (76%) (Pal *et al.* 2004). Sinha *et al.* (2015) have optimized a cationic liposomal formulation with sodium stibogluconate for the treatment of *L. donovani* infections sensitive or resistant to this drug. All cationic liposomes studied demonstrated leishmanicidal activity, with PC dimethyldioctadecyl ammonium bromide (DDAB) vesicles being the most effective formulations, followed by PC-SA liposomes. Administration of stibogluconate entrapped in PC-DDAB liposomes was able to induce significant ultrastructure alterations in promastigotes and eradication of parasites from the liver, spleen, and bone marrow of the animals.

The SA- and DDAB-containing liposomes mentioned above are also examples of detergent-modified liposomes. Then, another class of modified liposomal system that deserves to be mentioned is niosomes. The niosomes are lipid vesicles that contain a fraction of nonionic surfactants in their composition. The group of Baillie (Baillie *et al.* 1986, Hunter *et al.* 1988) found that the use of niosomes with sodium stiboglunate for the treatment of VL promoted high drug levels in the infected reticuloendothelial system. Niosomes have been also evaluated as vaccine carriers for CL, either using purified antigens, e.g. gp63 glycoprotein (Lezama-Dávila 1999) or inactivated whole parasites (Pardakhty *et al.* 2012).

## Liposomes modified with peptides for macrophage activation

Peptides similar to tuftsin (L-threonyl-L-lysyl-L-prolyl-L-arginine) have been employed as macrophage activators to improve the targeting of anti-leishmaniasis drugs because of their preferential binding property to such cells. In addition, such peptides also act as immunomodulators, by the nonspecific activation of mononuclear phagocytic cells against infection (Agrawal and Gupta 2000). Thus, peptide-modified liposomes were designed to be advantageous against parasitic infections. In an important study, Guru *et al.* (1989) evaluated the potential of tuftsin-coated liposomes against VL: with the encapsulation of SAG in such liposomes, a significantly greater effect was observed, in comparison with conventional (tuftsin-free) liposomal SAG. In other studies, the efficacy and toxicity of AmB were evaluated against *L. donovani* after administration in free form or encapsulated in conventional and tuftsin modified liposomes. Once again the liposomes with tuftsin were much more effective than the conventional liposomes and the free drug, besides showing no toxicity (Ahmad *et al.* 1991, Agrawal *et al.* 2002).

Primaquine, a compound of the 8-aminoquinoline group and with an antimalarial effect, has very small or discrete leishmanicity effect (New *et al.* 1983). However, when primaquine was encapsulated in liposomes modified with f-Met-Leu-Phe peptides, it was shown to be more effective in reducing the burden of parasites in hamster's spleen than free or primaquine encapsulated drug in conventional

liposomes. In addition, peptide-modified liposomes were not only capable of delivering the drug to macrophages, but also to activate macrophages leading to nonspecific killing of pathogens (Banerjee *et al.* 1998).

More recently, peptide 5, selected through Phage Display Technique demonstrated its ability to promote a state of immunity against *L. infantum* infection in murine model, after immunization using liposomes or aluminium hydroxide as vaccine carriers (Toledo-Machado *et al.* 2015).

### Immunoliposomes

Immunoliposomes are liposomes coated or bound to antibodies and have been tested and developed in experimental models with the aim of combating leishmaniasis, mainly because the surface of macrophages has receptors to bind to the Fc portion of antibodies. Antibodies have also shown synergistic leishmanicidal activity when administered with SAG (Kole *et al.* 1999). Thus, when antibodies are coupled to anti-leishmaniasis drug-containing liposomes or liposomes, it may be possible to achieve better targeting of the drug to macrophages coupled with a synergistic anti-leishmaniasis activity.

For example, Dasgupta and coworkers evaluated the utility of immunoglobulin G (IgG)-coupled liposomes, without any associated drug, in the treatment of VL. IgG-coupled liposomes were 2–3 times more active in killing several strains of *L. donovani* isolates, relatively to free IgG and decoupled liposomes. This improvement in anti-leishmanicidal activity is mainly due to the Fc receptor-mediated targeting. However, these investigators did not study the effect of liposome-coupled IgG containing any standard anti-leishmania drug, which could have helped to elucidate the mechanisms involved in cell targeting and the synergistic-targeting drug effect (Dasgupta *et al.* 2000).

Mukherjee *et al.* (2014) studied the effect of liposomal doxorubicin in the treatment of VL on liposomes coupled with *Leishmania* specific antibody. The liposome-bound antibody formulation containing doxorubicin was significantly more active and much less toxic than free doxorubicin or liposomal doxorubicin, when tested in *L. donovani*-infected mice.

In a recent report, the effectiveness of four adjuvants (alum, saponin, cationic liposomes, and monophosphoryl lipid-A), in combination with autoclaved *L. donovani* antigens was evaluated in a murine model. While the level of protection varied depending on the type of adjuvant used, all vaccine formulations presented a considerable protective efficacy, as shown by the significant reduction in parasite load, profound delayed type hypersensitivity responses, increased IgG2a titers and levels of Th1 cytokines (IFN-$\gamma$, IL-12) as compared to the nonvaccinated and infected controls (Thakur *et al.* 2015).

Also recently, liposomes carrying glycosylphosphatidylinositol (GPI)-anchored proteins from *Leishmania amazonensis* promastigotes where tested in a murine model. Mice inoculated with GPI-anchored and total proteins in constitutive proteoliposomes displayed a post-infection protection of about 70% and 90%, respectively (Colhone *et al.* 2015).

### Liposomes designed for immunization against leishmania

Despite the great effort made by researchers trying to develop prophylactic inoculations, there is no vaccine against leishmaniasis in routine use so far (Srivastava *et al.* 2016).

Lipid-encapsulated *L. donovani* antigens induced protective immunity in mice (Afrin *et al.* 2002), with best result occurring with positively charged liposomes, followed by neutral and negatively charged vesicles. The extent of protection induced by the same antigens varied depending on the vesicle load. Effective immunization against CL was also observed when specific parasite antigens were reconstituted into liposomes (Russell and Alexander 1988). Also liposomes coated with glycolipids (dipalmitoyl phosphatidyl ethanolamine with mannopentose) induced cellular immunity in *L. major*-infected mice, indicating that such modified liposomes could serve as a vaccine, inducing protection against infection (Shimizu *et al.* 2003).

Das and Ali (2014) evaluated the vaccine efficacies of Cysteine proteases (immunogenic proteins and key mediators of cellular functions in *Leishmania*) against VL, using cationic liposomes with Toll-like receptor agonists for stimulating host immunity against *L. donovani* in a hamster model. The survival analysis data indicated an appreciable long-term protective response generated by the liposome-entrapped cysteine proteases, against monophosphoryl lipid A-trehalose dicorynomycolate, which was sustained at least up to 180 days post infection, in hamsters.

Archaeosomes constitute a novel generation of liposomes with high stability at low or high temperatures, acid or alkaline pH, oxidative condition, high pressure, presence of phospholipase, bile salts and serum proteins. They have been studied for the potential applications in drug and vaccine delivery against different diseases and pathogens (Benvegnu *et al.* 2009). In a recent study on immunization for leishmaniasis with total antigens from *L. braziliensis* solubilized with sodium cholate within ultradeformable archaeosomes topically administered to mice, Higa and coworkers founded that such liposomes acted as penetration enhancers for parasite antigens, succeeding as topical adjuvants (Higa *et al.* 2016).

### Liposomes in clinical studies

Although multiple liposome-encapsulated drugs have been used for the treatment of different forms of leishmaniasis, most of recent reports involving humans have been related to L-AmB (Sundar *et al.* 2011, 2015, Lucero *et al.* 2015, Machado *et al.* 2015). Successful treatments have been reported with AmBisome for CL (Amato *et al.* 2004, Calderón Gómez *et al.* 2015), and for patients with VL (Minodier *et al.* 2003).

Recently, Machado *et al.* (2015) evaluated the treatment with L-AmB in an open clinical trial having 20 patients with disseminated leishmaniasis; a high cure rate (75%) was

achieved when a total dose above 30 mg/kg body weight was used. In another interesting study, doses of 10 mg/kg and 15 mg/kg body weight regimen of an indigenously manufactured L-AmB (Fungisome) for the treatment of 30 men and 30 women with VL, the authors reported an initial cure rate of 100% (at day 30) and a definitive cure rate of 93.3%, after six months (Sundar *et al.* 2015).

Finally, a study realized in Bangladesh to determine the effectiveness and safety of L-AmB treatment for VL included 1521 patients. The cure rates for 15 mg/kg AmBisome in three doses of 5 mg/kg body weight was higher than 95% and the treatment was considered safe (Lucero *et al.* 2015).

## Conclusions and perspectives

Leishmaniasis, despite its global importance, has been relatively under research by pharmaceutical companies, compared to other diseases, mainly due to the complexity of the parasitic infection and the lack of economic incentives. In such a scenario, drug delivery systems have a crucial role to play in the effective management of current and emerging anti-parasitic agents, enhancing their specificity, with a concomitant reduction in adverse effects associated therewith. In view of this, colloidal carriers such as liposomes have demonstrated good potential in improving the efficacy and tolerability of leishmanicidal agents because of their biodegradability, biocompatibility, nontoxic and non-immunogenic nature, ease of administration and sustained drug release. But despite extensive research, therapy in humans with liposome-encapsulated leishmanicidal drugs is not a reality. So far the only successful commercialized liposomal formulation is that for AmB, that has proved its usefulness. Advances in liposome preparation technologies, as well as the use of adjuvants to improve liposome targeting (peptides, sugars and positively charged lipids) is leading to the development of better formulations for the treatment of leishmaniasis.

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No potential conflict of interest was reported by the authors.

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