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Drug delivery systems against leishmaniasis? Still an open question

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Background: *Leishmania* amastigotes live inside resident macrophages in different anatomic sites. Their hidden location is responsible for impairing the accession of therapeutic drugs. Drug delivery systems (DDSs) should allow the adverse effects caused by problematic routes of administration to be avoided as well as enhancing the antileishmanial activity and reducing the toxicity of the medication. However, after 30 years of research in the field, and since leishmaniasis is mostly a disease affecting the poorest populations, currently AmBisome® is the only DDS used against the visceral form, and most experimental development only relates to parenteral administration. *Objective*: We critically review the main DDSs designed against the different clinical forms of leishmaniasis. *Methods*: A literature search was performed on PubMed and through Google. *Conclusions*: On reviewing the experimental and clinical therapeutic performance of former and current DDSs and considering the main obstacles to be overcome, we discuss how nanomedicine can contribute to the development of new and more efficient strategies.

Keywords: drug delivery systems, leishmaniasis, nanomedicine

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1. Introduction

Leishmaniasis is mostly linked to the poverty of developing countries and appears in different clinical manifestations: ulcerative skin lesions (cutaneous), destructive mucosal inflammation (muco cutaneous), and disseminated visceral infection (kala-azar) [1,2]. Visceral leishmaniasis is mortal if untreated; cutaneous leishmaniasis heals by reepithelisation with scarring, but it is treated to avoid disfiguring lesions; mucosal leishmaniasis can produce potentially life-threatening inflammatory disease and must be treated [3,4].

All clinical forms are caused by a flagellated promastigote of the *Leishmania* genus that is injected in the skin by sandflies. The parasites invade the local phagocytic host cells, including neutrophils. The promastigotes transform and replicate as amastigotes inside the phagolysosomes of resident macrophages; dissemination then occurs locally and distant macrophages are infected [5].

The amastigotes living inside the macrophage's phagolysosomes located in different anatomical areas present major structural and phenomenological barriers that antileishmanial drugs have to overcome. Drugs used in conventional treatments are poorly selective, or must be administered in repeated and high doses by parenteral routes [3,6-9]. These facts undoubtedly contribute to the high toxicity and in most cases to the poor compliance and efficacy of current conventional medication used against all the clinical forms [10-12].

In this review we first describe the attempts made to develop drug delivery systems (DDSs) to surpass these barriers. Second, we discuss the potential of new

technologies to improve the experimental issues of current delivery systems.

2. Review of antileishmanial drug delivery systems

2.1 1978, the beginnings with liposomes

Liposomes, vesicles made of one or more bilayers composed basically of phospholipids and cholesterol, have been the DDSs most used in the last 30 years [13,14]. Their application to the treatment of leishmaniasis has evolved together with advances in knowledge on relationships between liposomal structure – via administration – pharmacokinetics and biodistribution.

At the end of the 1970s, the groups of Ward and Hanson were the first to understand the strategic utility of the intravenous administration of liposomal pentavalent antimony (Sb^V) for selective delivery to the liver and spleen macrophages infected with visceral leishmaniasis (**Figure 1**) [15-17]. These liposomes also had antileishmanial activity against experimental cutaneous leishmaniasis but only if they were administered intravenously [18]. Excluding experimental treatments on canine visceral leishmaniasis, and further comparison with niosomes [19,20], the interest in liposomal Sb^V declined towards 1981 and commercial development was not encouraged because of its toxicity in monkeys [21]. These first works gave no major relevancy to the chemical composition of the liposomal matrix; the importance of such an issue became clear when dealing with hydrophobic drugs.

2.2 Liposomal amphotericin B for parenteral administration

2.2.1 Visceral leishmaniasis

The idea of loading amphotericin B (AmB) in liposomes to increase its selective uptake by accessible mononuclear phagocyte system (MPS) cells and to reduce its toxicity arose almost at the same time as for Sb^V. New *et al.* [22] were the first to survey the activity of different AmB liposomal matrices administered by the parenteral route against experimental visceral leishmaniasis. They found that sterol-containing liposomes were less toxic than their nonsterol counterparts and that saturated lipids reduced the toxicity of AmB to a greater extent than unsaturated compositions. Lopez-Berestein *et al.* [23] found that ^{99m}Tc-labelled multilamellar vesicles (MLVs) primarily accumulated in the liver (45%) and spleen (26%) upon intravenous administration. At the same time, liposomes loaded with AmB (AmB-MLV) improved the therapeutic efficacy in the treatment of cancer patients with systemic fungal infections (**Figure 1**) [24].

AmB-MLV did not cause damage in the liver or kidneys when administered in equal doses whereas free AmB caused nephrotoxicity in patients [24]. AmB-MLV was shown to be 170 – 750 and 60 times as active as Sb^V in *Leishmania donovani* infected hamsters and monkeys, respectively [25]. In spite of being successful against experimental visceral leishmaniasis, AmB-MLV never reached the market but set a precedent for the development of another liposomal formulation, with a lipid matrix adjusted to achieve maximal activity with minimal toxicity not against visceral leishmaniasis but against intra and extracellular mycosis. This formulation was launched onto the market in Europe in 1990 with the brand name AmBisome® (Astellas Pharma US, Inc.) (**Figure 2**). See [26-28] for a detailed description of the mechanism of action of AmB and on the structure of AmBisome.

Ten years after the first publication showing the advantages of liposomal AmB against experimental visceral leishmaniasis, AmBisome was administered to patients [29,30]. It was rapidly demonstrated that AmBisome eliminated liver parasites to the same extent but faster than Sb^V [31], and at higher doses than Fungizone™ (Bristol-Myers Squibb Co.), it presented higher activity without toxic effects in *L. infantum* infected mice [32]. Multicentre clinical trials showed that AmBisome was safe and effective for the treatment of visceral leishmaniasis, so its use in immunocompetent children suffering with visceral leishmaniasis caused by *L. infantum* was started [33]. AmBisome was shown to be effective in cases of Sb^V unresponsiveness in *L. infantum* and *L. donovani* foci, both in immunocompetent and in immunosuppressed patients, as well as being less toxic than other AmB lipid formulations such as Amphocil® (Cambridge Laboratories) [34]. European clinical trials have defined administration regimens for immunocompetent patients and have recommended higher doses for immunosuppressed patients who are infected with HIV and visceral leishmaniasis [35-37]. A comparison of the efficacy and pharmacology of AmB formulations in treatment of leishmaniasis has recently been reviewed [38]; for recommendations on AmBisome doses and use, see Bern *et al.* [39].

A significant regional variation in the response to AmBisome is reflected in the total dose required for 100% cure; it is low in India (6 mg/kg for *L. donovani*), higher in Kenya (14 mg/kg for *L. donovani*) and highest in Brazil (> 20 mg/kg for *L. chagasi*) [40].

In India, Fungisome, a liposomal AmB formulation, was developed and commercialised from May 2003 [41]. Preclinical pharmacological and postmarketing studies have reported its safe administration [42]. Although Phase II and III efficacy studies were done in patients with systemic fungal infections, it was reported that Fungisome was effective in patients with visceral leishmaniasis resistant to antimony, pentamidine and even to AmB [43-45]. Data are lacking on the amount of AmB retained in the liposomal matrix upon dilution and on interaction with plasma lipoproteins and biodistribution in humans. The previous administration sonication step can be a major safety concern for intravenous administration, instead of an advantage, since poorly controlled size of vesicles is extremely dependant on the equipment used for bath sonication (for comparison with AmBisome, see **Table 1**).

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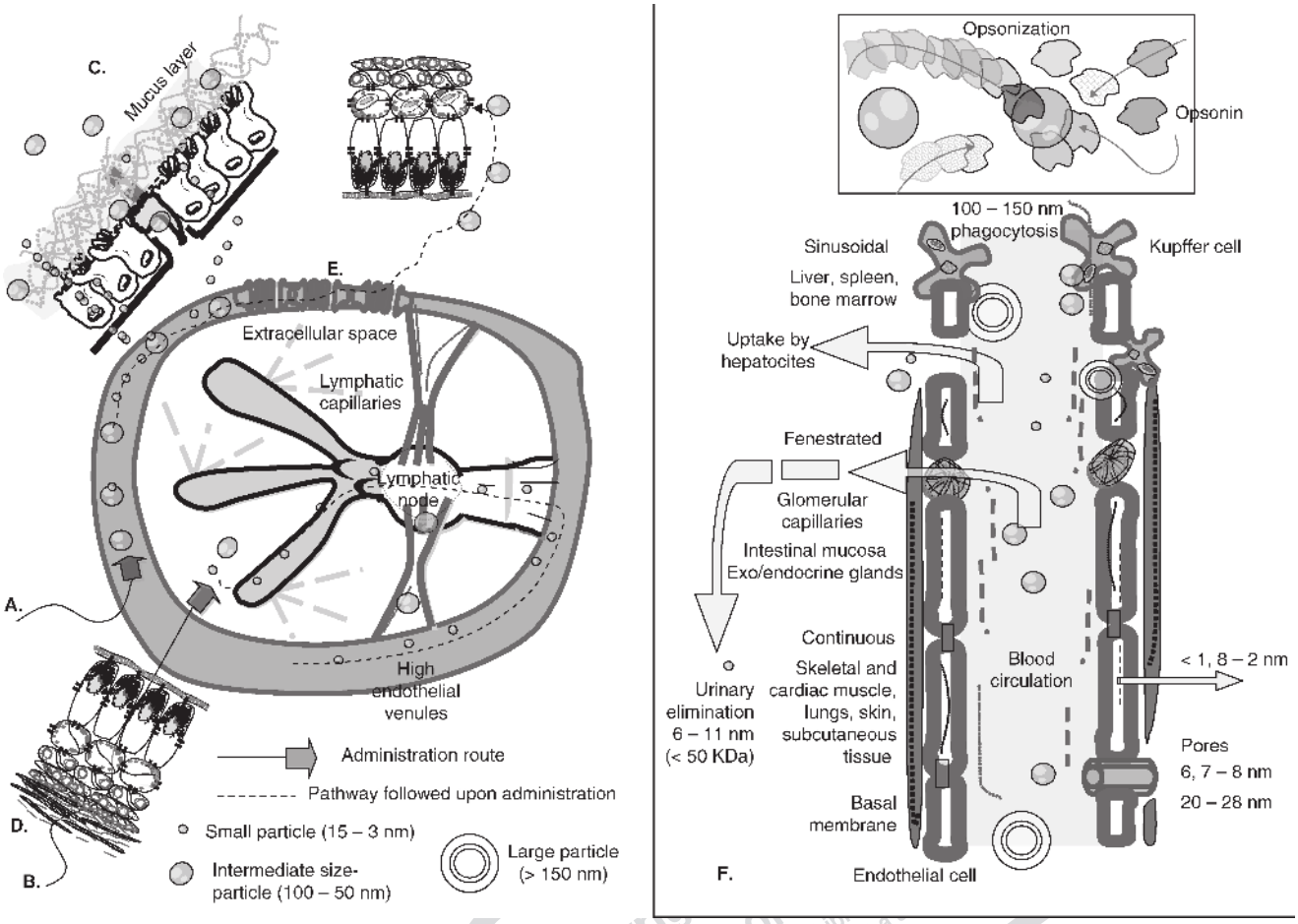

Figure 1. Routes of administration and anatomical barriers. A. Intravenous route: particles are injected directly into systemic blood circulation (rigid or solid particles smaller than 3×10^4 nm because of blockage of lung capillary bed). **B.** Subcutaneous, intramuscular and intraperitoneal route: particles are injected into the interstitial/extracellular space. The lymphatic system clears excess fluid and particulates from the interstitial tissue. Only small molecules (< 16 KDa) will enter the blood compartment bypassing through the pores in the blood capillary walls, whereas large molecules are mainly transported by the lymphatics. Small particles (< 100 nm) enter lymph capillaries and only a small fraction that avoid macrophage uptake at the lymph nodes can be drained into systemic blood circulation. Large particles remain in the extracellular space; and those made of biodegradable matrices first act as reservoirs for sustained release of the carried drug, and when size is decreased beyond $7 - 8 \times 10^3$ nm by biodegradation, can be taken up by phagocytosis [133]. **C.** Oral route: particles administered by the oral route are usually designed to protect the carried drug against the action of low pH, lipases, proteases and bile salts. Once in the small intestine, the particles should preferably be capable of attachment to the mucus layer (mucoadhesion). If mucoadhesive, particles can act as a reservoir for release of drug, which is taken up by enterocytes. No intact particle can be taken up by enterocytes, except the M cells from the Peyer's patch; in that case, transcitosed particles are delivered to the lymphatic circulation. **D.** Topical route: excluding ultradeformable vesicles (see **Figure 4**) and particles below 5 nm, any other particle, independently of shape or structure, cannot cross the dry surface of the stratum corneum cell layers. If the stratum corneum is absent, the diffusion barrier is decreased. **E.** Local inflammation in the cutaneous leishmaniasis is associated with leaky vasculature. In this case, particles in blood circulation can cross the permeability barrier to get close to the infected cell. **F.** Particles in blood circulation: continuous endothelium contains pores that allow the passage of small molecules (2 – 3 nm) outside the circulation; fenestrated endothelium in kidneys allow urinary elimination of small (< 5 nm), deformable, particles, depending on their hydrophilic/hydrophobic balance and electrical charge. Most of the particles are higher than 5 nm and remain confined to blood vessels, and their extravasation to peripheral tissues is impaired. While circulating, particles become coated by proteins; lipoproteins such as HDL adsorbed on liposomes, cause membrane destabilisation and leakage of carried hydrosoluble drugs. The adsorption of the proteins known as opsonins on the particle's surface promotes particle recognition and further removal from blood by the accessible cells from the mononuclear phagocyte system. The extent of opsonisation varies according to the nature of the particle surface and on its size. Opsonized particles are removed in organs vessel with fenestrate endothelium, mainly by Kupffer cells from liver vasculature, followed by macrophages in spleen, and to a lesser extent, depending on their size, by macrophages in lung and bone marrow. Particles designed to avoid opsonisation are less rapidly cleared from circulation. In this situation, particles have an increased chance to extravasate to peripheral tissues, in sites where increased permeability – with local destruction of the basal membrane of continuous endothelium – is caused by inflammation.

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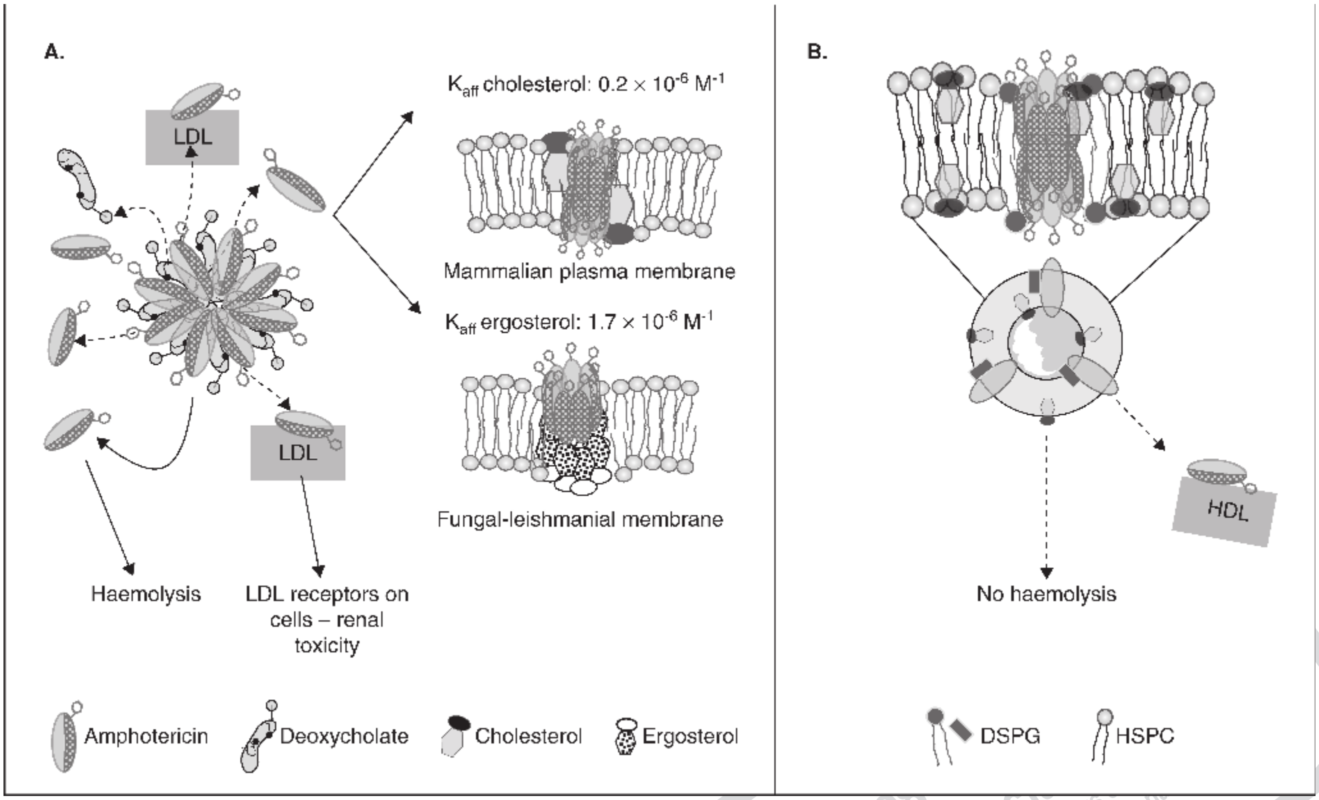

Figure 2. Structures of Fungizone and AmBisome. A. Fungizone: upon being administered intravenously, dilution in blood causes complete dissociation of the colloidal dispersion made of AmBisome (AmB) and deoxycholate. Conventional AmB causes haemolysis and extensively associates with plasma lipoprotein LDL to be taken up by cells exhibiting internalisable LDL receptors, for instance kidney cells. This mechanism is responsible for renal toxicity in mammals. AmB has less affinity for cholesterol than for ergosterol; the difference makes it useful against fungi and leishmaniasis. AmB inserts in ergosterol containing cell membranes to form aqueous pores permeable to small cations and anions, including H^+ and OH^-. Salt permeation across aqueous pores formed by AmB on *Leishmania* parasite membrane leads to rapid cell lysis via an osmotic mechanism. The same mechanism (also with increased permeability to H^+ and Ca^{2+}) is responsible for toxicity in cholesterol containing cell membranes from mammal cells. **B.** AmBisome lipid matrix (nearly 80 nm diameter, lipids in the gel phase at body temperature, presence of cholesterol) is refractory to opsonisation. Eight AmB molecules form a barrel pore that is strongly associated to the lipid matrix by two mechanisms: electrostatic interaction between the amino group of the mycosamine ring of AmB and the phosphate group of distearoylphosphatidyl glycerol (DSPG); hydrophobic interaction between AmB and cholesterol and between the stearyl residues of the DSPG and the polyene portion of AmB. Dilution upon administration does not dissociate AmB from the liposomal matrix. Additionally, DSPG from the AmBisome matrix is responsible for bridging the association of small amounts of AmB to HDL. This association modifies AmB biodistribution, decreasing its uptake by kidney cells and therefore reducing its toxicity. Note that *in vitro*, the macrophage uptake of conventional AmB is much higher than for AmBisome; conventional AmB is three to six times more active than AmBisome against both *Leishmania major* promastigotes in culture and amastigotes in murine macrophages [134]. *In vivo* AmBisome produces a higher AmB concentration in blood, liver and spleen, and decreased in kidney and lung compared with conventional AmB. Blood and tissues retain higher AmB concentration after administration of AmBisome at maximum total dose (higher than for conventional AmB); *in vivo* AmBisome is principally taken up by macrophages [135].

HSPC: Hydrogenated soybean phosphatidylcholine.

Table 1. Comparison between the two commercially available liposomal formulations of AmBisome.

	AmBisome	Fungisome
Formulation	213 mg HSPC, 84 mg DSPG, 52 mg cholesterol and 50 mg AmB	45 mg of PC : cholesterol (7 : 3 molar ratio) and 1 mg AmB
	14.3% w/w AmB/lipids	2.2% w/w AmB/lipids
	Others: α-tocoferol, sucrose, Na sucinato	
	SUV < 100 nm	MLV (2.743 – 3.454 μm)
Product	Lyophilized powder (50 mg AmB/vial)	Liquid (50, 25 and 10 mg AMB/vial)
	Storage 2 – 8°C	Unique packing allows uninterrupted cold chain maintenance from manufacturing to the end use
Administration	Reconstitute with 12 ml of water and filtered	The MLVs are converted to SUV 0.0884 – 3.00 μm through sonication for 45 min before administration
LD_{50} (mg/kg)	> 175 in mice, 50 in rats	17.6
Pharmacokinetic parameters		
Dose (mg/ml)	3 – 5	1 – 3
Cmax	83	1.012
AUC (mg h/l)	555	11.43
Vd (l/kg)	0.11	2.28
Biodistribution	Liver, spleen, lungs	Liver, spleen, lungs (mouse)
Dosage schedule	US FDA recomends [40]	1 mg/kg/day for 21 days (total dose 21 mg)
	Immunocompetent patients: 3 mg/kg/day (days 1 – 5) and 3 on days 14, 21 (7 days duration – total dose 21 mg)	2 mg/kg/day for 7 – 10 days (14 – 20 mg total dose)
	Immunocompromised patients: 4 mg/kg/day (days 1 – 5) and 4 on days 10, 17, 24, 31, 38 (total duration 10 days – total dose 40 mg)	3 mg/kg/day for 5 – 7 days (15 – 21 mg total dose)

AUC: Area under the curve; AmB: Amphotericin B; Cmax: Maximum concentration; DSPG: Distearoylphosphatidyl glycerol; HSPC: ; LD_{50}: Median lethal dose; MLV: Multilamellar vesicle; PC: Phosphatidylcholine; SUV: Small unilamellar vesicles; Vd: Volume distribution.

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2.2.2 *Cutaneous leishmaniasis*

From early on it was reported that activity of DDSs against cutaneous leishmaniasis is dependant on the route of administration. For instance, AmB loaded in small liposomes has no therapeutic effect when administered by the intraperitoneal route [46], whereas liposomal Sb^v is effective by the intravenous route (**Figure 1**) [21]. In 1997, intravenously administered AmBisome showed a dose (between 6.25 and 50 mg/kg) response effect against experimental cutaneous leishmaniasis (*L. major*); by the subcutaneous route, however, the treatment was ineffective. This was ascribed to the elimination of liposomes by local macrophages, whereas inflammation in the periphery of the skin lesions increased vascular permeability and allowed the local leakage of intravenously administered liposomes [47]. For experimental visceral leishmaniasis (*L. donovani*), the mean effective doses (ED_{50}) for AmBisome, the mixed micelle Amphocil and the ribbon-like lipid complex Abelcet when administered intravenously were 0.3, 0.7 and 2.7 mg/kg, respectively. For experimental cutaneous leishmaniasis (*L. major*), however, the ED_{50} for AmBisome was 25 mg/kg, Amphocil showed lower activity while Abelcet® (Enzon Inc.) was inactive [48]. These results show the importance of the size of the particulate material used (any structure > 2 nm). The success of cutaneous leishmaniasis treatment depends on how physically accessible the infected macrophages in the stratum spinosum are to the DDSs. Circulating DDSs can became close to the stratum spinosum only from local extravasation. The higher ED_{50} values for cutaneous leishmaniasis than for visceral leishmaniasis reflect the difficulty for particulate material to target the infected cells. The inactivity of large (in the order of micrometres) particles such as the ribbon-like formulation could be ascribed to impaired extravasation, which does not occur for the smaller AmBisome. The low physical stability of mixed micelles upon dilution, however, diminishes the amount of particulate AmB available for extravasation, and should explain the need for a higher Amphocil dose. A few clinical applications of intravenous AmBisome against cutaneous leishmaniasis have been reported [49-51].

3. Experimental approaches: plain and grafted liposomes

In the past 10 years, plain liposomes (conventional liposomes without *decoration*) loaded with drugs other than AmB (inosine analogs [52], atovaquone [53], harmine [54] and miltefosine [55]) have shown improved antileishmanial performance compared with free drugs upon intravenous administration against experimental visceral leishmaniasis. A different strategy was developed based on the use of cationic stearylamine-bearing liposomes. Both empty [56] or containing Sb^V stearylamine bearing liposomes (phosphatidylcholine: stearylamine, 7: 2) [57], burden parasites of liver and spleen were eliminated after a single dose in established and chronic experimental visceral leishmaniasis (*L. donovani*). There are issues on safety related to stearylamine-bearing liposomes, since they are known to be toxic in mammal cell cultures [58] and stearylamine incorporation lowers the median lethal dose (LD_{50}) of a single intravenous dose of MLV sevenfold [59].

Specifically recognised by receptors for terminal mannose residua on Kuppfer cells [60], mannose-grafted liposomes loaded with urea stibamine [61,62], hamycin [63], pentamidine isetionate and derivatives [64], natural agents from Indian medicinal plants such as andrographolide and piperine [65] and derivatives of a new antibiotic [66] have also shown improved activity over the free drugs on experimental visceral leishmaniasis, due to the increased macrophage uptake (**Figure 3**).

The peptide tuftsine specifically binds to macrophages and potentates their natural killer activity against pathogens [67]. Tuftsin-bearing liposomes loaded with sodium stibogluconate [68] and AmB [69] have shown higher activity against experimental visceral leishmaniasis compared with plain liposomes and free drugs. In another approach, empty liposomes grafted with IgG (immunoliposomes) resulted in two to threefold more activity than free IgG and plain liposomes in clearing *L. donovani* from macrophages, due to their increased uptake by the Fc receptor in macrophages [70].

None of these strategies have been clinically tested yet. Difficulties would probably arise from large-scale grafting, contributing to the problems associated with scaling up plain liposomes [71,72]. To overcome these problems, liposomes bearing different plant glycosides buried in the liposomal matrix have been assayed as antileishmanial agents. The aglycone moiety has antileishmanial activity and the sugar moiety flanges out of the liposomal surface and acts as a ligand for macrophages. These strategies showed improved efficacy in reducing spleen parasites on experimental visceral leishmaniasis [73].

Liposomal matrices bearing nonlipid components (grafted liposomes including stealth, immuno-stealth, immunoliposomes and also those bearing adsorbed/buried sugars) represent a safety concern because of the potential risk of producing immune responses or complement activation upon repeated parenteral administration, as was recently described for Peg grafted liposomes [74].

3.1 Control of zoonotic infection

In the Mediterranean zone, Asia, north of Africa and South America, dogs are the reservoirs of the viscerotropic species *L. infantum*/*L. chagasi*. Both meglumine antimoniate loaded in liposomes [75] and AmBisome [76] have been shown to reduce parasite burden in the liver, spleen and bone marrow in dogs with experimental visceral leishmaniasis. However, dosage schemes are based on frequent intravenous administration and long-term post-treatment control is impossible since clinically cured dogs frequently experience relapses and became infective shortly after treatment [77]. Additionally, veterinary use of AmB liposomal and other new antileishmanial drugs should be avoided to prevent the development of resistance [39,78]. These facts make it impractical to use therapeutic strategies on dogs with the idea of controlling the disease.

4. Others drug delivery systems for parenteral administration

During the past 10 years, other DDSs apart from liposomes have also been experimentally developed, mostly against visceral leishmaniasis. The most relevant approaches are briefly surveyed here; structural characteristics, doses and experimental models are detailed in **Table 2**.

4.1 Niosomes

Niosomes are liposome-like vesicles consisting of mixtures of cholesterol and nonionic surfactants such as monoalkyl or dialkyl polyoxyethylene ether and/or sorbitan esters [79]. Niosomes behave *in vivo* like liposomes, prolonging the circulation of entrapped drugs to alter organ distributions and metabolic stability. Niosomes do not require special conditions, such as low temperature, vacuum or inert nitrogen atmosphere for production and storage because there is no need to prevent oxidation of phospholipids. In addition, the materials are relatively low in cost. These facts make niosomes more attractive than liposomes for industrial manufacturing [80].

Niosomes and liposomes loaded with sodium stibogluconate (NI-SSG) were found to be equally active upon intravenous administration, but previous sonication was indispensable to fully eliminate not only the liver parasites but also those in the spleen and bone marrow in experimental visceral leishmaniasis, since only small particles can reach the macrophages in bone marrow [20,81]. Later, it was shown that a single dose of NI-SSG was sufficient to eliminate parasites in MPS organs to the same extent as AmBisome [82]; however, this did not protect against reinfection [83].

In a further work, the same group observed that NI-SSG and free SSG had similar pharmacokinetic

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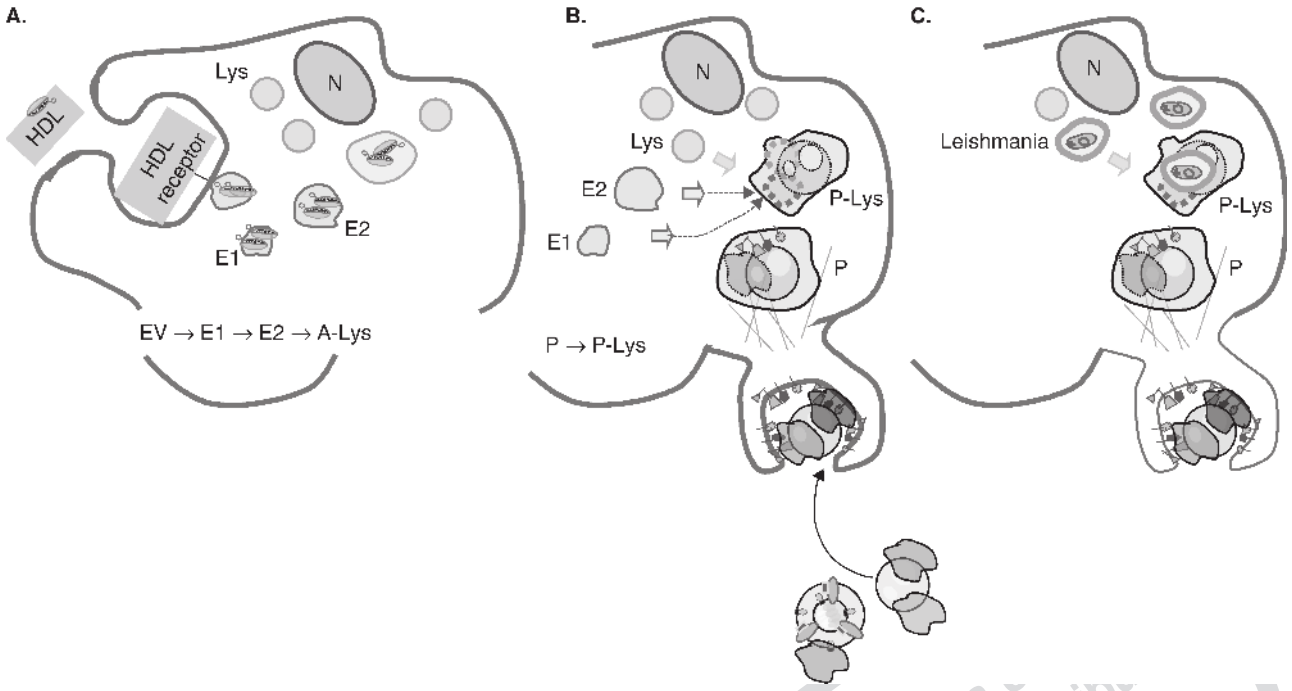

Figure 3. Cell uptake modalities for particles. A. Whether or not mediated by receptors, endocytosis is the process by which any cell ingests hydrophilic molecules, small and intermediate size particles. Endocytic vesicles interact with primary, recycling and secondary endosomes to follow an exocytic pathway (particle is released outside the cell) or to fuse with lysosomes. Intracellular vesicular traffic is mediated by tubulin filaments and tracked by motor proteins; the pH is decreased towards lysosomes that contain nucleases, proteases and lipases that destroy particulate matter. Intracellular traffic: EV (endosomal vesicle) → E1 (primary endosome) → E2 (secondary endosome) → A-Lys (lysosome). **B.** Phagocytosis, the process by which cells ingest large particles, is only performed by specialized cells called phagocytes, which include macrophages, monocytes, dendritic cells, and granulocytes. Opsonized intermediate/large particles are recognized by different receptors, the membrane folds around the particle by actin filaments and it is sealed off into a large vacuole known as a phagosome. By a mechanism known as kiss and run, constitutive early and secondary endosomes and lysosomes contribute with degradative enzymes (nucleases, proteases and lipases) to the phagolysosome, for ingested particles to be destroyed. Waste material is expelled or assimilated. Unless specially designed, phagocytosed particles remain inside the phagosomes and are not released to cytoplasm. **C.** *Leishmania* amastigotes live inside the phagolysosomal compartment of macrophages; upon particle phagocytosis, colocalisation of parasite and particles is straightforward. Intracellular traffic: P (phagosome) → P-Lys (phagolysosome).
N: Nucleus.

profiles in dogs; this was ascribed to the low entrapment efficiency (7%) of SSG. Pharmacokinetic differences between free and niosomal SSG, however, appeared when NI-SSG was covered with the polymer dextran (10 KDa). The anti-leishmanial efficacies were NI-SSG-dextran > NI-SSG > free SSG in experimental visceral leishmaniasis (*L. donovani*). No signs of toxicity were found in mice after NI-SSG and NI-SSG-dextran administration, although NI-SSG-dextran treatment was associated with short-term toxicity in dogs. Short-term toxicity in dogs was demonstrated by the development of chills and diarrhoea, which cleared by 24 h post dosing, and hepatic dysfunction at 24 h post dosing, but the levels of all the biochemical parameters had returned to normal at 1 month post dosing [84].

An elaborated approach consisted of the previous solubilisation of AmB in cyclodextrins as an inclusion complex and this was further loaded in niosomes. This formulation presented lower activity than AmBisome (= Amphocil) but higher than Abelcet and substantially higher than Fungizone to eliminate parasites from the liver, spleen, and bone marrow in experimental visceral leishmaniasis at the same AmB dose [85]. However, the AmB/niosome matrix ratio, stability, biodistribution and pharmacokinetics of niosomes were not reported, and such data could be useful in explaining the differences in activities.

4.2 Nanodisks

Nanometre-scale, apolipoprotein-stabilised phospholipid bilayer disk complexes or nanodisks are distinguished from liposomes in that they do not possess an aqueous core and are fully soluble in aqueous media. Their diameters range from 8 to 20 nm while apolipoproteins are an intrinsic structural element of the complex [86]. Recently, nanodisks loaded with AmB completely cleared parasites, with no lesions remaining, and no parasite was isolated in

Table 2. Others drug delivery systems for parenteral administration.

Drug delivery system	Drug	Properties	Preparation method	Animal model, administration route and doses	Ref.
Niosomes (NI)	AmB	Tetraethylene glycol mono-*n*-hexadecylether: cholesterol: dicetyl phosphate, 3 : 3 : 1, molar ratio 530 – 240 nm	The lipid was melted at 130°C for 5 min. The molted mixture was cooled to 70°C, hydrated with pre-heated AmB hydroxypropyl-γ-cyclodextrine complex or SSG solution and homogenised at 8000 rpm	*L. donovani* infected BALB/c mice, i.v., 2.5 mg AmB/kg on days 7 – 11 p.i.	[85]
	Sb^V			*L. donovani* infected BALB/c mice, i.v., single dose 296 mg Sb^V/kg vs AmBisome (8 mg AmB/kg)	[82]
				i.v., 100 mg Sb^V/kg, once a week, 4, 3, 2, 1 week before infection	[83]
				L. donovani infected BALB/c mice, SSG-NI (222 mg Sb^V/kg), SSG-NI-dextran (33 mg Sb^V/kg)	[84]
Emulsions	AmB	Nanodisk made of DMPC, DMPG and recombinant human ApoA-I	Phospholid vesicles and AmB were incubated with ApoA at 24°C, followed by dialysis	*L. major* infected BALB/c mice, i.p., 5 mg/kg, 1, 2, 10, 20, 30 and 40 days p.i.	[87]
	Piperine	Lipid nanospheres (o/w emulsions) made of soybean oil, eggPC, cholesterol, or pegylated lipid nanospheres (DSPE-PEG), or lipid nanosphere with stearylamine (LN-P-SA). Stable and sterile formulations 200 – 885 nm	Homogenisation followed by ultrasonication of oil and aqueous phases	*L. donovani* infected BALB/c mice, i.v., 5 mg/kg	[89]
	AmB	Trilaurin based nanosize lipid particles (emulsomes) stabilized by SPC	Prepared by cast film technique followed by sonication	*L. donovani* infected hamsters, i.v., 0.5 mg/kg	[90]
Conjugation to polymers	AmB	AmB–arabinogalactan conjugates	AmB was conjugated to oxidized arabinogalactan in borate buffer pH 11 for 24 h. Then, the solution was filtered, dialysed and lyophilized	*L. major* infected BALB/c mice, i.v. and s.c., 6 administrations from day 2 p.i. onward on alternate days, 6 mg/kg vs AmBisome (6 mg/kg), Fungizone (1 mg/kg)	[91]
	8-Aminoquinoline	HPMA copolymer quinoline conjugates with a content of 5% mole mannose targeting moiety and a lysosomally cleavable GFLG spacer	Polymerization	*L. donovani* infected BALB/c mice, i.v., 1 mg/kg on days 7 – 10 p.i.	[95]
Polymeric particles	AmB	Albumin microspheres 1 µm ± 0.7 µm, with 4% AmB	Spray-drying an aqueous solution of AmB and albumin	*L. infantum* infected hamsters i.v., 2, 10, 20 and 40 mg/kg	[100]

AmB: Amphotericin B; DMPC: Dimyristoylphosphatidylcholine; DMPG: Dimyristoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; GFLG: Glycylphenylalanylleucylglycyl; HPMA: *N*-(2-hydroxypropyl) methacrylamide; i.p.: Intraperitoneal; i.v.: Intravenous; p.i.: Postinfection; PLGA: Polylactic-co-glycolic acid; Sb^V: Pentavalent antimony; s.c.: Subcutaneous; SPC: Soybean phosphatidylcholine; SSG: Sodium stibogluconate.

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Table 2. Others drug delivery systems for parenteral administration (continued).

Drug delivery system	Drug	Properties	Preparation method	Animal model, administration route and doses	Ref.
	Pentamidine	Polymethacrylate-NP	Nanoprecipitation method and emulsion polymerisation	*L. infantum* infected BALB/c mice, i.v., 0.05, 0.09, 0.17 or 0.24 mg/kg on days 14, 16 and 18 p.i.	[109]
	Methoxy chalcones	Polymethacrylate-NP, 130 – 170 nm	Emulsion	*L. amazonensis* infected BALB/c mice, s.c., 1 mg/kg on days 27 and 54 and i.p. 10 mg/kg on days 42 and 48 p.i.	[110]
Others	Dihydroindolo-(2,3a) indolizines	Mannose-grafted microspheres made of PLGA and phosphatidylethanol amine in the molar ratio 1 : 71	Solvent evaporation	*L. donovani* infected hamsters, s.c., 3 mg/kg, 6 administrations every 3 days	[112]
	Bassic acid, an unsaturated triterpene acid isolated from *Mimusops elangii*	o/w microemulsions clove oil/Tween20/water (5/30/65), 16 – 20 nm and PLGA-NP 250 nm		*L. donovan* infected hamsters, s.c., 2 mg/kg every 3 days for a total 6 doses	[113]

AmB: Amphotericin B; DMPC: Dimyristoylphosphatidylcholine; DMPG: Dimyristoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; GFLG: Glycylphenylalanylleucylglycyl; HPMA: *N*-(2-hydroxypropyl) methacrylamide; i.p.: Intraperitoneal; i.v.: Intravenous; p.i.: Postinfection; PLGA: Polylactic-co-glycolic acid; Sb^V: Pentavalent antimony; s.c.: Subcutaneous; SPC: Soybean phosphatidylcholine; SSG: Sodium stibogluconate.

experimental cutaneous leishmaniasis in BALB/c mice upon intraperitoneal administration. AmBisome, however, was ineffective at an equivalent dosage and treatment frequency. Note that nanodisk doses are fivefold below those required for AmBisome to have a therapeutic effect by the intravenous route (25 mg/kg) [48]. Remarkably, AmB nanodisks were shown to have a long-term effect in that parasite burden continued to decline for > 100 days following the final treatment. The results obtained for intraperitoneal administration are probably due to the small size of the nanodisk complex. It was proposed that nanodisks are recognised by the class A scavenger receptor (SR-A) on macrophages, since apolipoproteins modified by acetylation, oxidisation or other modifications serve as ligands for SR-A [87].

4.3 Emulsions

An emulsion is a dispersion of two or more immiscible liquids stabilised by a surfactant or emulsifier coating the droplets and preventing coalescence by reducing interfacial tension or creating a physical repulsion between the droplets. Water in oil emulsions (w/o) only are used in sustained release of steroids and vaccines by intramuscular injection, meanwhile oil in water emulsions (o/w) can be administered by a variety of parenteral routes, including intravenously. The advantage of o/w emulsions is the solubilisation of low aqueous solubility drugs and the prevention of hydrolysis and oxidation [88].

The water insoluble piperine was formulated in stearylamine bearing o/w emulsions, and produced higher reduction in parasite burden in liver (90%) and spleen (85%) than plain and pegylated formulations, with no toxicity [89].

Recently, AmB was formulated in so-called trilaurin emulsomes that when coated with *O*-palmitoyl mannan showed higher accumulation in the liver, spleen and lungs and more efficient elimination of parasites from the spleen than free AmB (73.7 versus 30.4%, respectively) [90]. Data on acute and multiple dose toxicity as well as degree of AmB retention in emulsion after dilution have not been reported yet. However, lung accumulation is not necessary for visceral leishmaniasis.

4.4 Polymer conjugation

Aggregate forms of AmB in water are toxic for mammal cells [26]. AmB conjugation to a hydrosoluble polymer such as arabinogalactan was used to enhance its aqueous solubility. Arabinogalactan is a highly hydrosoluble and branched natural polysaccharide, available in a 99.9% pure form with reproducible molecular weight and physico-chemical properties. Its biocompatibility, biodegradability, simplicity of conjugation by reductive amination in an aqueous medium at room temperature, more cheaply than lipid formulations, makes arabinogalactan an attractive drug carrier. The degree of oxidation of the polymer can be

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varied to a large extent, thus leading to various amounts of bound drug. In 1999, AmB conjugated to arabinogalactan (AmB-arabinogalactan) resulted in significantly more effective reduction in the number of mice with lesions and reduction in lesion size than Fungizone and AmBisome, both by the intravenous and subcutaneous routes, in recently induced experimental cutaneous leishmaniasis. AmB-arabinogalactan was somewhat more effective than AmBisome in the treatment of established infection; lesions did not disappear but remained the same size, whereas size was increased in untreated lesions. Additionally, AmB-arabinogalactan was about 40 times less toxic than free AmB in mice [91].

An inherent problem of these coupling techniques is that if the excess oxidising agent is not removed from oxidised polysaccharide, susceptible drugs such as AmB can be oxidised and degraded during the coupling reaction; also the oxidation of the polysaccharide chain can degrade the polymer and change its natural structure and biodistribution [92]. Alternative conjugation methods rendered toxic tosilate or mesilate derivatives in organic solvents that required additional purification steps to remove residual solvents and tosilate or mesilate residues. Overall, the resulting conjugates were less effective and more toxic than those synthesised by reductive amination [93].

In another strategy developed with the aim of modifying a drug's biodistribution, increasing targeting to macrophages and decreased urinary elimination, the small hydrosoluble molecule (quinolone) was linked to a biocompatible hydrophilic lysosomotropic polymer (*N*-(2-hydroxypropyl)methacrylamide) bearing a 5% mole mannose targeting moiety. These conjugates showed higher *in vivo* antileishmanial activity than those with lower mannose content. The mannose-grafted polymers were designed on the premise that one of the routes of phagocytosis of *leishmania* is dependent on the interaction between the mannose-containing lipopolysaccharides on the parasite cell surface and the macrophage mannose receptors [94]. Therefore, by mimicking the invasion process (mannose-dependent receptor-mediated endocytosis) the systems can maximise the potential of the drug to destroy the parasite at the site where it resides [95].

4.5 Micro/nano-polymeric particles

Biodegradable polymers in biological fluids are degraded to produce biocompatible or nontoxic products that are removed from the body by normal physiological pathways and loaded drugs can be released at a controlled rate. Natural polymers such as albumin, cellulose derivatives, gelatine and polysaccharides biodegrade by solubilisation: the polymer itself does not disintegrate and its molecular weight remains unchanged; water diffuses into the polymer, leading to the formation of swollen systems, and ultimately dissolves [96].

Synthetic biodegradable polymers like poly(cyanoacrylates) and poly(esters) biodegrade by hydrolysis (bulk erosion mechanisms). Cleavage of the bonds transforms a water-insoluble polymer into water-soluble low molecular weight polymer fragments. The particles retain their original shape and mass until significant degradation has occurred (~ 90%), then it reaches critical molecular weight at which time solubilisation and mass loss begin. The poly(cyanoacrylates) are nontoxic, and the formaldehyde released as the degradation by-product creates a toxicity concern. Crystalline domains and stereoirregularity in lactides inhibit the degradation of the polymer: poly L-lactide (PLA) (crystalline, stereoirregular) degrading at a slower rate than poly DL-lactide (amorphous, stereoirregular) and polyglycolide (crystalline, stereorregular) [97].

Preparation technologies capable of producing larger amounts of micro/nano-polymeric particles in a safe, economic, robust and well-controlled manner are still lacking [98]. For instance, spray drying must not be used for highly temperature-sensitive compounds; coacervation is frequently impaired by residual solvents and none are well suited for producing particles in the low micrometre size range. Solvent extraction/evaporation (or the double emulsion technique) neither requires elevated temperatures nor phase-separating inducing agents. Controlled particle sizes in the nano- to micrometre range can be achieved but careful selection of encapsulation conditions and materials is needed to yield high encapsulation efficiencies and a low residual solvent content.

4.5.1 *Natural biodegradable particles*

AmB was loaded in albumine microspheres (A-MS) with the aim of developing a low-cost DDS, due to the cheap materials and manufacturing process, as well as the high chemical stability of A-MS [99]. Whereas Fungizone was lethal at 5 mg/kg, AmB A-MS showed no lethal effects up to 40 mg/kg in *L. infantum* infected hamsters, which are more susceptible to AmB acute toxicity than healthy animals [100]. The reduced toxicity was higher than that achieved with AmB loaded in emulsions and heated Fungizone (in the order of threefold). AmB A-MS administered at higher doses than Fungizone cause a higher reduction in parasite number in liver and spleen. AmB A-MS achieved higher tissue concentration, and in contrast to AmBisome, lower permanence in plasma [100]. Recently, the *in vitro* activity of three AmB aggregation forms (monomeric, dimeric and multi-aggregate) loaded in A-MS against *L. infantum* amastigotes and promastigotes was compared with that of the multi-aggregate AmB form loaded in poly-lactide co-glycolide (PLGA) particles. Whereas free multi-aggregate AmB was cytotoxic, monomeric AmB A-MS showed lower LD_{50} than free AmB (0.0037 versus 0.069 µg/ml, respectively) [101].

Biodegradable starch microparticles covalently linked to primaquine only were investigated for their *in vitro* antileishmanial activity [102].

4.5.2 *Synthetic biodegradable particles*

Primaquine loaded on polyalkylcyanoacrylate nanoparticles (PACA-NP) [103] and polyisohexylcyanoacrylate nanoparticles

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(PIHCA-NP) [104] exhibited increased activity compared with free primaquine. However, empty PACA-NP was found to activate respiratory burst in macrophages in the absence of primaquine [105] whereas PIHCA was toxic on infected mice. Further developments using these polymers were discontinued.

Primaquine loaded in (DL)PLA-NP was 3.3-fold more active in reducing liver amastigotes from experimental visceral leishmaniasis (*L. donovani*) than free primaquine [106]. Although when diluted in circulation primaquine loaded in (DL)PLA-NP is almost completely released from nanoparticle matrices, upon intravenous administration in healthy mice the LD_{50} was increased almost twofold compared with that of free primaquine [107].

Another hydrophilic drug, pentamidine (second-line treatment) also loaded on PLA-NP [108] and in poly-methacrylate-NP [109] resulted in 3.3 and 6 times more activity than free drug (ED_{50} 0.17 and 0.32 mg/kg versus 1.05 mg/kg) in experimental visceral leishmaniasis (*L. infantum*)

The natural antileishmanial agent 2′, 6′-dihydroxy-4′-methoxychalcone (DMC) loaded in PLA-NP reduced the amastigote number inside macrophages *in vitro*; and skin lesions were significantly reduced (60% the size of lesions in control animals) upon two subcutaneous and two intra-peritoneal administrations on experimental cutaneous leishmaniasis. Apparently PLA-NP reach the parasite site in the parasitophorous vacuole before their degradation, suggesting that DMC may be discharged close to the parasites, improving its bioavailability. The slow biodegradation rate of PLA-NP can be related to the fact that nanoparticles remain inside the phagolysosome system while in transit, as occurs for all PLA-NP. Remarkably, the DMC PLA-NP effect was comparable to that of equivalent doses of glucantime. The particles were well tolerated by mice upon intravenous administration [110].

Other polymeric nanoparticles such as poly (ε-caprolactone)-NP loaded with AmB were found to be two to three times more effective than free AmB in reducing parasite burden from experimental visceral leishmaniasis and also showed reduced side effects associated with AmB [111].

Other experimental drugs such as the poorly hydrosoluble dihydroindolo-(2,3a) indolizines incorporated in phospholipid microspheres prepared from a mixture of PLGA and pentamidine bearing a mannose arm were highly efficient in reducing spleen parasites (91%) [112]. Meanwhile bassin acid incorporated in PLA-NP resulted in less toxicity and improved leishmanicidal activity than o/w micro-emulsions, both subcutaneously injected in experimental visceral leishmaniasis [113].

5. Drug delivery systems for topical administration

To date, no strategy has been developed for topical treatment. Only a few attempts have been made to improve drug absorption. Using permeation enhancers like ethanol, significant improvements in lesion size on experimental cutaneous leishmaniasis (*L. major*) were observed upon topically applied dispersion of Amphocil and Abelcet in solution containing 5 – 25% ethanol, over animals treated with dispersed Fungizone [114].

Only *in vitro* permeation across rat skin studies were carried out with AmB loaded in different MLV matrices. Positively charged liposomes showed a high shelf life (1 year at 30°C), with a high flux of AmB across the stratum corneum (58 ng/cm^2/h), being maximal in viable epidermis for negatively charged liposomes (23 ng/cm^2/h). AmB loaded in liposomes is reported to be more stable than free AmB in solution and in powder forms [115].

The highly hydrophilic antibiotic paromomycin associated to the permeation enhancer methyl benzethonium chloride (MBC, responsible for adverse reactions) is the most efficient topical formulation for treatment of cutaneous leishmaniasis. The skin permeation of paromomycin loaded in unilamellar liposomes (soybean phosphatidylcholine : cholesterol 1 : 1 molar ratio) was increased compared with that of MBC aqueous solution of paromomycin [116].

6. Drug delivery systems for oral administration

Mucoadhesive nanosuspensions (hydrogels) containing chitosan were shown to adhere to the gastrointestinal mucosa, prolonging the contact time of released drugs. This property was formerly used for sustained drug release against *Cryptosporium parvum*, a parasite localised in the epithelial membrane of the gastrointestinal tract [117]. In a further work, nanosuspensions made of AmB in an aqueous solution of Tween 80, Pluronics F68 and sodium cholate were produced by a high-pressure homogenisation technique, showing long-term stability at 20°C, zeta potential of -36 mV and medium size of 620 nm. When orally administered against experimental visceral leishmaniasis (*L. donovani*), a significant reduction in the liver parasite load by nearly 29% occurred. Neither the oral administration of micronised AmB, AmBisome or Fungizone significantly reduced liver parasite load compared with untreated controls [118].

Another strategy for oral delivery used cyclodextrins, cyclic hydrophilic oligosaccharides with a hydrophobic core, composed of glucose units joined through α-1,4-glucosidic bonds, widely used as drug absorption enhancers for drug delivery. Meglumine antimoniate is a highly hydrosoluble and low membrane permeability class III drug, hence complexes formed with cyclodextrins were not expected to be conventional, such as those in which hydrophobic drugs are trapped inside the cyclodextrin core [119]. Meglumine antimoniate-β-cyclodextrin complexes were prepared by mixing β-cyclodextrins and meglumine antimoniate in distilled water at 1 : 1 cyclodextrin/Sb^V molar ratio and heating the mixture for 48 h at 55°C. A final

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freeze-drying step seems to be the key to promoting the formation of supramolecular nano-assemblies between meglumine antimoniate and β-cyclodextrins, which act as a sustained release system for meglumine antimoniate, following dilution in water. Plasma Sb^V levels were about threefold higher for the complex than for the free drug upon oral administration in Swiss mice. Significantly smaller skin lesions were developed on experimental cutaneous leishmaniasis (*L. amazonensis*) upon daily administration of the complex (32 mg/kg) than those treated with meglumine antimoniate (120 mg/kg) and control animals treated with saline. The effectiveness of the complex given orally was equivalent to that of the free drug given intraperitoneally at a twofold-higher Sb^V dose [120,121].

An interesting approach used cochleates, stable phospholipid-cation crystalline structures consisting of a spiral lipid bilayer sheet with no internal aqueous space, which have the potential for oral administration of hydrophobic drugs. Cochleates containing AmB orally administered demonstrated similar activity as deoxycholate AmB administered by the intraperitoneal route in a mouse model of systemic candidiasis [122]. However, the antileishmanial activity remains to be determined.

Recently, miltefosine (hexadecylphosphocholine, HePC), a molecule that forms micelles in aqueous media, has been used to solubilise AmB. The resulting mixed micellar system of 7 nm diameter and AmB in monomer form was less toxic toward mammalian cells than aggregated AmB [123]. HePC enhances the paracellular permeability across the intestinal epithelium and could therefore improve the transport of coadministered therapeutic agents [124]. *In vitro/in vivo* toxicity as well as antileishmanial activity remain to be determined.

7. Conclusion

7.1 First barrier: target or friendly administration?

We have seen that after 30 years of research it has not been possible to develop optimal DDSs against leishmaniasis. This is a disease linked to poverty and to practically nonexistent sanitary infrastructure, making it necessary to develop DDSs that could be administered by oral or topical routes. However, no DDSs can penetrate across the first entrance barriers such as the stratum corneum or the gastrointestinal tract to reach the enterocytes. In other words, DDSs responsible for targeting MPS cells cannot penetrate across the stratum corneum to access deeper epidermal layers since small hydrophobic molecules are only allowed to diffuse, and if given by the oral route, DDSs are destroyed. For instance, the recently developed nanosuspensions and cyclodextrins allow AmB [118] and Sb^V [120,121] to be administered by the oral route but it is the released drug and not the DDS that is absorbed across the mucosa. Having lost the DDS, probably biodistribution of the absorbed drug is no different to the intravenous administered free drug.

Most of the DDSs in the pipeline remain as injectable formulations that require the use of discarded or sterilised material, trained personnel for administration and to deal with complications such as infections and potential allergic reactions to high doses of lipids [3]. Loading antileishmanial drugs in DDSs such as polymeric particles, vesicles or emulsions, or the strategy of increasing the aqueous solubility of AmB by linkage to high molecular weight polymers (Sections 3 and 4) does not rule out the need for parenteral administration because injections are the only way to introduce particulate material into the bloodstream. Conversely, the DDS is lost when using friendly routes of administration, but neither biodistribution nor intracellular traffic can be modified. To date, it has not been possible to design a DDS that allows friendly administration and targeting at the same time; one appears to exclude the other.

7.2 Second and third barriers

In order to reach its target, DDSs in blood circulation must establish selective interaction with circulating proteins and target cells that constitute the second phenomenological barrier. Molecular tags (opsonins) on DDS surfaces must be exhibited to make them recognisable by phagocytes. To increase the selectivity and amount of uptake, specific ligands for internalisable receptors have been grafted on the surface of liposomes, emulsions and polymers (Section 3 [90,95]. The resulting constructs, however, are difficult to scale up and characterise, and present problems of chemical and physical stability. Some of these ligands are sugars (mannose surface in *Leishmania*); others are peptides or protein fragments such as tuftsine and IgG derivatives. Instead of artificial grafting, uptake could be improved if the adsorption of selective patterns of circulating opsonins on plain DDSs were favoured. To date, the knowledge states that protein adsorption is higher on hydrophobic, charged and larger particles than on hydrophilic, neutral or smaller surfaces. Hence, a deeper insight on matricial features responsible for selective adsorption of plasma proteins is needed; such a pattern is thought to influence the intracellular route followed by the DDS. For instance, albumine (not an opsonin) adsorption can be responsible for the DDS being processed along an exocytic route.

The intracellular pathways followed by DDSs are still poorly controlled and constitute the third phenomenological barrier. To optimise therapeutic action in visceral leishmaniasis, DDSs must deliver the drugs in the same spatial compartment as the target. To that aim, phagocytosed DDSs must be directed to phagolysosomes, where the amastigotes reside. However, a fraction of DDSs can be sorted back to the cell exterior through sorting/recycling endosomes; only those transported to secondary endosomes will fuse with phagolysosomes. Hence, exocytic routes reduce the DDS available to reach phagolysosomes. Again, deeper insight on the structural features conducting to one or other

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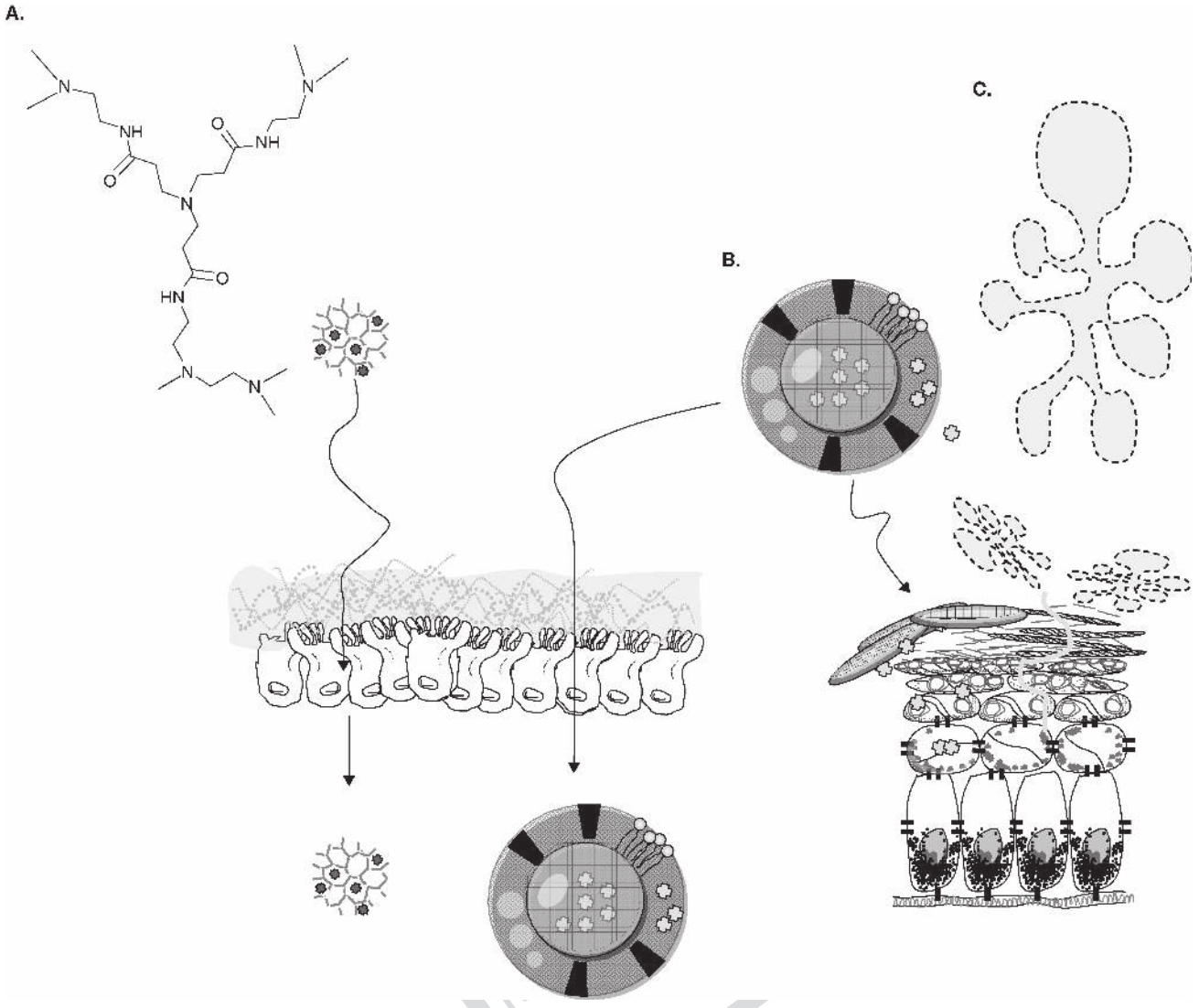

Figure 4. Novel nano drug delivery systems for potential use against leishmaniasis. A. Dendrimers: monodisperse polymers with high area/volume ratio, size between 2 and 8 nm, ranging from low generation (G0), to high generation (G8). Polyamidoamine dendrimers G2.5 – G3.5 can increase the paracellular passage across gastrointestinal mucosa, by sequestering Ca^{2+} to induce the opening of tight junctions. The complex formation mechanism is shared with chitosan-containing nanoparticles. Dendrimers are water soluble and act as unimolecular micelles to form complexes with hydrophobic drugs in their inner hydrophobic pockets. Because of their high structural stability dendrimers can be administered by the oral route [136]. Different to conventional polymeric particles, the issues associated with scaling up, presence of by-products and reproducible size are resolved for dendrimers. **B.** Solid and nanostructured lipid nanoparticles (SLNs and NLPs): solid hydrophobic core of variable crystallinity, stabilized by amphypatic surface. Drugs loaded in particles are retained and released in a controlled manner, as a function of core phase transitions in response to external stimuli such as changes in humidity, heat, light, or mechanical stress. SLNs and NPLs can be administered by the oral and topical route [137]. **C.** Ultradeformable lipid matrices: vesicles capable of experiencing spontaneous locomotion and penetration to deeper layers across water nano-channels in the stratum corneum. Ultradeformable liposomes do not fuse or coalesce on the surface of the stratum corneum, and penetrate without being destroyed. Ultradeformable liposomes could efficiently transport low or high molecular weight hydrophilic drugs across thickened lesions that represent an additional barrier to absorption in the cutaneous leishmaniasis [138].

intracellular pathways is needed. Most DDSs, such as conventional liposomes, niosomes, emulsions, lipid micelles, lysosomotropic polymers and PLA particles (which degrade slower than PLGA) remain confined inside the phagolysosomal pathway to finally localise with lysosomal amastigotes. Other DDSs made of polyester polymer PLGA experience a face charge reversal (cationisation) in the acidic pH of endosomes that leads to localised destabilisation of the membrane, fusion and subsequent release of the particle into the cytoplasm. These DDSs are thought to avoid exocytic routes; released early in the cytoplasm, the DDSs act as a source of sustained release. For this mechanism to have a therapeutic effect, however, the chemical nature of the carried drug must be accounted for. For instance, it is unlikely that the highly hydrophobic membrane pore forming AmB could achieve sustained released from cytoplasm to phagolysosomal amastigotes. However, the therapeutic effect of hydrosoluble drugs such as primaquine or pentamidine could be enhanced if achieving sustained released from the cytoplasm.

7.3 Structural constraints in cutaneous leishmaniasis

If administered by the parenteral route, and due to their special anatomical localisation, the access of DDSs to visceral leishmaniasis infected macrophages in liver, spleen and bone marrow is relatively straightforward, as is further DDS uptake and colocalisation of the delivered drug and the target. In cutaneous leishmaniasis, however, be it by the parenteral or topical route, access of DDSs to the stratum spinosum and colocalisation are hardly feasible. Administered by the intravenous route, it is believed that a fraction of small DDSs locally extravagates from systemic circulation [48]. Other smaller DDSs (nanodisks [87] and polymeric nanoparticles [110]) can be administered by the intraperitoneal route, probably because their smaller size allows them to get into systemic circulation from the extracellular space and from there further extravasation. To date, there are no studies proving that DDSs are taken up to reach the phagolysosomes in cutaneous leishmaniasis infected macrophages; probably the DDSs accumulate nearby the target stratum. For the topical route, depths of skin penetration of AmB in MLV and hydrophilic drugs in large unilamellar vesicles on skin surface have been assayed. However, the liposomal matrix does not penetrate the stratum corneum and fuses on the skin surface to act as a slow release reservoir. If the DDS matrix is absent, the chances of improving selectivity and colocalisation are also lost.

8. Expert opinion

To make DDSs capable of penetrating across mucosa or topical routes and at the same time allowing the modification of biodistribution and intracellular transit, new materials bringing new properties will be required since modification of a drug's chemical structure will be insufficient. Nanotechnology can provide the means and materials to build nano-DDSs in the size range 2 – 100 $\times$ 10^{-9} m (2 – 100 nm) [125], prepared from small biodegradable natural or synthetic blocks, preferably by cheap bottom-up (self-assembly) techniques (**Figure 4**). As for conventional DDSs, the pharmacokinetics and biodistribution of transported drugs also depend on the structure of nano-DDSs. However, structural features of nano-DDSs can be easily tailored to establish specific interaction with the close environment and to react with programmed structural changes to external signals. Being in the size of the nano-scale (below 100 nm), nano-DDSs can be taken up not only by phagocytosis but also by different pynocitic mechanisms. These properties enable nano-DDSs to surpass, for instance, the first entrance barriers (topical application across intact stratum corneum): self-locomotive nano-DDSs can penetrate across cell layers of stratum corneum across water nano channels; mucoadherent refractory to hydrolysis, oxidising agents and turbulent flux nano-DDSs could be delivered to blood circulation across the paracellular route; second barriers to achieve selective biodistribution and third barriers to intracellular traffic (up to now partly controlled by the size surface and the chemical nature of DDSs).

In particular, it is envisioned that the second and third barriers could be more efficiently crossed if precise control of the particle shape is exerted, an aspect that current fabrication methods for micro and nano-DDSs are unable to control [126]. For instance, the local shape of the particle at the point where the cell is attached, not the overall shape, dictates whether or not a macrophage begins internalisation [127]. The shape of nano-DDSs could affect the cell's ability not only to internalise successfully but also the transport and sorting of the particle once inside the cell [128]. Novel structures of nano-DDSs for specific intracellular targets have recently been described [129].

Paradoxically, AmBisome was the first nanomedicine to reach the pharmaceutical market. Today, however, only a small fraction of the diseased population can use AmBisome, in spite of its effectiveness with reduced toxicity, shorter treatment period and the response of a single dose clinically demonstrated [11,130]. In most countries it is the price of the medication that decides its applicability, independently of therapeutic and social advantages [131,132]. Hence, as long as nanomedicines remain unaffordable for most people, the benefits of the breakthrough started by AmBisome will be controversial.

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Declaration of interest

The authors declared that no competing financial interests exist.

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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