Nanotechnological strategies for treatment of leishmaniasis-a review

Review

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Nanotechnological Strategies for Treatment of Leishmaniasis—A Review

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The World Health Organization (WHO) estimates that more than one billion people suffer from neglected tropical diseases. Leishmaniasis is a widespread disease, affecting 12 million people around the world with about 1–2 million estimated new cases occurring every year. Although pentavalent antimonial drugs are the most frequently prescribed treatments for leishmaniasis, they produce severe side effects, including cardiotoxicity and hepatotoxicity. Other compounds, such as amphotericin B, pentamidine and miltefosine, are second choice drugs, but they also produce side effects that can endanger the patient's life. Nowadays, there are two approaches to develop new therapies: one is the search for new drugs and the other is the optimization of actual drug formulation. Traditional drug discovery takes 10 to 12 years in general and involves high costs; around one billion dollars on average to develop a drug. A possibility to improve leishmaniasis treatment would be the application of nanotechnology-drug delivery systems which can enhance the therapeutic potency of existing drugs by optimizing their adsorption, distribution, metabolism and excretion (ADME) and reducing toxicity. In this review we will discuss examples how nanotechnology-drug delivery systems have been used to improve the therapeutic aspects of existing antileishmanial drugs.

KEYWORDS: *Leishmaniasis, Nanotechnology, Liposomes, Lipid Nanocapsules, Solid Lipid Nanoparticles, Microemulsion.*

CONTENTS

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INTRODUCTION

Leishmaniasis is endemic in 98 countries, with 350 million people at risk, in Asia, Africa, Southern Europe and South and Central America. It is a complex of parasitic diseases with two major manifestations, visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL).[1] The estimated incidence for VL and CL is 0.3 million and 1 million cases, respectively.[2] Moreover, VL has emerged as an important opportunistic infection associated with HIV. In southern Europe, up to 70% of cases of visceral leishmaniasis in adults are associated with HIV infection.[3]

The etiologic agents are several different species, all belonging to the genus *Leishmania* which maintain their life cycle through transmission between an insect,

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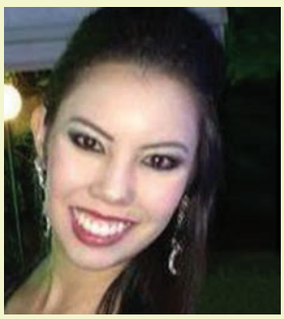

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a sandfly, and a mammalian host.[4,5] *Leishmania* organisms are dimorphic protists alternating between the promastigotes (in the insect vector) and the amastigotes (in vertebrate hosts)[6] (Fig. 1).

Pentavalent antimonials, the standard drugs for 70 years, remain being used as first-line drugs in several parts of the world as sodium stibogluconate (Pentostam®) or meglumine antimoniate (Glucantime®).[8,9] Amphotericin B (AmB) deoxycholate has been used since the early 1960s as second-line treatment for leishmaniasis in the New World, where resistance to pentavalent antimonials has emerged.[11] Alternatively, pentamidine isethionate, miltefosine and paromomycin are available, but their use is limited due to toxicity or high cost of treatment (Fig. 2).[12] Furthermore coinfection HIV-VL cases are less responsive to each of these antileishmanial therapies presenting high relapse rates or deaths due to concurrent illness complications and drug toxicity.[3] Moreover, strains of the most

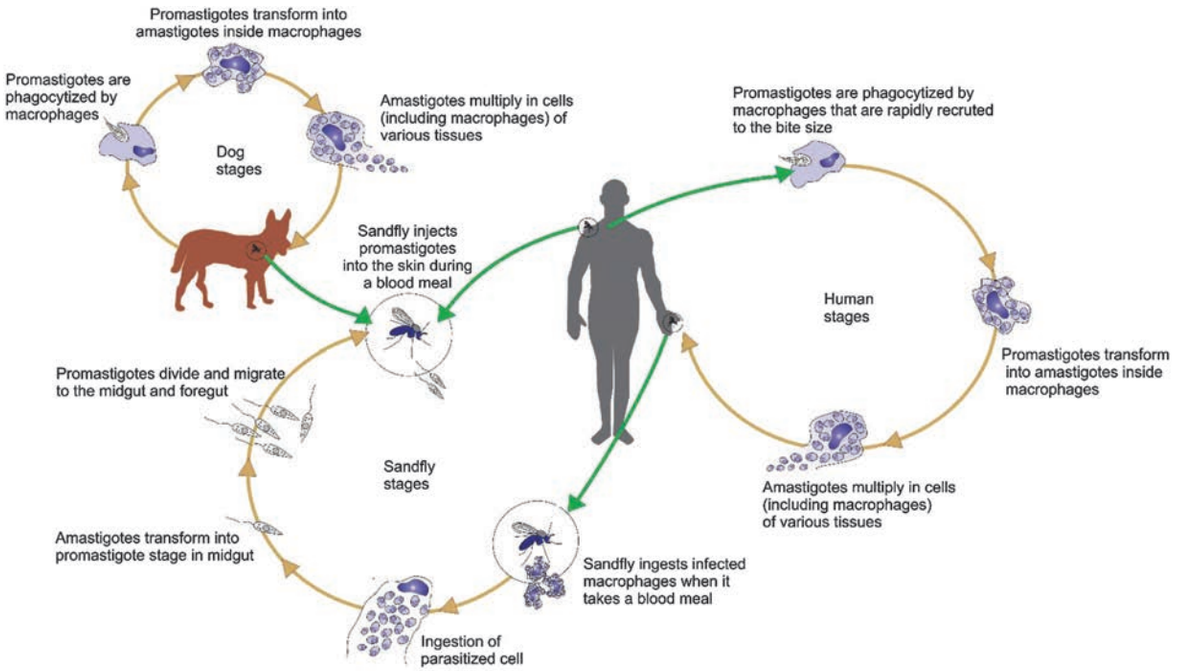

Figure 1. **Life cycle of Leishmania. Modified from [7], K. J. Esch and C. A. Petersen, Transmission and epidemiology of zoonotic protozoal diseases of companion animals. *Clinical Microbiology Reviews* 26, 58 (2013). © 2013.**

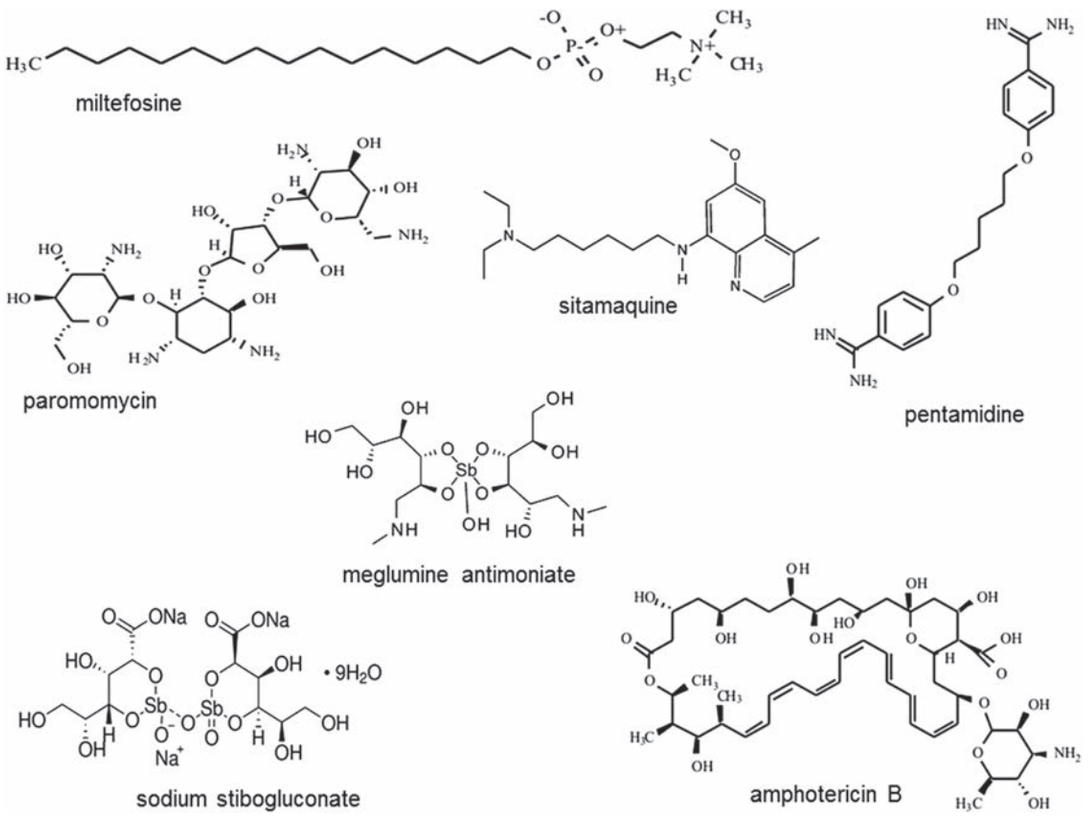

Figure 2. **Chemicals structures of miltefosine, pentamidine, paromomycin, meglumine antimoniate, sitamaquine, sodium stibogluconate and amphotericin B.**

Table I. Current antileishmanial drugs, associated limitations and (possible) mechanism(s) of action.

Drug	Administration	Associated problems	Mechanisms of action	Refs.
Sodium stibogluconate (Pentostam®) and Meglumine antimoniate (Glucantime®)	Parenteral	Hepatotoxic, nephrotoxic and potentially fatal cardiotoxic effects. Resistance in Bihar, India	Inhibition of glycolysis and fatty acid β-oxidation	[1, 8, 35]
Amphotericin B (Fungizone®)	Slow intravenous	Nephrotoxicity, hypokalemia, rigors and chills during infusion. Resistance not documented	Presents high affinity for ergosterol, the predominant sterol of the *Leishmania* cell membrane	[36, 37]
Pentamidine	Intramuscular	Pain, nausea, vomiting, dizziness, myalgia, hypertension, headache, hypoglycemia, and transient hyperglycemia	Interferes with DNA synthesis and modifies the morphology of the kinetoplast	[38]
Miltefosine	Oral	Contra-indicated in pregnancy. Gastrointestinal effects, nephrotoxicity, hepatotoxicity and possible teratogenicity. Resistance (due to inactivation of a *P*-type ATPase)	Associated with phospholipid biosynthesis and alkyl-lipid metabolism in *Leishmania*	[8, 39, 8]
Paromomycin	Topical for CL and parenteral for VL	Erythema, pain, oedema, and ototoxicity (damage to internal ear)	Inhibits translocation and recycling of ribosomal subunits and hence protein synthesis	[36, 38, 40]
Sitamaquine	Oral	Vomiting, abdominal pains, headache, methemoglobinemia (for individuals with glucose-6-phosphate dehydrogenase deficiency) and renal adverse effects	Unknown	[36, 41]

human-infective *Leishmania* species are reported to be resistant to standard chemotherapeutics[13] (Table I). Hence, development of new drugs for the treatment of leishmaniasis is urgent.

Nowadays, there are two approaches to develop new therapies: one is the search for new drugs and the other is the optimization of actual drug formulation.[14–22] Traditional drug discovery takes 10 to 12 years in general and involves high costs; around one billion dollars on average to develop a drug.[23] A possibility to improve leishmaniasis treatment would be the application of drug delivery systems which can enhance the therapeutic potency of existing drugs by optimizing their adsorption, distribution, metabolism and excretion (ADME) and reducing toxicity. However, *Leishmania* poses an additional challenge to drug delivery as the drug has to achieve therapeutic levels at multiple sites (liver, bone marrow, spleen, cutaneous lesions) and reach the parasites inside the phagolysosome of macrophages. Thus, the development of systems that are capable of delivering drugs and target parasites within the host cells is crucial. Novel advances in nanotechnology have proven beneficial in therapeutic fields such as drug-delivery and gene/protein delivery and its application in developing drug carriers has been useful for a range of different diseases caused by parasitic or bacterial pathogens such as leishmaniasis, malaria, Chagas disease and tuberculosis.[24–31] In this context, researchers have demonstrated the benefits of the use of nanotechnology to improve the efficacy as well as to reduce the side-effects and toxicity of the drugs used for treatment of these infectious diseases.

Nanotechnology involves the engineering of macromolecular devices in the nanometer range and has already been widely applied in medicine.[32, 33] There are some nanotherapeutics that have been approved by the FDA (Food and Drug Administration) and are currently available for clinical use for different diseases such as fungal infections and leishmaniasis (Abelcet®, AmBisome® and Amphotec®), hepatitis (Pegasys® and PegIntron™), HIV-associated sarcoma (DaunoXome®) and many other conditions.[34] In this review we will discuss examples how lipid-based colloid delivery systems have been used to improve the therapeutic aspects of existing antileishmanial drugs.

CURRENT STRATEGIES ON LEISHMANIASIS TREATMENT

The current treatment for leishmaniasis is based on chemotherapy and poses limitations such as toxicity, difficult route of administration and lack of efficacy on parasitic infections in some endemic areas. Despite considerable efforts to find new drugs against *Leishmania* spp., the treatment of leishmaniasis is still based on the use of the pentavalent antimonials sodium stibogluconate and meglumine antimoniate, developed during the 1920s.

These drugs are known to have severe side effects, including nausea, abdominal colic, diarrhea, skin rashes, hepatotoxicity, cardiotoxicity, nephrotoxicity and pancreatitis. Electrocardiogram evaluation (cardiotoxicity) as well as determination of serum levels of creatinine/urea (nephrotoxicity) aminotransferases/alkaline phosphatase (hepatotoxicity) and amylase (pancreatitis) all provide indications for the high incidence of adverse events, especially of pancreatitis, which in HIV and VL coinfection have often been associated with an increasing number of deaths.[42] Another problem associated to HIV-VL coinfection is the rise in the number of relapse cases after the use of antimonials.[43,44] Furthermore, resistance to antimonials has been a growing problem for approximately four decades.[45,46]

Among the chemotherapeutic agents used as second-line treatment for leishmaniasis, the polyene antibiotic AmB and its liposomal formulation have been introduced for use against VL.[47] Although it is highly effective, even in antimony-unresponsive patients, AmB has restrictions due to its renal toxicity and requirement for inconveniently slow intravenous administration.[48] Liposomal AmB is preferred over conventional AmB because of its milder toxicity profile, but its use remains very limited as a result of its high cost.[49] Only in South America, pentamidine, an aromatic diamine, has been used in the treatment of CL,[8] but severe adverse effects, including diabetes mellitus, hypoglycemia, shock, myocarditis and renal toxicity, limit its use.[50] Paromomycin is an aminoglycoside antibiotic with described leishmanicidal activity; however, this drug has been documented to have variable efficacy in different countries and is not commonly used or widely available outside Africa and the Indian subcontinent.[25,51] Miltefosine, registered in 2002, is the first, and remains the only, orally administered agent used for the treatment of all types of leishmaniasis,[38,45,52] even though gastrointestinal side-effects (anorexia, nausea, vomiting and diarrhea), hepatotoxicity and renal insufficiency have been reported.[53] Despite efforts to fight this disease for the past 10 years, and to allow the use of lipid formulations of AmB, miltefosine and paromomycin for the treatment of leishmaniasis, chemotherapy in many endemic countries, including Brazil, is still based on pentavalent antimonials or conventional AmB, notwithstanding their inherent toxicities and complex route of administration.[54]

NOVEL NANOTECHNOLOGY-BASED APPROACHES FOR ANTI-LEISHMANIAL DRUG DELIVERY

General Information About Nanotechnologies for Drug Delivery

Several authors reported the leishmanicidal activity of new compounds, but some of them showed toxicity when tested *in vivo*. Furthermore, the fact that the human pathogenic forms of *Leishmania*, the amastigotes, reside within the phagolysosome of macrophages can render it difficult for the drugs to access the parasites. In this sense, nanotechnological strategies have shown clear advantages both in efficacy to target monocytes/macrophages intracellularly and in overcoming toxicity problems (Table II).

In general, nanoformulations have been proposed primarily as means to decrease the toxic effects of available drugs, to provide sustained drug release in addition to improving their bioavailability, and to protect the incorporated drugs from being metabolized promptly, thus allowing prolonged drug residence in the human body, and therefore prolonging time between administrations.[33]

Nanomedicine is the field of science involved in the design and development of nanotechnology-based therapeutics and diagnostics with dimensions in the range of 1 nm to 1000 nm.[55,56] The design of nanometer-sized delivery systems for drugs represents one of the most promising developments for antimicrobial therapies because such systems may lead to treatments with higher target delivery effect, resulting in higher therapeutic efficacy and lower toxicity.[57] Moreover, such systems can improve bioavailability and protect the incorporated drugs from being metabolized, causing prolonged drug residence in the human body, and therefore allowing to increase the time between administrations.[33] Several nanotechnological strategies, such as lipid-based colloid systems,[37,58] nanostructured layered films,[59] polymeric nanoparticles[60,61] and silver nanoparticles[62] have been explored for precise drug delivery in leishmaniasis treatment, increasing effectiveness and safety of drug therapy.

Many drugs are poorly soluble in aqueous phase which compromise their distribution into and within the bloodstream. Solubilization of hydrophobic drugs in colloidal particles is a possible approach to overcome the problem of bringing a hydrophobic substance into the aqueous blood compartment.

Nanoparticles (NPs) can be classified either by the way in which they carry substances or by the characteristics of the matrix. According to the classification based on the type of material from which the matrix is made one can distinguish organic nanoparticles and inorganic nanoparticles.[63] Nanotechnology-drug delivery systems include organic NPs, liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), polymeric nanoparticles and microemulsions (Fig. 3). The inorganic nanoparticles involve the use of different inorganic oxides and have different sizes, shapes, solubility, and long-term stability.[64] They are usually synthesized by chemical reduction of the metallic salt (gold/silver/silica/aluminum/titanium) with a reducing agent.[65]

Liposomes

Liposomes are spherical vesicular structures that are composed of phospholipid bilayers concentrically oriented around an aqueous compartment that serve as carriers

Table II. Advantages and disadvantages of current nanomedicines.

Nanotechnology based-system	Advantages	Disadvantages	Reference
Liposomes	Liposome increased drug stability via encapsulation; Liposomes are non-toxic, flexible, biocompatible, completely biodegradable; Liposomes increased efficacy and therapeutic index of drug; Flexibility to couple with site-specific ligands to achieve active targeting; Use preferentially for parenteral or cutaneous routes	Short half-life; Leakage and fusion of encapsulated drugs; Production costs can be high	[132]
Polymeric nanoparticles	Avoid reticulo-endothelial system; High level of biocompatibility to reduce cytotoxicity and maximize tissue compatibility		[101]
Lipid nanocapsules	Easy to scale-up; Encapsulating lipophilic and hydrophilic drugs; Avoidance of organic solvents; Use for cutaneous route	Toxicity related to surfactant	[58]
Solid lipid nanoparticles	Easy to scale-up and sterilize; Improve stability of drugs; Encapsulating lipophilic and hydrophilic drugs; Avoidance of organic solvents; Use for oral, parenteral or cutaneous routes	Low drug loading and expulsion of matrix; Short half-life; Toxicity related to surfactant	[58]
Nanostructured lipid carriers	Improve stability of drugs; Improve loading of drugs when compared with SLN; Long shelf-life; Easy to scale-up and sterilize; Avoidance of organic solvents; Use for oral, parenteral or cutaneous routes	Toxicity related to surfactants	[133]
Microemulsions	Solubilize hydrophilic and lipophilic drugs; Easy preparation; Long shelf-life; Thermodynamically stables; Small size droplets ($<$0.22 μm) and can be sterilized by filtration; Use for oral, parenteral or cutaneous routes	Large amount of surfactants; Toxicity related to surfactant	

of lipophilic or hydrophilic drugs.[67] The main advantage of liposomes is their ability to provide sustained drug release, leaving the drug available in the blood circulation for a long period of time, which increases the prophylactic effect and reduces the drug dosage; additionally, these structures are biocompatible with biological fluids, decreasing the toxicity of drugs and limiting any local inflammatory reactions.[68–70] For antileishmanial therapy, drug targeting can be achieved by liposomal encapsulated drugs allowing them to reach the intracellular *Leishmania* amastigotes; the drug containing liposomes naturally enter in the macrophages by phagocytosis and hence deliver the drugs passively to the phagolysosome where they then can act directly on the parasites.[71–73]

Severe acute and chronic side effects have limited the use of AmB deoxycholate as antileishmanial agent.[74] Thus, in an attempt to improve the pharmacokinetic properties of the drug, the tolerability in the patient and to minimize the side effects, three lipid-associated formulations were developed and extensively used in clinical studies for treatment of leishmaniasis.[75–83]

The commercial liposomal AmB, Ambisome, produced by Gilead Sciences, is the only drug approved in 1997 by the FDA for VL treatment.[84] Ambisome presented low toxicity and high cure rate (above 90%) when compared to AmB deoxycholate (Fungizone)[85, 86] and is recommended by the WHO as first-line therapy for treatment of VL caused by *L. donovani* in India, Bangladesh, Bhutan and Nepal or *L. infantum* in the Mediterranean Basin, Middle East, Central Asia and South America.[87] This liposomal formulation is 350–750 times more active than meglumine antimoniate and 2–5 times more active than free AmB.[11, 86] Ambisome has been used to treat HIV-*Leishmania* coinfected patients;[84] however, this treatment has not been able to reduce relapse and mortality rates in cases of such coinfected patients[88] and unresponsiveness to Ambisome seemed to develop rapidly in co-infected patients.[89] Although Ambisome is the most efficacious AmB liposomal formulation, there are other formulations of this drug currently available on the market: the colloidal formulation Amphocyl in Europe (also called Amphotec in USA), the injectable suspension Abelcet (USA), Fungisome (India), and the emulsion Amphomul (India), but the administration and toxicity of the drug still pose problems in each of these therapeutic alternatives.

The improvement of antimonial chemotherapy is urgent; however, there are very few studies in experimental models regarding the use of liposomes containing antimonials for leishmaniasis treatment.[70, 90–95] The meglumine antimoniate liposomal formulation was 10-fold more effective than the free drug against intracellular amastigotes of *L. major* and five-fold more selective to the parasite with reduced macrophage toxicity. It was observed that the uptake of meglumine antimoniate containing liposomes was higher

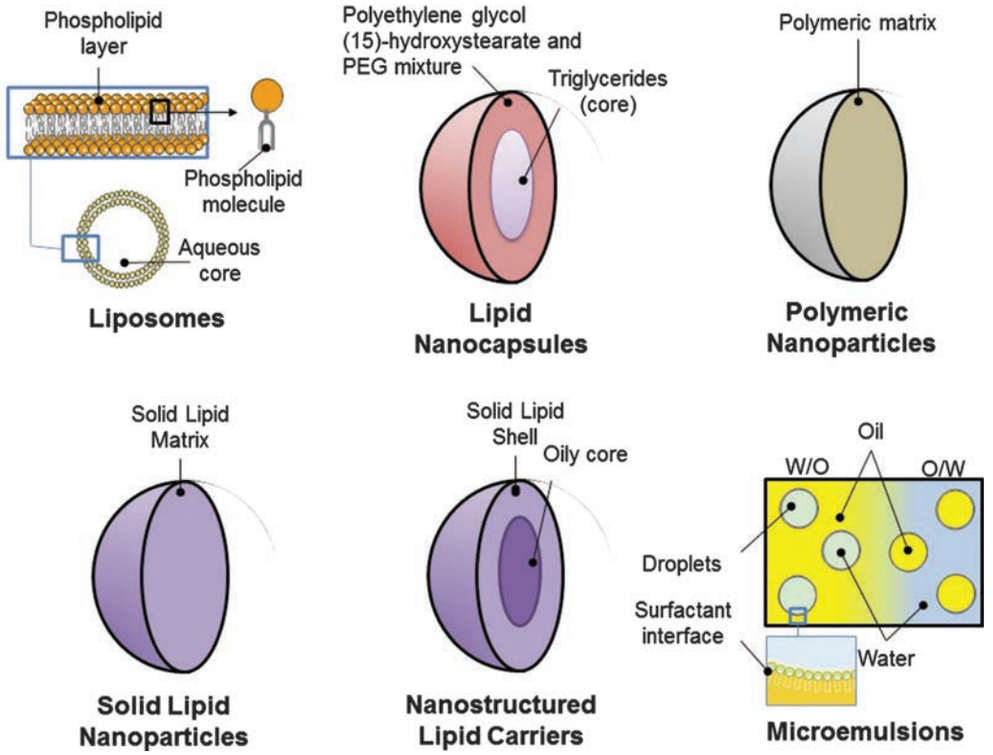

Figure 3. **Conventional lipid-based colloidal carriers. Schematic differences between liposomes, lipid nanocapsules, polymeric nanoparticles, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and microemulsions drug delivery systems. Modified from [66], B. Fonseca-Santos, et al., Nanotechnology-based drug delivery systems for the treatment of alzheimer's disease. *International Journal of Nanomedicine* 10, 4981 (2015). © 2015.**

than that observed for non-infected macrophages which could have contributed to the improvement of the selectivity index.[91]

A macrophage possesses many receptors on its surface involved in control of its cellular processes such as activation, recognition, endocytosis and secretion. Incorporation of ligands into liposomes capable of interacting with macrophage receptors has been used to enhance the uptake of liposomal content by the macrophages.[96] Mannose and 4-sulphated *N*-acetyl galactosamine bearing liposomes showed increased antileishmanial activity and enhanced intracellular localization of AmB compared to unmodified liposomes.[97] Other macrophage–specific ligands such as tuftsin residues and phosphatidylserine have also been incorporated into liposomes resulting into improved macrophage targeting of antileishmanial agents.[91, 98, 99]

Polymeric Nanoparticles

Polymeric nanoparticles are composed of a polymeric matrix, which can be made of synthetic or natural polymers.[100] They present a high level of biocompatibility to reduce cytotoxicity and maximize tissue compatibility, by their formulation from either natural or synthetic polymers.[101] The synthetic polymers most widely used are polyesters such as polylactide (PLA), polylactide–polyglycolide copolymers (PLGA), polycaprolactones (PCL), and polyacrylates (PCA); examples of natural polymers employed are albumin and chitosan.[102] The most commonly used polymer is PLGA and several PLGA-drug delivery systems have been approved by the FDA.[103] Polymeric nanoparticles are prepared by several methods, such as solvent evaporation, spontaneous emulsification, solvent diffusion or polymerization.[104]

The first researchers who evaluated the potential of polymeric nanoparticles against leishmaniasis were Gaspar and coworkers.[105] They developed primaquine-loaded poly-alkylcyanoacrylate nanoparticles against *L. donovani*-infected macrophages. This formulation was shown to be 21 times more effective than the free drug in clearance of the parasite.[105] AmB has also been employed in the development of some polymeric nanoparticles. In some studies, PLGA nanoparticles with AmB have been developed and were tested against *in vitro* cultured promastigotes and intracellular amastigotes of *L. infantum*. Moreover, their *in vivo* biological activity was determined in a mouse model and compared with that of the commercial solution AmBisome in doses of 2.5 and 5.0 mg/Kg AmB equivalents by intravenous administration. PLGA nanoparticles appeared to be equally effective as AmBisome against the different

Leishmania life-cycle stages in the *in vitro* assays and superior in *in vivo* efficacy.[106] Another study developed AmB nanoencapsulated in PLGA and dimercaptosuccinic acid (DMSA) nanoparticles. Their efficacy was evaluated in the treatment of experimental cutaneous leishmaniasis in C57BL/6 mice. AmB-coated PLGA–DMSA nanoparticles showed the same efficacy as free AmB to reduce paw diameter; however, the treatment with these nanoparticles also promoted a significantly greater reduction in parasite number and cell viability compared with the free drug.[107]

Lipid Nanocapsules

Lipid nanocapsules (LNs) are biomimetic carriers that mimic lipoprotein with size ranges from 20 to 100 nm. These carriers are composed of a lipid core surrounded by a membrane made from surfactant characterized by a hybrid structure between polymer nanocapsules and liposomes. Their preparation involves a method based on the phase inversion principle of an oil/water (O/W) system upon thermal manipulation. Unlike liposomes which are manufactured through processes involving organic solvent and are leaky and unstable in biological fluids, LNs are prepared by solvent-free methods, and "soft-energy" technology and present great stability (with physical stability up to 18 months). In addition, besides the advantage of high drug bioavailability, it is important to emphasize that LNs containing drugs are able to deliver their content directly to the target site at a reducible dosage (10,000 fold), thus leading significantly to the prevention or at least reduction of any side effects to acceptable levels.[108, 109]

The potential of targeting and drug delivery of LNs to specialized phagocytes via phosphatidylserine (PS)-specific ligand-anchored nanocapsules bearing doxorubicin (DOX) has been evaluated. Kansal and coworkers showed that LN containing doxorubicin (LN-DOX) had a 1.75-fold higher uptake if PS is anchored onto its surface compared with non-PS containing LN-DOX and that the former favored delivery into the cell cytoplasm.[24] PS containing LN had a lower IC50 value against *L. donovani*, probably as a consequence of better uptake of PS-containing LN as both formulations had similar entrapment efficiencies (80%). Therefore, PS can improve uptake by macrophages by binding to ligands present on the surface of the cell or the carrier system.[24]

Miltefosine is a membrane-active alkylphospholipid administered orally for treatment of leishmaniasis. Its mechanism of action may involve disturbance of calcium homeostasis in *Leishmania*.[110] Electron paramagnetic resonance (EPR) spectroscopy studies showed that this drug causes dramatic injuries in the membrane of *Leishmania* promastigotes.[111] In studies of drug delivery of miltefosine-loaded LNs, these formulations showed high entrapment efficiency, good colloidal properties, sustained release of the drug and physical stability. It is possible to design unique nanodevices combining the biological activity of a drug with the biopharmaceutical advantages of LNs, and allowing targeting via the oral route. From a clinical point of view it is important that available data suggest the feasibility of a single-dose oral nanomedicine for enhanced therapy of schistosomiasis caused by *Schistosoma mansoni* and possibly other diseases like leishmaniasis.[112]

Although there are few studies regarding the effectiveness of this nanocarrier against *Leishmania* spp, its structure has shown advantages such as the ability to deliver hydrophobic/hydrophilic drugs, the increased uptake of drugs by the cell, the possibility for both parenteral and oral delivery, and the feasibility to target drugs.[90, 113]

Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs)

Among the nanocarriers, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are the most promising.[114] SLNs are prepared by using physiologically tolerated solid lipid components and are applied for administration of lipophilic drug molecules. However, SLNs have major drawbacks of low drug loading and possible drug expulsion due to a propensity for lipid crystallization or transformation during storage procedures (Table II). To overcome these disadvantages, NLCs have been introduced with a matrix made up of a mixture of solid and liquid lipids. SLNs and NLCs have several advantages such as low cost and safety compared to other colloidal carriers including liposomes, microemulsions and nanocapsules.[115] Although NLCs possess a higher capacity for drug incorporation than SLNs,[58, 113, 116] this formulation has not yet been evaluated as alternative administration for therapy of leishmaniasis.

Paromomycin has been the most intensively studied compound with regard to the potential topical treatment for leishmaniasis.[117] Conventional topical dosage forms of paromomycin had already been tested previously, but despite the promising results obtained, the efficacy was limited due its physicochemical properties and different constituents present in ointments formulations which contribute to insufficient concentration of paromomycin at the sites of infection after topical administration.[118–125] For the treatment of CL, drugs topically applied must be able to cross the skin and reach the *Leishmania* amastigotes within the phagolysosomes of infected macrophages localized in the deep dermal layer of the skin.

The therapeutic potential of SLNs has been evaluated in different studies such as cancer[126] and parasitic diseases such as malaria,[127] human African trypanosomiasis[128] and CL.[129] Particularly for CL, SLNs could improve the interaction of the drug with the stratum corneum and other layers of the skin and thus provide the possibility of topical administration with controlled release, reduced drug toxicity and so to optimize its treatment.[129, 130] Paramomycin-containing SLN has been previously described[130] and

showed potent activity against *L. major* and *L. tropica* intracellular amastigotes when compared to free paromomycin; the cytotoxicity of paramomycin-SLN formulation is size dependent.[131] These results showed that delivery of drugs to macrophages via nanoparticles is feasible; however, additional studies are necessary in order to investigate the *in vivo* antileishmanial potential of this antibiotic formulation.

In a similar way, Gupta and coworkers developed AmB containing SLN as well as AmB loaded in modified SLN coated with the macrophage-specific ligand, O-palmitoyl mannan.[10] The antileishmanial activity of free and SLNs entrapped AmB was tested *in vitro* against a *L. donovani* infected macrophage-amastigote system (J774A.1 cells), which showed higher efficacy of the AmB-modified SLNs over AmB-SLNs and free drug. Both formulations were also able to target infected macrophages in the liver and spleen of mice which demonstrates the potential of these formulations as therapeutic alternative to treat VL.[10]

Among all its sophisticated mechanisms of evasion of the host immune system[134–142] is *Leishmania*'s capability of preventing the generation of pro-inflammatory cytokines[143] and activating a Th2 type immune response. Therefore, stimulation of Th1 and suppression of Th2 immune responses are considered a promising therapeutic strategy for leishmaniasis.[144] It is known that the aminopolysaccharide chitosan stimulates macrophages to produce several proinflammatory cytokines including IL1, IL6, TNF-α and nitric oxide (NO).[144–146] Thus, AmB-SLN bound to chitosan successfully exhibited low toxicity and improved the therapeutic effect against *L. donovani* intracellular amastigotes, probably because of an enhancement of NO production during the TH1 cell response which could explain its efficacy over amphotericin loaded SLN and free AmB.[129]

Microemulsions

Microemulsions are translucent, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant, with a size of approximately 100 nm. Such a mixture may form hydrophilic aggregates in oil (water-in-oil system, W/O), hydrophobic aggregates in water (oil-in water system, O/W), and also bi-continuous systems, such as a mixture of W/O and O/W, in which the dispersed phase is in the nanometer size range. Microemulsions have generated considerable interest over the years as potential drug delivery systems.[147, 148] The existence of microdomains of different polarity within the same single-phase solution improves drug solubilization and protection against enzymatic hydrolysis, while a potential to increase drug absorption is provided by the surfactant that induces membrane fluidity and thus permeability. Despite the huge therapeutic potential of microemulsions in leishmaniasis treatment, there are few reports about it in the literature, most of them merely describing the preparation of AmB or the promising experimental drug buparvaquone loaded microemulsions rather than their potential biological application.[149]

Other Nanocarriers

Currently, new nanosystems are being developed. One of the most popular technologies involves carbon nanostructures such as nanotubes.[63] Carbon nanotubes have engrossed remarkable attention as the most promising nano-material in the 21st century for numerous applications.[152, 153]

AmB has been attached to functionalized carbon nanotubes. This new formulation of AmB (f-CNT–AmB) exhibited significantly higher efficacy against *in vitro* intracellular amastigotes of *L. donovani* than that of free AmB. The *in vivo* toxicity assessment of the compounds in BALB/c mice revealed no hepatic or renal toxicity. Moreover, f-CNT-AmB inhibited the amastigote replication in hamsters by 90%, instead of treatment with free AmB that caused 69% inhibition.[154]

Another study involved the test of a formulation of betulin (BET) attached to functionalized carbon nanotubes (f-CNTs) against *L. donovani*. The fCNT-BET was 12-fold more effective against intracellular amastigotes than BET alone with no significant cytotoxicity observed on host cells.[155]

ABSORPTION AND DISTRIBUTION OF NANOPARTICLES

The absorption and distribution profile of a nanoparticle is greatly determined by physicochemical properties such as size, charge, hydrophobicity, and targeting molecules, and these properties are dependent on the type of the delivery system[156] (Fig. 4).

The size of the nanoparticle is important for its entry into cells, its interactions with the immune system, and its clearance.[158] Cell uptake mechanisms are partially dependent on size. Endocytosis is the process through which nanoparticles or small molecules enter cells; the specific type of endocytosis through which the nanoparticle enters the cell determines the translocation of the entrapped molecule inside the endosome.[159] Hydrophobicity affects cellular uptake, distribution, interaction with immune cells and plasma proteins, and clearance from the body.[158, 160] Charge is important for mucoadhesion or diffusion, cellular uptake, and toxicity. Targeting affects biodistribution and immune responses.[158]

These physicochemical characteristics of the nanoparticle surface also play a decisive role in uptake. Passive vectoring is related to the inherent capacity of phagocytic cells when they recognize substances foreign to the organism.[63] According to the physicochemical characteristics of the nanocarrier and the nature of the target cells, two main internalization pathways may occur: either

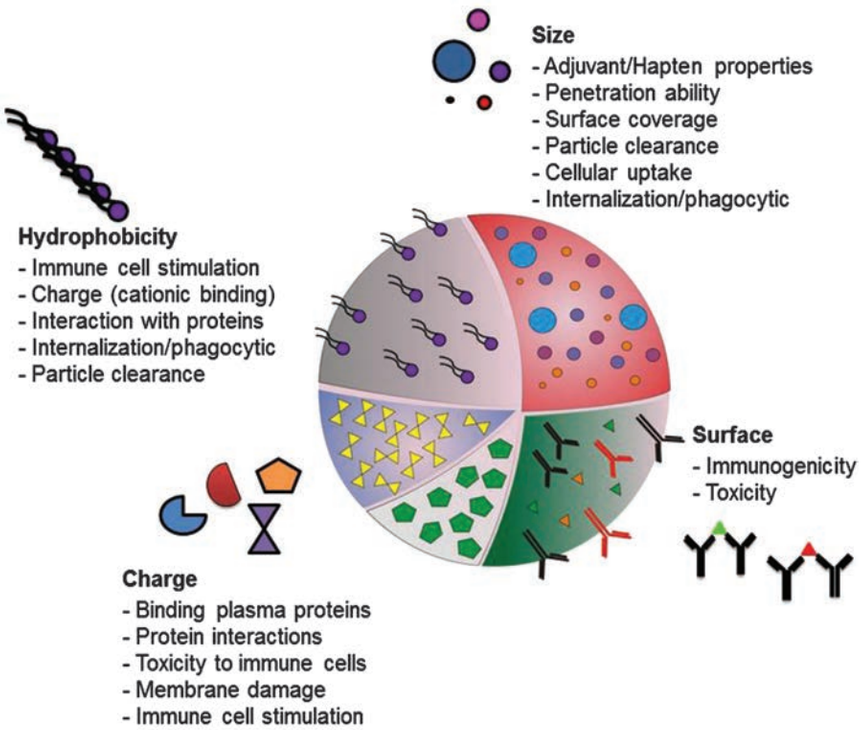

Figure 4. Properties of nanoparticles such as size, surface, charge and hydrophobicity that each, by different mechanisms, may affect the efficacy of drug delivery. Modified from Dobrovolskaia and McNeil, 2007 and [157], J. Lazarovits, et al., Nanoparticle-blood interactions: The implications on solid tumour targeting. *Chemical Communications* 51, 2756 (2015). © 2015.

phagocytosis or other endocytic pathways (macropinocytosis or endocytosis) (Fig. 5).[102]

Distribution and uptake can also be driven by active vectoring. This involves the addition of specific compounds (ligands) to the nanoparticle surface. Such ligands favor interactions with the cell membrane, thus enhancing the recognition of nanoparticles by cells.[161, 162]

The ligand is chosen dependent on its stability and selectivity with regard to the target cells. Other factors such as availability and interactions with immunologic cells or with membranes are also taken into account.[63] It is noteworthy that polymeric nanoparticles and liposomes, whose structure and chemical composition strongly differ, still show similar interactions with macrophages, based on their surface electric charge. Liposomes displaying a negatively charged surface, generally containing the negatively charged phospholipids phosphatidylserine (PS) and phosphatidylglycerol (PG), exhibit a much higher binding to and phagocytosis by macrophages as compared to neutral vesicles.[163, 164] Indeed, up-regulation of the level of negatively charged PS in the outer leaflet of the *Leishmania* spp. amastigote plasma membrane was recently demonstrated. This seems to be highly relevant for their attachment to macrophages,[165] as negatively charged phospholipids expressed in the outer membrane leaflet of cells are recognized by macrophages (via scavenger receptors) as apoptotic cells.[166]

COST EFFECTIVENESS ANALYSIS OF THERAPY-BASED NANOTECHNOLOGY AND DRAWBACKS

Commercial feasibility is the most desired characteristic of any novel delivery system and it is governed by the cost of the material and the ease of manufacturing and scale up.

Although liposomal AmB is currently the most effective strategy, this drug is quite expensive resulting in higher cost-effectiveness.[168] The higher the dosage, the more expensive the treatment. Indeed, the cost (estimates presented in 2008 US dollars, US$) per patient treated with Ambisome varied from $153.4 (10 mg/kg, single dose) to $300 (20 mg/kg of L-AmB for 4 days). In order to reduce the cost of treatment, clinical trials involving coadministration of Ambisome with other antileishmanial drugs were conducted. The estimated total cost of the treatment for combination therapy per patient is $129 and $132 for Ambisome along with miltefosine and Ambisome along with paromomycin, respectively, compared with $153 for a single dose of Ambisome (10 mg/kg).[168, 169] The cost limitations of Ambisome were counter balanced to some extent by a donation program involving Gilead Sciences and WHO in 2012 that involved the donation of 450,000 vials of Ambisome with their distribution to all beneficiary countries in 2013. However, the lyophilized Ambisome is not stable at temperatures above 25 °C (kept lyophilized) or even at 2–8 °C (when reconstituted in sterile water)

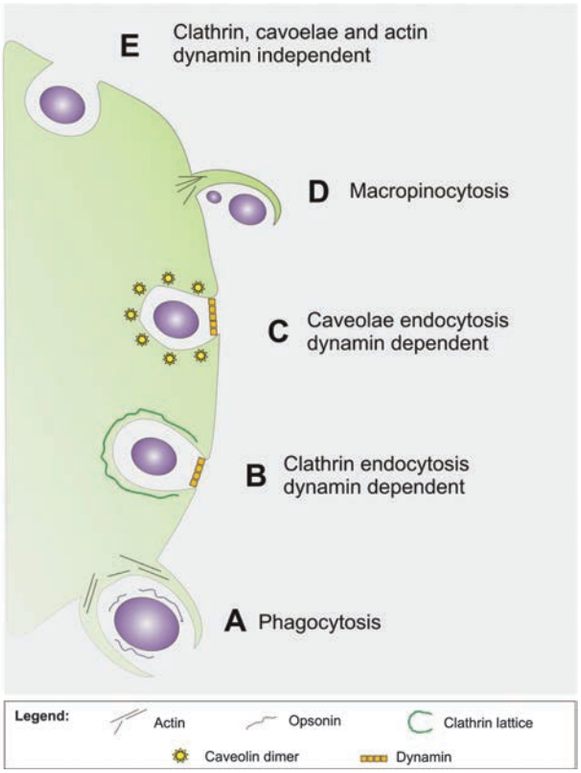

Figure 5. Main internalisation pathways of mammalian cells. (a) Phagocytosis is an actin-based mechanism occurring primarily in macrophages, and fully associated with opsonization. (b) The process by which clathrin-coated vesicles are produced involves interactions of multifunctional adaptor proteins with the plasma membrane, with the formation of a clathrin lattice and a dependence on the GTPase dynamin. (c) Caveolae are one source of clathrin-independent or raft-dependent endocytosis. Caveolae-mediated endocytosis occurs in typical flask-shaped invaginations of the membrane coated with caveolin dimers, also with a dependence on dynamin. (d) The formation of macropinosomes involves actin-based deformation and remodeling of the cell membrane and is regulated by mechanical stimuli to which cells are exposed. Macropinocytosis is an actin-based pathway, engulfing extracellular milieu with nanoparticles; it has a poor selectivity. (e) Other endocytosis pathways can be involved in the nanoparticle internalization, independent of clathrin, caveolae and actin. Modified from [102, 167], H. Hillaireau and P. Couvreur, Nanocarriers' entry into the cell: Relevance to drug delivery. *Cellular and Molecular Life Sciences* 66, 2873 (2009). © 2009 and K. Murugan, et al., Parameters and characteristics governing cellular internalization and trans-barrier trafficking of nanostructures. *International Journal of Nanomedicine* 10, 2191 (2015). © 2015.

which limits its use in the field in most of the affected countries.[170, 171]

The commercial scenario for SLN is promising. SLNs are based on triglyceride lipids which are less expensive than phospholipids and their manufacturing process and scaling up are feasible.[113] The scale up and manufacture process are also possible when microemulsions are used as template for SLN production.[172]

PERSPECTIVES AND CONCLUSION

Targeting of antileishmanial drugs to infected macrophages via novel drug delivery systems presents a promising approach to overcome the limitations associated with the current treatment protocols. The unmodified and surface engineered drug carriers resulted in the reduced toxicity and increased effect of drug on the *Leishmania* parasites. The development of drug delivery systems will allow to overcome not only toxicity and drug effectiveness but also contribute to lower costs which will not only benefit the treatment of patients with leishmaniasis but also accelerate the development of systems for other serious (sub)tropical infectious diseases such as malaria, Chagas disease, tuberculosis, schistosomiasis among others.

Despite the promising results involving nanotechnology-drug delivery systems in the treatment of leishmaniasis, the results obtained so far are mainly based on preclinical studies and, consequently, patients have not entirely yet been benefited. The next step will thus be to conduct clinical trials to confirm the results already obtained. Indeed, despite the need to improve antimonial chemotherapy and the extremely promising results obtained with liposomes in experimental models of leishmaniasis, no pharmaceutical composition associating lipid formulations with an antimonial has reached commercialization so far. The meglumine antimoniate, for instance, presents low drug encapsulation efficiency in liposomes due to its hydrophilic property as well as short-term stability, thus it is necessary to develop other nanotechnology-based drug delivery systems to overcome these problems.

Conflicts of Interest

The authors declare no conflict of interest.

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