Chapter 11

Nanomedicines for Cutaneous Leishmaniasis

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Abstract

Leishmaniasis is a vector-borne disease caused by *Leishmania* parasites, which cause a range of clinical manifestations in man. These are didactically classified into cutaneous leishmaniasis (CL), the most common form of the disease, and visceral leishmaniasis (VL), the life-threatening form. There are so far no vaccines approved for humans. Conventional drugs pose limitations ranging from low efficacy and high cost to systemic toxicity. Low efficacy derives in part from difficult drug access to the parasites, which rides themselves inside macrophage phagosomes. This prompts to high dosage, with consequent increased toxicity. Difficult intracellular drug access can be overcome with nanomedicines such as biocompatible lipid and polymeric nanoparticles that can be phagocytosed by the infected macrophages. Besides cell membranes, appropriate drug nanostructuring may allow tissue barrier penetration and drug administration through higher compliance routes such as skin and intestine, in contrast to the usual intravenous and intramuscular routes. In general, CL and VL are both treated with toxic systemic injections, disregard of disease severity. This chapter will review and discuss studies with nanomedicines that have reached the market such as liposomal amphotericin B for intravenous administration, and innovative preclinical studies aiming at developing effective cutaneous and oral drugs with focus on CL.

Keywords: *Leishmania*, cutaneous leishmaniasis, chemotherapy, drug delivery systems, nanodrugs, liposomes, solid lipid nanoparticles, polymeric nanoparticles, nanoemulsions

1. Introduction

Leishmaniasis is a complex of neglected tropical diseases (NTDs) caused by intracellular protozoans of the genus *Leishmania*, transmitted to humans and other animals by the bite of infected female phlebotomine sand flies. Once in the vertebrate skin, the flagellated

promastigote forms are phagocytosed by local macrophages. Once inside macrophage phagolysosomes, the parasites survive enzyme digestion, transform into amastigote forms and multiply. Dermotropic parasite species causing cutaneous leishmaniasis (CL) remain in the skin, whereas viscerotropic species causing visceral leishmaniasis (VL) migrate to deeper macrophage-rich organs such as liver, spleen, and bone marrow.

Although not fatal as VL, CL is the most common form of leishmaniasis and a serious public health problem. According to the World Health Organization (WHO) estimates, CL is endemic in 87 countries, with almost 200,000 new cases reported in 2015 [1]. From 2005 to 2013, CL-associated morbidity increased by 175% of disability-adjusted life years (DALYs) [2]. The impact of CL may be much greater considering the high under-reported cases and estimation that one fourth of the world population (1.7 billion people) live in area at risk of infection [3]. In addition, inadequate disease control may promote the progression of CL to more morbid and undefined subforms, such as diffuse CL and mucosal leishmaniasis (ML).

In the great majority (>90%) of cases worldwide, CL is of the uncomplicated type, with 1–4 localized skin ulcers, not larger than 3–4 cm diameter, with a raised border and central depression [4]. Even with localized manifestation, current treatment is normally based in the daily administration of intramuscular or intravenous injections with antimonials, pentamidine, or amphotericin B for 20–30 days. Besides limited to few drugs, and occurrence of drug resistance, available CL treatment produces unacceptable systemic toxicity [5].

Ideally, CL chemotherapy as proposed by Drugs for Neglected Diseases initiative (DNDi) should be efficacious against all species, compatible in combination therapy, safe in pregnant and breastfeeding women, and administered by oral or topical route [6]. However, oral and topical therapies have shown limited efficacy.

The major challenge in CL treatment is the preferred intracellular parasite location in macrophage phagolysosomes. That hinders drug access, making treatment with conventional formulations especially difficult [7].

Thus, the search for new drugs with different mechanisms of action and innovative forms of drug delivery systems appropriate for the effective treatment of CL is urgently needed. In that context, nanotechnology has emerged as an interesting strategy to increase drug potency and reduce toxicity.

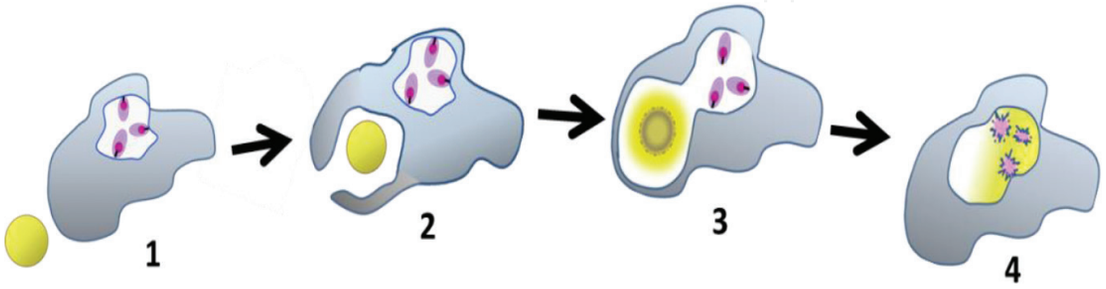

Figure 1. Nanoparticle drug delivery to intracellular parasites. A drug-loaded lipid or polymeric nanoparticle (Np, yellow) reaches the *Leishmania*-infected macrophage (1). The Np is actively phagocytosed by the infected macrophage (2). The Np-containing phagolysosome fuses with the amastigote-containing parasitophorous vacuole (3). Drug is released from digested Np to kill amastigotes (4).

Nanotechnology consists of the development of systems, structures, or devices in the nanometric scale, presenting at least one novel/superior characteristic or property over the original [8]. The use of nanostructured particles for drug delivery is a promising strategy due to their versatility. Besides, they may: (i) protect the drug against physical, chemical, and/or enzymatic degradation, (ii) enhance the pharmacokinetic properties, and (iii) improve bioavailability. They may also be functionalized for drug release at a specific site and thereby reduce systemic toxicity [7]. Furthermore, leishmaniasis is a particularly interesting disease to be treated with drug-loaded nanoparticles since the parasites almost exclusively infect the highly phagocytic macrophages. In this way, the infected cells of the skin (CL) or deep organs (VL) take up the nanoparticulated drug, which will reach the parasitophorous vacuole and act directly on the parasite (**Figure 1**). This allows the drug to reach an effective intracellular concentration, allowing dose and toxicity reduction. Particle uptake may be further increased with surface functionalization with receptor-binding ligands like mannose or mannan [9].

Interest in designing nanomedicines for CL has grown over the years, as seen by the steady increase in scientific publications. Several nanosystems, such as liposomes [10–20], solid lipid nanoparticles [21, 22], lipid complexes [23, 24], lipid-core nanocapsules [25], polymeric particles [26–31], inorganic nanoparticle [32–35], cyclodextrins complexes [36, 37], and drug nanoparticles [38] have been tested *in vivo* by different routes in experimental mouse and hamster models to improve CL treatment as summarized in **Table 1**. Of those, only liposomal

Routes	Drug	Nanosystem	Parasite	Efficacy	Ref
Parenteral	Amphotericin B	Chitosan and chondroitin sulfate nanoparticles	*L. amazonensis*	Yes	[26]
	Amphotericin B	Poloxamer 407-micelles	*L. amazonensis*	Yes	[27]
	Amphotericin B	PLGA-DMSA nanoparticles	*L. amazonensis*	Yes	[28]
	Amphotericin B	Liposome	*L. tropica*	No	[10]
	Amphotericin B	Liposome (Ambisome®)	*L. major*	Yes	[11]
	Amphotericin B	DSHemsPC-liposome	*L. major*	Yes	[12]
	Amphotericin B	Nanodisks	*L. major*	Yes	[23]
	Amphotericin B	PADRE-derivatized-dendrimer complexed with liposome	*L. major*	Yes	[13]
	Chalcone DMC	PLA Nanoparticles	*L. amazonensis*	Yes	[29]
	Nanoselenium	Inorganic nanoparticle	*L. major*	Yes	[33]
	Paromomycin	Solid lipid nanoparticle	*L. major*	Yes	[21]
	Paromomycin	Solid lipid nanoparticle	*L. tropica*	Yes	[22]
	Pentamidine	Methacrylate nanoparticles	*L. major*	Yes	[30]
	Pentavalent antimonial	Nanohybrid hydrosols	*L. amazonensis*	Yes	[38]
	Sodium stibogluconate	Liposome	*L. mexicana* / *L. major*	Yes	[14]

Routes	Drug	Nanosystem	Parasite	Efficacy	Ref
Oral	Quercetin	Lipid-core nanocapsules	*L. amazonensis*	Yes	[25]
	Meglumine antimoniate	Beta-cyclodextrin	*L. amazonensis*	Yes	[36]
	Meglumine antimoniate	Polarity-sensitive nanocarrier	*L. amazonensis*	Yes	[24]
Topical	Amphotericin B	Liposome	*L. mexicana*	No	[15]
	Amphotericin B	Gamma-cyclodextrin	*L. amazonensis*	Yes	[37]
	Chalcone CH8	Liposome	*L. amazonensis*	Yes	[16]
	Paromomycin	Liposome	*L. major*	Yes	[17]
	Paromomycin	Liposome	*L. major*	Yes	[18]
	Meglumine antimoniate	Liposome	*L. major*	Yes	[19]
	Nanosilver	Inorganic nanoparticles	*L. major*	No	[32]
	Nanosilver	Inorganic nanoparticles	*L. major*	No	[34]
Intralesional	Amphotericin B	Liposome (Ambisome®)	*L. major*	No	[11]
	Chalcone CH8	PLGA microparticles	*L. amazonensis*	Yes	[31]
	Nanosilver	Inorganic nanoparticles	*L. amazonensis*	Yes	[35]
	Meglumine antimoniate	Liposome	*L. major*	No	[20]
	Miltefosine	Liposome	*L. major*	Yes	[20]
	Paromomycin	Liposome	*L. major*	No	[20]
	Paromomycin	Solid lipid nanoparticle	*L. tropica*	Yes	[22]
	Sodium stibogluconate	Liposome	*L. mexicana* / *L. major*	Yes	[14]

Note: Chalcone DMC – 2′,6′-dihydroxy-4′-methoxychalcone; Chalcone CH8 – 3-nitro-2′-hydro-4′,6′-dimethoxychalcone; DMSA – dimercaptosuccinic acid; DSHemsPC – 1,2-distigmasterylhemi-succinoyl-sn-glycero-3-phosphocholine; PADRE – pan DR-binding epitope; PLA – poly(D,L-lactide); PLGA – poly(lactic-co-glycolic acid); UVB – ultraviolet B radiation.

Table 1. Experimental studies using nanosystems for CL treatment.

amphotericin B has been approved for human leishmaniasis so far, but that is restricted to VL and the more severe mucosal form of CL. Additional studies and clinical trials are needed to validate the potential of those experimental nanomedicines in human CL.

Advances and challenges of nanotechnology use in leishmaniasis treatment, especially for VL, have been extensively reviewed recently [7, 39, 40]. Here, we attempted to identify some of the opportunities and challenges of using nanotechnology to improve CL treatment. For that, mainly *in vivo* studies were considered.

2. Systemic therapies

2.1. Parenteral treatments

For more than 70 years, injectable pentavalent antimonials such as meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) have been the first-choice drugs in most countries. The paucity of new effective drugs in the market is due to lack of investment/economic interest for the discovery of therapeutic alternatives. The therapeutic regimen consists of intramuscular or intravenous daily injections for 20–30 days. The long period of treatment leads to the accumulation of antimony (Sb) in the tissues, producing myalgia, pancreatitis, pancytopenia, hepatic and cardiotoxicity [41]. Other limiting factors are drug resistance and increased therapeutic failure [42]. In India, its use in VL has been contraindicated due to the appearance of resistant *L. donovani* strains [43].

In Sb-refractory cases, injectable pentamidine, amphotericin B or paromomycin are used. Pentamidine acts on the DNA synthesis of the parasite and has similar efficacy to antimonials, but also produces side effects such as hypoglycemia, diabetes, tachycardia, hypotension, nephrotoxicity and pain at the site of administration [44]. Like antimonials, cases of pentamidine resistance have been increasing, compromising their use in many endemic regions [45].

Amphotericin B is a polyene antibiotic mostly used in VL and in the disfiguring CL form, mucosal leishmaniasis, administered intravenously for 20 days, usually under hospital admission. This is the most efficacious antileishmanial drug, but it produces serious side effects due to its low solubility (nephrotoxicity), and secondary affinity not only for the parasite ergosterol but also for the host cholesterol, causing hypokalemia and cardiotoxicity [5]. Formulations of amphotericin B in lipids have led to a marked improvement in their plasma solubility and bioavailability. Three lipid formulations are commercially available: unilamellar liposomes (Ambisome®), lipid complex (Abelcet®) and colloidal cholesterol suspension (Amphocil®). Among these, Ambisome® has the highest plasma half-life, lowest toxicity, and highest efficacy against VL and CL models [46, 47]. In some countries, Ambisome® is already recommended as the first-choice drug for the treatment of VL and ML difficult cases. However, its high cost, the undefined optimum dosing regimen, toxicity, and the greater uptake of liposomes by the liver make its widespread use in the treatment of CL unfeasible [48].

The interest in the administration of nanosystems by parenteral routes has been increased, mainly for VL, since they increase the drug bioavailability and depending on the charge, size and composition accumulate preferentially in organs such as liver. In addition, nanosystems can be conjugated to biological compounds, such as peptides, antibodies and mannose, favoring their targeting to macrophages [9]. Thus, even with the dose reduction, the encapsulated drugs present greater efficacy and reduction of toxic effects. To date, most experimental studies are conducted parenterally that include chitosan and chondroitin sulfate nanoparticles, Poloxamer 407-micelles, PLGA-DMSA nanoparticles, PADRE-derivatized dendrimer complexed with liposomes, PLA nanoparticles, solid lipid nanoparticles, methacrylate nanoparticles, and liposomes (**Table 1**). Since amphotericin B is currently the most potent antileishmanial

agent, most studies have used it in order to improve its specificity and reduce its adverse effects [10–12, 23, 26–28]. Despite the promising effects of nanomedicines obtained so far, Ambisome® remains the only nanomedicine approved for leishmaniasis parenteral treatment.

Another interesting strategy in the treatment of CL is the use of inorganic nanoparticles, such as nanoselenium, nanosilver and nanotitanium dioxide. Despite the promising efficacy of injected nanosilver [35] and nanoselenium [33] in CL models, the use of nanosilver by topical route was ineffective [32, 34], probably due to the lack of nanoparticle permeation through the infected skin, since those particles were directly active against culture parasites. Nanosilver may act directly on the *Leishmania* parasite by different mechanisms including: (i) increased cell cycle S phase length; (ii) inhibition of trypanothione/trypanothione reductase (TR) redox system; and (iii) cell necrosis [49].

The experimental parenteral routes are normally intravenous or intraperitoneal, the latter not applicable in clinical usage. An important issue to be considered when nanoparticles are intravenously injected is the possibility of thrombosis induction [50]. However, small and submicrometric they may be, larger aggregates can form and clog small veins [51]. Therefore, for safety reasons, intralesional, topical and oral routes should be preferable for CL treatment.

2.2. Oral treatment

The oral route is recommended for both CL and VL due to the ease of administration, high patient compliance, and versatility to increase drug bioavailability. However, systemic adverse effects cannot be precluded.

Miltefosine, a hexadecylphosphocholine previously used to treat cancer, is the only oral drug approved in VL treatment, with good cure rates in India, Nepal, and Bangladesh. However, its teratogenic potential, poor efficacy in patients coinfected with VL and human immunodeficiency virus and recently high rates of clinical failures have increasingly restricted its use in combination therapy [52]. Data on the efficacy of miltefosine in CL treatment are inconclusive, with a large variation depending on the parasite species and geographical area [53].

Another oral drug, allopurinol, an inhibitor of xanthine oxidase, has been explored since 1982 when its activity was demonstrated *in vitro*. Despite the promising results in the oral treatment of CL in Asia, it does not appear to be as effective in Latin America [54]. The azoles act directly on the parasite, blocking the synthesis of ergosterol, and have good pharmacokinetic profile. However, clinical studies with fluconazole, ketoconazole, and itraconazole have shown controversial efficacy, suggesting that the effect is species-dependent [55–57]. The main limiting factor for an oral drug is its low intestinal absorption. Nanosystems can overcome this problem by increasing aqueous solubility and epithelial barrier permeation. In addition, nanosystems can protect drugs from physical, chemical, and biological degradation. In this sense, a few studies have attempted to improve miltefosine and amphothericin B oral efficacy in VL models by encapsulation in nanosystems [58, 59]. For example, PLGA nanoparticles have been used to increase the oral bioavailability of the immunomodulator curcumin and the efficacy of miltefosine in hamsters infected with *L. donovani* [60]. However, only a few studies in the literature have used different nanosystems to increase drug efficacy in CL. In

L. amazonensis-infected BALB/c mice, nanoassemblies formed by two different complexes with N-Octanoyl-N-methylglucamide and β-cyclodextrin were used to increase intestinal permeability of a highly water-soluble meglumine antimoniate drug [24, 36]. More recently, quercetin, a poorly water-soluble plant flavonoid with promising antileishmanial activity [61], was successfully encapsulated in lipid-core poly-e-polycaprolactone (PCL) nanocapsules [25]. Nanoparticle encapsulation increased by more than 40-fold drug oral efficacy in BALB/c mice infected with *L. amazonensis*. The enhancing effect was possibly due to quercetin protection against extensive gastric and intestinal degradation [62]. Besides, PCL nanocapsules were shown to be absorbed intact by mouse intestinal epithelia [63] and also taken up by M cells [64]. Whether or not absorbed quercetin-loaded particles reach the circulation [65] and *Leishmania*-infected skin macrophages remained to be determined.

3. Localized skin therapies

Local therapies are the ideal way to treat uncomplicated CL, as they avoid unnecessary systemic side effects. This topic was subdivided in topical and intralesional treatments due to their different delivery approaches.

3.1. Topical treatment

Topical CL treatment may be provided with chemical drugs or physical methods, such as thermotherapy and cryotherapy. Thermotherapy is the application of high temperature (>50°C) at the center and border of each lesion, based on the inability of *Leishmania* to multiply at temperatures higher than 39°C. Its use has been restricted to the Old World, where 70% efficacy in repeated applications was shown to be similar to intramuscular or intralesional antimony [66]. Presently, thermotherapy is under clinical trial in Colombia in combination with a short course of oral miltefosine [67]. Cryotherapy is the application of liquid nitrogen (−195°C) in the center and border of the lesion once or twice a week for 6 weeks. This treatment has also shown ~70% efficacy [4]. Both therapies are well accepted by the patient, but the difficult access to the specific device (Thermomed), liquid nitrogen, and trained personnel for subjective applications limits their use.

Topical drug treatment of CL normally involves administration of drugs in the form of ointments, creams or gels. These should be ideal for uncomplicated CL due to reduced hospital costs, since it can be auto-applied [44]. The most studied topical formulations are paromomycin creams and gels. The low skin permeation of paromomycin requires association with strong permeants, such as methylbenzethonium chloride, urea and surfactant-associated gentamicin (WR-279396), which may produce local burn and skin irritation [68]. To circumvent that, some formulations have used the milder urea permeant; however, clinical efficacy remains variable depending on the parasite species and geographical area [4]. The results with the WR-279396 formulation are also conflicting, showing high efficacy in patients infected with *L. panamensis* in Panama [69], but not in patients infected with *L. major* in Tunisia [70].

Recently, DNDi supported a Phase Ib and II clinical study in Colombia evaluating the safety, pharmacokinetics, and efficacy of Anfoleish, a cream formulation containing 3%

amphotericin B [71]. However, limited efficacy was found after topical application in patients infected with *L. braziliensis* and *L. panamensis*.

For an optimal topical formulation, the drug should be highly effective and have a high permeation through the skin, reaching the parasite in the deep dermal layer in effective concentrations. For the drug to successfully permeate the stratum corneum, it must possess adequate lipophilicity and a molecular size below 500 Da. The failure or partial success of the topical formulations of paromomycin (615 Da) and amphotericin B (924 Da) is directly related to the low permeability of these drugs through the skin, probably due to their high molecular size [72]. In addition, the typical morphology of CL ulcer with necrotic center and high borders influences the permeation of drugs. Although local inflammatory reaction may facilitate the permeation of more hydrophilic drugs [68], infected macrophages are located in the border of the lesions where epidermal thickening occurs, with hyperplasia and increased number of cell layers, which may hamper drug permeation.

Topical liposomes have emerged as an advantageous way to overlay this problem by increasing drug skin permeation. In fact, some studies have shown the efficacy of liposomes loaded with paromomycin [17, 18] or meglumine antimoniate [19] in *L. major*-infected BALB/c mice, although even with the use of liposomes only 1.5% of antimoniate and a range of 4.8 to 15% of paromomycin were able to permeate through the skin. The use of liposomes was also shown to increase, the *in vitro* permeation and activity of amphotericin B in *L. braziliensis* promastigotes and intracellular amastigotes [73]. Nonetheless, *in vivo* another amphotericin B liposomal formulation did not show effectiveness in the topical treatment of CL caused by *L. mexicana* using ulcerated (BALB/c) and non-ulcerated (129SVE) experimental mice models [15]. On the other hand, in two different clinical studies conducted by the same research group in an endemic area for *L. tropica* and *L. major* at Ghaem Hospital in Iran, liposomes loading amphotericin B [74] and azithromycin [75] when administrated topically demonstrated the same efficacy as intralesional meglumine antimoniate.

In the search for new active drugs for leishmaniasis, our group has been studying the chalcone CH8 (3-nitro-2′-hydro-4′,6′-dimethoxychalcone), a nitrosylated derivative of the plant-derived chalcone (DMC – 2′,6′-dihydroxy-4′-methoxychalcone), which demonstrated a high selectivity index (SI = 143) and antileishmanial activity *in vitro* (IC_{50} = 0.7 μM) and *in vivo* against *L. amazonensis* [76]. In addition, the CH8 molecule exhibits physicochemical characteristics favorable to encapsulation with high efficiency in different nanosystems such as liposomes and polymeric particles. Indeed, CH8 loading into cationic liposomes interferes with the lipid structure rendering it more elastic, enhancing formulation permeation through the skin and increasing CH8 topical efficacy in *L. amazonensis* murine model [16].

Notwithstanding, the high phospholipid cost and liposomal instability hinder their use for CL. Thus, other nanosystems such as gamma-cyclodextrin have been studied for amphotericin B skin delivery to improve drug solubility and topical efficacy in *L. amazonensis*-infected golden hamsters [37]. Other interesting nanosystems are solid lipid nanoparticles (SLN), which can improve drug interaction with the stratum corneum facilitating permeation and improving the efficacy of the drug. The better activity of paromomycin entrapped in SLN was already described against *L. major* and *L. tropica* intracellular amastigotes [77]. Despite the

promising results found with the different nanosystems, additional *in vivo* studies are necessary in order to develop an effective topical treatment for CL.

3.2. Intralesional treatments

Intralesional drug administration is an alternative local treatment for CL. This is especially appropriate for patients with uncomplicated localized CL—up to four lesions, each no more than 3 cm in diameter, as well as parenteral medication restrictions due to systemic toxicity. Besides the lesser toxicity, local subcutaneous injections can accelerate clinical cure and reduce hospital costs as less injections are needed [4]. Pentavalent antimonials are the most used drugs, showing 68–100% efficacy in different clinical studies, depending on the size of the lesions [78–81]. Repeated injections are required due to the high solubility that favors rapid absorption into the circulation. Treatment generally consists of 1–5 injections around each lesion per day, twice a week. In addition to the pain inflicted, adverse effects like local hyperpigmentation and anaphylactic shock have been reported [82].

Amphotericin B has also been tested by intralesional route in Iran in patients refractory to antimony therapy, leading to complete lesion remission in 61% of the cases [83]. Due to the necrotizing effect of deoxycholate surfactant in amphotericin B formulation, the amount injected has to be as low as possible, reducing effectiveness. Thus, despite its high potential in CL, intralesional treatments need improvement, particularly as regards dose number reduction.

Intralesional drug-loaded nanoparticles have appeared as interesting drug delivery systems in CL due to direct drug delivery to the infected macrophages. However, for the formulation to be effective, drug chemistry, nanosystem choice, and treatment schedule must be finely adjusted. Lipid systems such as SLN loaded with paromomycin have been tested intralesionally in *L. tropica*-infected BALB/c mice and shown increased drug efficacy by 2-fold [22]. On the other hand, in another study comparing the efficacy of liposomal formulations of Glucantime®, miltefosine and paromomycin in *L. major*-infected BALB/c mice, only liposomal miltefosine was shown to have therapeutic effect compared with control group [20]. Interestingly, intralesional Ambisome® was not effective in *L. major*-infected mice [11]. Additionally, intralesional Pentostam® liposomes were only effective if given at the time of infection with *L. major* or *L. mexicana* in TFW mice [14].

In this context, polymeric particles have emerged as an interesting strategy for CL intralesional and single-dose treatment. The advantage of this system is that particles smaller than 6 μM can be easily phagocytosed by infected macrophages releasing the drug directly into the target, whereas the larger microparticles form a depot slowly releasing the drug into the site, allowing at only one dose the drug to remain in the site of infection for the time needed for healing. The size of the microparticles and their polymer composition ensures retention of the drug in the lesion and determines its release time. In this way, adverse systemic effects are avoided and the effectiveness of the drug is increased. Recently, the safety and efficacy of PLGA microparticles containing chalcone CH8 in the intralesional treatment was demonstrated in *L. amazonensis*-infected BALB/c mice. Even a single subcutaneous injection with CH8-loaded particles was effective in controlling parasite growth, superior than three injections with the free drug or Glucantime®, demonstrating the promising use of these systems in local and single-dose treatment of CL [31].

4. Conclusion

Since nanomedicines can be more efficiently taken up by the infected macrophages than free drugs, and also be designed to cross skin and epithelial barriers, they have emerged as promising strategies to allow novel topical and oral treatments for CL. Noteworthy is the possibility to treat the disease with a single local injection with biodegradable polymeric particles. Despite the promising results obtained with the different nanomedicines in pre-clinical studies, so far none has so far progressed to clinical trials in CL. Therefore, further efforts must be made in order to have them in the near future in the antileishmanial therapy arsenal.

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Conflict of interest

The authors have no conflict of interest to declare.

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References

[1] WHO. Global leishmaniasis update, 2006-2015: A turning point in leishmaniasis surveillance. Weekly Epidemiological Record [Internet]. 2017;**92**:557-572. Available from: http://www.who.int/wer/2017/wer9238/en/ [Accessed: 2017-11-16]

[2] WHO. Leishmaniasis in high-burden countries: An epidemiological update based on data reported in 2014. Weekly Epidemiological Record [Internet]. 2016;**91**:285-296. Available from: http://www.who.int/wer/2016/wer9122/en/ [Accessed: 2017-11-16]

[3] Pigott DM, Bhatt S, Golding N, Duda KA, Battle KE, Brady OJ, et al. Global distribution maps of the leishmaniases. eLife. 2014;**3**:1-21. DOI: 10.7554/eLife.02851.001

[4] Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. Expert Opinion on Pharmacotherapy. 2014;**16**:1-16. DOI: 10.1517/14656566.2015.973850

[5] Uliana SRB, Trinconi CT, Coelho AC. Chemotherapy of leishmaniasis: Present challenges. Parasitology. 2017;**20**:1-17. DOI:10.1017/S0031182016002523

[6] DNDi. DNDi's Strategy for Cutaneous Leishmaniasis. 2017. Available from: https://www.dndi.org/diseases-projects/leishmaniasis/dndi-strategy-cl/ [Accessed: November 16, 2017]

[7] Gutiérrez V, Seabra AB, Reguera RM, Khandare J, Calderón M. New approaches from nanomedicine for treating leishmaniasis. Chemical Society Reviews. 2016;**45**:152-168. DOI: 10.1039/C5CS00674K

[8] Bawa R, Melethil S, Simmons WJ, Harris D. Nanopharmaceuticals: Patenting issues and FDA regulatory challenges. The SciTech Lawyer. 2008;**5**:6-13

[9] Gustafson HH, Holt-Casper D, Grainger DW, Ghandehari H. Nanoparticle uptake: The phagocyte problem. Nano Today. 2015;**10**:487-510. DOI: 10.1016/j.nantod.2015.06.006

[10] Panosian CB, Barza M, Szoka F, Wyler DJ. Treatment of experimental cutaneous leishmaniasis with liposome-intercalated amphotericin B. Antimicrobial Agents and Chemotherapy. 1984;**25**:655-656

[11] Yardley V, Croft SL. Activity of liposomal amphotericin B against experimental cutaneous leishmaniasis. Antimicrobial Agents and Chemotherapy. 1997;**41**:752-756

[12] Iman M, Huang Z, Alavizadeh SH, Szoka FC, Jaafari MR. Biodistribution and in vivo Antileishmanial activity of 1,2-distigmasterylhemisuccinoyl-sn-glycero-3-phosphocholine liposome-intercalated amphotericin B. Antimicrobial Agents and Chemotherapy. 2017;**61**:1-16. DOI: 10.1128/AAC.02525-16

[13] Daftarian PM, Stone GW, Kovalski L, Kumar M, Vosoughi A, Urbieta M, et al. A targeted and adjuvanted nanocarrier lowers the effective dose of liposomal amphotericin B and enhances adaptive immunity in murine cutaneous leishmaniasis. The Journal of Infectious Diseases. 2013;**208**:1914-1922. DOI: 10.1093/infdis/jit378

[14] New RRC, Chance ML, Heath S. The treatment of experimental cutaneous leishmaniasis with liposome-entrapped Pentostam. Parasitology. 1981;**83**:519-527. DOI: 10.1017/S0031182000080501

[15] Varikuti S, Oghumu S, Saljoughian N, Pioso MS, Sedmak BE, Khamesipour A, et al. Topical treatment with nanoliposomal amphotericin B reduces early lesion growth but fails to induce cure in an experimental model of cutaneous leishmaniasis caused by *Leishmania mexicana*. Acta Tropica. 2017;**173**:102-108. DOI: 10.1016/j.actatropica.2017.06.004

[16] Rossi-Bergmann B, Falcao CAB, Zanchetta B, Bentley MVLB, Santana MHA. Performance of elastic liposomes for topical treatment of cutaneous Leishmaniasis. In: Beck R, Guterres S, Pohlmann A, editors. Nanocosmetics Nanomedicines New Approaches Ski. Care, Berlin, Heidelberg: Springer; 2011. pp. 181-196. DOI: 10.1007/978-3-642-19792-5_9

[17] Carneiro G, Santos DC, Oliveira MC, Fernandes AP, Ferreira LS, Ramaldes GA, et al. Topical delivery and in vivo antileishmanial activity of paromomycin-loaded liposomes for treatment of cutaneous leishmaniasis. Journal of Liposome Research. 2010;**20**:16-23

[18] Jaafari MR, Bavarsad N, Bazzaz BSF, Samiei A, Soroush D, Ghorbani S, et al. Effect of topical liposomes containing paromomycin sulfate in the course of *Leishmania major* infection in susceptible BALB/c mice. Antimicrobial Agents and Chemotherapy. 2009; **53**:2259-2265. DOI: 10.1128/AAC.01319-08

[19] Moosavian Kalat SA, Khamesipour A, Bavarsad N, Fallah M, Khashayarmanesh Z, Feizi E, et al. Use of topical liposomes containing meglumine antimoniate (Glucantime) for the treatment of *L. major* lesion in BALB/c mice. Experimental Parasitology. 2014;**143**:5-10. DOI: 10.1016/j.exppara.2014.04.013

[20] Momeni A, Rasoolian M, Momeni A, Navaei A, Emami S, Shaker Z, et al. Development of liposomes loaded with anti-leishmanial drugs for the treatment of cutaneous leishmaniasis. Journal of Liposome Research. 2013;**23**:134-144. DOI: 10.3109/08982104.2012.762519

[21] Heidari-Kharaji M, Taheri T, Doroud D, Habibzadeh S, Badirzadeh A, Rafati S. Enhanced paromomycin efficacy by solid lipid nanoparticle formulation against *Leishmania* in mice model. Parasite Immunology. 2016;**38**:599-608. DOI: 10.1111/pim.12340

[22] Heidari-Kharaji M, Taheri T, Doroud D, Habibzadeh S, Rafati S. Solid lipid nanoparticle loaded with paromomycin: In vivo efficacy against *Leishmania tropica* infection in BALB/c mice model. Applied Microbiology and Biotechnology. 2016;**100**:7051-7060. DOI: 10.1007/s00253-016-7422-y

[23] Nelson KG, Bishop JV, Ryan RO, Titus R. Nanodisk-associated amphotericin B clears *Leishmania major* cutaneous infection in susceptible BALB/c mice. Antimicrobial Agents and Chemotherapy. 2006;**50**:1238-1244. DOI: 10.1128/AAC.50.4.1238-1244.2006

[24] Lanza JS, Fernandes FR, Corrêa-Júnior JD, Vilela JM, Magalhães-Paniago R, Ferreira LA, et al. Polarity-sensitive nanocarrier for oral delivery of Sb (V) and treatment of cutaneous leishmaniasis. International Journal of Nanomedicine. 2016;**11**:2305-2318. DOI: 10.2147/IJN.S105952

[25] Sousa-Batista AJ, Poletto FS, Philipon CIMS, Guterres SS, Pohlmann AR, Rossi-Bergmann B. Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis. Parasitology. 2017;**144**:1-6. DOI: 10.1017/S003118201700097X

[26] Ribeiro TG, Franca JR, Fuscaldi LL, Santos ML, Duarte MC, Lage PS, et al. An optimized nanoparticle delivery system based on chitosan and chondroitin sulfate molecules reduces the toxicity of amphotericin B and is effective in treating tegumentary leishmaniasis. International Journal of Nanomedicine. 2014;**9**:5341-5353. DOI: 10.2147/IJN.S68966

[27] Mendonça DVC, Lage LMR, Lage DP, Chávez-Fumagalli MA, Ludolf F, Roatt BM, et al. Poloxamer 407 (Pluronic® F127)-based polymeric micelles for amphotericin B: In vitro biological activity, toxicity and in vivo therapeutic efficacy against murine tegumentary leishmaniasis. Experimental Parasitology. 2016;**169**:34-42. DOI: 10.1016/j.exppara.2016.07.005

[28] de Carvalho RF, Ribeiro IF, Miranda-Vilela AL, de Souza Filho J, Martins OP, de Oliveira Cintra e Silva D, et al. Leishmanicidal activity of amphotericin B encapsulated in PLGA-DMSA nanoparticles to treat cutaneous leishmaniasis in C57BL/6 mice. Experimental Parasitology. 2013;**135**:217-222. DOI: 10.1016/j.exppara.2013.07.008

[29] Torres-Santos EC, Rodrigues JM, Moreira DL, Kaplan MAC, Rossi-Bergmann B. Improvement of in vitro and in vivo antileishmanial activities of 2′,6′- dihydroxy-4′-methoxychalcone by entrapment in poly(D,L-lactide) nanoparticles. Antimicrobial Agents and Chemotherapy. 1999;**43**:1776-1778

[30] Fusai T, Deniau M, Durand R, Bories C, Paul M, Rivollet D, et al. Action of pentamidine-bound nanoparticles against *Leishmania* on an in vivo model. Parasite. 1994;**1**:319-324. DOI: 10.1051/parasite/1994014319

[31] Sousa-Batista AJ, Pacienza-Lima W, Arruda-Costa N, Falcão CAB, Rossi-Bergmann B. Depot subcutaneous injection with chalcone CH8-loaded PLGA microspheres aiming at a single-dose treatment of cutaneous leishmaniasis. Antimicrobial Agents Chemotherapy. 2018;**62**: 1-11. DOI: 10.1128/AAC.01822-17

[32] Mohebali M, Rezayat MM, Gilani K, Sarkar S, Akhoundi B, Esmaeili J, et al. Nanosilver in the treatment of localized cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): An in vitro and in vivo study. Daru. 2009;**17**:285-289

[33] Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, et al. Efficacy of biogenic selenium nanoparticles against *Leishmania major*: In vitro and in vivo studies. Journal of Trace Elements in Medicine and Biology. 2013;**27**:203-207. DOI: 10.1016/j.jtemb.2012.11.002

[34] Nilforoushzadeh MA, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Jafari R, Heidari-Beni M, Siadat AH, et al. Topical effectiveness of different concentrations of nanosilver solution on *Leishmania major* lesions in Balb/c mice. Journal of Vector Borne Diseases. 2012;**49**:249-253

[35] Rossi-Bergmann B, Pacienza-Lima W, Marcato PD, De Conti R, Duran N. Therapeutic potential of biogenic silver nanoparticles in murine cutaneous leishmaniasis. Journal of Nano Research. 2012;**20**:89-97

[36] Demicheli C, Ochoa R, da Silva JBB, Falcão CA, Rossi-Bergmann B, de Melo AL, et al. Oral delivery of meglumine antimoniate-beta-cyclodextrin complex for treatment of leishmaniasis. Antimicrobial Agents and Chemotherapy. 2004;**48**:100-103. DOI: 10.1128/AAC.48.1.100

[37] Ruiz HK, Serrano DR, Dea-Ayuela MA, Bilbao-Ramos PE, Bolás-Fernández F, Torrado JJ, et al. New amphotericin B-gamma cyclodextrin formulation for topical use with synergistic activity against diverse fungal species and *Leishmania* spp. International Journal of Pharmaceutics. 2014;**473**:148-157. DOI: 10.1016/j.ijpharm.2014.07.004

[38] Franco AMR, Grafova I, Soares FV, Gentile G, Wyrepkowski CDC, Bolson MA, et al. Nanoscaled hydrated antimony (V) oxide as a new approach to first-line antileishmanial drugs. International Journal of Nanomedicine. 2016;**11**:6771-6780. DOI: 10.2147/IJN.S121096

[39] Akbari M, Oryan A, Hatam G. Application of nanotechnology in treatment of leishmaniasis: A review. Acta Tropica. 2017;**172**:86-90. DOI: 10.1016/j.actatropica.2017.04.029

[40] Bruni N, Stella B, Giraudo L, Della Pepa C, Gastaldi D, Dosio F. Nanostructured delivery systems with improved leishmanicidal activity: A critical review. International Journal of Nanomedicine. 2017;**12**:5289-5311. DOI: 10.2147/IJN.S140363

[41] Haldar AK, Sen P, Roy S. Use of antimony in the treatment of leishmaniasis: Current status and future directions. Molecular Biology International. 2011;**2011**:1-24. DOI: 10.4061/2011/571242

[42] Castro MDM, Cossio A, Velasco C, Osorio L. Risk factors for therapeutic failure to meglumine antimoniate and miltefosine in adults and children with cutaneous leishmaniasis in Colombia: A cohort study. PLoS Neglected Tropical Diseases. 2017;**11**:1-14. DOI: 10.1371/journal.pntd.0005515

[43] Sundar S. Drug resistance in Indian visceral leishmaniasis. Tropical Medicine & International Health. 2001;**6**:849-854

[44] Iqbal H, Ishfaq M, Wahab A, Abbas MN, Ahmad I, Rehman A, et al. Therapeutic modalities to combat leishmaniasis: A review. Asian Pacific Journal of Tropical Medicine. 2016; **6**:1-5. DOI:10.1016/S2222-1808(15)60975-6

[45] Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in *Leishmania mexicana* involves exclusion of the drug from the mitochondrion. Antimicrobial Agents and Chemotherapy. 2002;**46**:3731-3738. DOI: 10.1128/AAC.46.12.3731

[46] Yardley V, Croft SL. A comparison of the activities of three amphotericin B lipid formulations against experimental visceral and cutaneous leishmaniasis. International Journal of Antimicrobial Agents. 2000;**13**:243-248. DOI: 10.1016/S0924-8579(99)00133-8

[47] De Almeida L, Fujimura AT, Del Cistia ML, Fonseca-Santos B, Imamura KB, Michels PAM, et al. Nanotechnological strategies for treatment of leishmaniasis – A review. Journal of Biomedical Nanotechnology. 2017;**13**:117-133. DOI: 10.1166/jbn.2017.2349

[48] Wortmann G, Zapor M, Ressner R, Fraser S, Hartzell J, Pierson J, et al. Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. The American Journal of Tropical Medicine and Hygiene. 2010;**83**:1028-1033. DOI: 10.4269/ajtmh.2010.10-0171

[49] Zahir AA, Chauhan IS, Bagavan A, Kamaraj C, Elango G, Shankar J, et al. Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in *Leishmania donovani*. Antimicrobial Agents and Chemotherapy. 2015;**59**:4782-4799. DOI: 10.1128/AAC.00098-15

[50] Agallou M, Margaroni M, Athanasiou E, Toubanaki DK, Kontonikola K, Karidi K, et al. Identification of BALB/c immune markers correlated with a partial protection to *Leishmania infantum* after vaccination with a rationally designed multi-epitope cysteine protease a peptide-based nanovaccine. PLoS Neglected Tropical Diseases. 2017;**11**:1-27. DOI: 10.1371/journal.pntd.0005311

[51] Mayer A, Vadon M, Rinner B, Novak A, Wintersteiger R, Fröhlich E. The role of nanoparticle size in hemocompatibility. Toxicology. 2009;**258**:139-147. DOI: 10.1016/j.tox.2009.01.015

[52] Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clinical Infectious Diseases. 2012;**55**:543-550. DOI: 10.1093/cid/cis474

[53] Monge-Maillo B, López-Vélez R. Miltefosine for visceral and cutaneous leishmaniasis: Drug characteristics and evidence-based treatment recommendations. Clinical Infectious Diseases. 2015;**60**:1398-1404. DOI: 10.1093/cid/civ004

[54] Vélez I, Agudelo S, Hendrickx E, Puerta J, Grogl M, Modabber F, et al. Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis: A randomized, controlled trial. Annals of Internal Medicine. 1997;**126**:232-236

[55] Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF. Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) vs. ketoconazole for teating cutaneous leishmaniasis in Guatemala. The Journal of Infectious Diseases. 1992;**165**:528-534

[56] Alrajhi A, Ibrahim E, De Vol E, Khairat M, Faris R, Maguire J. Fluconazole for the treatment of cutaneous leishmaniasis caused by *Leishmania major*. The New England Journal of Medicine. 2002;**346**:891-895. DOI: 10.1056/NEJM200208013470517

[57] Nassiri-Kashani M, Firooz A, Khamesipour A, Mojtahed F, Nilforoushzadeh M, Hejazi H, et al. A randomized, double-blind, placebo-controlled clinical trial of itraconazole in the treatment of cutaneous leishmaniasis. Journal of the European Academy of Dermatology and Venereology. 2005;**19**:80-83. DOI: 10.1111/j.1468-3083.2004.01133.x

[58] da Gama Bitencourt JJ, Pazin WM, Ito AS, Barioni MB, de Paula Pinto C, dos SMA, et al. Miltefosine-loaded lipid nanoparticles: Improving miltefosine stability and reducing its hemolytic potential toward erythrocytes and its cytotoxic effect on macrophages. Biophysical Chemistry. 2016;**217**:20-31. DOI: 10.1016/j.bpc.2016.07.005

[59] Serrano DR, Lalatsa A. Oral amphotericin B: The journey from bench to market. Journal of Drug Delivery Science and Technology. 2017;**42**:75-83. DOI: 10.1016/j.jddst.2017.04.017

[60] Tiwari B, Pahuja R, Kumar P, Rath SK, Gupta KC, Goyal N. Nanotized curcumin and miltefosine: A potential combination for treatment of experimental visceral leishmaniasis. Antimicrobial Agents and Chemotherapy. 2017;**61**:1-13. DOI: 10.1128/AAC.01169-16

[61] Muzitano MF, Falcão CAB, Cruz EA, Bergonzi MC, Bilia AR, Vincieri FF, et al. Oral metabolism and efficacy of Kalanchoe pinnata flavonoids in a murine model of cutaneous leishmaniasis. Planta Medica. 2009;**75**:307-311. DOI: 10.1055/s-0028-1088382

[62] Weiss-Angeli V, Poletto FS, De Marco SL, Salvador M, Da Silveira NP, Guterres SS, et al. Sustained antioxidant activity of quercetin-loaded lipid-core nanocapsules. Journal of Nanoscience and Nanotechnology. 2012;**12**:2874-2880. DOI: 10.1166/jnn.2012.5770

[63] Rodrigues SF, Fiel LA, Shimada AL, Pereira NR, Guterres SS, Pohlmann AR, et al. Lipid-core nanocapsules act as a drug shuttle through the blood brain barrier and reduce glioblastoma after intravenous or oral administration. Journal of Biomedical Nanotechnology. 2016;**12**:986-1000. DOI: 10.1166/jbn.2016.2215

[64] Lopes M, Abrahim B, Cabral L, Rodrigues C, Seiça R, de Baptista F, et al. Intestinal absorption of insulin nanoparticles: Contribution of M cells. Nanomedicine. 2014;**10**:1139-1151. DOI: 10.1016/j.nano.2014.02.014

[65] Li H, Chen M, Su Z, Sun M, Ping Q. Size-exclusive effect of nanostructured lipid carriers on oral drug delivery. International Journal of Pharmaceutics. 2016;**511**:524-537. DOI: 10.1016/j.ijpharm.2016.07.049

[66] Cardona-Arias JA, Darío Vélez I, López-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: A meta-analysis of controlled clinical trials. PLoS One. 2015; **10**:1-15. DOI: 10.1371/journal.pone.0122569

[67] DNDi D for ND initiative. Thermotherapy + A Short Course of Miltefosine for the Treatment of Uncomplicated Cutaneous Leishmaniasis in the New World. ClinicalTrials.gov. 2017. Available from: https://www.clinicaltrials.gov/ct2/show/NCT02687971 [Accessed: January10, 2018]

[68] Van BK, Yardley V, Murdan S, Croft SL. Drug permeation and barrier damage in *Leishmania*-infected mouse skin. The Journal of Antimicrobial Chemotherapy. 2016;**71**:1578-1585. DOI: 10.1093/jac/dkw012

[69] Sosa N, Capitán Z, Nieto J, Nieto M, Calzada J, Paz H, et al. Randomized, double-blinded, phase 2 trial of WR 279,396 (paromomycin and gentamicin) for cutaneous leishmaniasis in Panama. The American Journal of Tropical Medicine and Hygiene. 2013;**89**:557-563. DOI: 10.4269/ajtmh.12-0736

[70] Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. The New England Journal of Medicine. 2013;**368**:524-532. DOI: 10.1056/NEJMoa1202657

[71] DNDi D for ND initiative. Topical 3% Amphotericin B Cream for the Treatment of Cutaneous Leishmaniasis in Colombia (Anfoleish). ClinicalTrials.gov. 2017. Available from: https://clinicaltrials.gov/ct2/show/NCT01845727 [Accessed: January 10, 2018]

[72] Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Experimental Dermatology. 2000;**9**:165-169. DOI: 10.1034/j.1600-0625.2000.009003165.x

[73] Perez AP, Altube MJ, Schilrreff P, Apezteguia G, Celes FS, Zacchino S, et al. Topical amphotericin B in ultradeformable liposomes: Formulation, skin penetration study, antifungal and antileishmanial activity in vitro. Colloids Surfaces B Biointerfaces. 2016; **139**:190-198. DOI: 10.1016/j.colsurfb.2015.12.003

[74] Layegh P, Rajabi O, Jafari MR, Emamgholi Tabar Malekshah P, Moghiman T, Ashraf H, et al. Efficacy of topical liposomal amphotericin B versus intralesional meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. Journal of Parasitology Research. 2011;**2011**:1-5. DOI: 10.1155/2011/656523

[75] Rajabi O, Layegh P, Hashemzadeh S, Khoddami M. Topical liposomal azithromycin in the treatment of acute cutaneous leishmaniasis. Dermatologic Therapy. 2016;**29**:358-363. DOI: 10.1111/dth.12357

[76] Boeck P, Bandeira Falcão CA, Leal PC, Yunes RA, Filho VC, Torres-Santos EC, et al. Synthesis of chalcone analogues with increased antileishmanial activity. Bioorganic & Medicinal Chemistry. 2006;**14**:1538-1545. DOI: 10.1016/j.bmc.2005.10.005

[77] Kharaji MH, Doroud D, Taheri T, Rafati S. Drug targeting to macrophages with solid lipid nanoparticles harboring paromomycin: An in vitro evaluation against *L. major* and *L. tropica*. AAPS PharmSciTech. 2016;**17**:1110-1119. DOI: 10.1208/s12249-015-0439-1

[78] Sharquie KE, Al-Talib K, Chu A. Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony. The British Journal of Dermatology. 1988;**119**:53-57

[79] Tallab TM, K a B, Mirdad S, Johargi H, Mourad MM, Ibrahim K, et al. Cutaneous leishmaniasis: Schedules for intralesional treatment with sodium stibogluconate. International Journal of Dermatology. 1996;**35**:594-597

[80] Oliveira-Neto MP, Schubach A, Mattos M, da Costa SC, Pirmez C. Intralesional therapy of American cutaneous leishmaniasis with pentavalent antimony in Rio de Janeiro, Brazil – An area of *Leishmania* (V.) *braziliensis* transmission. International Journal of Dermatology. 1997;**36**:463-468

[81] Soto J, Rojas E, Guzman M, Verduguez A, Nena W, Maldonado M, et al. Intralesional antimony for single lesions of Bolivian cutaneous leishmaniasis. Clinical Infectious Diseases. 2013;**56**:1255-1260. DOI: 10.1093/cid/cit049

[82] Esfandiarpour I, Farajzadeh S, Rahnama Z, Fathabadi EA, Heshmatkhah A. Adverse effects of intralesional meglumine antimoniate and its influence on clinical laboratory parameters in the treatment of cutaneous leishmaniasis. International Journal of Dermatology. 2012;**51**:1221-1225. DOI: 10.1111/j.1365-4632.2012.05460.x

[83] Goyonlo VM, Vosoughi E, Kiafar B, Nahidi Y, Momenzadeh A, Taheri AR. Efficacy of intralesional amphotericin B for the treatment of cutaneous leishmaniasis. Indian Journal of Dermatology. 2014;**59**:631-636. DOI: 10.4103/0019-5154.143571

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