EXPERT OPINION

Drug delivery systems for the topical treatment of cutaneous leishmaniasis

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Introduction: The parenteral administration of pentavalent antimonials for the treatment of all forms of leishmaniasis, including cutaneous leishamniasis (CL), has several limitations. Therapy is long, requiring repeated doses and the adverse reactions are frequent. Topical treatment is an attractive alternative for CL, offering significant advantages over systemic therapy: fewer adverse effects, ease of administration, and lower costs.

Areas covered: This review covers, from 1984 to the present, the progress achieved for the development of topical treatment for CL, using different drugs such as paromomycin (PA), imiquimod, amphotericin B (AmB), miltefosine, and buparvaquone. PA is the most commonly studied drug, followed by AmB and Imiquimod. These drugs were incorporated in conventional dosage forms or loaded in lipid nanocarries, which have been used mainly for improved skin delivery and antileishmanial activity.

Expert opinion: Developing an effective topical treatment for CL using these antileishmanial drugs still remains a great challenge. Insights into the most promising delivery strategies to improve treatment of CL with PA and AmB using conventional dosage forms, lipid nanocarriers, and combined therapy are presented and discussed. The results obtained with combined therapy and alternative delivery systems are promising perspectives for improving topical treatment of CL.

Keywords: amphotericin B, cutaneous leishmaniasis, lipid nanocarriers, liposomes, paromomycin, skin penetration, topical treatment

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1. Introduction

Leishmaniasis is caused by protozoan parasites that are transmitted by the bite of phlebotomine sandflies that injects infective metacyclic promastigotes under the vertebrate host skin. In vertebrate hosts, Leishmania survive and multiply as non-motile amastigotes in cells of the phagocytic mononuclear system at the site of infection [1]. Eventually, depending on several factors, parasites may spread to other cutaneous sites or to visceral organs [2]. Therefore, upon infection, patients may develop symptoms that range in severity from skin lesions to serious disfigurement and fatal systemic infection [3]. Based on this, leishmaniasis has been classified into two major clinical entities: i) tegumentary, which comprises cutaneous, cutaneous diffuse, and mucocutaneous leishmaniasis (MCL) and ii) visceral leishmaniasis (VL). Each syndrome depends on both the infecting parasite species and the host immune responses [3-5].

Cutaneous leishmaniasis (CL) results in formation of skin ulcers at the site of the sandfly bite. CL is caused mainly by *Leishmania (Leishmania) major*, *L. (L.) tropica*, *L. (L.) aethiopic*a in the Old World, and by *L. (Viannia) braziliensis*, *(L.) (V.)*

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Article highlights.

- General information is provided about cutaneous leishmaniasis, its causative organisms, its symptoms, and the methods of conventional (pentavalent antimonials) or alternative (topical) treatment of this disease.
- Key information is arranged about the chemistry of the main drugs used for the topical treatment of cutaneous leishmaniasis, the actual knowledge of their mechanism of action, and their pharmacokinetics parameters.
- The structure and permeability of the healthy skin as well as changes in skin permeability during the progression of the disease and skin conditions in the topical treatment of the cutaneous leishmaniasis are discussed.
- The main conclusions of clinical studies or studies on animals using conventional dosage forms containing topical drugs used for the treatment of cutaneous leishmaniasis are presented.
- The advantages of using lipid nanocarriers loaded with paromomycin and amphotericin B for the topical treatment of cutaneous leishmaniasis are presented.

This box summarizes key points contained in the article.

guyanensis L. (V.) panamensis, *L. (L.) amazonensis* and *L. (L.) mexicana* in the New World. The disease is usually self-limiting, but infection by New World species, for example, may spread to lymph nodes and other cutaneous sites. It is generally assumed that MCL, often caused by *L. (V.) braziliensis*, develops after cure of the initial skin lesions. The late development of metastatic lesions leads to partial or total destruction of the mucous membranes, progressing to severe deformities or even death [3].

Considering the diversity of clinical manifestations, immunopathogical responses, and parasite species associated with leishmaniasis, the concept that leishmaniasis may be more properly defined as a diverse group of syndromes has recently emerged [3] and, certainly, has implications for development of effective treatment alternatives.

Meglumine antimoniate and sodium stibogluconate have been used for more than half of a century in the therapy of all forms of leishmaniasis. These drugs have to be given parenterally, daily, for at least 3 weeks (typically, 20 mg/kg/day for 20 – 30 days). The treatment with antimonials is long, requiring repeated doses. The adverse reactions (arthralgia, myalgia, nausea, vomiting, elevated liver and pancreatic enzymes, etc.) are frequent, the most serious one on the cardiovascular system [6,7].

Alternative therapies for the treatment of all forms of leishmaniasis have been intensively investigated, including the identification of drugs and formulations that can be administered topically for the treatment of CL [8,9].

Topical treatment is an attractive alternative for CL [6]. Topical formulations offer significant advantages over systemic therapy: fewer adverse effects, ease of administration and reduced cost. Among the different drugs that have been studied for the topical treatment of CL are paromomycin (PA), imiquimod, amphotericin B (AmB), miltefosine, and buparvaquone. PA is the most commonly studied drug for topical treatment of CL caused by different species [10-12].

Most studies evaluating formulations containing antileishmanial drugs for topical treatment of CL have investigated conventional dosage forms such as ointments, creams, and gels. Although experimentally infected animal studies have shown promising results, clinical trials have shown variable efficacy and sometimes disappointing results [13,14]. This may be attributed, at least in part, to low skin penetration of antileishmanial drugs. Therefore, novel drug delivery systems such as lipid nanocarriers present the potential to improve drug penetration into or across skin [15,16].

Among the lipid nanocarriers of antileishmanial drugs, liposomes, the most extensively investigated, provide improved efficacy, probably due to enhanced drug skin penetration [16]. When applied to the intact skin, liposomes can enhance dermal or transdermal drug penetration depending on the composition and size of the vesicles [17]. Different mechanisms of action for liposomes as skin drug delivery systems have been suggested: penetration enhanced by liposome components individually; vesicle adsorption to and/or fusion with the stratum corneum (SC); intact vesicle penetration into and through the intact skin; and follicular penetration (**Figure 1**) [18].

The rationale for the use of liposomes in the topical treatment of CL when compared with conventional formulations can be schematized as shown in **Figure 2**. When applied to the skin with normal or impaired barrier, liposomes can increase the drug skin penetration [16-19]. In addition, when applied to the totally damaged skin (open lesions or full-thickness skin wound), liposomes showed the ability to accelerate wound healing when compared to conventional formulations [20-22].

Following a brief description of the more studied drugs for the topical treatment of CL, as well as on the nature of the CL lesion and intact skin barrier, this review critically evaluates conventional dosage forms and novel drug delivery systems, concentrating primarily on lipid nanocarriers used for the topical treatment of CL.

2. Drugs used for topical treatment of CL

2.1 Paromomycin (PA)

The aminoglycoside antibiotic PA is a highly hydrophilic drug with a high molecular weight and relative lipid insolubility (**Table 1**). PA has variable activity against different *Leishmania* species [23]. Studies on PA have demonstrated that it was able to inhibit protein synthesis *in vivo*, though in the promastigote stage of the parasite [24]. PA has low bioavailability when administered orally. When administered intramuscularly, PA is quickly absorbed, reaching peak plasma levels within 1 h [25,26]. The nephrotoxicity and ototoxicity, adverse events commonly associated with the

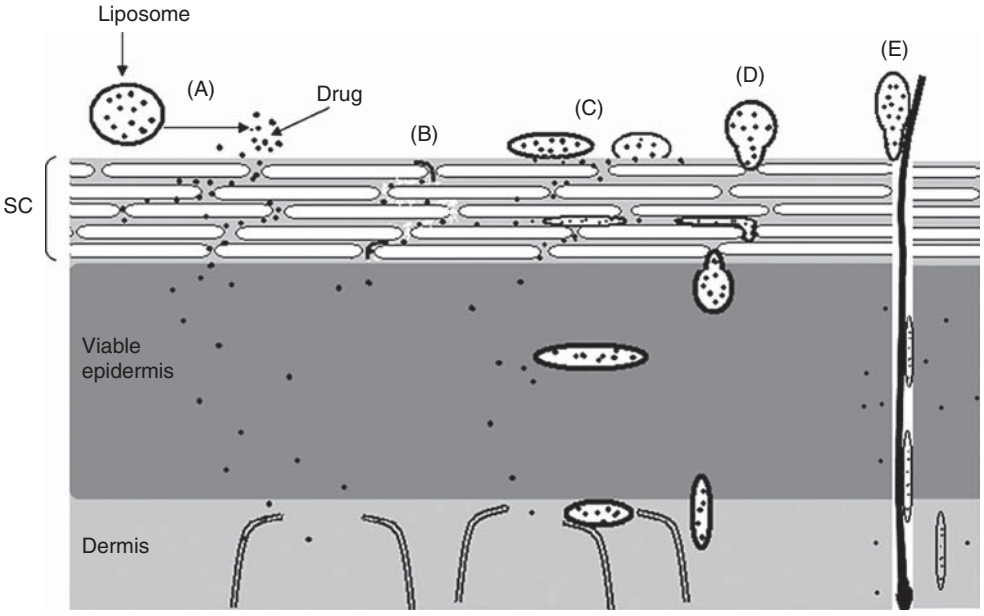

Figure 1. Possible mechanisms of action of liposomes as skin drug delivery systems. (A) is the free drug mechanism, **(B)** is the penetration enhancing process of liposome components, **(C)** indicates vesicle adsorption to and/or fusion with the stratum corneum (SC) and **(D)** illustrates intact vesicle penetration into or into and through the intact skin (not to scale).
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aminoglycosides, occurred in about 1.4% of patients, after parenteral administration [23]. The PA percutaneous absorption after topical application was investigated in ulcerated CL lesions of BALB/c infected mice. The PA concentration in plasma after 1 h ranged from 2 to 22 μg/mL, suggesting that topically applied PA can be absorbed systemically [27].

2.2 Miltefosine

Miltefosine, an alkylphosphocholine (**Table 1**), originally developed for the topical treatment of cutaneous metastases of breast cancer, was the first oral drug with antileishmanial activity. Miltefosine is registered for the oral treatment of VL in India and Germany, and for CL in Colombia [28,29]. Miltefosine can act through different mechanisms on *Leishmania* spp., altering the metabolism of lipids and inducing the production of interferon γ (IFN-γ) [30,31]. Adverse reactions of miltefosine over the gastrointestinal system include nausea, vomiting, and diarrhea [32-34]. Toxicity studies have shown that the other organs most affected are the eyes, kidneys, and the reproductive system [7].

2.3 Amphotericin B

AmB is a highly hydrophobic drug with a high molecular weight that exhibits amphiphilic and amphoteric properties (**Table 1**). It is a polyene antifungal produced by *Streptomyces nodosus* that was introduced originally as a drug to combat systemic fungal infections. AmB also exhibits antiparasitic activity and is a second-line therapy useful for the treatment of CL, VL, and MCL [35-37]. The possible mechanism of action of AmB over *Leishmania* occurs through the formation of channels that are permeable to small cations and anions [36,38]. However, medical application of AmB is limited by its toxicity. Required hospitalization, constant monitoring of patients, prolonged duration of treatment, and infusion-related adverse events are the drawbacks of AmB deoxycholate [39].

2.4 Imiquimod

Imiquimod, a nucleoside analogue of the imidazoquinoline family, was the first immune response modifier used for the treatment of infectious skin conditions (**Table 1**). It was approved originally for the topical treatment of external anogenital warts [40]. There are case reports and preliminary studies suggesting its effectiveness in the treatment of CL [41,42]. The effects of imiquimod on innate immune responses, via toll-like receptors, suggest a potential antileishmanial activity that was demonstrated by inducing the release of nitric oxide [43,44]. Less than 1% of the drug is recovered in urine after topical application. Topical imiquimod (5% cream) is only mildly irritating and does not lead to systemic toxic effects [43].

2.5 Buparvaquone

Buparvaquone (BPQ), a hydroxynaphthoquinone (**Table 1**), is currently marketed for the treatment of theileriosis in cattle (as Butalex®). It has several physicochemical properties suitable for topical delivery (low molecular weight, low melting point, etc.). However, it has low aqueous solubility and high

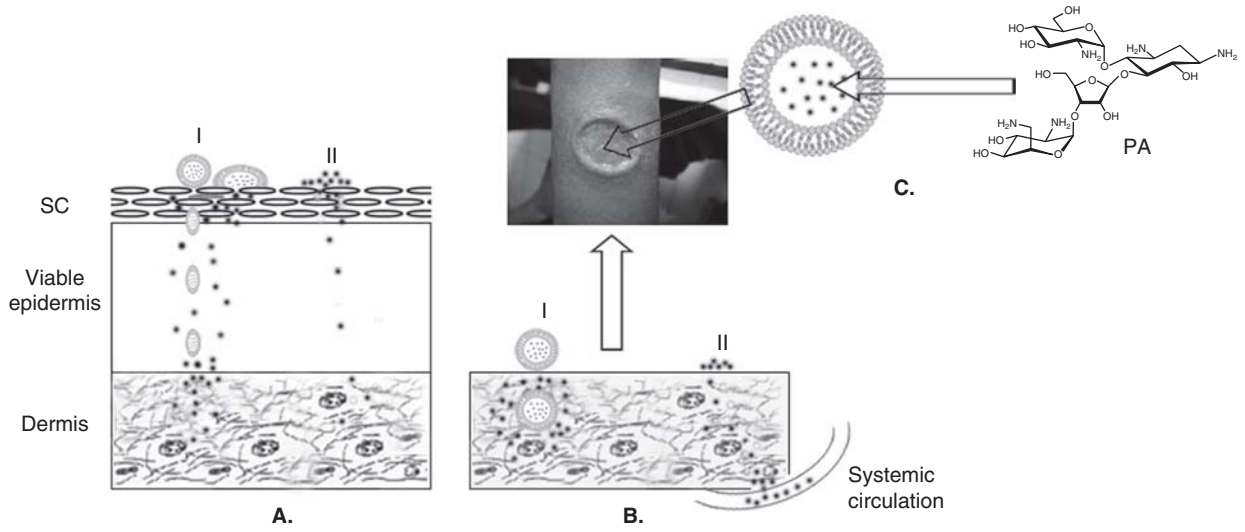


Figure 2. Rationale for the use of liposomes in topical treatment of CL. Liposomes (I) or free drug (II) can be applied either to the intact skin (A) or to the ulcerated skin (B). When applied on the intact skin, liposomes can improve drug penetration in comparison with the free drug; when applied on the highly damaged skin, liposomes provide targeting skin avoiding systemic absorption. Hydrophilic drugs such as PA loaded in liposomes can be applied in the leishmaniasis lesions (C), in which skin is ulcerated.

PA: Paromomycin; SC: Stratum corneum.

lipophilicity. Attempting to increase aqueous solubility and absorption in topical treatment for CL, a phosphate prodrug approach has been investigated [45,46].

3. Skin structure and skin permeability in CL lesions

Human skin is composed of two parts: the epidermis and the dermis. The superficial layer, and also the thinner one, is the epidermis, whereas the deeper layer is the dermis. The epidermis or, specifically, the SC, its outermost layer, is considered the main barrier to the passage of substances through the skin.

The SC is formed by dense, functionally dead, keratinized cells, named the corneocytes. These cells are surrounded by the intercellular lipid matrix composed of non-polar lipids organized in lamellar lipid layers. Thus, the SC is mainly a hydrophobic layer. Although the SC is 10 – 20 µm thick, it is also considered responsible for the barrier function of the skin. It hampers the skin penetration of drugs, other chemicals, and microorganisms besides being involved in the regulation of transepidermal water loss [47,48].

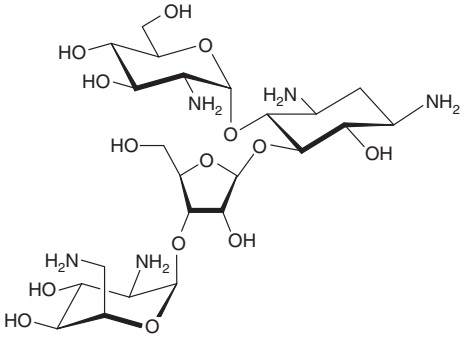
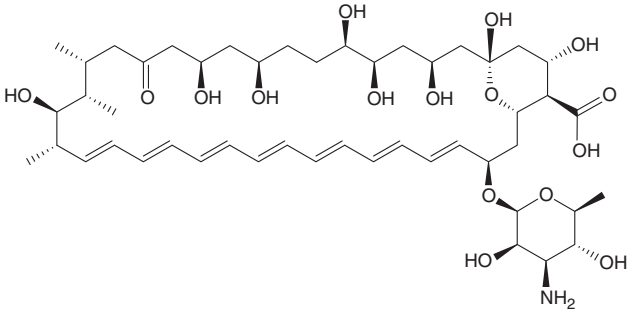
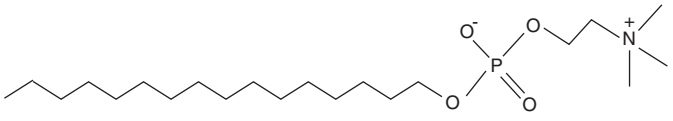
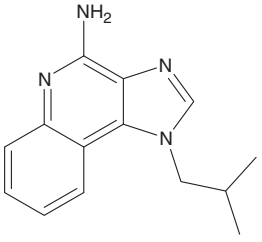
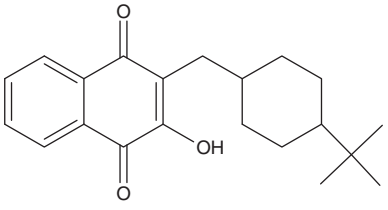
Three possible pathways for drug skin delivery have been considered: the intercellular route, between the corneocytes, sinuously through the lipid matrix; the transcellular route, through the corneocytes and the lipid matrix; and the transappendageal route, through the cutaneous appendices (hair follicles, sebaceous and sweat glands). The intercellular route is considered the most important one to drug penetration, since on the transcellular route, the drug has to cross the skin passing through both the lipid structures and the cytoplasm of the corneocytes [49,50]. Although cutaneous appendices occupy less than 0.1% of the total skin surface, this path can circumvent the low diffusivity of the SC, acting as a diffusional shunt [47,49].

To facilitate drug absorption through the skin, it is also important to select an adequate molecule. An ideal molecule would have to possess a low molecular mass, preferably less than 600 Da; solubility in oil and water, i.e., balanced partition coefficient; and a low melting point [15]. Thus, the skin penetration of drugs such as PA, which is highly hydrophilic and has a high molecular weight, is hampered.

In CL, the permeability of the skin is highly variable in comparison to the normal skin. CL typically begins with a small erythema and swelling which increases in size at the area where an infected sandfly has bitten the host. Then, the erythema develops into a papule, which increases in size, becoming a nodule that progressively ulcerates over a variable period ranging from 2 weeks to 6 months. After this time, it becomes the characteristic lesion of CL [51,52], with the loss of epidermis and part of the dermis. Therefore, since the skin is damaged, the barrier function exerted by the SC is absent and the absorption of any kind of drug used in topical treatment can be enhanced, especially the hydrophilic ones [53].

The size and evolution of lesions vary with the infecting strain of *Leishmania*. Old World CL and *L. mexicana* lesions tend to heal spontaneously after 6 – 12 months, but *L. braziliensis* may take years to heal. New World CL tends to be more severe and spontaneous healing is rare, with lesions which vary widely, ranging from small, dry lesions to large ulcers. In severe disease, keratotic nodules can also develop. Both in

Table 1. Structural formula and physicochemical characteristics of drugs used for the topical treatment of CL.

Drug	Molecular structure	Physicochemical characteristics
Paromomycin		Very soluble in water, insoluble in alcohol, chloroform and ether [99]
Amphotericin B		Insoluble in water, soluble dimethyl sulfoxide and propylene glycol, very slightly soluble in methanol [100]
Miltefosine		Solubility: > 10 g/100 mL in water, methanol or ethanol; < 10 g/100 mL in: acetone, n-Pentane [33]
Imiquimod		Crystalline odorless solid, poorly soluble in water [43]
Buparvaquone		Solubility in water: < 0.03 μg/mL, in pH 3.0, 5.0 or 7.4 [88]

keratotic nodules and in scar tissues formed during the treatment, the SC barrier function is recovered or even more pronounced. Thus, the drug skin penetration may become hindered at the end of treatment in healing lesions [52,54].

Therefore, skin conditions represent important considerations in the topical treatment of CL. Formulations can be applied either to thickened lesions, with an additional barrier to absorption or, more commonly, to open lesions (e.g., ulcers), in which the barrier properties of the epidermis have been completely lost [6]. In addition, the topical treatment of CL can last for 20 – 30 days [8]. Therefore, re-epithelization of open lesions can be observed, at least partially, during therapy, whereas the renewal of the epidermis contributes to a decrease in drug penetration due

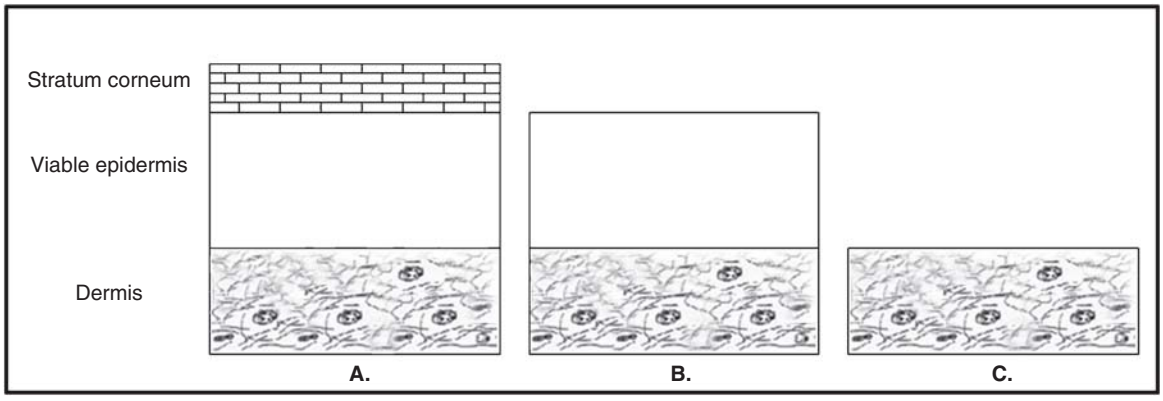


Figure 3. Schematic diagram of the skin conditions during the CL. Formulations can be applied to thickened lesions, in which the barrier properties are normal or even higher than the intact skin, or to open lesions, in which the barrier properties of the epidermis have been completely lost. Application on partially damaged skin (skin without SC) is also possible. **(A)** Intact skin: with SC, viable epidermis and dermis; **(B)** Partially damaged skin: without SC; **(C)** Highly damaged skin: tissue without epidermis (open leishmaniasis lesion; ulcerated skin).
SC: Stratum corneum.

to barrier repair. An ideal formulation for the topical treatment of CL should be used to provide improved topical delivery in all antagonistic situations (i.e., intact, partially, or completely damaged barrier) (**Figure 3**). Finally, drug delivery systems should promote drug penetration into the deeper dermal layer, as the parasites in CL are mainly located in the dermis.

4. Conventional drug delivery systems

Ointments, creams, and hydrophilic gels are the conventional drug delivery systems most frequently tested for the topical treatment of CL.

4.1 Conventional PA delivery systems

Petrolatum ointments, containing PA associated with methylbenzethonium chloride (MBCL), an ammonium quaternary surfactant, which also has antileishmanial activity, have been evaluated with favorable results, in a series of studies in animal models infected with different species causing CL [55-58]. Several clinical trials have also been performed to test this formulation in CL patients from different geographical regions of the Old and New World (**Table 2**) [59-62].

However, MBCL induces local irritation, which has prevented its use in some cases [55,57]. The local side effects due to MBCL have led to the development of alternative topical formulations, replacing the MBCL by urea, which has lower toxicity when compared to MBCL. The ointment PA/urea cured all L. major lesions on BALB/C mice [13]. However, clinical trials have shown that this formulation is not as effective as that containing PA/MBCL (**Table 2**) [10,14,63-65].

Replacement of MBCL by addition of 0.5% of gentamicin has also been evaluated as an alternative PA topical formulation (cream) [58]. Based on the data obtained in animal models, studies were conducted to evaluate the clinical efficacy [8,12]. However, variable cure rates were reported in comparison to placebo (**Table 2**) [8,12].

Considering that parasites in CL are located mainly in the dermis, drug penetration into and across skin would be desirable. To investigate the influence of different formulations (oil/water cream, ointment and hydrophilic gel) on the skin permeation of PA, *in vitro* studies on Franz cells were performed. As an attempt to mimic the conditions of skin lesions observed in CL, the permeation studies were conducted using two skin models: intact mouse skin and stripped mouse skin (removal of the SC), which was used to mimic the damaged skin. PA permeation across intact skin was low, regardless of the formulation tested. When the SC was removed, PA permeation from hydrophilic formulations (cream and gel) was enhanced significantly [66]. However, studies were carried out on hairless mice skin, a model that cannot be easily extrapolated to human skin [67]. Thus, additional permeation studies were performed using pig ear skin, a more relevant model for human skin [68]. Three models were tested: intact and stripped skin and dermal membranes (pig skin without epidermis). The dermal membrane has been considered a good model for ulcerated skin [69], which is frequently observed in CL. PA permeation across intact pig skin was low, regardless of formulation tested (gel, water/oil emulsion and ointment) (data not published). In contrast, PA permeation across dermal membranes from hydrophilic gel was higher than that observed for other formulations (**Figure 4**). These findings are consistent with data obtained previously, which showed that PA penetration into or across skin from gels was higher than that observed for hydrophobic formulations.

Since CL lesions often develop into an ulcer without epidermis, one may consider that the principal barrier to

Table 2. Animal studies and clinical trials using conventional dosage forms containing PA for topical treatment of CL.

Concentration (%)	Formulation	Composition	Studies in animals	Clinical trials
15	Hydrophobic ointment	White soft paraffin + 12% MBCL	[42,55-58]	[10,59-62]
15	Hydrophobic ointment	White soft paraffin + 10% urea	[13]	[10,14,63-65]
15	Cream (WR 279.296)	0.5% gentamicin in a cream vehicle	[58]	[8,12]
10	Hydrogel	Hydroxiethylcellulose	[27,72-74]	[11]

CL: Cutaneous leishmaniasis; PA: Paromomycin; US: United States.

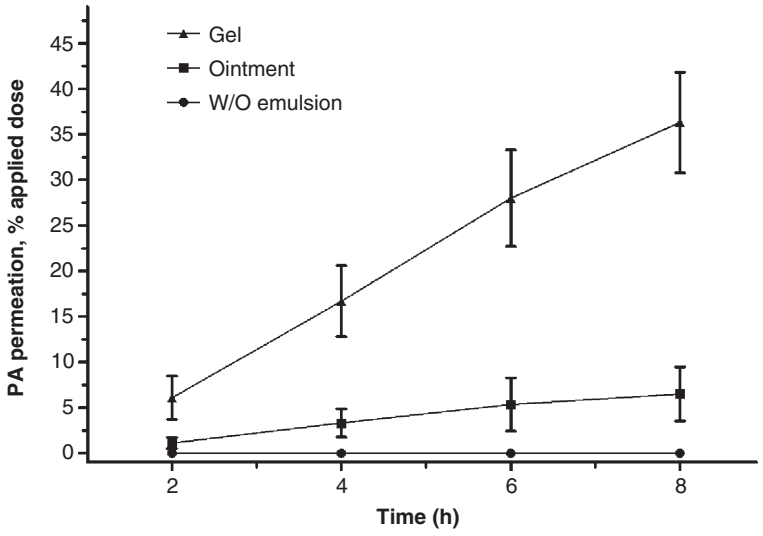


Figure 4. *In vitro* penetration of PA from topical formulations (gel, ointment and w/o emulsion) containing PA at 1% across dermal membranes. The formulations were applied over biopsies of pig skin without epidermis (dermal membranes) mounted in Franz cells. Each point represents means ± SD (n = 3).
PA: Paromomycin; SD: Standard deviation; W/O emulsion: Water-in-oil emulsion.

drug skin penetration is absent [70]. For the hydrophilic gel, PA is dissolved and available allowing its prompt release and penetration into or across the skin. In contrast, PA is insoluble in hydrophobic ointments and therefore drug release depends strongly on the particle size. For the water/oil emulsion, although the drug is soluble in the internal aqueous phase, drug diffusion across the external oil phase is the rate-limiting step. Lalor *et al.* have shown that, in an emulsion, the interaction of the drug between the aqueous and oily phase can determine the extent in which the thermodynamic activity is decreased in the external phase in contact with the membrane [71].

These findings prompted investigations on the efficacy of this new formulation (hydrophilic gel) in animal models. Accordingly, the studies performed have demonstrated that a PA gel formulation was highly effective to treat lesions in *L. (L.) majo*r, *L. (L.) amazonensis*-infected mice or *L. (V.) braziliensis*-infected hamsters [27,72,73]. This formulation was also recently tested in CL patients infected by *L. (V.) braziliensis* that could not be submitted primarily to meglumine antimoniate therapy [11]. Treatment was well tolerated and patients who did not present healing after a 20-day course continued spontaneously treating themselves. The average time of treatment was 30 days and a cure rate of 60% (9/15) was observed. These findings suggest that the efficacy of this formulation should be further evaluated in clinical trials.

Efficacy of the topical treatment of CL may not be attributed, however, solely to drug skin delivery. Other variables may significantly affect cure rates and reduction of tissue parasitism. *Leishmania* species differ significantly in their susceptibility to distinct drugs, including PA. In addition, based on the fact that infection by New World *Leishmania* species may disseminate reaching other distant body sites, combined alternative therapies have been advocated [37]. In agreement, findings from pre-clinical trials revealed that combined topical PA-gel and oral miltefosine is highly effective regardless of the parasite species susceptibility to both drugs, promoting complete elimination of parasites in infected mice at the site of infection as well as in internal organs [27,74].

Finally, although the drug penetration has been improved through the use of the gel, it is noteworthy that PA

permeation across the stripped pig skin, a damaged skin model, was still quite low. In this context, drug nanocarriers such as liposomes have demonstrated the capacity to increase drug penetration into and across the skin, when compared to conventional formulations [75,76]. In addition, application on partially damaged tissue (stripped skin) provided targeted, sustained topical delivery, [77] whereas on open lesions, liposomes, as compared to conventional formulations, showed the ability to accelerate wound healing [20-22]. Thus, PA-loaded liposomes could provide a reliable alternative for the topical treatment of the CL.

4.2 Conventional AmB delivery systems

A topical preparation of Fungizone® (conventional formulation of AmB) in white paraffin associated with the MBCL was evaluated in experimental CL. However, this formulation was not effective [55]. This could be explained by the fact that to obtain effective skin penetration of AmB, an adequate drug-carrier system is required. Thus, a drug-carrier system loaded with AmB may also prove to be an effective alternative for the topical treatment of CL.

4.2 Conventional miltefosine delivery systems

The potential of oral miltefosine against CL has been demonstrated [78,79]. In agreement, Miltex®, a micellar solution containing miltefosine at 6%, which was originally used for the treatment of cutaneous metastases of breast cancer, efficiently reduced the parasite burden in experimentally infected mice after topical administration [80].

4.3 Conventional imiquimod delivery systems

The imiquimod at 5%, which is available as a cream (Aldara®), has demonstrated antileishmanial activity for the treatment of CL, both in experimentally infected mice [42,81] and in humans [41,82-85]. These studies suggest that Imiquimod in combination with pentavalent antimonials presents potential to reduce the therapy course with antimony alone. However, the results of human clinical trials of Aldara®, as a primary treatment for CL, were of limited success [43,86].

4.4 Conventional buparvaquone delivery systems

Different formulations (solution, gels, and w/o emulsion) containing BPQ and 3-phosphonooxymethyl-buparvaquone (3-POM-BPQ), a prodrug of BPQ, were evaluated in the topical treatment of CL. The prodrug was rapidly hydrolyzed in the skin, being used to improve aqueous solubility and enhance skin absorption [87]. Studies of *in vitro* permeation demonstrated that BPQ and 3-POM-BPQ can penetrate the skin when applied in different formulations. The BPQ penetration was highest from isopropyl myristate, hydrophilic gel, and w/o emulsion, whereas 3-POM-BPQ penetration was greatest from an anhydrous gel [88]. The efficacy of these formulations in *in vivo* models of CL was evaluated [45]. The formulations showed antileishmanial activity reducing the cutaneous parasite burden and lesion size.

5. Novel drug delivery systems

The topical treatment of CL using novel drug delivery systems consists of the incorporation of the drug in nanostructured carrier systems (NCS), i.e., complex particles in the nanometer range. NCS have been proposed with the main objective to enhance skin penetration, to protect the drug against degradation, and to promote controlled release and follicular targeting. Lipid nanocarriers such as liposomes loaded with PA as well as nanoemulsions or lipid complexes (Amphocil®) loaded with AmB are the most frequently evaluated NCS for the topical treatment of CL.

Liposomes are spherical vesicles formed basically by phospholipids which associate themselves spontaneously and form a bilayer containing a centralized aqueous cavity (**Figure 2**). They are most commonly formed by phospholipids of natural, synthetic, or semi-synthetic origin and possess great potential for the delivery of hydrophilic, lipophilic, and amphiphilic drugs. The hydrophilic drugs can be encapsulated in the aqueous cavity, while the lipophilic ones are located in the non-polar portion of the bilayers. Amphiphilic drugs can be loaded in the hydrophilic–lipophilic interface [89]. Depending on the composition of the phospholipids (saturated or unsaturated) and the presence of co-surfactants (cholesterol, sodium cholate), rigid, fluid or elastic vesicles can be obtained and dermal or transdermal delivery can be favored [15,90].

Nanoemulsions are lipid formulations constituted by oil and aqueous phases with droplet size in the nanometric scale. Surfactants and co-surfactants are often used to stabilize these systems. Due to their oil phase, nanoemulsions are well established carriers for lipophilic drugs [91]. Other nanocarriers studied include ribbon-like lipid complex with dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol (Adelcet®) or even disc-like colloidal dispersions with cholesteryl sulfate (Amphocil®).

There are few studies involving lipid nanocarriers loaded with antileishmanial drugs for the topical treatment of CL. However, these systems have been proposed as carriers of hydrophilic PA or hydrophobic AmB, which are both high molecular weight drugs and, therefore, difficult to penetrate the skin.

5.1 Liposomes loaded with PA

The first efforts to obtain PA encapsulation into liposomes were reported by Ferreira et al. [92]. The PA penetration from fluid liposomes such as large unilamellar vesicles (LUV), through intact and stripped mouse skin, was investigated. In intact skin, PA penetration into and across skin from LUV liposomes was significantly higher than that observed for the free drug solution. As expected, PA permeation across stripped skin was higher than that observed across intact skin, regardless of the tested formulation. Although permeation from solution was also high, liposomes promoted skin targeting when compared to the free drug. However, these studies have been

Table 3. PA permeation and penetration across pig skin from PC, PC/Chol liposomes and aqueous solution after 8 h.

Formulations	Intact skin		Stripped skin* (Permeation)	
	Penetration‡	Permeation	μg/cm²	% applied dose
PC	7.2 ± 0.2	ND	14.6 ± 0.6	19.6 ± 0.8
PC/Chol	4.8 ± 0.2	ND	4.5 ± 0.4	6.1 ± 0.5
Solution	1.9 ± 0.1	ND	0.9 ± 0.5	1.0 ± 0.6

Data represented as percent applied dose.

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Values represent means ± SD (n = 3 – 5).

*SC was removed by stripping 30 times the skin with adhesive tape (Book Tape no. 845, Scoth 3M, EUA).

‡Penetration studies were not conducted with stripped skin.

Chol: Cholesterol; ND: Not detected; PC: Phosphatidylcholine; SC: Stratum corneum; SD: Standard deviation.

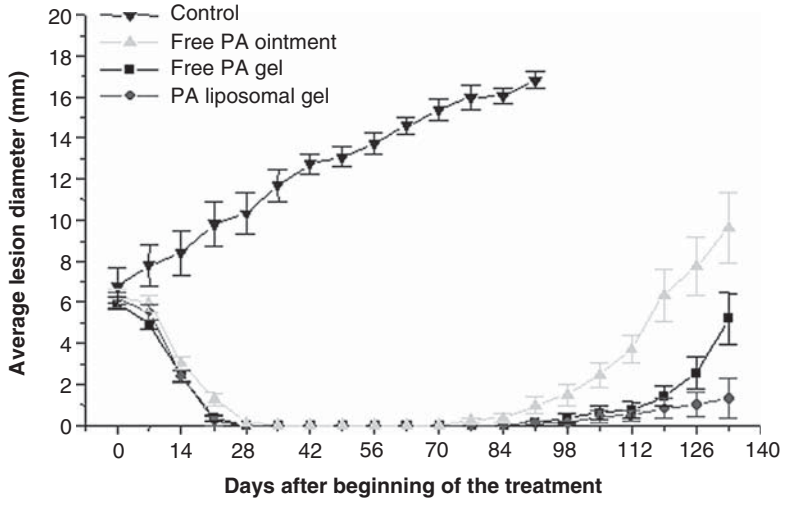

Figure 5. Evaluation of treatment efficacy in female BALB/c mice infected with *L. (L.) major*. Animals (n = 5) were treated topically with formulations containing PA at 5%: free PA ointment, free PA gel, PA liposomal gel or placebo gel for 12 days, twice a day. Lesion diameters are shown as averages and standard deviation. Mean lesion diameter was monitored for 133 days.

PA: Paromomycin.

carried out on hairless mice skin, a model that cannot be easily extrapolated to human skin [67]. Thus, more recently, the potential of liposomes in the delivery of PA was investigated by conducting permeation studies on pig ear skin, using two *in vitro* models: intact and stripped skin [19]. PA permeation across intact skin was low, regardless of the formulation tested. However, drug penetration into intact skin from liposomes was significantly higher than that from solution. Unexpectedly, PA permeation across stripped pig skin from solution was also low, but a remarkable increase in permeation was observed for PA-loaded liposomes (**Table 3**).

Topical treatment of *L. major* infected BALB/c mice resulted in a decrease in lesion size in animals treated with PA-loaded liposomes and free PA gel (**Figure 5**). However, local relapse, characterized by the reappearance of ulcers, occurred faster in animals treated with free PA than in those treated with liposomes. These findings suggest that liposomes represent a promising alternative for the topical treatment of CL using PA [19].

Jaafari et al. also evaluated liposomes loaded with PA at 10% and 15%. Both liposomes promoted high permeation and retention of PA in the mouse skin [93]. These PA liposomal formulations were three to four times more effective against *L. major* amastigotes and promatigotes than PA solution. Treatment of *L. major* infected BALB/c mice with topical liposomes loaded with PA induced the complete cure of the lesions, and the mice had significantly reduced lower parasite burdens in the spleen than the control mice. However, in this study comparison of topical treatment with free PA was not reported.

5.2 Lipid nanocarriers loaded with Amphotericin B

Despite its amphiphilic nature, AmB has low solubility due to the asymmetric distribution of hydrophilic and hydrophobic groups and presents itself as a zwitterion compound [94].

Table 4. *In vitro* permeation and penetration of AmB from different formulations across dermal membrane skin after 24 h.

Formulation	Permeation	Penetration*	
		($\mu g/cm^2$)	(% applied dose)
Solution	< DL‡	17.5 ± 4	31 ± 5
Amphocil®,§	< DL	15.2 ± 3	27 ± 4
Amphocil®¶	< DL	9.6 ± 3	17 ± 3
Fungizone®	< DL	3.5 ± 1	6 ± 2
FLmix	< DL	1.7 ± 0.3	3 ± 0.5
NE	< DL	1.1 ± 0.1	2 ± 0.15

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*Values represent the mean ± SD (n = 4).
‡DL = detection limit (0.02 $\mu g/cm^2$).
§Aqueous Amphocil®.
¶Hydroalcoholic Amphocil®.

These hydrophobic properties limit the skin penetration of AmB, mainly into the dermal layer. Lipid nanocarriers loaded with AmB constitute, therefore, a credible alternative to improve topical delivery.

Frankenburg et al. have reported the antileishmanial effects of topically applied lipid-based formulations loaded with AmB in *L. major* experimentally infected mice. The three tested preparations (Fungizone®, Amphocil®, and Adelcet®) were ineffective when applied topically, except when Amphocil® and Adelcet® were dispersed in 5% ethanol. No relapse was observed during the follow-up period after treatment [95].

Subsequently, *L. major*-infected patients were treated with an Amphocil® dispersion in 5% ethanol in a prospective placebo-controlled study. A significant improvement was demonstrated in Amphocil®-treated lesions compared with the placebo-treated lesions. This formulation promoted complete healing of all lesions with no evidence of relapse on follow-up visits [96]. This modality was also used for the topical treatment of an infant patient who had not responded to the topical application of a PA ointment [97], resulting in resolution of the skin lesions and absence of local or systemic side effects.

Considering that the development of lipid nanocarriers loaded with AmB for the topical treatment of CL still remains an opened question, a cationic nanoemulsion was developed and its potential for drug topical delivery was investigated through studies with dermal membranes, a damaged skin model [98]. Cationic nanocarriers were selected as carriers of drugs, since these systems have been used for improved skin delivery [90]. Other lipid nanocarriers (Amphocil® in water or in ethanol, Fungizone®, a Fungizone®-Lipofundin® mixture – FLmix) were also evaluated. Regardless of the formulation, the AmB permeation across the dermal membrane was low. In contrast, AmB penetration into the dermal membrane from Amphocil® (aqueous or hydroalcoholic) was significantly higher than that observed for other formulations, while the dermal uptake either from FLmix or nanoemulsion was lower (Table 4). Amphocil® provided the best results, highlighted by its high improvement of dermal penetration of AmB [98].

6. Conclusion

Topical treatment is an attractive alternative for CL, offering significant advantages over systemic therapy. As detailed in this review, the most important drugs utilized in the topical treatment of CL include PA, imiquimod, AmB, miltefosine, and buparvaquone. Among these, PA is the most commonly studied, followed by AmB, which is useful as the second-line therapy for systemic treatment of CL. Animal and clinical studies using conventional topical formulations containing PA showed variable results, which can be attributed to low skin penetration of PA, a highly hydrophilic drug. Although PA penetration across highly damaged skin has been enhanced from hydrophilic formulations, PA permeation across the intact or partially damaged skin was still quite low. Liposomes loaded with PA improved drug skin penetration in these conditions. In agreement, PA encapsulated in liposomes has shown enhanced efficacy for the treatment of experimental CL. Topical use of AmB is also quite limited because of its low skin penetration. In this context, lipid nanocarriers can also improve topical treatment since they can enhance drug skin penetration. Among these, Amphocil® has provided the best results, highlighted by its high improvement in dermal penetration and antileishmanial activity of AmB. In synthesis, the evidence gathered from tests performed with topical formulations containing PA or AMB, two rather distinct chemical entities, indicate that lipid nanocarriers may be useful to circumvent drug properties and skin permeability, the two main aspects that limit the efficacy of CL topical treatment.

7. Expert opinion

The topical pharmacotherapy of CL includes a variety of drugs with well-established antileishmanial activity; however, an effective topical treatment of CL using these antileishmanial agents still remains a great challenge. PA is the most commonly studied drug for the topical treatment of CL, followed by Imiquimod, AmB, and buparvaquone. Miltefosine has been used mainly for oral administration, and concerns about drug resistance, which could be even greater after topical application, constitute a major limitation for its use in the topical therapy of CL. Imiquimod occupies a prominent place in combination with antileishmanial drugs, such as pentavalent antimony, aiming mainly at shortening the antimony therapy course. However, its efficacy when used alone is usually low.

As previously pointed out, PA is the drug most frequently studied in the topical treatment of CL, although its physicochemical properties (high hydrophilicity and high molecular weight) are not considered ideal for skin penetration. One possible explanation for the promising findings obtained with topical PA could be the high penetration of this

hydrophilic drug into and across damaged skin (open lesions), in which the barrier properties of the epidermis have been completely lost. Moreover, some disappointing results have been attributed to low skin delivery of the drug and the use of MBCL has been proposed as an alternative to improve PA skin penetration, although this has not been experimentally investigated.

The majority of studies evaluating the efficacy of PA for the topical treatment of CL used conventional drug delivery systems such as ointments, creams, and hydrophilic gels. Few studies have compared the efficacy among these systems, although the PA delivery across the damaged skin has been improved with the use of hydrophilic formulations (creams and gels). However, PA penetration into and across intact or partially damaged (without SC) pig skin was still very low, regardless of the formulation tested and this could partially explain some disappointing results observed in clinical trials. It has not been investigated whether conventional skin penetration enhancers (ethanol, fatty acids, etc.) may enhance drug penetration and, consequently, the antileishmanial activity of topical PA, has not yet been investigated. Nanocarriers have demonstrated the capacity to increase drug skin penetration, when compared with conventional formulations, and, therefore, could also provide a credible alternative for the topical treatment of the CL. Among the nanocarriers for the topical treatment of CL, liposomes are considered the most promising and several studies have reported the advantages of these systems when compared with conventional formulations.

Liposomes are known to enhance skin penetration and pharmacological activity of highly hydrophilic drugs with a high molecular weight. In agreement, liposomes loaded with PA increased drug penetration into and across intact and partially damaged skin when compared with conventional formulations. The very high water solubility of PA makes liposomes the most suitable colloidal carrier for achieving maximal drug-to-carrier ratio. Moreover, leakage of the encapsulated drug from the internal to the external aqueous phase is expected to be low due to the high hydrophilicity of PA. Although preliminary studies of efficacy in experimentally infected mice have shown the potential of these systems, this alternative should be investigated further in different models. Moreover, progress towards clinical trials is still lacking. As previously pointed out, PA-loaded topical liposomes combined with other antileishmanial drugs, administered orally or parenterally, may also represent an effective alternative to be investigated for an effective treatment for CL.

Costs and technological problems related to production of liposomes must be considered in the rational design of these formulations. One limitation is the fact that their fabrication often requires the use of organic solvents; thus, further investigation is needed to allow their large-scale production at lower costs. This is a serious limitation for the treatment of such a neglected disease as CL, which depends essentially on therapeutic options at low costs. Alternative procedures have been described for the production of liposomes without using organic solvents as well as ways in which this could be applied to PA (Jaari and coworkers).

Although AmB is a second-line therapy useful for CL treatment, few studies have described the topical use of this antileishmanial drug. This can be attributed to the low skin penetration of such a highly hydrophobic drug with a high molecular weight. Lipid nanocarriers are often used for parenteral administration of AmB and these systems have also been described for the topical treatment of CL. Among these systems, Amphocil® occupies a prominent place in that it improved the skin penetration and antileishmanial activity of AmB when compared with other nanocarriers. Curiously, the liposomal form of AmB (Ambisome®) has not been tested for the topical treatment of CL and this should be regarded as an interesting issue in future investigations.

CL may not be taken as a single disease entity, as it is caused by rather distinct *Leishmania* species worldwide. Therefore, several factors, including the considerable variability in drug sensitivity among the infecting *Leishmania* species, ability to disseminate to distant body sites, and problems related to skin delivery, may affect treatment effectiveness. These aspects should be taken into consideration when interpreting efficacy results of chemotherapeutical studies. The variable sensitivity among species can be overcome, at least in part, by combined therapy. The usefulness of this approach, as well as that of the combination of a PA topical gel and oral miltefosine, was demonstrated experimentally for the treatment of CL. Nevertheless, the effectiveness of this approach and that of the combination between PA and other topical antileishmanial drugs in other experimental models awaits further investigation. Finally, it is highlighted that the conceptual scenario for the treatment of CL has changed significantly in the past few decades with the availability of additional drugs, routes of administration, and the knowledge of species diversity and host immune responses. This has broadened the alternatives for investigations, and in this context the results obtained with combined therapy and alternative delivery systems offer promising perspectives on improving the topical treatment of CL.

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