Topical treatment for cutaneous leishmaniasis

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Parenteral pentavalent antimonials remain the standard therapy for cutaneous leishmaniasis. More effective and patient-compliant topical treatments are an important alternative treatment for the localized, self-limiting forms of this disease. Two paromomycin ointments are commercially available but their use is limited by either toxicity or lack of efficacy. Other topical formulations have been in clinical trials, but many results have been equivocal and no major breakthroughs have been achieved. The focus of this review is on recent developments in the field of topical treatment for cutaneous leishmaniasis and rational approaches to enhance topical drug absorption.

Keywords Amphotericin B, cutaneous leishmaniasis, imiquimod, licochalcone A, miltefosine, paromomycin, topical treatments

Introduction

Leishmaniasis is a widespread disease with both visceral and cutaneous manifestations. Cutaneous leishmaniasis (CL) is the most common form of this disease. It has an annual incidence of 1 to 1.5 million cases and is endemic in 88 countries; 90% of cases are reported in just six countries, Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria [1]. These numbers are probably an underestimate as leishmaniasis is a notifiable disease in only 40 of the 88 countries. There is evidence to suggest that the worldwide incidence is increasing, probably due to improved reporting, population migration, seasonal/climatic changes, HIV co-infection and agro-industrial development in endemic areas [2].

The clinical manifestations of CL result from the interaction of factors including, parasite species, site of inoculation and host immune status. Although the disease is normally localized to the site of infection within dermal macrophages, metastasis to lymphatic, mucosal and bone marrow sites can occur [3]. After parasites are inoculated into the mammalian host by the sandfly vector, they are engulfed by macrophages where the intracellular amastigotes survive and divide within the parasitophorous vacuole. The manifestations of CL encompass a wide spectrum of severities and present in a range of clinical forms [4]. Lesions are characterized by development of nodules, which progress to ulcerative lesions, lasting from between 3 months to three years. Epidermal changes reflect the immune response to the infection, resulting in hyperplasia and epidermal thickening. Within the dermis the collagen matrix is disrupted and fibroblasts are eventually recruited during the healing process [5]. Epidermal disruption results in discharge and eventually dries to form an encrusted ulcer, with a central depression and raised border [6]. It is in this latter region where parasites are present in dermal macrophages. Resolution usually occurs following the generation of the appropriate Th1 response and the resulting cytokines (IFNγ, TNFα, IL-12) that confer resistance to infection with leukocyte migration resulting in necrosis and formation of a healing granuloma [7]. In the majority of cases of CL there is a single self-limiting lesion.

Over 17 species of *Leishmania* cause CL (Table 1) [8,9]. Acute CL, caused by *L major, L tropica* and *L aethiopica* in the Old World and *L braziliensis, L panamenis* and *L mexicana* in the New World are the most common causes of infection. Complex and rare manifestations of CL include: (i) mucocutaneous leishmaniasis (MCL) found in > 5% *L braziliensis* cases, often several years after the initial lesion; (ii) diffuse cutaneous leishmaniasis (DCL), a rare disseminating anergic disease caused by *L aethiopica* and *L amazonensis*; and (iii) '*recidivans*' leishmaniasis (LR) a chronic disease caused by *L tropica* and *L amazonensis*. Parasites that normally cause visceral leishmaniasis (VL) can also cause CL; some strains of *L infantum* can give rise to simple lesions, whereas post-kala-azar dermal leishmaniasis (PKDL) is caused by *L donovani,* usually two years after a visceral cure [10]. Identifying the causative organism of CL is important as there is significant variation in the drug sensitivity of *Leishmania* species [11].

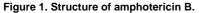**Table 1. Species of Leishmania causing CL as either primary or secondary manifestations.**

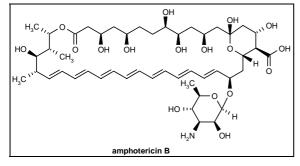| *Leishmania* species	Primary pathology	Secondary pathology
L(viannia) braziliensis (NW)	cutaneous	5% mucocutaneous (espundia)
L(v) peruviana (NW)	cutaneous (uta)	
L(v) panamensis (NW)	cutaneous (ulcera de bejuco)	5% mucocutaneous
L(v)guyanenesis (NW)	cutaneous ('pian-bois')	
L mexicana (NW)	cutaneous (chiclero's ear)	
L amazonensis (NW)	Cutaneous	diffuse CL
L venezuelensis	Cutaneous	
L pifanoi	Cutaneous	
L garnhami	Cutaneous	
L tropica (OW)	Cutaneous	*recidivans*
L aethiopica (OW)	Cutaneous	diffuse CL
L major (OW)	Cutaneous	
L donovani (OW)	visceral (kala-azar)	PKDL
L infantum (OW)	Visceral	CL
L chagasi (NW)	Visceral	CL

NW New World, **OW** Old World

Over the past decade, co-infection with HIV has become an increasing problem, particularly in southern Europe. The HIV virus hastens the spread of *Leishmania* and can re-awaken latent infections, while *Leishmania* accelerates AIDS onset [12,13]. It was recently estimated that CL occurs in 2 to 3% of HIV-infected patients [14].

Chemotherapy of cutaneous leishmaniasis

CL is a disfiguring disease that normally resolves within 3 to 18 months of initial infection. Treatment aims to cure as well as prevent the development of more complex manifestations like MCL and DCL. Generally the New World species tend to cause more severe and longer infections than the Old World species [15,16]. Therapeutic response is dependent on efficiency of host immune response and genetic makeup. Unfortunately, many published reports on treatment are based upon small, uncontrolled clinical trials which give equivocal results with differences dependent upon patient selection, parasite strain/virulence, drug regimen, evaluation criteria and site of infection. Moreover, spontaneous healing in CL causes additional difficulties in interpreting treatment data without adequate control (placebo) groups and trial design.

The standard therapy for CL involves daily injections of pentavalent antimony for 20 days, either as Pentosam (sodium stibogluconate) or Glucantime (meglumine antimonate); clinical use and problems are described elsewhere [17]. Studies in the Old World have show that intralesional administration gives superior healing rates compared to intramuscular antimonials [18,19]. Advantages of this route of administration include, targeting higher drug concentrations to the site of infection, lower systemic toxicity, decreased cost and faster healing time. However, local therapy alone is not appropriate for the New World *L braziliensis* species, which can potentially disseminate. The use of second line drugs, amphotericin B (AmB; Figure 1) and pentamidine isethionate, and the parenteral formulation of the aminoglycoside paromomycin (aminosidine; Figure 2) have also been described elsewhere [20,21].

Figure 1. Structure of amphotericin B.

Attempts to investigate possible new therapies have focused on modestly active oral agents, eg, antifungal azoles and allopurinol, and local therapy, including cryotherapy, thermotherapy, surgery and electrotherapy [22]. An open-label phase I/II clinical trial of oral miltefosine (an alkylphosphocholine; Figure 3) against CL in South America gave a 94% cure rate when a dose of 133 to 150 mg/day was used [23]. Mixed results have been achieved with oral itraconazole and ketoconazole in clinical trials [24-27].

Immunomodulators including BCG, IFNγ and GM-CSF have been used alone and in combination with antimonials for the treatment of CL [28], however, recent trials with a synthetic immunomodulator, imiquimod (Figure 4), offer a more realistic option.
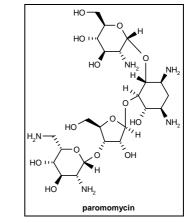
Figure 2. Structure of paromomycin.

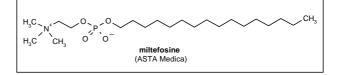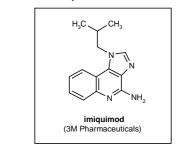Figure 3. Structure of miltefosine.

Figure 4. Structure of imiquimod.

Topical treatments

Topical formulations offer significant advantages over systemic therapy, such as, ease of administration, fewer adverse effects and cost-effectiveness. Local treatment remains an attractive approach for simple localized forms of CL that do not pose a risk of developing complications that require systemic therapy. This paper reviews the current status of topical treatments and looks at developments over the past decade since those described in reference [29].

Renewed interest in the topical treatment of CL began with the studies of El-On *et al* on the aminoglycoside, paromomycin (aminosidine, monomycin, neomycin E) (Figure 1) [30]. They showed that a 15% paromomycin sulfate (PM) plus 12% methyl benzethonium chloride (MBCl) ointment effected a complete cure of *L major* lesions in BALB/c mice. In the same series of experiments, paromomycin in combination with other quaternary

ammonium disinfectants or DMSO (a known penetration enhancer), gave significant levels of activity. Two other aminoglycosides, kanamycin and gentamycin in the MBCl formulation also had some activity, but three others, neomycin, amikacin and tobramycin, were not active. The success of this approach in demonstrating the potential of aminoglycosides as topical antileishmanials contrasted with parallel studies that showed other antileishmanial drugs (sodium stibogluconate, pentamidine, AmB, emetine, metronidazole, co-trimoxazole and allopurinol) to have no significant activity in this model. Rifampin was the only other drug that demonstrated some activity. It should be noted that MBCl alone also has antileishmanial activity [31]. These studies led to a series of small clinical trials of the PM-MBCl ointment against cutaneous leishmaniasis caused by *L major, L tropica, L aethiopica, L braziliensis* and *L mexicana*. Initial studies were mostly carried out in Old World CL [32,33••]. In a randomized, double-blind, crossover study in patients with *L major* infection there was a 74% cure rate, versus 27% in the placebo control group [33••]. A placebo-controlled trial in Guatemala [34] and uncontrolled trial in Ecuador [35] reported 85.7% and 85% cure rates, respectively (twice daily applications for 20 days), one year post-treatment. These results contrast with the apparent lack of efficacy in the placebo-controlled trial in Colombia [36]. This ointment is commercially available and marketed by TEVA Pharmaceuticals, Jerusalem. However, early studies also reported severe irritancy and intolerance (burning, pruritis and vesicle formation), most probably due to the high concentration of MBCl, a cationic surfactant that is used as a disinfectant at 0.1 to 0.2%. Concerns over metastasis led to a trial of 15% paromomycin/5% MBCl with concomitant systemic antimony, which gave a 90% cure rate [36].

To circumvent the problem of irritation, other formulations were studied, most of which contained a penetration enhancing agent. A formulation of 15% aminosidine with 10% urea in white soft paraffin was the most efficacious in experimental and clinical studies [37,38]. However clinical trials of this formulation (twice daily applications for 14 days) in Tunisia [39] and Iran [40] were not effective in accelerating cure. A clinical trial in Honduras similarly reported lack of efficacy following three-times daily applications for 4 weeks, where infection was caused by *L chagasi* and *L mexicana* [41]. More recent studies in Iran have identified differences between treated and untreated groups following topical application twice a day for 28 days [Modabber F, unpublished data]. A formulation of 15% PM with 10% urea in a cetomagrocol/mineral oil/white petroleum vehicle is produced by Razak Laboratories, Tehran, Iran.

In an experimental study using *L major*-infected BALB/c mice, PM-MBCl ointment plus gentamicin (an aminoglycoside that when used alone has limited antileishmanial activity) was significantly more active than the PM-MBCl ointment alone [42]. Subsequently, a group at the Walter Reed Army Institute of Research reported the antileishmanial activity of 15% PM and 0.5% gentamicin (WR-279396, PM-G) in a vehicle containing ten surfactants (unnamed, US patent pending). In experimental models the PM-G ointment gave a 100% cure of *L major* and *L mexicana* lesions on mice following twice-daily treatment for 10 days; there was no relapse up to 70 days after the completion of

treatment [43•]. The antileishmanial activity proved better than the PM-MBCl formulation against *L panamenis* and *L amazonensis* and equivalent to the PM-MBCl formulation against *L major* and *L mexicana*. Importantly, the PM-G formulation also proved less toxic than the PM-MBCL formulation. However, preliminary results from a clinical trial involving 45 CL patients in Colombia reported a 65% cure rate after 20 days of treatment with the PM-G ointment, in comparison to a 90% cure rate with the PM-MBCl ointment after 12 month follow-up [44].

AmB is a recommended second line drug for the treatment of VL, CL and MCL. Lipid formulations of AmB have reduced toxicity compared to the parent drug formulation, AmB deoxycholate (Fungizone). Several AmB liposomal formulations have proved effective for the treatment of VL [28] with AmBisome approved for use in the treatment of adult VL [45]. The activities of lipid AmB formulations have been compared in experimental *L major* infections in mice [46] and intravenous AmBisome has been used to treat MCL in Brazil [47]. When dispersed in an aqueous solution with 5 to 25% ethanol, topical AmB formulations, such as Amphocil (colloidal dispersion of cholesterol sulfate and AmB) and Abelcet (phospholipid/AmB complex), had a curative effect on lesions caused by *L major* in mice following 21 days administration [48]. Fungizone (micellar AmB and sodium deoxycholate) was ineffective. Subsequently, 17 patients in Israel were treated with Amphocil dispersed in a 5% ethanol aqueous solution. The AmB-treated lesions healed faster than placebo-treated ones in all but one case, who had a deep ulcerated lesion [49]. A patent has been filed for addition of ethanol to lipid AmB in topical formulations.

Miltefosine (hexadecylphosphocholine), an alkylphosphocholine originally developed as an oral antineoplastic agent, is an active antileishmanial in experimental models [50] and is now in clinical trials for VL in India [51]. A topical formulation of miltefosine, Miltex, has been approved for use in 22 countries for the treatment of cutaneous malignancies [52]. Miltex contains 6% miltefosine in a vehicle (Kasacade) comprised of 3-propyloxypropylene glycol, 3-hexylpropylene glycol and 3-nonylpropylene glycol. There have been two clinical trials with Miltex for CL, one in Syria involving 16 patients with nodular CL (applied twice daily) and a second trial in Colombia involving 19 patients (applied once-daily for 4 weeks). Neither trial demonstrated efficacy against CL [Bachmann P, personal communication], although the potential of oral miltefosine against CL has since been demonstrated [23]. The absence of a significant response of CL patients to Miltex contrasts with reported experimental studies using *L mexicana*- and *L major*-infected BALB/c, CBA/J and C57BL/6 mice where the formulation reduced lesion size and healed established lesions, although relapse did occur [53].

Other approaches have exploited improved understanding of the immunology of leishmaniasis. The generation of nitric oxide (NO) is an important mechanism by which activated macrophages kill intracellular *Leishmania* parasites. *Leishmania* sensitivity to NO has been exploited in a number of clinical studies. In a trial in Ecuador in 16 patients with *L braziliensis* infections, administration of an *S*-nitroso-*N*-acetylpenicillamine NO-generating cream led to clinical

improvements being reported [54]. In contrast, a study in Syria using a different NO-generating cream on 40 patients had poor results [55]. This formulation used potassium nitrite in aqueous cream to generate NO when acidified with various agents. More promisingly, the immunomodulator imiquimod (Figure 2), an imidazoquinazoline, and its analog resiquimod (3M Pharmaceuticals/Eli Lilly & Co), stimulated NO synthesis *in vitro* and killed *Leishmania* amastigotes in macrophages. Imiquimod, commercially available as Aldara in a 5% cream for the topical treatment of genital warts, proved effective in treating experimental murine CL caused by *L major* [56]. In a subsequent study on antimony-resistant CL cases in Peru, 12 patients were treated with a combination of topical 5% imiquimod cream + parenteral Glucantime for 20 days. All patients responded well to this combination therapy and there was a 90% cure at the 6 month follow-up [57•].
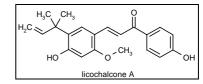
Several studies have focused on drugs interfering with sterol biosynthesis. One double-blind trial in Saudi Arabia investigated the clinical efficacy of topical 1% clotrimazole (Canesten) and 2% miconazole (Daktarin). Both treatment groups showed improvements, with clotrimazole being most effective [58]. Another study in mice examined the topical versus oral delivery of terbinafine and itraconazole in BALB/c mice infected with *L major* [59].

Other experimental studies with *L amazonensis*-infected BALB/c mice proved the phenothiazine chlorpromazine and the tricyclic antidepressant amitriptyline, as 10% ointments in petroleum, to be ineffective [60]. However, the antimycobacterial drug clofazamine and the naphthoquinone plumbagin had a suppressive effect on lesion growth [61,62]. Elsewhere, the dinitrolalanine herbicide trifluralin, a compound
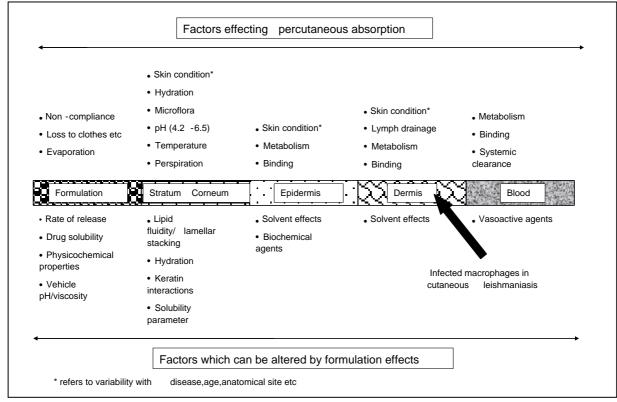
with proven *in vitro* antileishmanial activity, in a 15% topical formulation had suppressive effects on *L major* and *L mexicana* lesions in BALB/c mice [63]. More promising experimental studies have been reported with the plant product licochalcone A (Central Drug Research Institute; Figure 5), which had activity against *L donovani* and *L major* in experimental models [64]. 150 Synthetic derivatives have since been tested against *Leishmania in vitro*. Two of these compounds, PH-81 and PH-104 (Royal Danish School of Pharmacy), have been further tested against *L major* CL in BALB/c mice. The topical application of an ointment containing these two chalcones (50 mg/ml, twice daily for 10 days) in white petroleum caused a pronounced suppressive effect on lesion growth, but did not result in a cure, although suppression continued for several weeks after completion of dosing [65].

Figure 5. Structure of licochalcone A.

licochalcone A

The skin and its barrier properties

Topical formulations are faced with a number of barriers, some of which are shown in Figure 6. The skin consists of: (i) the stratum corneum (SC); (ii) the viable epidermis, which generates the SC at the germinative layer and also functions in metabolism and melanin synthesis; (iii) the dermis, which provides both mechanical and nutritional support to the epidermis; and (iv) the appendages - hair follicles, sebaceous glands, eccrine glands and apocrine glands [66].

Figure 6. Considerations in topical delivery.

Representing the least permeable late stage of differentiation, the SC is the greatest barrier to percutaneous absorption. Most molecules permeate the skin via a tortuous but continuous lipid intercellular route [67]. An important consideration in CL is the skin condition. For example, topical formulations may be applied to open lesions that have lost the SC barrier property or epithelial thickening may present an additional hindrance to absorption.

Numerous strategies have been devised to enhance topical absorption and some are listed in Table 2. These either deal with optimizing properties for absorption or aim to transiently disrupt SC barrier properties. Physical methods for enhancing topical absorption include, iontophoresis, electroporation and ultrasound [68,69], however, these are in early stages of development and are not suitable for treating simple CL. Due to the continuity of the lipid pathway, chemicals altering the lipid structure can have a significant effect on percutaneous absorption [70]. Known penetration enhancers have been used in topical formulations for CL, eg, DMSO, urea, ethanol and polyglycols. However, most studies fail to discuss the rational approach to developing the formulation. Attempts to predict permeability are similarly crucial in selecting likely candidates for topical delivery [71]. Successful development of topical formulations for CL necessitates both a rational choice of drug and topical delivery vehicle [72].

Table 2. Enhancing percutaneous absorption.

Strategy	Example
Optimizing physicochemical properties	Prodrugs
Penetration enhancers	DMSO, oleic acid, urea
Optimizing formulation characteristics	Eutectic mixtures, saturated systems
Physical methods	Sonophoresis, electroporation, iontophoresis
Particulate carriers	Transferosomes, liposomes, cyclodextrans, emulsions

Metabolic skin activity has been reviewed elsewhere [73,74], highlighting not only the potential for metabolic inactivation, but also the possibility for prodrug delivery. Prodrugs can be used to mask functional groups in an attempt to alter solubility and permeability characteristics. The modified drug is rendered pharmacologically inactive and penetrates the SC to undergo cutaneous biotransformation within the viable tissues. This reverses the modification and essentially releases the active drug. A number of studies have been carried out on non-steroidal anti-inflammatory prodrugs [75,76]. Targeted drug delivery necessitates identifying features unique to the invading parasite or lesion. One study in *L mexicana*-infected mice found stage-specific cysteine proteases within lesions [77]. Interestingly, some cysteine protease inhibitors have been active against BALB/c mice infected with *L major* [78]. Full enzymatic analysis of *Leishmania*-infected skin could identify specific enzymes, which could allow targeting of prodrugs to infected lesions.

Conclusions

CL is an increasingly prevalent disease causing ulcerative lesions that are often disfiguring and can leave permanent scars for which drug therapy is largely inadequate. Topical treatment offers few adverse effects, better compliance, reduced costs and is feasible for a rural setting. Currently, only two marketed topical formulations of an antileishmanial drug, paromomycin, are available. Studies are underway to investigate the topical potential of other candidate antileishmanial drugs. Often these drugs have undesirable physicochemical properties so novel strategies are required to enhance absorption. Total flux depends on drug physicochemical properties combined with the influence of the delivery vehicle on penetration profiles. Future development of topical drugs must employ rational drug design to take account of physicochemical properties and their effect on percutaneous absorption and retention or drug release at the sites of infection in the dermis. Therapeutic efficacy depends on both adequate permeation and pharmacological potency.

Acknowledgements

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