Research Signpost
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences IV, 2014: 181-201 ISBN: 978-81-308-0554-2
Editors: Diego Muñoz-Torrero, Manuel Vázquez-Carrera and Joan Estelrich

12. Review and new therapeutic alternatives for the treatment of cutaneous Leishmaniasis

Alba Pujol and Cristina Riera

Unitat de Parasitologia, Departament de Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n. E-08028 Barcelona, Spain

Abstract. Leishmaniasis comprises a group of diseases caused by protozoa of the genus *Leishmania* and has two basic clinical forms, visceral *Leishmania*sis and cutaneous *Leishmania*sis. The clinical features of *Leishmania*sis depend on the species of *Leishmania*, the interaction between host and parasite and the immune response.

This work focuses on cutaneous leishmaniosis because although it is not a deadly disease it results in significant scars and facial disfigurements, thus being clinically important. Furthermore, the first-line treatment consists of intravenous or intramuscular administration of intralesional pentavalent antimonials, which are highly toxic, making hospitalization of patients compulsory during treatment, with the associated financial costs. Herein, we review studies on drugs and treatments with fewer side effects and easier routes of administration such as topical administration. Recent research shows that the topical route of administration holds promise for the future treatment of cutaneous leishmaniosis.

Correspondence/Reprint request: Dr. Cristina Riera, Unitat de Parasitologia, Departament de Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, Universitat de Barcelona. Av. Joan XXIII s/n, 08028 Barcelona Spain. E-mail: mcriera@ub.edu

Introduction

1.1. Overview of Leishmaniasis

Leishmaniasis comprises a group of diseases caused by protozoan parasites of the genus *Leishmania*. There are two main clinical forms, visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). It is transmitted by vectors of the genus *Phlebotomus* and *Lutzomyia* in Europe and America respectively, and a large number of vertebrates act as reservoirs. It is considered a zoonosis in most cases, and can affect around 100 species of animals including humans [1].

The World Health Organization (WHO) has reported a wide distribution of leishmaniasis, extending from South America, Africa and Asia to Europe. The disease is endemic in 88 countries, 72 of which are developing countries (Fig. 1 and 2). According to the WHO, as many as 12 million people are believed to be currently infected, with about 1–2 million estimated new cases occurring every year of which 500,000 are VL (90% in India and Sudan) and 1,000,000 are CL (90% Afghanistan, Saudi Arabia, Algeria, Brazil, Iraq, Iran, Syria and Sudan). Furthermore, a total of 350 million people live in risk areas.

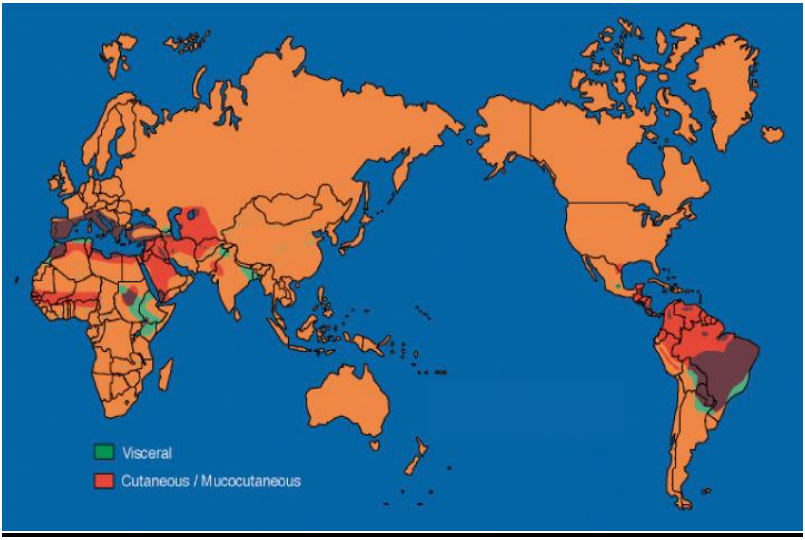

Figure 1. Areas of the world affected by leishmaniasis. The visceral form is shown in green and the cutaneous and mucocutaneous form in red (WHO).

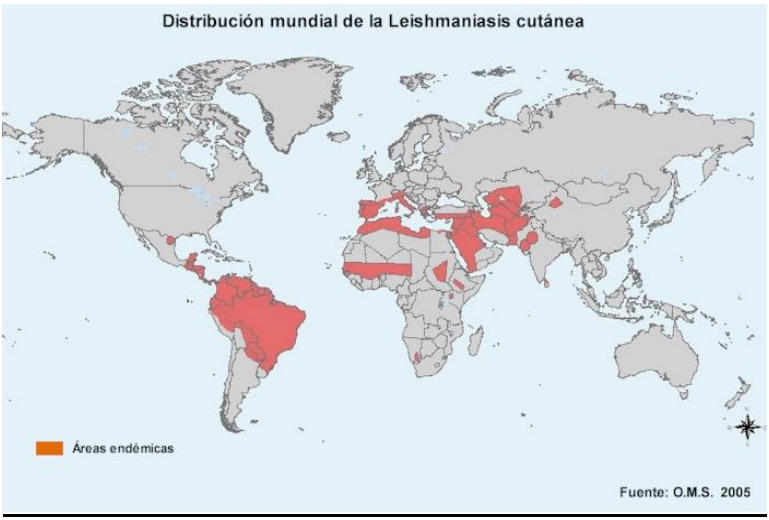

Figure 2. Global distribution of cutaneous Leishmaniasis (WHO).

Aetiological agent

The genus *Leishmania* contains a number of species. Its taxonomic status is as follows (Lainson and Shaw, 1987):

Phylum: Sarcomastigophora (Honigberg & Balamuth, 1963).
Subphylum: Mastigophora (Diesing, 1866).
Class: Zoomastigophorea (Calkins, 1909).
Order: Kinetoplastida (Vickerman, 1976).
Suborder: Trypanosomatina (Kent, 1880).
Family: Trypanosomatidae (Gobben, 1905).
Genus: *Leishmania* (Ross, 1903).

About 29 species of *Leishmania* have been described, of which 21 have been isolated from humans. These are responsible for producing the different clinical forms of the disease with a wide range of clinical symptoms. The species are grouped according to their geographical distribution in the Old and the New World, and their main clinical form: visceral, cutaneous or mucocutaneous. Cutaneous leishmaniasis is the most common form. Visceral leishmaniasis is the most severe form, in which vital organs of the body are affected. The same species can be found in both geographical areas, and can be responsible for more than one clinical form (Table 1).

Table 1. Classification of different species of *Leishmania* in human species, geographical distribution and clinical form (WHO, 2010).

***Leishmania* found in humans**

Subgenus	***L.(Leishmania)***	***L.(Leishmania)***	***L. (Viannia)***	***L. (Viannia)***
Old World	*L. donovani*	*L. major*		
	L. infantum	*L. tropica*		
		L. killicki[a]		
		L. aethiopica		
		L. infantum		
New World	*L. infantum*	*L. infantum*	*L. braziliensis*	*L. braziliensis*
		L. mexicana	*L. guyanensis*	*L. panamensis*
		L. pifanol[a]	*L. panamensis*	
		L. gamhami[a]	*L. shawi*	
		L. amazonensis	*L. naiffi*	
			L. lainsoni	
			L. lindenbergi	
			L. peruviana	
			L. colombiensis[b]	
Principal tropism	Viscerotropic	Dermotropic	Dermotropic	Mucotropic

[a]Species status is under discussion
[b]Taxonomic position is under discussion

1.2. Morphology and life cycle

The life cycle of *Leishmania* involves transfer between a vertebrate host and an invertebrate host, with two main morphological forms:

- **Amastigote form:** it is reproduced within macrophages and cells of the mononuclear phagocyte system of vertebrate host. It is oval, without a flagellum, and is therefore immobile. It measures about 2.5 to 7 µm in diameter. The cytoplasm contains a nucleus and kinetonuclei (Fig. 3).

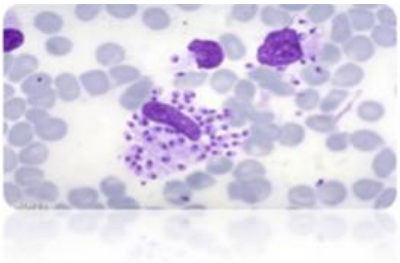

Figure 3. Amastigote form (WHO).

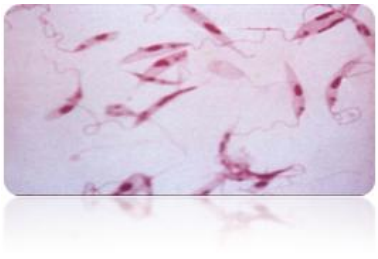

Figure 4. Promastigote form (WHO).

- **Promastigote form:** the parasite is a motile form with an anterior flagellum that develops in the sandfly, the insect vector. It is larger than the amastigote form. It has a central oval nucleus and prenuclear kinetonuclei (Fig. 4).

The cycle begins when the vector of female sand flies (invertebrate host) ingests blood of the vertebrate host and inoculates infective promastigotes, also called metacyclics. These promastigotes are phagocytized by macrophages in the skin, mucosal membranes and viscera. Within the cytoplasm of macrophages, the promastigotes transform into amastigotes. The amastigotes multiply until the cell explodes. Free parasites invade other macrophages or spread directly through the skin or the systemic circulation. Macrophages loaded with amastigotes can be re-ingested by the vector at a recent sting and then transform into promastigotes in the digestive tract. The promastigote form develops into a metacyclic infectious form over approximately 10 days.

1.3. Clinical manifestations of Leishmaniasis

The clinical manifestations of leishmaniasis are varied and depend on the species of *Leishmania*, the interaction between host and parasite and the host immune response [2-4]. This polymorphism is grouped into three different clinical forms:

Cutaneous Leishmaniasis

There is a wide variety of clinical presentations. A ‘classical’ lesion starts as a papule or nodule at the site of inoculation; it grows slowly, taking at least 1 week to reach its final size. Generally it is a single lesion located on the face, arms or legs. A crust develops centrally, which may fall away, exposing

an ulcer up to 5 cm in diameter with a raised edge and variable surrounding induration, which heals gradually over months or years, leaving a depressed scar with altered pigmentation. Satellite nodules at the edge of the lesion are common. Occasionally, it may be complicated with bacterial superinfection or regional lymphadenopathy [5] (Fig. 5A).

Mucocutaneous Leishmaniasis

This clinical form occurs in just a few species of the New World. Studies in Brazil have shown that mucocutaneous leishmaniasis can present from several months to 20 or more years after a cutaneous lesion. Nasal lesions are always present, with nodules and infiltration of the anterior cartilaginous septum, leading to obstruction of the nostril and, later, perforation of the septum with collapse and broadening of the nose. In one third of patients, other sites are involved, in the following descending order of frequency: the pharynx, palate, larynx, trachea and upper lip. Mucocutaneous leishmaniasis almost never heals spontaneously. Secondary bacterial infections are frequent, intercurrent pneumonia being the commonest cause of death (Fig. 5B).

Visceral Leishmaniasis or Kala azar

This is the most severe form of leishmaniasis. Clinically it is characterized by the appearance of fever, vomiting, shivering, diarrhoea, weight loss and anaemia. The common clinical signs are non-tender splenomegaly, with or without hepatomegaly, wasting and pallor of mucous membranes. In more advanced cases clotting disorders occur. Without treatment, it can lead to death (Fig. 5C).

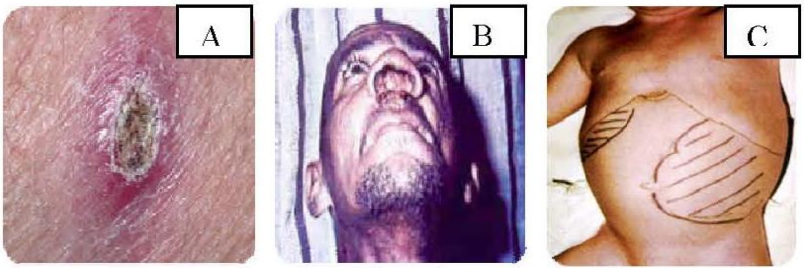

Figure 5. Cutaneous Leishmaniasis (A), mucocutaneous leishmaniasis (B), visceral leishmaniasis [6] (C).

1.4. Cutaneous Leishmaniasis: Its problematic healthcare

Cutaneous leishmaniasis is not deadly, and some forms may heal spontaneously with time, but others may leave significant scarring, resulting in disfigurements to the face that are a barrier to finding work or marriage, especially for women. This in turn leads to social and economic consequences and increased poverty [7].

Another important point to remember from health and epidemiological points of view is that patients with lesions act as reservoirs of infection, and thus without treatment the potential for transmission increases. Unfortunately the high cost of treatment means that in many cases it does not reach poor people [7].

According to the WHO (2010), the main problem with cutaneous leishmaniasis relates to access to treatment, because the cost of hospitalization is added to the drug and may not be affordable in developing countries. For this reason and because leishmaniasis is one of a group of neglected diseases, the Drugs For Neglected Disease Initiative (DNDi) has highlighted the urgent need for affordable and effective drugs with short treatment schedules and uncomplicated management (such as topical administration) that do not require complex health infrastructures.

The first-line treatment of cutaneous leishmaniasis is based on pentavalent antimonial drugs that can be administered in intravenous or intramuscular form or by intralesional injection, depending on the severity of the injury and the species responsible. These drugs are highly toxic, so it is necessary to hospitalize patients, which is very expensive in developing countries. Drugs with fewer toxic effects, such as liposomal amphotericin B (Ambisome®), can be administered in cases of complicated cutaneous and mucocutaneous leishmaniasis, but these are costly and are not widely available in countries with a high poverty index.

Herein we review existing conventional treatments currently available for cutaneous leishmaniasis and describe the different drugs and therapies currently used, as well as the different treatment regimens specific to the Old World and the New World. Also, we report on new treatments for cutaneous leishmaniasis that are currently being tested and studied: studies of new drugs, formulations, and drug combinations.

2. Conventional treatments for cutaneous Leishmaniasis

There are currently several therapies for the treatment of leishmaniasis. The most commonly used compounds are pentavalent antimonials, and these are considered the drugs of first choice in the treatment and control of

leishmaniasis. Alternatives include the liposomal amphotericin B, the pentamidine paramomycin, drugs derived from imidazole, and an orally administered drug, miltefosine [8].

Treatment of cutaneous leishmaniasis involves a combination of topical, systemic and non-pharmacological approaches. The therapeutic decision depends on the infecting species and geographic region.

2.1. Pentavalent antimonials

Two pentavalent antimonials are available: sodium stibogluconate (SSG, Pentostam®) (Fig. 6A) and meglumine antimoniate (AMG, Glucantime®) (Fig. 6B). These have been used since the 1940s and are the first-line therapeutic agents for cutaneous leishmaniasis.

They cannot be administered orally. The recommended dose is 20 mg/kg/day sodium stibogluconate intramuscularly or intravenously for 20 days or 60 mg/kg/day of meglumine antimoniate. The principal problems encountered are their cost, toxicity and emergence of resistance. Intralesional administration at a dose of 0.5 to 5 mL injected at the base of the lesion is also an option [9].

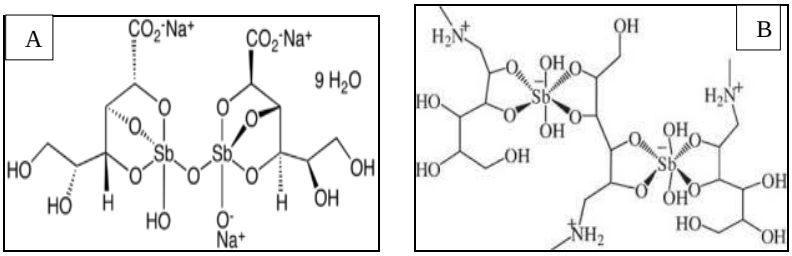

Figure 6. A. Sodium stibogluconate (Pentostam®). **B.** Meglumine antimoniate (Glucantime®).

2.2. Amphotericin B

This drug has low antifungal spectrum activity and is derived from *Streptomyces nodosus*. It shows high leishmanicidal activity by joining the parasite lipids. It is a second-line drug (Fig. 7).

A test dose of 1 mg, given by infusion, is recommended, followed by a full dose 4–6 h later. Infusion reactions are common. Nephrotoxicity is also common. Treatment should always be given in hospital to allow continuous monitoring of patients [9].

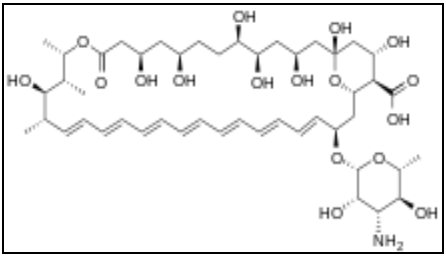

Figure 7. Amphotericin B.

2.3. Pentamidine

This drug is an antiprotozoal compound that is considered a second-line drug with significant toxic effects (damage to the pancreas, kidney and bone marrow). The drug is given intramuscularly or, preferably, by intravenous infusion (Fig. 8).

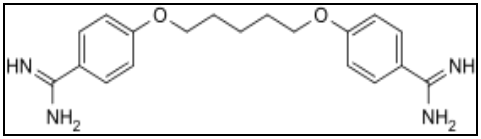

Figure 8. Pentamidine.

2.4. Paromomycin

Paramomycin (aminosidine) is an aminoglycoside antibiotic, first isolated from *Streptomyces krestomuceticus* in the 1950s. It is usually administered intramuscularly. Its mechanism of action is inhibition of mitochondrial activity of *Leishmania*. It acts synergistically with antimonials and is often used in combination or as the therapy of second choice (Fig. 9).

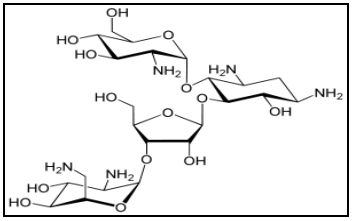

Figure 9. Paromomycin.

2.5. Medicines derived from imidazole

– **Ketoconazole:** This is an antifungal drug that inhibits the synthesis of ergosterol in the membrane of the parasite. It has been proven effective in the treatment of cutaneous leishmaniasis (Berman, 1997) (Fig. 10).

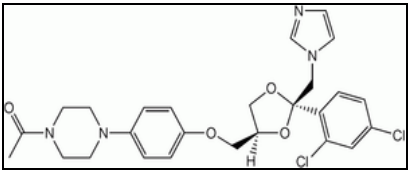

Figure 10. Ketoconazole.

– **Itraconazole:** This is another antifungal drug with a similar effect to ketoconazole (Davidson, 1998), but improved pharmacokinetics (Fig. 11).

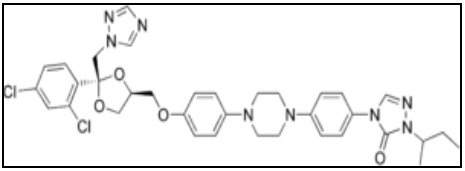

Figure 11. Itraconazole.

2.6. Miltefosine

This is an alkyl phospholipid (hexadecylphosphocholine) that was originally developed as an oral anticancer drug but was shown to have antileishmanial activity. It was the first oral agent effective against leishmaniasis and should only be administered once a day. Unlike other antileishmanial drugs, its mechanism of action involves disrupting cellular signaling pathways and the synthesis of cell membranes of the parasite through different mechanisms. Unfortunately the development of resistance is common [10, 11] (Fig. 12).

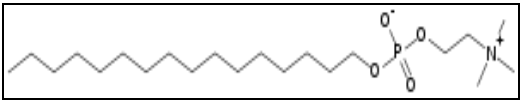

Figure 12. Miltefosine.

3. Treatment of cutaneous Leishmaniasis in the Old and the New World

The choice of treatment is based mainly on the risk-benefit ratio for each patient, with different therapeutic interventions in the Old and the New World [8].

3.1. Cutaneous Leishmaniasis in the Old World

Old World cutaneous leishmaniasis is caused by five species of *Leishmania*: *L. infantum*, *L. tropica*, *L. major*, *L. aethiopica* and *L. donovani*. These are mainly species of the Mediterranean coast, the Middle East and Africa.

Cutaneous lesions caused by *L. infantum* are seen especially in the Mediterranean basin. *L. infantum* is the most frequent cause of cutaneous leishmaniasis in southern Europe. The lesions are usually single nodules, with little inflammation but triggering an ulcer. The lesions heal spontaneously within about 1 year and seem to confer immunity [8].

L. tropica, which causes anthroponotic or urban anthroponotic cutaneous leishmaniasis, is distributed mainly in Morocco, Saudi Arabia and West Asia. This leishmaniasis is characterized by the presence of multiple painless and dry ulcers of the skin. These generally heal spontaneously within a year, often leading to disfiguring scars [1].

Cutaneous leishmaniasis caused by *L. major* (previously known as zoonotic or rural zoonotic cutaneous leishmaniasis) is a rural zoonosis typical of North Africa, West Asia and Asia Minor. Like other forms of cutaneous leishmaniasis it is characterized by painless lesions and often severe inflammation and ulceration. These lesions heal after 2–8 months. Multiple lesions may occur, especially in nonimmune immigrants; these are slow to heal and can leave large disfiguring or disabling scars [1].

Finally, cutaneous leishmaniasis, caused by *L. aethiopica* (Ethiopia, Kenya and Sudan), results mainly in localized cutaneous nodular lesions. A less frequent complication is mucocutaneous lesions of the oropharynx, causing a distortion of the nostrils of the nose and lips. Most lesions develop slowly and can spread locally. Ulceration is late or absent. Spontaneous healing usually occurs within 2–5 years.

Established criteria for topical treatment

According to WHO guidelines, local treatment of the lesion is indicated in patients who meet the following requirements [8]:

- Confirmed or strongly suspected infection with *L. major*.
- Fewer than four lesions requiring immediate treatment.
- Lesions < 5 cm in diameter.
- No potentially disfiguring or disabling lesions (face, joints, toes, fingers).
- No immunosuppression.
- Possibility of follow-up.

The options for topical treatment are (Table 2) [8]:

Intralesional therapy with pentavalent antimonials: injected at a dose of 0.5 to 5 mL at the base and margins of the lesion between 3 and 7 days. Infiltration can be performed daily, on alternate days or weekly until healing of the lesion. These injections are effective but repetitive administration may lead to resistance.

Table 2. Recommendations for treatment and dosage of Old World cutaneous leishmaniasis (WHO 2010).

Recommended tretment regimens for Old World cutaneous leishmaniasis (not ranked for preference)

Local therapy

L. major

- 15% paromomycin/12% methylbenzethonium chloride ointment twice daily for 20 days.
- Intralesional antimonials, 1-5 mL per session plus cryotherapy (liquid nitrogen: –195 °C), both every 3-7 days (1-5 sessions).
- Thermotherapy, 1-2 sessions with localized heat (50 °C for 30 s).

L. tropica, L. aethiopica[*] **and** *L. infantum*[*]

- 15% paromomycin/12% methylbenzethonium chloride ointment, as above.
- Intralesional antimonials plus cryptherapy, as above.
- Thermotherapy, as above.
- Intralesional antimonials, alone, as above.
- Cryotherapy, alone, as above.

Systemic therapy

L. major

- Fluconazole, 200 mg oral daily for 6 weeks.
- Pentavalent antimonials, 20 mg Sb^v/kg per day intramuscularly or intravenously for 10-20 days.
- Pentavalent antimonials, 20 mg Sb^v/kg per day intramuscularly or intravenously plus pentoxyfyline, 400 mg three times a day for 10-20 days.

L. tropica **and** *L. infantum*[*]

- Pentavalent antimonials, 20 mg Sb^v/Kg per day intramuscularly or intravenously for 10-20 days.
- Pentavalent antimonials, 15-20 mg Sb^v/Kg per day intramuscularly or intravenously for 15 days plus oral allopurinol 20 mg/Kg for 30 days, to treat leishmaniasiss recidivans caused by *L. tropica*.

L. aethiopica

- Pentavalent antimonials 20 mg Sb^v/Kg per day intramuscularly or intravenously plus paromomycin, 15 mg (11 mg base)/kg per day intramuscularly for 60 days or longer to treat difuse cutaneous leishmaniasis.

[*] Few data are available on therapy for cutaneous leishmaniasis due to *L. infantum* and *L. aethiopica*.

Paromomycin ointments: either a formulation of 15% paromomycin plus 12% methyl benzethonium chloride (CLMB) or a formulation of 0.5% gentamicin and 15% paramomycin in paraffin, twice daily for up to 20 days.

Thermotherapy: it consists of one or two applications of localized heat at 50 °C for 30 seconds. One of the drawbacks of this therapy is the high cost of the device and also the second-degree burns that occur. Local anaesthesia is therefore necessary.

Cryotherapy: cryotherapy with liquid nitrogen (−195 °C) applied once or twice a week for up to 6 weeks. The application of liquid nitrogen requires specific devices and expensive specialist medical equipment. Although it is highly effective it is less effective than intralesional therapy with pentavalent antimony.

Local therapy is an attractive option with little toxicity, but thermotherapy and especially intralesional treatment cause significant discomfort.

3.2. Cutaneous Leishmaniasis in the New World

Cutaneous leishmaniasis of the New World refers to infection caused by species found in Mexico, Central America and South America. These species belong to both the *Leishmania* and the *Vianna* subgenera, as follows: *L. (L.) mexicana, L. (L.)* amazonensis *L. (V.) brazilensis, L. (V.) panamensis, L. (V.) guyanensis* and *L. (V.) peruviana* [1].

The clinical manifestations are similar to cutaneous leishmaniasis in the Old World but are usually more aggressive and tend to become chronic. Lesions caused by *L. mexicana* generally have a benign evolution, whereas *L. amazonensis* causes a single or multiple lesions that are resistant to treatment and have a high tendency to evolve to diffuse lesions.

On the other hand, species of the subgenus *Vianna* tend to form mucocutaneous lesions. These are normally single or multiple lesions that heal routinely, in the case of *L. brazilensis*, or have a low cure rate (*L. panamensis, L. guyanensis*). If the lesion is located near a lymphatic chain or if treatment is inadequate the mucocutaneous form can develop.

Finally, *L. peruviana* is the most benign species because healing is spontaneous and there is no tendency to cause the mucocutaneous form.

Local therapy is considered inadequate for the treatment of cutaneous leishmaniasis in the New World when caused by *L. brazilensis* or *L. panamensis*, due to the potential risk of spread to mucous membranes. Topical treatment is currently considered acceptable in specific cases of cutaneous leishmaniasis in the New World.

Experience with local therapy for cutaneous leishmaniasis in the New World is limited. The criteria for the use of local treatment are similar to those of the Old World. The proposed options are thermotherapy and paramomycin. One to three applications of localized heat (50 °C for 30 seconds) proved to be 70% effective in studies in Colombia and Guatemala, 3 months after treatment. On the other hand, 15% and 12% paramomycin ointment applied twice daily for 20 days was 70–90% effective against cutaneous leishmaniasis caused by *L. mexicana*, *L. panamensis* and *L. brazilensis* in Ecuador and Guatemala.

The options for systemic treatment are (Table 3) [8]:

Pentavalent antimonials: The cure rate is very high at around 77% and 90% when pentavalent antimonials are administered at a dose of 20 mg/kg/day for 20 days intravenously or intramuscularly respectively. They are most effective against cutaneous leishmaniasis caused by *L. braziliensis* and *L. panamensis*.

Pentamidine: Administered at a dose of 3–4 mg/kg on alternate days for a total of three or four doses intramuscularly, and is as effective as pentavalent antimonials against cutaneous leishmaniasis caused by *L. panamensis* or *L. guayanensis*. However, it is less effective than antimonials in the case of *L. brazilensis*.

Miltefosine: Oral miltefosine at a dose of 2 mg/kg/day for 28 days is effective against cutaneous leishmaniasis caused by *L. panamensis* but not against *L. brazilensis* or *L. mexicana*, for which the cure rate is less than 60%. The response of *L. brazilensis* cutaneous leishmaniasis to miltefosine may vary depending on the area.

Ketoconazole: At a dosage of 600 mg daily for 28 days this is highly effective (76–90%) against *L. panamensis* and *L. mexicana* in Guatemala and Panama.

Table 3. Recommendations for treatment and dosage of New World cutaneous leishmaniasis (WHO, 2010).

Recommended tretment regimens for New World cutaneous leishmaniasis (not ranked for preference)

Local therapy, all species

- 15% paromomycin and 12% methylbenzethonium chloride ointment twice daily for 20 days.
- Thermotherapy: 1-3 sessions with localized heat (50 °C for 30 s).
- Intralesional antimonials: 1-5 mL per session every 3-7 days (1-5 infiltrations).

Systemic

L. mexicana

- Ketoconazole: adult dose, 600 mg oral daily for 28 days.
- Miltefosine: 2.5 mg/Kg per day orally for 28 days

L. guyanensis* and *L. panamensis

- Pentamidine isothionate, intramuscular injections or brief infusions of 4 mg salt/kg per dose every other day for 3 doses*
- Pentavalent antimonials: 20 mg Sb^v/Kg per day intramuscularly or intravenously for 20 days*
- Miltefosine: 2,5 mg/kg per day orally for 28 days.

L. braziliensis

- Pentavalent antimonials: 20 mg Sb^v/kg per day intramuscularly or intravenously for 20 days.
- Amphotericin B deoxycholate: 0.7 mg/kg per day, by infusion, for 25-30 doses.
- Liposomal amphotericin B: 2-3 mg/kg per day, by infusion, up to 20-40 mg/kg total dose.

L. amazonensis, L. peruviana* and *L. venezuelensis

- Pentavalent antimonials: 20 mg Sb^v/kg per day intramuscularly or intravenously for 20 days.

Relapse treatment:

- Amphotericin B deoxycholate, as above.
- Pentavalent antimonials: as above plus topical imiquimod every other day for 20 days.
- Liposomal amphotericin B: 3 mg/kg per day, by infusion, up to 20-40 mg/kg total dose may be considered.

* The efficacy of pentamidine, and pentavalent antimonials depends on the geographical area.

4. New treatments for cutaneous Leishmaniasis

4.1. New drugs

Many of the drugs mentioned in the previous section have led to resistance in many species of *Leishmania* and consequently their effectiveness has diminished in areas in which the parasite was previously susceptible to such treatment. For this reason, many research groups have focused on investigating new therapeutic alternatives against leishmaniasis. The following table provides a summary of various natural products that have been reported to have leishmanicidal properties [12] (Table 4):

Table 4. Sources of essential oils with leishmanicidal properties [12].

Family	Species	*Leishmania* species
Lauraceae	*Ocotea duckei*	*L. infantum* and *amazonensis*
Rutaceae	*Dictyoloma peruviana*	*L. amazonensis* and *brazilensis*
Verbenaceae	*Lyppia alba*	*L. infantum*
Zingiberaceae	*Curcuma longa*	*Leishmania spp.*
Myristicaceae	*Myristica malabàrica*	*L. donovani*
Pinaceae	*Cedrus atlantica*	*Leishmania spp.*

Moreover, the importance of metal compounds in antiparasitic development is well known. For example, in vitro and in vivo studies of *L. major* and *L. tropica* showed greater susceptibility to zinc than pentavalent antimony [13].

Finally, a recent study examined the antileishmania effectiveness of a drug called buparvacuona, a hydroxynaphthoquinone used for the treatment of theileriosis in cattle. Specifically, the efficacy and toxicity of different topical formulations of buparvacuona against *L. major* were tested in vivo in BALB/c mice. The results of this study were encouraging, as parasite load and lesion size were reduced, and therefore the introduction of a topical formulation of this type would be a significant advance for the treatment of simple cutaneous lesions [14].

The search for new drugs with antileishmanial activity is essential for new treatment options for cutaneous leishmaniasis. Avoiding the use of parenteral antimony would greatly increase patient compliance and reduce treatment costs.

4.2. New formulations

As mentioned above, the standard topical treatment used at present is intralesional therapy with pentavalent antimony. However, this requires multiple injections, which are painful and not tolerated by most patients. Furthermore, they are not always effective, have many adverse effects and can lead to the development of resistance. Alternative topical therapies mainly contain paramomycin e.g. a formulation called WR 279,396, a hydrophilic formulation of paramomycin 15% and gentamicin 0.5% that is currently in a Phase III trial in Tunisia [15].

Other researchers studied the permeability of the skin and the leishmanicidal activity of a hydrophilic gel containing 10% paramomycin in

vivo in experimental mice infected with *L. major* and compared its activity with parenteral pentavalent antimony. The gel showed higher efficiency than the pentavalent antimony administered intravenously. The same formulation was tested on hamsters infected with *L. brazilensis* and showed similar efficacy to that of parenteral antimony [16].

Other authors studied a new topical formulation containing amphotericin B and evaluated its leishmanicidal effectiveness in CBA mice as a model for human cutaneous leishmaniasis. For the first time, they showed that amphotericin B administered topically as a complex with phospholipids or with cholesterol sulphate in the presence of ethanol can penetrate the skin and act in a localized manner even at very low concentrations, which therefore makes it less toxic [17].

Liposomes have been widely used as drug delivery systems to treat infections caused by bacteria, fungi, viruses and parasites. These are nanometric spherical vesicles containing one or more phospholipid bilayers [18]. Their molecular architecture can capture a wide variety of active agents, thus providing a means of transport to bring drugs to their sites of action at appropriate concentrations, in turn ensuring a safe and effective pharmacological response. Furthermore, they are biodegradable, non-toxic and non-immunogenic. A further advantage is the ability of liposomes to isolate active substances during their passage through the organism, protecting them from enzymatic actions and also protecting the organism against toxic effects.

Liposomic formulations have demonstrated the ability to increase the penetration of drugs through the skin in comparison to conventional formulations. In addition, in open injuries, liposomes show the ability to accelerate healing [19].

Various studies have shown promising results when investigating liposomal formulations containing sulphate of paramomycin (PM). In one study liposomal formulations of PM were assessed as topical delivery systems. Two types of liposomes were used: large multilamellar vesicles (MLVs) and large unilamellar vesicles (LUVs). Skin permeation experiments across stripped and normally hairless mouse skin were performed in modified Franz diffusion cells, revealing higher entrapment of paramomycin in LUV compared to MLV and better penetration into the skin with paromomycin liposomal encapsulation. The study concluded that liposomal formulations of paramomycin are an interesting alternative for the treatment of cutaneous leishmaniasis as they increase the skin penetration of paramomycin [20].

In another study, liposomal formulations containing 10% and 15% paramomycin sulphate were developed to evaluate the antileishmanial activity of paramomycin against *L. major*-infected BALB/c mice. The study

also evaluated the penetration properties of PM into the skin, as this antibiotic shows a low level of penetration. Such low penetration reduces the effectiveness of paramomycin treatment of some injuries, particularly those that are not ulcerated, and is therefore a major drawback. The overall results of the study showed that liposomes are interesting paramomycin carriers and that liposomal PM could be a suitable candidate for the treatment of cutaneous leishmaniasis [21].

4.3. Combined treatment

Combination therapy provides several advantages: it reduces the duration of treatment, increases compliance, and reduces the dose of each drug, which helps to reduce the cost and side effects of therapy, as well as reducing the risk of emergence of resistance. Various authors have investigated different combination therapies with favorable results. One study verified the effectiveness of a combination of paramomycin gel and oral miltefosine in BALB/c mice infected with *L. major* for the treatment of cutaneous leishmaniasis. Miltefosine was administered orally at 2 mg/kg/day for 10 days while the 10% paramomycin gel was applied twice daily for 10 days. This combination was highly effective, significantly reducing the size of the lesion and the load of parasites in the skin, and leading to complete healing of ulcers, in comparison with oral miltefosine alone [22].

Other drugs such as azithromycin and allopurinol have been studied in the search for new therapies for cutaneous leishmaniasis. One study compared the efficacy and tolerance of azithromycin plus allopurinol with intramuscular Glucantime®, and found similar results. One of the benefits of allopurinol and azithromycin is that they are less expensive than Glucantime® and more readily available in developing countries. Furthermore, they are administered orally and therefore can be considered an alternative option to Glucantime® treatment in patients who do not tolerate the latter [23].

Another study evaluated the efficacy of combination with pentoxifylline and Glucantime®. Pentoxifylline is an inhibitor of TNF-α that has been shown to enhance the beneficial effects of antimonial compounds. The results of this study indicated that Glucantime® with pentoxifylline is more effective than Glucantime® alone. However, more tests are needed with different populations of patients with CL to demonstrate the real efficacy of the combination of pentoxifylline and Glucantime® and develop specific treatment guidelines [24].

The results of a study that evaluated the effectiveness of a combination of oral ketoconazole with intralesional estibogluconat sodium for the treatment of localized cutaneous leishmaniasis were promising. Furthermore, this

combination is easier to use, less aggressive and free of side effects compared to treatment with intralesional pentavalent antimonial compounds [25].

A compound called Imiquimod, an inhibitor of tumor necrosis factor TNF-alpha, is useful in patients with moderate to severe psoriasis and other skin diseases and is currently used for the treatment of cervical warts caused by human papillomavirus infection. In vitro, this drug has proven to be an immunomodulator that activates macrophages to release nitric oxide, thus killing intracellular *Leishmania* amastigotes. A study evaluating combination therapy with Imiquimod and meglumine antimoniate in patients with cutaneous leishmaniasis who previously had not responded to treatment with meglumine antimoniate alone showed promising results, as all patients responded well to combination therapy [26].

5. Conclusions

Intralesional and systemic therapy with pentavalent antimonials has been the treatment of choice for cutaneous leishmaniasis to date.

The application of paramomycin ointments to localized lesions and those less than 5 cm in diameter was the first attempt at local therapy and indicated the potential benefit of this type of administration.

The search for new drugs with anti-*Leishmania* activity is important for new treatments for CL. Avoiding the administration of derivatives of parenteral antimony greatly increases patient compliance and reduces treatment costs.

Studies of formulations based on liposomes and hydrophilic gels containing paromomycin indicate the effectiveness of these formulations in the topical treatment of cutaneous leishmaniasis.

Combination treatment has the potential advantages of: shortening the duration of treatment, thereby increasing compliance; reducing the overall dose of medicines, thereby reducing their toxic effects and cost; and reducing the probability of selection of drug-resistant parasites, thereby prolonging the effective life of the available medicines.

Due to the importance of this parasitic disease and the low efficiency of the limited therapies available, the search for new therapeutic alternatives against leishmaniasis is very important for the future treatment of cutaneous leishmaniasis.

Acknowledgements

I would like to thank Marta Mas for her assistance in bibliographic searches, and Dra. Ana Cristina Calpena and Mireia Mallandrich (*Unitat de Tecnologia Farmacèutica, Departament de Farmàcia i Tecnologia*

Farmacèutica, Universitat de Barcelona), for their encouragement and help regarding new formulations.

References

1. Alvar, J., Las leishmaniasis, de la biologia al control. 1997. Ed. Junta de Castilla y León, Madrid
2. Cunningham, A.C., 2002, *Exp. Mol. Pathol.*, 72, 132.
3. Reed, S.G., Scott, P., 1993, *Curr. Opin. Immunol.*, 5, 524.
4. Mansueto, P., Vitale, G., Di Lorenzo, G., Rini, G.B., Mansueto, S., Cillari, E., 2007, *Int. J. Immunopathol. Pharmacol.*, 20, 435.
5. Pujol, A., Fisa, R., Riera, C., Rocamora, V., Boteller, D., 2012, *Piel Formacion continuada en Dermatologia*, 27, 527.
6. Murray, H.W., Berman, J.D., Davies, C.R., Saravia, N.G., 2005, *Lancet*, 366, 1561.
7. Modabber, F., Buffet, P.A., Torreele, E., Milon, G., Croft, S.L., 2007, *Kinetoplastid Biol. Dis.*, 6:3.
8. WHO. CONTROL OF THE LEISHMANIASES. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. WHO Technical Report Series
9. Richard, J.V., Werbovetz, K.A, 2010, *Curr. Opin. Chem. Biol.*, 14, 447.
10. Choudhury, K., Zander, D., Kube, M., Reinhardt, R., Clos, J., 2008, *Int. J. Parasitol.*, 38, 1411.
11. Seifert, K., Matu, S., Pérez-Victoria, F.J., Castanys, S., Gamarro, F., Croft, S.L., 2003, *Int. J. Antimicrob. Agents*, 22, 380.
12. Arévalo, Y., Robledo, S., Muñoz, D.L., Granados-Falla, D., Cuca, L.E., Delgado, G., 2009, *Rev. Colomb. Cienc. Quím. Farm.*, 38, 131.
13. Minodiera, P., Parolab, P., 2007, *Travel Medicine and Infectious Disease*, 5, 150.
14. Garnier, T., Mäntylä, A., Järvinen, T., Lawrence, J., Brown, M., Crofts, S., 2007, *J. Antimicrob. Chemother.*, 60, 802.
15. Ben Salah, A., Ben Messaoud, N., Guedri, E., Zaatour, A., Ben Alaya, N., Bettaieb, J., Gharbi, A., Belhadj Hamida, N., Boukthir, A., Chlif, S., Abdelhamid, K., El Ahmadi, Z., Louzir, H., Mokni, M., Morizot, G., Buffet, P., Smith, P.L., Kopydlowski, K.M., Kreishman-Deitrick, M., Smith, K.S., Nielsen, C.J., Ullman, D.R., Norwood, J.A., Thorne, G.D., McCarthy, W.F., Adams, R.C., Rice, R.M., Tang, D., Berman, J., Ransom, J., Magill, A.J., Grogl, M., 2013, *N. Engl. J. Med.*, 368, 524.
16. Gonçalves, G.S., Fernandes, A.P., Souza, R.C. Cardoso, J.E., de Oliveira-Silva, F., Maciel, F.C., Rabello, A., Ferreira, L.A., 2005, *Acta Tropica*, 93, 161.
17. Frankenburg, S., Glick, D., Klaus, S., Barenholz, Y., 1998, *Antimicrob. Agents Chemother.*, 42, 3092.
18. Ball, E., 1995, *Derm. Venez.*, 33, 15.
19. Carneiro, G., Santos, D.C.M., Oliveira, M.C., Fernandes, A.P., Ferreira, L.S., Ramaldes. G.A., Nunan, E.A., Ferreira, L.A.M., 2010, *J. Liposome Res.*, 20, 16.

20. Ferreira, L.S., Ramaldes, G.A., Nunan, E.A., Ferreira, L.A., 2004, *Drug Dev. Ind. Pharm.*, 30, 289.
21. Jaafari, M.R., Bavarsad, N., Bazzaz, B.S.F., Samiei, A., Soroush, D., Ghorbani, S., Lofti Heravi, M.M., Khamesipour, A., 2009, *Antimicrob. Agents Chemother.*, 53, 2259.
22. Aguiar, M.G., Silva, D.L., Nunan, F.A., Nunan, E.A., Fernandes, A.P., Miranda Ferreira, L.A., 2009, *J. Antimicrob. Chemother.*, 64, 1234.
23. Dastgheib, L., Naseri, M., Mirashe, Z., 2012, *Int. J. Dermatol.*, 51, 1508.
24. Sadeghian, G., Nilforoushzadeh, M.A., 2006, *Int. J. Dermatol.*, 45, 819.
25. El-Sayed, M., Anwar, A.E., 2010, *J. Eur. Acad. Dermatol. Venereol.*, 24, 335.
26. Arevalo, I., Ward, B., Miller, R., Meng, T.-C., Najar, E., Alvarez, E., Matlashewski, G., Llanos-Cuentas, A., 2001, *Clin. Infect. Dis.*, 33, 1847.