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REVIEW

Leishmaniasis treatment—a challenge that remains: a review

Dilvani O. Santos · Carlos E. R. Coutinho · Maria F. Madeira · Carolina G. Bottino · Rodrigo T. Vieira · Samara B. Nascimento · Alice Bernardino · Saulo C. Bourguignon · Suzana Corte-Real · Rosa T. Pinho · Carlos Rangel Rodrigues · Helena C. Castro

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Abstract Leishmaniasis is a disease caused by flagellate protozoan *Leishmania* spp. and represents an emergent illness with high morbidity and mortality in the tropics and subtropics. Since the discovery of the first drugs for Leishmaniasis treatment (i.e., pentavalent antimonials), until the current days, the search for substances with antileishmanial activity, without toxic effects, and able to overcome the emergence of drug resistant strains still remains as the current goal. This article reports the development of new chemotherapies through the rational design of new drugs, the use of products derived from microorganisms and plants, and treatments related to immunity as new alternatives for the chemotherapy of leishmaniasis.

D. O. Santos (✉) · C. E. R. Coutinho · C. G. Bottino · R. T. Vieira · S. B. Nascimento · S. C. Bourguignon · H. C. Castro
Laboratório de Biopatógenos e Ativação Celular (LaBiopAc), Departamento de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense (UFF), Av. Barros-Terra s/n Valonguinho, Niterói, Rio de Janeiro CEP 24001-970, Brazil
e-mail: santosdilvani@gmail.com

M. F. Madeira
Instituto de Pesquisa Clínica Evandro Chagas (IPEC), Fundação Oswaldo Cruz- FIOCRUZ, Av. Brasil, 4365, Manguinhos, Rio de Janeiro CEP 21045-900, Brazil

A. Bernardino
Instituto de Química, UFF, Av. Barros-Terra s/n Valonguinho, Niterói, Rio de Janeiro CEP 24001-970, Brazil

S. Corte-Real · R. T. Pinho
Laboratório de Imunologia Clínica, Instituto Oswaldo Cruz, FIOCRUZ-Av. Brasil 4365, Manguinhos, Rio de Janeiro CEP 21045-900, Brazil

C. R. Rodrigues
Laboratório de Modelagem Molecular e QSAR (ModMolQSAR), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, CEP 21941-590 Rio de Janeiro, Rio de Janeiro, Brazil

Introduction

Among all the emergent diseases, the ones caused by protozoans have great importance. Leishmaniasis is a disease caused by a parasite member of the *Leishmania* genus and presents high morbidity and mortality levels. This disease affects around 12 million people worldwide and is present in 88 countries, mainly in tropical and subtropical areas. The annual incidence of approximately two million new cases and around 350 million people that are living in endemic areas reveals the importance of this neglected disease (Grimaldi et al. 1983; Deane and Grimaldi 1985; Green et al. 1990; Barral et al. 1991; Mcgregor 1998; Weniger et al. 2001; Gontijo and Carvalho 2003; WHO 1990, 1991, 2001) (Fig. 1).

Leishmania is a protozoan and a compulsory intracellular parasite in the mammalian host (Bogdan et al. 1990; Green et al. 1990; Chang 1990; Alexander and Russel 1992; Corte-Real et al. 1995; Hespanhol et al. 2005; Bogdan et al. 1996; Ritting and Bogdan 2000; Sereno et al. 2005). This parasite is involved in pathologies that range from the cutaneous to the visceral forms, depending on the species of *Leishmania* and the host immune response (Barral et al. 1991; Grimaldi et al. 1991; Liew and O'Donnel 1993).

The tegumentary form or American tegumentary leishmaniasis (ATL; Fig. 1) has increased in the last 20 years in almost all Brazilian states, and epidemic outbreaks have

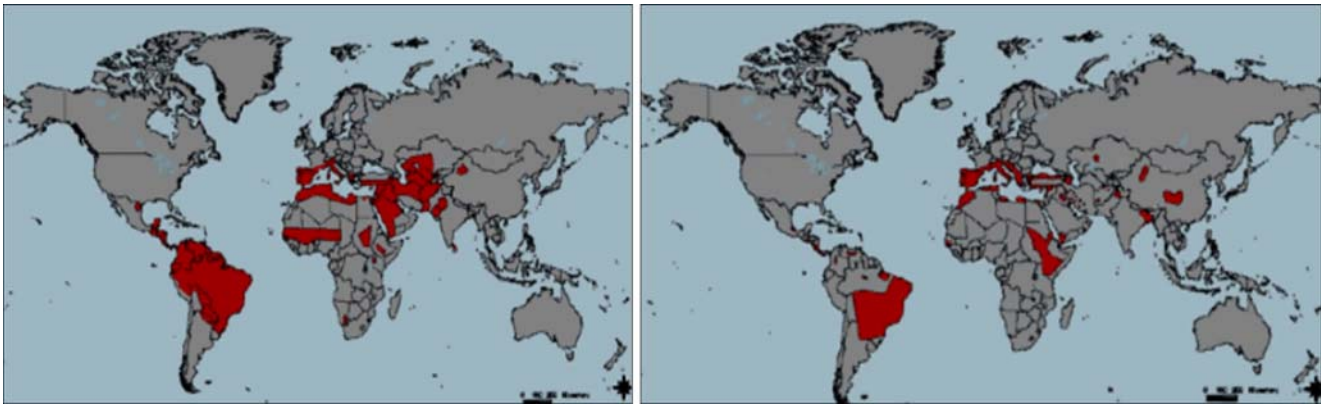


Fig. 1 Distribution in Old World and New World of cutaneous (*left*) and visceral (*right*) leishmaniasis. The affected areas are marked in *red* according to the World Health Organization

occurred at Southeast, Middle West, Northeast, and more recently, Amazon regions, due to the predatory process of colonization (Delorenzi et al. 2001; Gontijo and Melo 2004). Historically, ATL has been a rural disease affecting farmers, military group, and other people from rural areas. However, this epidemic profile has changed, and the transmission of this disease has been related to the interface of rural and urban areas among people of all sex and ages (Weniger et al. 2001; Gontijo and Carvalho 2003; Pal et al. 2004; Oliveira et al. 2004).

Leishmaniasis may be caused by different *Leishmania* species such as *Leishmania (viannia) braziliensis*, *Leishmania (viannia) guyanensis*, *Leishmania (viannia) naiffi*, *Leishmania (viannia) shawi*, *Leishmania (viannia) lainsoni*, *Leishmania (leishimania) amazonensis*, *Leishmania (l.) mexicana*, *Leishmania (v.) panamensis*, and *Leishmania pifanoi.* The species involved depends on the geographical distribution, and the disease may appear as simple or diffuse ulcerations on skin, mainly in the face, causing mutilation and disfiguration of the patient. The disease may regress spontaneously or evolve, thus requiring treatment (Grimaldi et al. 1991).

The visceral leishmaniasis (VL) or Calazar is more severe than cutaneous leishmaniasis (CL) and may be caused by *Leishmania (leishmania) donovani* and *Leishmania infantum* (similar to *Leishmania chagasi* in Brazil). These agents affect around 500 thousand people per year; mainly in India, Sudan, and Latin American countries (Weniger et al. 2001; Gontijo and Melo 2004). The agent affects mainly liver and spleen, determining hepatomegaly, splenomegaly, and consequent lost of their function, among other severe alterations, which may be fatal if an efficient treatment is not established (Carvalho et al. 2000; Croft et al. 2006). The situation becomes critical in immune-depressed patients where *Leishmania* appears as an important opportunist agent (Coura et al. 1987; Soong et al. 1995; Carvalho et al. 2000; Silva et al. 2002; Desjeux and Alvar 2003). In that case, it leads to uncommon clinical forms and resistance against the current treatments (i.e., tegumentary form; Dey et al. 2000). The immunesuppression may be due to viral infections (i.e., HIV), which increased the number of VL reported cases in countries where the disease was rare such as France, Italy, Spain, and Portugal (Desjeux and Alvar 2003; Molina et al. 2003). Epidemiological evidences indicate that within this group the transmission is high among drug addicts due to contaminated syringes. Another possibly affected group involves the increasing number of organ transplants throughout the world whose recipients need the continuous use of immunosuppressive drugs. The risks to such individuals are multiple. Clinical leishmaniasis may result from the activation of occult infections in patients undergoing organ transplants with contaminated organs or with high susceptibility to natural transmissions (Berman 1988; Murray et al. 2003; Berengener et al. 1998). In addition to exacerbating occult infections, immunesuppression may alter the clinical symptoms more commonly associated with some *Leishmania* species such as *L. (viannia) braziliensis*, which may cause visceral infections (Soong et al. 1995) or disseminated skin lesions (Coura et al. 1987) in HIV patients. Transmission levels may also increase when the vector feeds from immune-depressed individuals with high parasitemias (Morales et al. 2003).

The transmission of leishmaniasis occurs through hematophagus vectors from the *Phlebotomus* genus in the old world and *Lutzomyia* in the new world (Weniger et al. 2001) (Fig. 2). *Leishmania* multiplies in the vector digestive tract, and the parasites are transmitted to the mammalian host during vector blood feeding. Inside the vectors, the *Leishmania* parasites are in the promastigote forms, which are long, flagellate, and extracellular (Fig. 3). The amastigote forms are spherical, intracellular, without flagellum, and found in the vertebrate hosts (Chang 1990; Green et al. 1990; Ritting and Bogdan 2000). In that case,

Fig. 2 Life cycle of *Leishmania* sp. causing leishmaniasis. (World Health Organization)

the multiplication of the parasites occurs inside the macrophages, which are their main targets (Figs. 2 and 3). After macrophages lyses, *Leishmania* new phagocytosis episodes spread in the organism (Fig. 2). However, the establishment of the disease depends on the success of the parasite to differentiate to the amastigote form (Bogdan et al. 1990, 1996; Chang 1990; Corte-Real et al. 1995; Kayser et al. 2001; Sereno et al. 2005).

Classic treatments

Currently, the drugs used in leishmaniasis treatment present several problems, including high toxicity and many adverse effects, leading to patients withdrawing from treatment and emergence of resistant strains. In addition to these problems, the high cost of the compounds makes the treatment far from suitable and, regrettably, it has been increasing gradually throughout the years (Yardley et al. 2002; Singh and Sivakumar 2004). The primary treatment against leishmaniasis includes pentavalent antimonial, mostly in sodium stibogluconate and *N*-methylglucamine antimoniate forms, used since the 1940 decade (Berman 1988; Olliaro and Bryceson 1993; Raht et al. 2003) (Table 1). In some cases, other drugs, such as pentamidine, amphotericin B, and paromomycin are used as a second option in resistant cases, despite their great toxicity to the host (Ramos et al. 1990; Kuhlencord et al. 1992; Escobar et al. 2001; Bray et al. 2003; Rosa et al. 2003). Recently, pentamidine resistance was also described by the literature (Bray et al. 2003) as well as difficulties on treating immune-depressed patients (i.e., HIV), in whom conventional drugs are less efficient and higher drug doses and a long treatment period are commonly necessary (Escobar et al. 2001).

Several researches have been carried out to develop new protocols and chemotherapies for leishmaniasis treatment. Their purpose is to reduce the problems related

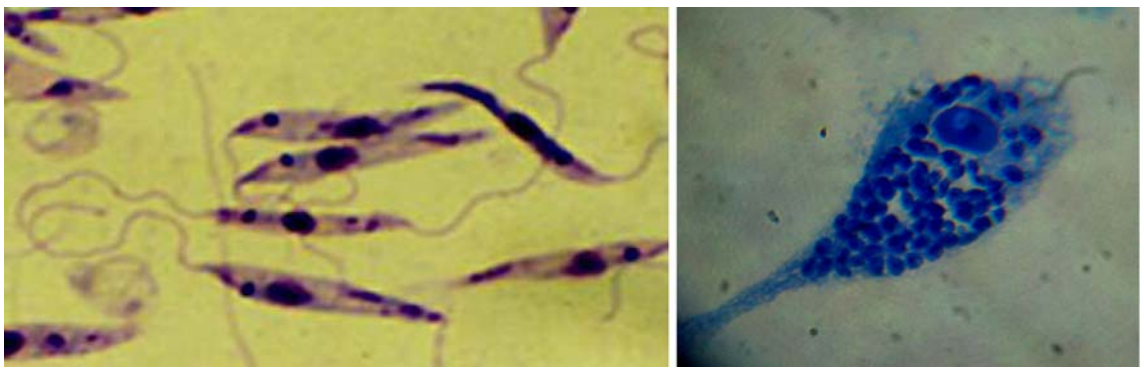
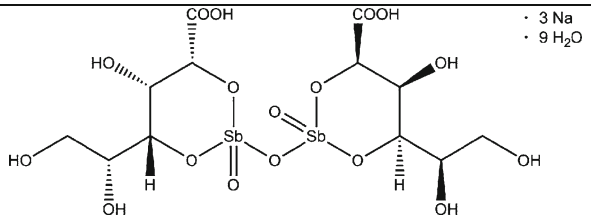
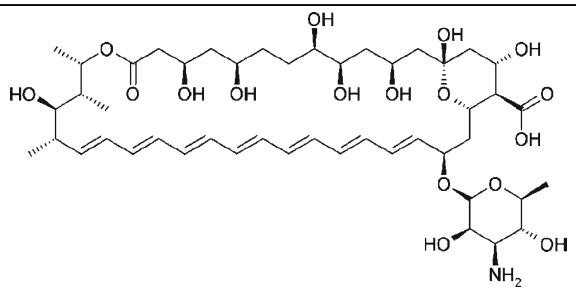
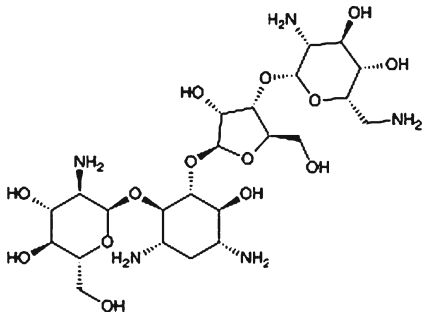
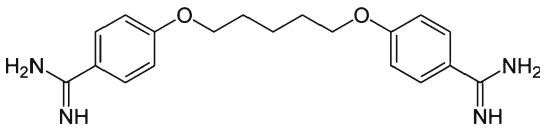
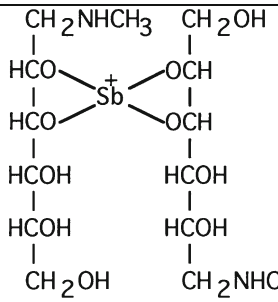


Fig. 3 *Leishmania amazonensis* promastigote (×1,000; *left*) and amastigote (*right*) forms. Amastigotes are infecting a macrophage (×1,000)

Table 1 Drugs most commonly used for leishmaniasis treatment

Name		2D - structure	Chemical formula	Molecular Mass (g/mol)
Commercial	Chemical			
Pentostam®	Sodium stibogluconate		$C_{12}H_{38}Na_3O_{26}Sb_2$	910.9
Fungizone	Amphotericin B		$C_{47}H_{73}NO_{17}$	924.084
Humatin	Paromomycin sulfate		$C_{23}H_{47}N_5O_{18}S$	615.629
Pentamidine	Pentamidine isethionate		$C_{19}H_{24}N_4O_2$	340.42
[a]Glucantime	Meglumine antimoniate		$C_{14}H_{29}O_{10}N_2Sb$	507.01

[a] Structure may considerably vary (Roberts et al. 1998)

to medicines already in use and increase their efficiency (Ma et al. 2004). Figure 4 illustrates different drugs for leishmaniasis treatment including: (a) antimonial that is not approved by Food and Drugs Administration (FDA-EUA), (b) Pentostam allowed by Center for Disease Control and Prevention (CDC-Atlanta) and used mainly in English language countries, and (c) Glucantime that is used in Brazil and other countries of Latin America.

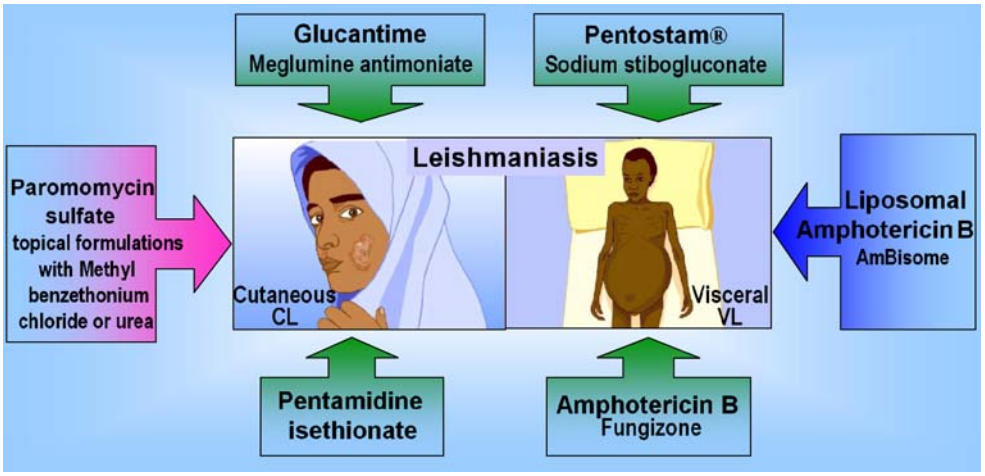


Fig. 4 Treatment used in cutaneous (*CL*) and visceral leishmaniasis (*VL*). *Green arrows* indicate drugs used in both CL and VL treatment, *blue* only for VL and *red* for CL

According to WHO, Glucantime therapeutic efficiency varies depending on the country, and treatment protocols are determined depending on the geographical area (Carvalho et al. 2000).

Different clinical responses to the VL, CL, and ATL treatment using pentavalent antimonials are an issue for leishmaniasis patient healing process. It includes the accumulation of drug in tissues such as spleen and liver, and even myalgia, pancreatitis, cardiac arrhythmia, and hepatitis, which may lead to reduction of or withdrawal from the treatment (Croft et al. 2006) and even to acquired resistance to these compounds (Escobar et al. 2001).

New alternatives: associating drugs to liposomes

The association of some drugs used in leishmaniasis treatment within liposomes is one of the alternatives used to reduce the undesirable effects. This procedure not only reduced the drugs' toxicity but also increased their efficiency and the concentration in the tissues (Desjeux and Alvar 2003; Gontijo and Carvalho 2003). The hypothetical mechanism of action of the liposomes involves the parasite membrane and the inhibition of oxygen consumption (Rosa et al. 2003). According to Dey et al. (2000), liposomes consisting of estearilamine (SA) and phosphatidylcholine (PC) affect other species of the protozoan. They show that SA–PC is effective against *L. donovani* promastigotes and amastigote forms when tested in both infected macrophage cultures and Balb/c mice. They also showed a therapeutical effect to recent and late leishmaniasis infections with a considerable reduction of parasites in the liver and spleen of infected mice and of hepatomegaly and splenomegaly with no toxic effects to host cells (Dey et al. 2000).

Amphotericin B is a compound used for the treatment of calazar patients that are clinically resistant to pentamidine. This molecule preferentially binds to ergosterol at the *Leishmania* plasmatic membrane but also recognizes the human cell cholesterol in a lower extent. Aiming to decrease the adverse effect resulting from this inappropriate recognition, there are some commercial formulations that associate amphotericin B to lipids (Ambisome, Abelcet, and Amphotec). Unfortunately, the production is very expensive, which makes their use difficult in poor countries (Golenser et al. 1999). There are studies based on the development of low-cost methods of producing these drugs as described by Larabi et al. (2003). They tested a formulation with a similar lipid composition to Abelcet, but with different conformation and molecular weight that may influence the drug release and action in the organism. They observed that the new formulation was 27% more efficient and less toxic than Abelcet in vivo and in vitro. Despite its less efficiency compared to Ambisome, this formulation is of low cost and less expensive for use in leishmaniasis treatment. These results show that the inclusion of drugs such as amphotericin B or even pentamidine in these vesicles may be an alternative treatment due to the synergistic action of these substances. These liposomes may also be used in combination with the antimonium, sodium gluconate, by increasing its efficiency as Pal et al. (2004) described it. By testing the combination of PC–SA + sodium antimony gluconate (SAG), in a single dose, against *L. donovani*-infected macrophages in vitro, they verified its better efficiency compared to SAG monotherapy. They also noticed that this combination reduced the parasites in mice liver (98%) and spleen (97%), compared to the monotherapy (76% and 65%, respectively) with no toxic effects. Three months after the treatment, all parasites were completely eliminated as observed by histopathological analyses.

According to Golenser et al. (1999), the conjugation with a polymeric loader is also another alternative to reduce toxicity and improve drug effect. This approach increases drug solubility in water, the time of circulation in the organism, and its accumulation in damaged tissues. These authors noticed that the association of amphotericin B with

arabinolactam, a polysaccharide soluble in water, was more efficient in the treatment of recent and late infections by *L. infantum* and *L. major* compared to the drugs with lipid conjugation. The advantages of this association described by these authors include its physical and chemical stability when lyophilized or soluble, the easy sterilization by filtration, the drug release profile in the circulation and, consequently, good elimination by the organism, in addition to the possibility of intravenous and subcutaneous administration.

New drugs from microorganisms and plants

Some researchers have been looking for new alternatives for leishmaniasis treatment in nature, an important source of drugs for the treatment of several diseases (Kayser et al. 2001; Silva et al. 2002). Searching for better effects and less toxicity in substances derived from microorganisms or plants (Kayser et al. 2001) based on the use by people from endemic areas, these authors studied the extracts of these organic materials for treating lesions (Weniger et al. 2001). Weniger et al. (2001) tested plant extracts of native species from the Pacific Colombian coast that are popularly used for leishmaniasis treatment by local people. Among the extracts tested, four of five species traditionally used against leishmaniasis were active (80%) in vitro at 100 μg/mL against promastigote forms of *Leishmania*, whereas three of the five extracts showed good activity against amastigotes of *L. (v.) panamensis.*

Kayser et al. (2001) tested the action of afidicoline against promastigotes and amastigotes of *L. donovani*. This metabolite isolated from the fungus *Nigrospora sphaerica* is described as an inhibitor of DNA polymerase thus affecting the cellular division. These authors observed that, from the 18 derivatives based on this metabolite, only three acted against resistant parasites compared to other drugs (i.e., amphotericin B and miltefosine) with moderate toxicity against the macrophages, although with the toxicity profile these compounds may be an alternative for treatment.

Other substances such as the pigments hypocrellins A and B, isolated from the fungus *Hipocrella bambusae* and used in the Chinese Traditional Medicine for treating other diseases, were tested by Ma et al. (2004) against *L. donovani*. In the in vitro tests, hypocrellin A was more active against the parasite than amphotericin B and pentamidine, whereas hypocrellin B showed moderate activity, pointing them as feasible treatment alternatives for leishmaniasis.

Plant derivatives are among the most active agents against different infections (Delorenzi et al. 2001). Nerolidol is present in essential oils of some plants and apparently inhibits the earlier steps of ergosterol and dolicol synthesis, which are important for parasite membrane constitution (Arruda et al. 2005). Arruda et al. (2005) tested nerolidol against *L. amazonensis*, *L. chagasi*, and *L. braziliensis* promastigotes and amastigotes forms and observed that it reduced ergosterol and dolicol synthesis of the parasites. The molecule was also efficient when intraperitoneally injected in infected mice. In contrast, topic applications did not lead to satisfactory effects and, after the end of the treatment, there were lesion recurrences, suggesting the need of a longer treatment. Other drugs present a similar nerolidol mechanism but against the last steps of ergosterol synthesis. These drugs are inefficient to some *Leishmania* species, as they use host sterols (Arruda et al. 2005).

Another study against leishmaniasis using plant-derived products is about *Tanacetum parthenium* from the Asteraceae family, which is commonly used in medicine for treating different diseases (Tiuman et al. 2005). Tiuman et al. (2005) tested the plant extract in vitro against *L. amazonensis* promastigotes and amastigotes forms. They observed that the pure extract and the fractions diluted in solvents reduced the parasite internalization in macrophages up to 84%. In addition, they showed no toxic effects to host cells compared to other drugs used in leishmaniasis treatment. Among the solvents used in this study, a dichloromethane fraction was more efficient than the whole plant extract.

Rosa et al. (2003) investigated the biological profile of the essential oil extracted from *Croton cajucara*, a plant used in folk Brazilian medicine for treating gastrointestinal disorders and inflammation processes, against *L. amazonensis* promastigotes and amastigotes forms. Interestingly, the *C. cajucara* oil is rich in linalol, a terpenic alcohol that is also present in other plants. The authors observed that even in small doses the extract and the isolated Linalol were able to kill 100% of the parasites without cell toxicity. The macrophages' pretreatment with the oil decreased the parasites' infection to these cells, while it increased the nitric oxide production, which has an important role in the defense against the parasites.

Peschiera australis is found in Brazil and other South American countries, and its extract was tested in vitro against *L. amazonensis* promastigotes and amastigote forms by Delorenzi et al. (2001). These authors observed the inhibition of promastigote growth (100%) by this plant extract. The dilution of the *P. australis* extract with solvents generated three different fractions, and the chloroform one was the most efficient against amastigotes in culture and in macrophages, either in single or multiple doses, reducing up to 99% of the infection. The anti-leishmanial profile of the extract is attributed to the indolic alkaloid, which was shown to be effective against amastigotes and mainly promastigotes, pointing this molecule and its derivatives as promising agents in leishmaniasis treatment.

Rational drug design

The Medicinal Chemistry is a recent applied science directed to the development of new drugs that evolved significantly due to recent technological advances, mainly in molecular, structural biology and computational chemistry areas (Liñares et al. 2006). Currently, the rational development of medicines is a reality that offers new perspectives for discovering new drugs and/or improving those that already exist (Lima and Barreiro 2005).

Some of the approaches used in this area consist of generating structural modifications in an initial molecule (called leading compound), which may be of natural or synthetic origin, for obtaining a new derivative series. Then, the compounds are tested in pharmacological assays performed in vitro and in vivo for detecting new compounds with the expected biological activity over the chosen therapeutical target. In this process, knowledge about the physic-chemical and structural properties of the leading compound and its relation to the pharmacological target (i.e., enzyme or receptor) as well as to preserve the initial pharmacophoric characteristics of this lead compound is essential (Liñares et al. 2006).

Once determined (the pharmacological target and the leading compound), different strategies of molecular modifications may be applied. Among them are (a) the molecular simplification that allows the synthesis of compounds structurally less complex from the original active prototypes (Liñares et al. 2006) and (b) bioisosterism, which is the substitution of parts, radical, or even atoms of a molecule to obtain derivatives that may present better pharmacotherapeutical activity (Lima and Barreiro 2005).

Several parasite biosynthetic pathways have been investigated as target for drug design. Recently, Liñares et al. (2006) used ergosterol biosynthesis as an example for showing the usefulness of drug design. There are many enzymes involved in this biosynthetic pathway such as squalene synthase, farnesylpyrophosphate synthase, and other enzymes that are able to deplete parasite endogenous sterols. The enzymes involved in trypanothione biosynthesis, glutathionyl spermidine synthetase, and trypanothione synthetase do not have an equivalent in mammals and, therefore, low toxicity for compounds that are able to produce highly selective inhibition can be predicted.

The design of specific inhibitors of such metabolic activities as possible means of controlling the parasites without damaging the hosts and the recent advances in the biochemistry of pathogenic parasites including the discovery of novel organelles may act together in improving leishmaniasis treatment. In fact, during the past 21 years, the leishmaniasis treatment success was based on reformulation of old drugs, researches of rational drug design, and the better understanding of the immunology of the disease. In the 1980 decade, pyrazolopiridines (i.e., alopurinol and derivatives), which affect nucleic acid biosynthesis, and C14-α demethylase and sterol synthesis inhibitors (i.e., antifungal azoles—ketoconazol, itraconazol) were one of the most promising molecules, but their low efficiency and their pharmacokinetic properties compromised their potentiality (Tempone et al. 2005), indicating the need for further research in this area since then.

Croft et al. (2006) reported the biological tests required in the process of discovering new drugs against leishmaniasis that include (a) in vitro assays against the extracellular stage of the protozoan (promastigote) and against different species of *Leishmania* using as host cells the murine peritoneal macrophages or human monocytes turned into macrophages as well as in axenic cultures of amastigotes and (b) in vivo tests that allow the determination of drug absorption (route of administration) and distribution (sites of infection, metabolism, excretion, and toxicity levels) profiles. All together, these studies and reports of these authors may help to orient the scientific research currently in progress in rational drug design and in other areas.

New synthetic drugs

Hexadecylphosphocoline (HPC or miltefosine) is a widely studied drug for leishmaniasis treatment but originally developed for cancer treatment. Apparently, it stimulates the hematopoietic and immune system, with T cells and macrophage activation and with the increase of the interferon-γ production, thus helping against *Leishmania* infection (Escobar et al. 2001). Nevertheless, there are disadvantages such as teratogenic effects and emergence of resistance.

Interestingly, there are studies that report this drug effects directly to the parasite (Escobar et al. 2001). Escobar et al. (2001) verified no direct correlation of HPC antileishmania activity to B and T cells in contrast to the immune dependence of stibogluconate. HPC presented similar antileishmania effects in both immune-deficient and normal infected mice, different from stibogluconate. The same effect was also observed in vitro using macrophages. Kuhlencord et al. (1992) verified in their studies that the HPC orally administrated presented good distribution in the organism and was more efficient in reducing parasites in spleen, liver, and bone marrow than other drugs (i.e., stibogluconate). Although there are some adverse effects, these results pointed HPC as an alternative in the treatment of HIV patients. The feasibility of oral administration, as it presented good distribution and efficacy in the organism by this route, makes its use easier by the patients.

Mai et al. (2004) tested the action of some compounds derived from hydroxamate against *L. donovani* in vitro.

Their antileishmanial profile may be related to the inhibition of the enzyme histone deacetylase, similar to pentamidine, suberoilanilidine hydroxamic acid (SAHA), and trichostatin A (TSA). Interestingly, all compounds were active against the parasite, and two were as efficient as pentamidine with lower cytotoxic profile but not as good as SAHA and TSA.

Sereno et al. (2005) described nicotinamide (NAM) affecting *Leishmania* through not only the inhibition of a deacetylase but also the synthesis of a protein called S1R2 important to parasite survival in the amastigote form (Silva et al. 2002). They observed that NAM inhibited the amastigotes but not promastigote growth in cultures of *L. infantum* and *L. amazonensis*. It was observed that NAM inhibited the proliferation of amastigotes in infected macrophages with no host cytotoxicity. In addition, this drug may be orally used and associated with other antileishmanial drugs that reinforced it as a potential choice for leishmaniasis treatment.

Kar et al. (1993) studied the effects of trans-acotinic acid (TAA), an aconitase inhibitor, against *L. donovani* due to its action in the fatty acid oxidation. This metabolism has great importance at the energy supply to the parasite development. They observed that the TAA inhibited the in vitro growth of promastigotes and amastigotes in macrophages and in vivo (i.e., infected hamsters) without toxicity to host cells. TAA acted synergistically when associated with other drugs, thus reducing drug active doses.

Recently, some drugs that are already used in other pathology treatment are being tested against leishmaniasis such as risedronate and pamidronate. The molecules are from the biphosphonate class and are commonly used in the treatment of bone diseases. They were tested in *L. donovani*- and *L. mexicana*-infected mice and reduced over 99% of the parasite infection in the animal livers with no toxic effects. According to Tempone et al. (2005), the quinolones and their derivatives were active against protozoans, also including *Leishmania*. They tested four compounds derived from quinolones and using pentamidine as control and observed that all compounds showed greater efficiency and less toxicity against *L. chagasi* promastigotes. Differently, only one compound was effective against amastigotes in infected macrophages without toxic effects, killing 100% of the parasites.

Treatment and the immune system

One alternative in leishmaniasis treatment is the association of drugs with some cytokines that stimulate the immune system. As the host immune system and its components play a crucial role in this infection healing process (Murray et al. 2003), the purpose of this protocol is to enhance the immune response by the activation of macrophages and the increase of the nitric oxide production, among other mechanisms, to eliminate the infection (Shapiro et al. 1991; Tiuman et al. 2005). Murray et al. (2003) tested this protocol in *L. donovani*-infected mice by the association of amphotericin B with one cytokine and observed that, despite this drug's direct action against parasites and its independence from host immunity, the combination was more efficient than the monotherapy and led to the reduction of the amphotericin B dose. The use of amphotericin B with IL12, anti-CD40, or anti-IL10 increased parasite death in 63%, 62%, and 56%, respectively.

The development of vaccines against leishmaniasis is directly related to host immunity and to the development of immunity after the patient healing from VL or CL (Mukhopadhyay et al. 2000). Based on attenuated, dead, or ruptured parasites, isolation and purification of antigens, and parasites encapsulated in liposomes, this process has been widely investigated (Soong et al. 1995; Mukhopadhyay et al. 2000). Soong et al. (1995) isolated antigens P4 and P8 from *L. pifanoi* amastigotes and used them to immunize infected mice. They verified that the use of P8 with adjuvant (*C. parvum*) fully protected the animals against the infection by *L. pifanoi* and cross-protected it against *L. amazonensis*, whereas P4 only protected against *L. pifanoi*. Mukhopadhyay et al. (2000) used a UR6 atypical promastigote, administered alive, centrifuged, killed, and treated with formol, to immunize mice against *L. donovani*. These promastigotes reduced, respectively, 91%, 99%, 88%, and 93% of parasites present in the spleen and the liver of the inoculated animals. The authors still observed differences depending on the route of administration and concluded that the subcutaneous route was the most efficient without needing the protector effect of the adjuvants.

Conclusion

All the studies described so far and those still in process aim to obtain new drugs to be used in leishmaniasis treatment with low or no toxic effects compared to that of current treatment, but keeping its efficiency or overcoming their benefic effects. Importantly, the costs of the treatment should be minimized to allow its dissemination and use mainly in poorer countries, where there is a high incidence of this disease.

The current investigations that may allow the development of new medicines are also based on testing (a) drugs commonly used in other pathologies, (b) substances isolated from microorganisms and plants, and (c) substances used in popular medicine. The scientific advances, mainly related to the knowledge of host immune response mechanisms against the parasites, from their antigenic characteristic to their

metabolism, may allow a better understanding of the mechanisms of action of antileishmanial drugs and their interaction with hosts and parasites. Despite all of this knowledge, this review pointed out that leishmaniasis treatment still remains as a challenge that needs urgent solution and further researches to achieve more specific and efficient medicines.

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References

Alexander J, Russel DG (1992) The interaction of *Leishmania* species macrophages. In: Baker JR, Muller R (eds) Advances in parasitology. vol. 131. Academic, New York, USA, pp 175–254

Arruda DC, Dalexandri FL, Katzin AM, Uliana SRB (2005) Antileishmanial activity of terpene nerolidol. Antimicrob Agents Chemother 49:1679–1687

Barral A, Pedral-Sampaio D, Grimaldi G Jr., Momen H, Mc Mahon-Pratt D, Ribeiro de Jesus A, Almeida R, Badaró R, Barral-Neto M, Carvalho EM, Johnson WD Jr (1991) Leishmaniasis in Bahia, Brazil: evidence that *Leishmania amazonensis* produces a wide spectrum of clinical disease. Am J Trop Med Hyg 44:536–546

Berengener J, Gomez-Campdera F, Padilha B (1998) Visceral leishmaniasis (Kala-Azar) in transplant recipients: case report and review. Transplantation 65:1401–1404

Berman JD (1988) Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy and future strategies. Rev Infect Dis 10:560–586

Bogdan C, Rollinghoff M, Solbato W (1990) Evasion strategies of leishmania parasite. Parasitol Today 6:183–187

Bogdan C, Gessner A, Solbach W, Rollinghoff M (1996) Invasion, control and persistence of leishmania parasites. Curr Opin Immunol 8:517–525

Bray PG, Barrett MP, Ward SA, Koning HP (2003) Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. Trends Parasitol 19:232–239

Carvalho PB, Arribas MAG, Ferreira EI (2000) Leishmaniasis. What do we know about its chemotherapy? Braz J Pharm Sci 36:69–96

Chang KP (1990) Cell biology of leishmania. In: Wyler DW (ed) Modem parasite biology cellular, immunological and molecular aspects. Freeman, New York, pp 79–90

Corte-Real S, Santos CB, Meirelles MNL (1995) Differential expression of the plasma membrane Mg^{2+} ATPase and Ca^{2+} ATPase activity during adhesion and interiorization of *Leishmania amazonensis* in fibroblasts in vitro. J Submicrosc Cytol Pathol 27(3):359–366

Coura JR, Galvão-Castro B, Grimaldi JG (1987) Disseminated American cutaneous leishmaniasis in a patient with AIDS. Mem Inst Osw Cruz 82:581–582

Croft Sl, Seifert K, Yardley V (2006) Current scenario of drug development for leishmaniasis. Indian J Med Res 123(3):399–410

Deane LM, Grimaldi G (1985) Leishmaniasis in Brazil. In: Chang KP, Bray RS (eds) Leishmaniasis. Elsevier, Amsterdam, pp 247–281

Delorenzi JC, Attias M, Gattass C, Andrade M, Rezende C, Pinto AC, Henriques AT, Bou-Habib DC, Saraiva EM (2001) Antileishmanial activity of na índole alkaloid from *Peschiera australis*. Antimicrob Agents Chemother 45(5):1349–1354

Desjeux P, Alvar J (2003) Leishmania/HIV. Co-infections: epidemiology in Europe. Ann Trop Med Parasitol 97(suppp.1):3–15

Dey T, Anam K, Afrin F, Ali N (2000) Antileishmanial activities of stearylamina-bearing liposomes. Antimicrob Agents Chemother 44(6):1739–1742

Escobar P, Yardley V, Croft SL (2001) Activities of hexadecylphosphocholine (miltefosine), ambisome, and sodium stibogluconate (Pentostam) against *Leishmania donovani* in immunodeficient *scid* mice. Antimicrob Agents Chemother 45(6):1872–1875

Golenser J, Frankenburg S, Ehrenfreund T, Domb AJ (1999) Efficacious treatment of experimental leishmaniasis with amphotericin b-arabinogalactan water-soluble derivatives. Antimicrob Agents Chemother 43(9):2209–2214

Gontijo B, Carvalho MLR (2003) Leishmaniose Tegumentar Americana. Revista de Sociedade Brasileira de Medicina Tropical 36 (1):71–80

Gontijo CMF, Melo MN (2004) Leishmaniose Visceral no Brasil: Quadro Atual, Desafios e Perspectivas. Rev Bras Epidemiol 7 (3):338–349

Green SJ, Meltzer MS Jr, Hibbs JB, Nacy CA (1990) Activated macrophages destroy intracellular *Leishmania major* amastigotes by an L-arginine-dependent killing mechanism. J Immunol 144:278–283

Grimaldi G Jr., Mc-Mahon-Pratt D, Sun T (1991) Leishmaniasis and its etiologic agents in the New World: an overview. Prog Clin Parasitol 2:73–118

Grimaldi G Jr., Corte-Real S, Pinho RT (1983) Interactions between *Leishmania mexicana mexicana* promastigotes and amastigotes and murine peritoneal macrophages in vitro. Mem Inst Osw Cruz 78:136–146

Hespanhol RC, Soeiro MNC, Corte-Real S (2005) The expression of mannose-receptor in skin fibroblast and their involvement in *Leishmania (L.) amazonensis* invasion. J Histochem Cytochem 53(1):35–44

Kar S, Kar K, Bhattacharya PK, Ghosh DK (1993) Experimental visceral leishmaniasis: role of trans-aconitic acid in combined chemotherapy. Antimicrob Agents Chemother 37(11):2459–2465

Kayser O, Kiderlen AF, Bertels S, Siems K (2001) Antileishmanial activities of aphidicolin and its semisynthetic derivatives. Antimicrob Agents Chemother 45(1):288–292

Kuhlencord A, Maniera T, Eibl H, Unger C (1992) Hexadecylphosphocholine: oral treatment of visceral leishmaniasis in mice. Antimicrob Agents Chemother 36(8):1630–1634

Larabi M, Yardley V, Loiseau PM, Appel M, Legrand P, Gulik A, Bories C, Croft SL, Barratt G (2003) Toxicity and antileishmanial activity of a new stable lipid suspension of amphotericin B. Antimicrob Agents Chemother 47(12):3774–3779

Liew FY, O'Donnell CA (1993) Immunology of leishmaniasis. Adv Parasitol 32:161–259

Lima LM, Barreiro EJ (2005) Bioisosterism: a useful strategy for molecular modification and drug design. Curr Med chem 12 (1):23–49

Liñares GE, Ravaschino EL, Rodriguez JB (2006) Progresses in the field of drug design to combat tropical protozoan parasitic diseases. Curr Med Chem 13:335–360

Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, Walker LA, Khan IA (2004) Antimicrobial and antileishmanial activities of hipocrellins A and B. Antimicrob Agents Chemother 8(11): 4450–4452

Mai A, Cerbara I, Valente S, Massa S, Walker LA, Tekwani BL (2004) Antimalarial and antileishmanial activities of aroyl-pyrrolyl-hydroxyamides, a new class of histone deacetylas inhibitors. Antimicrob Agents Chemother 48(4):1435–1436

Mcgregor A (1998) WHO warns of epidemic *Leishmania*? Lancet 351:575–575

Molina R, Gradoni L, Alvar J (2003) HIV and the transmission of *Leishmania*. Ann Trop Med Parasitol 97(Suppl. 1):29–45

Morales P, Torres JJ, Salavert M, Peman J, Lacruz J, Sole A (2003) Visceral leishmaniasis in lung transplantation. Transplant Proc 35:2001–2003

Mukhopadhyay S, Bhattacharyya S, Majhi R, De T, Naskar K, Majumdar S, Roy S (2000) Use of attenuated leishmanial parasite as an immunoprophylactic and immunotherapeutic agent against murine visceral leishmaniasis. Clin Diagn Lab Immunol 7 (2):233–240

Murray HW, Brooks EB, Devecchio JL, Heinzel FP (2003) Immunoenhancement combined with amphotericin B as treatment for experimental visceral leishmaniasis. Antimicrob Agents Chemother 47(8):2513–2517

Oliveira CC, Lacerda HG, Martins DR, Barbosa JD, Monteiro GR, Queiroz JW, Sousa JM, Ximenesf MF, Jerônimo SM (2004) Changing epidemiology of American cutaneous leishmaniasis (ACL) in Brazil: a disease of the urban–rural interface. Acta Trop 90(2):155–162

Olliaro PL, Bryceson ADM (1993) Practical progress and new drugs for changing patterns of leishmaniasis. Parasitol Today 9:323–328

Pal S, Ravindran R, Ali N (2004) Combination therapy using sodium antimony gluconate in stearylamine-bearing liposomes against established and chronic *Leishmania donovani* infection in BALB/c mice. Antimicrob Agents Chemother 48(9):3591–3593

Raht S, Trivellin A, Imbrunito TR, Tomazela DM, Jesus MN, Marzal P, Junior HFA (2003) Tempone, A.G. Antimoniais Empregados no Tratamento da Leishmaniose: Estado de Arte. Quim Nova 26:550–557

Ramos H, Milhaud J, Cohen BE, Bolard J (1990) Enhanced action of anphotericin B on *Leishmania mexicana* resulting from heat transformation. Antimicrob Agents Chemother 34(8):1584–1589

Ritting MG, Bogdan C (2000) Leishmania host–cell interaction: complexities and alternative views. Parasitol Today 16:292–297

Roberts W, McMurray W, Rainey P (1998) Characterization of the antimonial antileishmanial agent meglumine antimonate (Glucantime). Antimicrob Agents Chemother 42(5):1076–1082

Rosa MSS, Mendonça-Filho RR, Bizzo HR, Rodrigues IA, Soares RM, Padrón TS, Alviano CS, Lopes AHCS (2003) Antileishmanial activity of a linalool-rich essential oil from *Cróton cajucara*. Antimicrob Agents Chemother 47(6):1895–1901

Sereno D, Alegre AM, Silvestre R, Vergnes B, Ouaissi A (2005) In vitro antileishmanial activity of nicotinamide. Antimicrob Agents Chemother 49(2):808–812

Shapiro TA, Were JB, Danso K, Nelson DJ, Desjardins RE, Pamplin CL (1991) Pharmacokinetics and metabolism of allopurinol riboside. Clin Pharmacol Ther 49(5):506–514

Silva ES, Pacheco RS, Gontijo CM, Carvalho IR, Brazil RP (2002) Visceral leishmaniasis caused by *Leishmania (viannia) braziliensis* in a patient infected with human immunodeficiency virus. Rev Inst Med Trop São Paulo 44:145–149

Singh S, Sivakumar R (2004) Challenges and new discoveries in the treatment of leishmaniasis. J Infect Chemother 10(6):307–315

Soong L, Duboise SM, Kima P, Mcmahon-Pratt D (1995) *Leishmania pifanoi* amastigote antigens protect mice against cutaneous leishmaniasis. Infection and Imunnity 63(9):3559–3566

Tempone AG, Silva ACMP, Brandt CA, Martinez FS, Borborema SET, Silveira MAB, Andrade HF Jr (2005) Synthesis and antileishmanial activities of novel 3-substituted quinolones. Antimicrob Agents Chemother 49(3):1076–1080

Tiuman TS, Nakamura TU, Cortez DAG, Filho BPD, Diaz JAM, Souza W, Nakamura CV (2005) Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium*. Antimicrob Agents Chemother 49(1):176–182

Weniger B, Robledo S, Arango GJ, Deharo E, Aragon R, Munoz V, Callapa J, Lobstein A, Anton R (2001) Antiprotozoal activities of Colombian plants. J Ethnopharmacol 78(2–3):193–200

WHO (2001) Tropical disease research: progress 1999–2000. World Health Organization, Geneva

WHO (1990) Tropical disease research progress. AIDS, leishmaniasis dangers of clash highlighted. TDR News. 36:1–11. World Health Organization

WHO (1991) Tropical disease research progress. Antimonials large-scale failure in Leishmaniasis “alarming”. TDR News. 34:17. World Health Organization

Yardley V, Khan AA, Martin MB, Slifer TR, Araujo FG, Moreno SNJ, Docampo R, Croft SL, Oldfield E (2002) In vivo activities of farnesyl pyrophosphate synthase inhibitors against *Leishmania donovani* and *Toxoplama gondii*. Antimicrob Agents Chemother 46(3):929–931