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Therapeutic options and vaccine development in the treatment of leishmaniasis

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Abstract

Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease. Untreated Leishmaniasis- cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis - results in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world. However, cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance. New therapeutic targets for medications and vaccine development are under investigation to provide improved healing and efficacy for the treatment of *Leishmania spp*.

Key words: Leishmania; Visceral; Cutaneous; Mucocutaneous; Amphoterecin; Vaccine

Core tip: Leishmaniasis is an old disease, hard to diagnose and even harder to treat. Limited treatment is available. Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease. Untreated Leishmaniasis - cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis - results in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world. Cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance. There is an emergent need for development of new therapeutic options with improved tolerability, improved healing process minimizing scarring, and improved efficacy amongst all *Leishmania spp*.

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INTRODUCTION

Leishmania spp. are intracellular protozoa transmitted between mammals by the bite of a female sandfly, genus *Phlebotmus* in the Old World (the Middle East, Asia, Eastern Europe, Western Europe, and Africa) and genus *Lutzomyia* in the New World (Central and South America)[1,2]. A variety of animals, including humans, can be infected with *Leishmania spp.* and many animals serve as natural reservoirs[1]. Leishmaniasis is endemic in 98 countries with an estimated prevalence of 12 million people infected and 350 million people at risk of infection[1-4]. There are more than 20 known *Leishmania spp.* that cause human disease[1,5,6]. *Leishmania spp.* cause four main human syndromes: Cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). The clinical syndrome varies based on the *Leishmania spp.*, the geographic location and the host immune system[1,6,7]. However, all forms of leishmaniasis are severely debilitating and affect the livelihood of those living in endemic areas of the world. An estimated loss of 2357000 disability-adjusted life years (DALYs) is attributed to leishmaniasis alone[7,8].

CL accounts for approximately 1.2 million new cases of Leishmaniasis per year reported in 83 countries[4]. The majority of CL cases occur in Afghanistan, Algeria, Brazil, Colombia, Iran, Peru, Ethiopia, Costa Rica, North Sudan, Saudi Arabia and Syria[3,6,8,9]. CL is typically caused by *Leishmania major* (*L. major*), *Leishmania tropica* (*L. tropica*), *Leishmania infantum* (*L. infantum*) and *Leishmania donovani* (*L. donovani* in the old world and *L. mexicana*, *L. amazonensis*), *Leishmania guyanensis* (*L. guyanensis*), *Leishmania panamensis* (*L. panamensis*) and *Leishmania braziliensis* (*L. braziliensis*) in the new world[1,8]. It may present as a single ulcerative or nodular lesion near the site of the sandfly bite on uncovered areas of the body[1]. In some cases, however, individuals may have a more severe diffuse infection called DCL, with nodular lesions of variable size in various locations (DCL)[1,10]. Lesions evolve over weeks to months and may resolve spontaneously over months to years. Treatment of primary CL depends on the *Leishmania spp*., the geographic region, and the clinical presentation[9]. For many species of leishmaniasis, cutaneous disease is self-limiting and will be cured over time. In Old World leishmaniasis, *L. major* spontaneously heals in 40%-70% of cases at 3 mo and close to 100% of cases by 12 mo, whereas *L. tropica* spontaneously resolves in less than 1% of cases at 3 mo and close to 100% by 3 years[9]. In New World leishmaniasis, *L. mexicana* may resolve spontaneously within 3-4 mo but *L. braziliensis*, *L. panamensis, L. guyanensis* and *L. peruviana* may take more than 6 mo to self-resolve[9]. After resolution patients may be left with disfiguring cutaneous scars[1]. Scarring caused by CL has a distinctive appearance particularly when involving sensitive areas such as the face. Scars often have a central depressed surface that is covered by rounded hyper-pigmented skin[11]. Years after spontaneous resolution, CL lesions have the potential to relapse, a condition known as leishmaniasis recidivans[1]. Despite the possibility a lesion will self-heal, initiation of treatment, either systemic or local therapy, may hasten resolution of disease and may prevent further transformation to MCL[1].

MCL occurs most commonly due to progression of CL caused by *L. braziliensis*. Metastasis of the parasite into the mucosal tissue causes significant tissue destruction and disfigurement[1,9]. Almost 90% of MCL occurs in Bolivia, Brazil, and Peru; up to 30% of *L. braziliensis* cases progress to mucocutaneous disease[6,9]. MCL typically involves the nose, palate, pharynx, and larynx and occurs months to years after resolution of the primary lesions[1]. Ulcerated lesions of the nasal septum, which may lead to perforation and deformities of the nasal pyramid, larynx, and pharynx, can cause significant morbidity and social rejection[12]. Mucocutaneous disease always requires treatment for cure; however, it may be refractory to current available therapeutic chemotherapy. With continued destruction of mucosal membranes, patients are at risk for secondary super-infections and severe malnutrition[1]. Because of the risk of secondary morbidity and mortality, systemic treatment is preferred[9].

VL, also known as Kala azar, is caused by *L. donovani* in India, Pakistan, China and several countries in Africa and by *L. infantum* in the Mediterranean region and in the New World[1,8]. VL occurs secondary to proliferation of parasites in macrophages in the liver, spleen and bone marrow which causes hepatosplenomegaly and bone marrow suppression with subsequent pancytopenia and immunosuppression[1]. There are an estimated 200000-400000 new cases of VL each year with a case fatality rate of more than 10%[3,4,6]. Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan report over 90% of all VL cases worldwide[4,6]. Without treatment, VL is almost universally fatal[1,8]. Systemic therapy is the current standard of care.

An appropriate cellular immune response is essential for the control and eradication of leishmania in the human host. With exposure to leishmania, the host T cells produce cytokines, specifically interferon gamma (INF-γ) and tumor necrosis factor-alpha (TNF-α), that activate host macrophages[12]. The activation of host macrophages produces nitric oxide (NO), peri-nitrate and oxygen derivates that are directly involved with leishmania killing and eradication[7,12]. Patients with immunocompromised conditions such as human immunodeficiency virus (HIV) are at increased risk of progressive, debilitating disease states. Interestingly, patients who recover from leishmaniasis often have a spectrum of resistance to re-infection or acquired anti-leishmanial immunity. Host resistance is mediated by both innate and adaptive immune responses including activation of macrophages, dendritic cells, and antigen specific CD4 and CD8 T cells[7]. These host responses allow for immunity against re-infection and

highlight possible new avenues for therapeutic drug development.

CURRENT TREATMENT

Leishmania spp. vary in their sensitivity to available drugs[8]. The choice of treatment is based on the region where the infection was acquired, local experience with treatment, and known species resistance patterns[8]. Currently the gold standard therapy for most forms of leishmaniasis remains pentavalent antimony (Sb^v), meglumine antimoniate, or sodium stibogluconate[1]. The mechanism of action against *Leishmania spp*. is still poorly understood and thought to act on the parasite indirectly through augmentation of the host's macrophage parasiticidal activity[1]. Sb^v can be administered by intravenous (typical dose of 20 mg/kg), intramuscular or intra-lesion route and typically requires at least 20-28 d course of treatment[1,8,9,13]. Despite its common use, Sb^v have different cure rates between species, ranging from 60%-80%[8,9,11]. Furthermore, recent studies involving the use of Sb^v in children showed significantly lower cure rates and significantly higher metabolic elimination of the drug compared with adults[14]. Adverse effects are also common with Sb^v and include cardiotoxicity such as arrhythmias, QTc prolongation, and sudden cardiac death; elevated aminotransaminases; elevated pancreatic enzymes; pancytopenia; and electrolyte abnormalities[1]. Because of these adverse effects, administration of Sb^v is highly restricted in pregnant and lactating women, infants, and patient with drug sensitivities[15]. Intralesional injections of Sb^v are the most established local therapy available for the treatment of CL and do not cause the same systemic adverse effects as intravenous and intramuscular formulations. However, there is a lack of standardization of dosing and treatment regimens with varying cure rates among geographic regions due to the development of resistance[16]. While intralesional injections do not cause significant systemic adverse effects, local therapy can cause itching, erythema, pain, and hyperpigmentation of the lesion, and put the patient at increased risk of bacterial super infection[9]. Sb^v chemotherapeutic agents currently available for the treatment of Leishmaniasis are toxic, costly, and not readily available in every community, and require a long duration of therapy as well as daily systemic administration with medical monitoring. These limitations promote poor treatment adherence within a community[1,17]. Due to the wider geographic distribution of leishmaniasis, the toxic chemoprophylaxis treatment available and the emergence of drug resistant *Leishmania* strains, new antimicrobial therapies and strategies are being developed to address the growing problem[1,3,7].

SYSTEMIC TREATMENT

Systemic therapies are recommend in complex CL, MCL and VL[9] (Table 1). However, current alternative systemic agents to Sb^v are limited.

Table 1 Common therapeutic options for treatment of leishmaniasis

Medication	Disease	Dosing	Adverse effects	Ref.
Antimony Sodium stibogluconate	CL, VL	IM, IV, IL	IM/IV: Cardiotoxicity, elevated aminotransaminases, elevated pancreatic enzymes, pancytopenia, electrolyte abnormalities IL: pain, hyperpigmentation, risk of bacterial infection	[1,13,15,89]
Amphotericin	CL, MCL, VL	IV	Renal insufficiency, electrolyte abnormalities	[1,13,18,20]
Pentamidine	CL, VL	IM, IV	Hypoglycemia, elevated amiontransaminases, nausea, vomiting, bone marrow toxicity, nephrotoxicity, cardiotoxicity	[1,13,22,23]
Miltefosine	VL, CL, MCL	PO	Vomiting, nausea, diarrhea, teratogen	[1,13,15,18]
Paromomycin	CL, VL	IM, PO, topically	Ototoxicity, vestibular instability, nephrotoxicity	[1,22]
Pentoxyfylline	CL, MCL	PO	Nausea, vomiting, headache, dizziness	[27]
Azoles	CL, VL	PO	Hepatic toxicity	[1,17]
Imiquimod	CL	Topical	Irritation at site of application	[1,11]
Thermotherapy	CL	Topical	Pain, post-inflammatory hyperpigmentation	[16,17,30]
Cryotherapy	CL	Topical	Local blistering, secondary bacterial infection	[8,16]
Phototherapy	CL	Topical	Pain	[30]

CL: Cutaneous leishmaniasis; VL: Visceral leishmaniasis; MCL: Mucocutaneous leishmaniasis; IM: Intramuscular; IV: Intravenous; IL: Intralesional; PO: Oral.

Amphotericin B

More recent clinical trials and clinical experience have highlighted the use of polyenes such as Amphotericin B in the treatment of leishmaniasis. Both liposomal and deoxycholate formulations have been found to have high affinity to the ergosterol membrane of *Leishmania spp.* and create membrane instability[1,18]. In areas of India, Bangladesh, Bhutan and Nepal where high resistance of Sb^v exists, Amphotericin B is the therapeutic drug of choice for VL. In these studies, using high doses of Amphotericin 10 mg/kg and 15 mg/kg demonstrated cure rates of 96% and 100% after a single dose[8,18]. In other areas where resistance to Sb^v is not as high but drug toxicity is a concern, such as in patients co-infected with *Leishmania spp.*

and HIV, and in travelers returning from regions where VL is endemic, Liposomal Amphotericin B again is the recommended drug of choice[8]. Treatment of CL and MCL caused by *L. braziliensis* requires systemic therapy. Studies comparing Liposomal Amphotericin B to Sb^v have shown superior results when treated with Liposomal Amphotericin B[19,20]. There is no standard dosing regimen for treatment of VL with Amphotericin B; however, lower dosing using Liposomal Amphotericin B at 3 mg/kg per day administered intravenously for days 1-5, 14 and 21 (total 21 mg/kg) has been used to treat CL, although further studies to evaluate optimal dose and duration are still needed[13,20,21]. Despite the promising results of Amphotericin formulations there remain many limitations including the need for intravenous administration, the significant cost of the medication, the limited availability of the medication, the emergence of *Leishmania spp*. resistance, and the significant adverse effect profile including renal insufficiency and electrolyte abnormalities[1,18]. Despite its high cost, cost analysis studies have shown the expense for total treatment with a shorter duration of therapy with Liposomal Amphotericin is less than with full treatment with Sb^v[19].

Pentamidine

Pentamidine isethionate is an intravenous or intramuscular formulation used to treat cutaneous Leishmaniasis caused by *L. amazonensis, L. guyanensis, L. panamensis* and *L. peruviana*. Pentamidine also serves as an alternative agent for patients with recurrence of cutaneous *L. braziliensis* or an alternative agent for recurrent VL after treatment failure with Sb^v or amphotericin[9,11]. The mechanism of action remains unknown but studies suggest the drug may target protozoa mitochondria and interfere with biosynthesis of macromolecules[1,9,22]. The optimal dosing of pentamidine is currently unknown with proposed dosing of 2-4 mg/kg per day *im* or *iv* for 21 d[13]. Adverse effects of Pentamidine include hypoglycemia, worsening of diabetes, elevated aminotransaminases, musculoskeletal pain, anorexia, nausea, vomiting, headaches, bone marrow toxicity, nephrotoxicity, and cardiotoxicity with arrhythmias, heart failure and hypotension[1,22,23]. The extensive side effect profile limits the use of pentamidine.

Paromomycin

An alternative systemic agent against leishmaniasis is Paromomycin, an aminoglycoside antibiotic that blocks protein synthesis through binding of 16S ribosomal RNA. Paromomycin can be administered intramuscularly, orally, or topically[1,22]. Paromomycin has been shown to be effective against CL and VL in areas with susceptible protozoa, although cure rates vary greatly amongst geographic locations[16]. It can be used alone or in combination with Sb^v or amphotericin B and has been associated with increasing time to resolution of lesions caused specifically by *L. major*[1]. A Phase 3 clinical study evaluating the efficacy of topical combination therapy with 15% Paromomycin and 0.5% Gentamicin applied to each lesion once a day for 20 d to treat CL has shown promise in advancing cure rates with reduced systemic absorption[24,25]. Phase 2 trials using topical paromomycin plus gentamicin formulation showed a 6 mo cure rate of 87% compared to paromomycin alone at 60%[26]. Despite these advancements, paromomycin has limiting adverse effects of systemic formulations, which include ototoxicity, vestibular instability and nephrotoxicity, as well as with the topical formulations, which include erythema, pain, edema as well as ototoxicity[1,22].

Miltefosine

Miltefosine, an alkylphosphocholine, is a promising oral agent, recently approved by the United States Food and Drug Administration (FDA) to treat VL, complicated CL and MCL cases that do not respond to first line therapeutics[1,23-25,27]. In adults, the treatment regimen consists of one 50-mg oral capsule twice a day for 28 consecutive days[13]. The oral formulation of miltefosine alleviates the risk, cost, and time demands of daily intramuscular or intravenous injections[14,18]. The mechanism of action is associated with interruption of phospholipid biosynthesis and alkyl-lipid metabolism in specific *Leishmania spp*[22]. As with other therapeutics to treat leishmania, the efficacy of Miltefosine is variable based on species and geographic location[14]. Studies in children specifically showed promising results comparing Miltefosine to the standard of care, Sb^v, for treatment of CL[14]. Miltefosine has been shown to be effective against CL by *L. major* but may also be effective in new world CL with *L. panamensis*[1]. Additional studies have shown improved cure rates in treating VL in India particularly when used in combination with paromomycin[1,15]. However, other New World studies have shown inferiority of miltefosine to Sb^v in the treatment of CL in Colombia. The finding of inferiority in this particular study was thought to be species specific. Treatment of CL with Miltefosine in Colombia demonstrated a cure rate of only 69.8%, which fell to 49% when administered to patient with lesions caused by *L. braziliensis*[15]. Miltefosine tends to be well tolerated with minimal non-specific adverse effects, including vomiting, nausea, diarrhea, and headache. However, miltefosine is a teratogen and an abortifacient and must be used with caution in females of reproductive age[1,18]. Females of reproduction age who are taking Miltefosine should be provided with contraception during the course of treatment as well as for 5 mo post-therapy[13,15]. Miltefosine also remains costly and requires prolonged therapy presenting additional barriers to therapeutic adherence[15].

Pentoxifylline

Pentoxyfylline, a xanthine derivative, is an orally administered immunomodulator that is an attractive therapeutic alternative for CL and MCL. *In vitro* there is

no evidence that pentoxifylline directly kills *Leishmania spp*. but the major contribution of pentoxifylline is reduction of the TNF-α mediated tissue damage caused by *Leishmania spp*[28]. Pentoxyfylline blocks the transcription of TNF-α mRNA from macrophages leading to reduction in TNF-α synthesis, decreases leukocyte migration, and decreases leukocyte adhesion[28]. Pentoxifylline also causes significant vasodilation and increase in red blood cell flexibility for improved circulation and migration of host defense cells to the damaged tissue[12,28]. While Pentoxyfylline has been demonstrated to reduce the concentration of TNF-α in damaged tissue caused by *Leishmania spp*. in CL and MCL, monotherapy has not been associated with cure[28]. Pentoxyfylline is more commonly used as an adjuvant immunomodulating therapeutic agent[28]. In combination therapy regimens, pentoxifylline allows for reduction in the inflammatory response and promotes improved defense against protozoa by Sb^v[12]. Recent studies have shown higher cure rates and reduction of time to cure using combination of Sb^v and pentoxifylline. The reduction in time to cure has allowed for shorter Sb^v dosing regimens reducing the risk of adverse effects, the total cost of therapy, and the total hospital stay associated with prolonged Sb^v[8,12,28]. Along with the improved efficacy, pentoxifylline is associated with minimal adverse effects even with chronic use[12]. Adverse effects including nausea, vomiting, dizziness and headache occur in less than 2.2% of patients[28]. Additionally there are reports of safe use in children although large clinical trials are currently not available[12].

Azoles

Azoles, *e.g.,* posaconazole, itraconazole, fluconazole and ketoconazole, are oral therapeutic alternatives for treatment of Leishmaniasis. Azoles inhibit ergosterol synthesis through alteration of sterol demethylation causing the accumulation of sterols[1,17,27,29]. Decreased production of ergosterol, which composes the cell wall, inhibits leishmania growth and causes structural instability of the protozoa[27,29]. *In vitro* murine studies suggest azoles have anti-parasitic activity against certain *Leishmania spp.* causing VL such as *L. infantum* but are less active against *L. donovani*[27]. Ketoconazole and fluconazole have also been shown to hasten healing of CL caused by *L. mexicana, L. panamensis* and *L major*[1,29]. While several *in vitro* studies demonstrate effective anti-parasitic activity, clinical studies have not been as promising[1]. One clinical study did show comparable outcomes of Ketoconazole to standard Sb^v in the treatment of *L. panamensis* CL, although more recent studies have shown clinical benefit is achieved only with high dosing[29]. Azoles given at high doses expose patients to significant hepatic toxicity[1,17]. In order to reduce the high dosing, further studies evaluating azoles in combination with other therapeutic options may provide increased efficacy at lower dosing[17,27]. Topical imiquimod in combination with itraconazole has been shown to have better cure rates when either of the therapeutics were used alone[17].

LOCAL TREATMENTS

Local treatments can be used to treat CL when the *Leishmania spp.* has low potential to advance to MCL; there are a limited number of lesions (less than four); the lesions are small (< 4-5 cm); the lesions are not localized on delicate areas of the body; and the host is not immunosuppressed[9,23]. The use of local agents is favorable in these circumstances as they tend to have less systemic toxicity and allow for outpatient treatment regimens[9]. Local therapies are currently considered first line treatment in most cases of CL[9]. Despite these advantages, there is a need for standardization and highly scrutinized efficacy studies for the use of local therapies[23].

Imiquimod

Imiquimod, a topical imidazole quinolone cream, is a potent immune-modulator and Toll-like receptor 7 agonist that induces macrophage activation through production of pro-inflammatory cytokines interleukin-2, INF-γ and TNF-α[1,11]. Direct activation of macrophages mediates intracellular killing of *Leishmania spp.*[11]. Topical imiquimod can be used as monotherapy; however, the rate of treatment failure is currently unknown. When used alone imiquimod has demonstrated rapid initial healing but failed to maintain response after treatment was stopped. As a result, when imiquimod is used as monotherapy, patients may need a prolonged treatment course to ensure therapeutic cure[11]. More commonly imiquimod is added in combination with Sb^v[1,11]. Addition of imiquimod cream to a Sb^v based regimen to treat Sb^v-resistant CL showed increased rate of cure and higher sustained treatment response compared with persons treated with Sb^v alone[11,23]. Combination therapy also had increased rates of healing and an improved overall cosmetic effect with reduced scarring and reduced hyperpigmentation of the wounds compared to Sb^v therapy alone[11,23]. Imiquimod has been associated with lower treatment cost and fewer adverse effects compared to standard of care due to reduced need for prolonged Sb^v[11]. Imiquimod is generally well tolerated with the main adverse effect being irritation at the site of application[1].

Cryotherapy

Cryotherapy uses liquid nitrogen applied directly to CL lesions and has been proven effective in Old World CL including *L. tropica*, *L aethiopica* and *L infantum*, as well as New World CL that has low potential to progress to MCL such as *L. mexicana, L. panamensis and L. amazonensis*[1,8]. Application of liquid nitrogen is completed 2-3 times each session and repeated every 1-4 wk until complete healing of the lesion is achieved[9]. When used as monotherapy, cryotherapy has shown cure rates superior to spontaneous healing

and comparable to intralesional Sb^v[16]. However, superior results are observed with Cryotherapy in combination with intralesional Sb^v, with a cure rate of 89% compared to cryotherapy alone (75%) or intralesional Sb^v alone (67.8%)[1,9,16,17]. Cryotherapy, while safe and effective, can be painful and cause post-inflammatory hyperpigmentation[16,17,30]. The availability of cryotherapy in endemic regions of the world as well as unknown relapse rates further limit its consistent use as a therapeutic option for CL[16,17].

Thermotherapy

Thermotherapy, *i.e.,* heating the CL lesion to 50 degree Celsius for 30 s once weekly for 4 wk, has been used for the treatment of New world CL caused by *Leishmania spp.* with low likelihood of progression to MCL, such as *L. mexicana, L. panamensis, L. amazonensis*[1,8,9]. Through application of heat radiofrequency, the protozoa are directly killed[9]. Compared to intralesional or parenteral Sb^v, the duration of therapy and the adverse effects were reduced when using thermotherapy monotherapy[16]. Thermotherapy may put patients at risk for local blistering and secondary bacterial infection during the healing period[16]. CO2 Laser is a type of thermotherapy which operates through thermolysis on damaged tissues without causing damage to the surrounding healthy tissue. The CO2 laser is used in one single session and has been shown to be more effective than combined therapy of cryotherapy plus intralesional Sb^v[9]. With disfiguring facial lesions or lesions at sites at risk of significant scaring, CO2 thermotherapy may be an alternative therapeutic option[8]. Despite the positive effects of thermotherapy on healing of wounds, cure rates remain variable from 48%-83% amongst different *Leishmania spp*[16]. While shown to be effective in certain species, thermotherapy requires costly advanced technology equipment and adjuvant medications including local anesthetic and prophylactic antibiotics that are not readily available in endemic areas[8,16].

Phototherapy

Photodynamic therapy is an additional new treatment modality that uses light-mediated cytolysis of protozoa. The photodynamic therapy is applied once weekly for a total of 4 wk and does not induce drug resistance even after repeated applications[9,30]. Conventionally, photodynamic therapy requires activation of a topical photosensitizer, usually aminolevulinic acid (ALA) or methyl aminolevulinate, followed by irradiation by a visible light source[30]. Activation of the photosensitizer in the presence of oxygen results in the generation of reactive oxygen species, activation of host macrophages and subsequent destruction of the infected tissue[30]. This process can be time consuming and expensive and requires specialized technology[30]. New technology is emerging that uses daylight activation of the topical photosensitizers, abolishing the need for specialized light sources[30]. It has proven to be effective in the treatment of CL caused by both *L. major* and *L. tropica*, with an overall cure rate of 88.9%; however, efficacy is dependent on weather conditions in geographic locations[30]. Adverse effects associated with phototherapy include pain caused by the sudden activation of the photosensitizer[30].

Approach to chemotherapeutics selection

Choosing the appropriate initial therapy for a patient with leishmaniasis is dependent on the disease (CL, MCL, DCL or VL), the geographic location, the *Leishmania spp.*, and the state of the host immune response.

Currently the WHO recommends pentavalent antimonial, sodium stibogluonate 20 mg/kg per day for 21 d, and IV, as first line therapy for CL and VL[10,21]. However, Liposomal amphotericin B has been found to be as effective in treatment of VL, and superior in treatment for MCL, and better tolerated compared to Sb^v. As a result the US FDA has approved amphotericin B as first line therapy for VL caused by *L .infantum* and *L. donovani*[21]. Patients with CL, DCL and MCL caused by *L. braziliensis*, patients in the New World with leishmaniasis of unknown species, and patients with complicated CL including lesions on the face or lesions over the joints should also be treated with Liposomal amphotericin B 3 mg/kg on Days 1-5, 14, 21[20,21,30]. All patients with VL, CL, DCL or MCL who are immunocompromised should be treated with systemic therapy, either antimony or amphotericin B, as treatment failure and disease progression is more common in this group[21]. Due to the reduced side effects and reduced duration of therapy, Liposomal Amphotericin B should be the first line therapy in immunocompromised patients if available. Miltefosine is an appropriate alternative to Amphotericin B in DCL, MCL and VL caused by *L. donovani* and *L. infantum*[10,21].

For cutaneous disease that has low potential to advance to MCL; is caused by species other than *L. braziliensis*; where the patient has a limited number of lesions (less than four); where the lesions are small (< 4-5 cm); where the lesions are not localized on delicate areas; and where the host is not immunosuppressed; topical therapies such as intra-lesional chemotherapeutics, thermotherapy, phototherapy or cryotherapy or combination therapies should be used as first line therapy are to minimize adverse effects[20,31].

Vaccines

Preventative and therapeutic vaccines are recognized as the most efficacious and most cost-effective protection against leishmaniasis. Currently there is no licensed vaccine against human leishmaniasis; however, several vaccine candidates have been tried and several others are currently under further investigation. Vaccine development has been challenging due to the complexity of the protozoa pathogenesis and the interaction with the host cell-mediated immune response[2,7]. Despite the complexity of vaccine development, the cost-

effectiveness of leishmania vaccines makes further investigation, production and clinical development an attractive endeavor. Cost-analysis studies have shown that a vaccine even with a relatively short duration of protection will affect cost savings and prevent cases of leishmaniasis. The study found that a vaccine with 10 years protection used in endemic areas such as Brazil, Bolivia, Colombia, Ecuador, Peru and Venezuela that have a country-wide incidence of at least 0.03% in a total population of approximately 308 million people could prevent 41000-144784 CL cases at a cost less than the cost of chemotherapy[10]. This held true for vaccines with 5 years of protection as well[10]. Leishmania vaccines currently receiving attention include a live leishmania vaccine, whole killed or fractions of leishmania, live attenuated and DNA vaccines.

Live parasites were first tried for vaccine development by isolating *L. major* promastigotes from free culture and injecting into the patient. While promising results from live parasite exposure were identified, the standardization and quality control were lacking and concerns about possibility of transmission remain valid[7]. While live vaccines may prevent future infection, they are not currently reasonable options for vaccine development.

First generation vaccines consisting of whole killed leishmania or fractions of the protozoa have also been explored. Killed isolated *L. amazonensis* has been used as a therapeutic vaccine in combination with chemotherapy and has been shown to reduce the required dose of Sb^{v} to achieve cure[7]. Furthermore, in Venezuela autoclaved killed *L. mexicana* has been used to treat patients with Sb^{v} non-responsive CL[7]. Killed vaccines are valuable due to their safety in administration[32]. Despite the potential therapeutic value and minimal safety profile of killed leishmania vaccines, preventive vaccines have not shown significant protection[32]. In studies of autoclaved *L. major* vaccine, the host did not mount a robust immunogenic response. However, with better adjuvants that are able to maintain effector memory cell activation to achieve protection, the vaccine potency increases[32]. Addition of different adjuvants including alum, saponin, cationic liposomes and MPL-A have all been studied and are associated with significant cell mediated immune response, humoral immune response and reduced parasite load[32]. If an adequate adjuvant is used to produce improved immunogenicity with standardized preparation, it is possible that killed leishmaniasis vaccines may be candidates for further vaccine discovery as they are safe, low cost, stable, and composed of the complete protozoa spectrum of antigens[7,32,33].

Live attenuated, recombinant proteins and DNA vaccines are new vaccine strategies under consideration[7]. While some target proteins are conserved proteins across species, others are species and life cycle stage specific, making them limited in use[7]. Important recombinant protein candidate vaccines to date include surface expressed glycoprotein leishmaniolysin (gp63); Leishmania activated C kinase (LACK); parasite surface antigen (PSA); Leishmania-derived recombinant polyprotein (Leish-111f); serine proteases; LEISH-F1; and LEISH-F2[7,33]. LEISH-F1, three recombinant proteins conserved in *L. donovani*, *L. chagasi* and *L. braziliensis*, respectively, and LEISH-F2 re-designed recombinant protein have undergone phase 1 and phase 2 clinical trials with significant success against CL and VL in multiple target locations[33]. Both LEISH-F1 and LEISH-F2 have proven to be immunogenic, safe and well tolerated[33]. The next generation LEISH-F3, another recombinant protein vaccine, is currently under investigation in phase 1 clinical trials for VL[32-34]. Mucosal vaccination through oral and intranasal vaccine, using Leishmanial antigen, has shown promise in mice with *L. amazonensis* in protection against developing CL[35]. Additional mechanisms of combining recombinant parasite-derived nucleoside hydrolase with antigens from the sand fly genus *Lutzomyia* for *L. mexicana* CL have also been under investigation with initial successful results[10,33]. Naked DNA vaccines are another new approach that have shown promise in animal models[7,33]. Cloned genes encoding the target proteins are expressed in mammalian plasmids and injected intra-dermally or intramuscularly[7]. Replication within the host leads to expression of the recombinant proteins for longer periods of time in order to sustain a more robust immunologic response[7]. As no pathogenic organisms are used, the potential for infection is non-existent. It is possible that these DNA vaccines may be used therapeutically for CL cure as well[7]. Studies of live-attenuated leishmaniasis and naked DNA vaccines are limited, as vaccine development is still in its early stages. However great strides have been achieved recently in the development of safe, immunogenic vaccines[7].

Lastly, to achieve control of Leishmaniasis, control of animal reservoirs must also be addressed. *L infantum* is a primarily zoonotic disease, affecting millions of dogs around the world, and remains a source of leishmania transmission. To break the cycle of transmission new canine vaccine candidates are also under further investigation[7].

CONCLUSION

Early treatment of leishmaniasis is critical to achieve cure, prevent psychological and social distress, and prevent transmission of disease[17]. Untreated Leishmaniasis - CL, MCL and VL - result in disfiguring scars and high rates of morbidity and mortality in highly endemic regions of the world[11]. Cure rates with available therapeutics are limited due to cost, therapeutic toxicity and the growing rate of resistance[11]. The growing rate of drug resistance amongst all therapeutic options is of particular concern as little is known about the mechanism of resistance[22]. There is an emergent need for development of new therapeutic

options with improved tolerability, improved healing process minimizing scarring, and improved efficacy amongst all *Leishmania spp*[11]. Despite this need, the challenges associated with therapeutic development are vast due to parasite diversity across continents, the complexity of the host response, and the lack of full understanding of protozoa pathogenesis[23]. Gaining greater understanding on the pathogenesis of the disease and the interaction with host immune response might unveil new therapeutic targets, particularly for vaccine development.

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