Part One
Disease Burden, Current Treatments, Medical Needs,
and Strategic Approaches

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Visceral Leishmaniasis – Current Treatments and Needs

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Abstract

The last decade has seen significant advances in the treatment of visceral leishmaniasis. Two new drugs (miltefosine and paromomycin) have been registered for the treatment of visceral leishmaniasis since 2002. Multidrug treatments have been investigated in systematic clinical studies and are now recommended as a new treatment approach for visceral leishmaniasis. However, the range of available drugs is still limited. Regional differences in response rates to anti-leishmanial available drugs as well as treatment of post-kala-azar dermal leishmaniasis, a complication of visceral leishmaniasis, are examples of the continued need for improved treatments for visceral leishmaniasis. In this chapter we discuss current treatments for visceral leishmaniasis and needs for drug discovery and development and translation to the clinic.

Introduction

Visceral leishmaniasis belongs to the group of neglected tropical diseases (NTDs), a group of chronic parasitic and related bacterial and viral infections that promote poverty [1]. Visceral leishmaniasis is caused by different species of the intracellular protozoan parasite *Leishmania*; predominantly by *L. donovani* in Asia and Africa, *L. infantum* in Europe and Latin America, and to a lesser extent in Africa [1–4]. Parasites, promastigotes as the insect stage, are transmitted by the bite of female phlebotomine sandflies to mammalian hosts [5]. Transmission can be zoonotic (transmission from animal to vector to human) or anthroponotic (transmission from human to vector to human). Inside the host the parasites invade monocytes and macrophages of the mononuclear phagocyte system and transform to the mammalian stage, intracellular amastigotes, which survive and multiply within host cell phagolysosomes. Parasite dissemination occurs through lymphatic and vascular systems. Clinical features of established visceral leishmaniasis include fever, abdominal pain, weight loss, splenomegaly, hepatomegaly, and lymphadenopathy [6]. It should be noted that infection can remain subclinical or develop into clinical disease. The latter displays fatality rates of 100% if untreated. Risk factors to develop

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clinical disease include malnutrition and immune suppression, and are often linked to the overarching factor of poverty [7–9]. Visceral leishmaniasis is also an important infection associated with HIV/AIDS [10].

Major geographical areas affected are South Asia, which carries around 60% of cases worldwide [1], East Africa [4], North Africa and the Middle East [11], Latin America [2], and Southern Europe [3]. There are an estimated 50 000 new visceral leishmaniasis cases and 59 000 deaths per year [12]. However, under-reporting, misdiagnosis, and forced human migration obscure the establishment of exact numbers [4,6,12]. Importantly 90% of cases occur in only six countries: India, Bangladesh, Sudan, Brazil, Nepal, and Ethiopia [13].

Post-kala-azar dermal leishmaniasis (PKDL) is a complication of visceral leishmaniasis characterized by a spectrum of skin lesions following a visceral leishmaniasis episode mainly in areas where *L. donovani* is endemic. Reported incidence and time of onset of PKDL vary between countries; from 50 to 60% of cured visceral leishmaniasis cases within weeks to few months in Sudan to 5–10% generally after 2–4 years in India. There are sporadic reports of PKDL cases with no previous recorded history of visceral leishmaniasis. PKDL lesions contain parasites and are seen as an important reservoir for transmission [14].

In the following sections we will address (i) treatment options for visceral leishmaniasis and geographical differences in treatment response (see the "Current Anti-Leishmanial Drugs and Treatment Options for Visceral Leishmaniasis" section), and (ii) pathology, immunopathology, and treatment options for PKDL (see the "PKDL" section).

Current Anti-Leishmanial Drugs and Treatment Options for Visceral Leishmaniasis

Available Drugs

Pentavalent antimonials (sodium stibogluconate (SSG), generic sodium antimony gluconate, and meglumine antimoniate depending on country and region) have been the standard drugs for the last 60 years. Due to high rates of clinical unresponsiveness their use has been largely abandoned in Bihar state, India [15], but they continue to be used in other endemic areas [16,17]. SSG is still used to a wide extent in Africa, where limited availability of other drugs persists. Toxicity, lot-to-lot variations and need for hospitalization are severe limitations of antimonial treatment [16]. The polyene antibiotic amphotericin B is highly effective. It is used as amphotericin B deoxycholate, which suffers from toxicity [16], and as a liposomal formulation (AmBisome®) approved for the treatment of visceral leishmaniasis [18]. Notably, liposomal amphotericin B is the safest and most effective drug available [19]. Recently, a preferential pricing agreement for developing countries has reduced the cost of liposomal amphotericin B from US$200 to 20 per 50 mg vial [20]. Following this agreement a single infusion of liposomal amphotericin B at a dose of 10 mg/kg body weight was shown to be non-inferior and less expensive than treatment with conventional

amphotericin B deoxycholate [21]. A single infusion of 10 mg/kg liposomal amphotericin B is now recommended as first-line treatment for anthroponotic visceral leishmaniasis in the Indian subcontinent [22] and one component in recently trialed short-course multidrug treatment regimes [23,24]. Sadly, despite the price reduction, cost is still an inhibiting factor for use of liposomal amphotericin B in some endemic areas. Another limitation is temperature stability as temperatures above 25 °C and below 0 °C can alter liposome characteristics, and impact on drug efficacy and toxicity [19]. Miltefosine, an alkylphosphocholine, was the first oral anti-leishmanial drug registered for visceral leishmaniasis in India in 2002 following clinical trials with 94% cure rates [25]. It is used as a potential tool in the visceral leishmaniasis elimination program in India, Bangladesh, and Nepal [16,17]. The gastrointestinal tract is the main target organ for side-effects [26] and gastrointestinal symptoms were recognized as the most common adverse effect in clinical trials [27]. The major limitation of miltefosine is its contraindication in pregnancy, and mandatory contraception for women in child-bearing age for the duration of therapy and 2–3 months beyond. This restriction is based on a teratogenic effect seen in one species (rat) in preclinical studies and the pharmacokinetic profile of miltefosine [26]. Paromomycin, an aminoglycoside antibiotic, is the latest drug registered for visceral leishmaniasis in India in 2006. Non-inferiority of paromomycin to amphotericin B was shown in a phase III trial in India [28]. Safety and efficacy of both miltefosine [27] and paromomycin [29] were confirmed in phase IV studies in an outpatient setting.

Current drugs and treatment regimes are summarized in Table 1.1.

New Treatment Regimes

Treatment courses for visceral leishmaniasis have been long (3–4 weeks) with a negative impact on compliance and cost. Experimental resistance to the new drugs, miltefosine [30–33] and paromomycin [34,35], was easily generated in the laboratory. Antimony-resistant Indian visceral leishmaniasis parasites show increased tolerance to miltefosine and amphotericin B, but not to paromomycin [36,37]. In addition, miltefosine has a long terminal half-life and concerns have been raised about the emergence of resistance when used as monotherapy [38]. Drug combinations and multidrug treatment regimes are in practice for infectious diseases such as malaria and tuberculosis. The rational for use of combination chemotherapy or multidrug treatments is reduction of treatment duration and total drug doses (resulting in decreased toxicity, higher compliance, and less burden on health systems), and delay of emergence of resistance (and hence an increase of a drug's lifespan) [39,40].

Multidrug treatments against visceral leishmaniasis have been trialed earlier on smaller scales with a limited number of drugs available. Examples are SSG plus paromomycin in Sudan (which became standard treatment used by Médecins Sans Frontières) and India [41,42], and SSG plus allopurinol in Kenya [43] in the 1990s and 1980s. With the registration of new drugs and the efficacy of single-dose treatment of liposomal amphotericin B, multidrug treatment regimes became a real possibility for systematic use in visceral leishmaniasis. Non-overlapping drug toxicities and

Table 1.1 Current drugs for visceral leishmaniasis and treatment regimes in Europe, Middle East, Latin America, South Asia, and East Africa.

Drug	Dosage	Regimen and clinical efficacy			Limitations
		South Asia: visceral leishmaniasis due to L. donovani	East Africa: visceral leishmaniasis due to L. donovani	Europe, Middle East, and Latin America: visceral leishmaniasis due to L. infantum	
SSG	20 mg/kg intramuscular/intravenous injection	30 d; effective in Bangladesh, Nepal, and Indian states of Jharkhand, West Bengal, and Uttar Pradesh	30 d; effective and first-line treatment; >95% cure rate	28 d; effective >95% cure rate	Liver and cardiac toxicity; widespread resistance in Indian state of Bihar
Amphotericin B deoxycholate	0.75–1 mg/kg slow infusion	Daily or alternate days infusion 15–20 d, total dose 15 mg/kg; 99% cure rate in India	Daily or alternate days infusion 15–20 d, total dose 15 mg/kg; 97% cure rate in Uganda	Daily or alternate days infusion 20–30 d, total dose 2–3 g effective, but not used	Renal and cardiac toxicity
Liposomal amphotericin B	3–5 mg/kg infusion in 5% dextrose	Daily infusion given over 3–5 d; total dose 15 mg/kg; >90% cure rate; single-dose infusion of 10 mg/kg recommended as first line, 95% cure rate	Daily infusion given over 6–10 d; total dose 30 mg/kg; 88% cure rate in Sudan. 93% in Ethiopia; single-dose infusion drug trials ongoing	Daily infusion given over 3–6 d; total dose 18–24 mg/kg; >95% cure rate and recommended as first-line drug	Significantly less toxic than Amphotericin B, but highly expensive, temperature stability
Miltefosine	2–11 yr: 2.5 mg/kg; >12 yr and <25 kg: 50 mg daily; 25–50 kg: 50 mg b.i.d.; >50 kg: 50 mg thrice orally	28 d; used as first line in Indian control program in Bihar, 94% cure rate; 85% cure rate in Bangladesh	28 d; 90% cure rate in Ethiopia	Not used so far for primary visceral leishmaniasis, recommended as an alternative drug for relapse treatment in visceral leishmaniasis–HIV coinfection	Potential teratogenicity implicates cautionary use in women of child-bearing age; gastrointestinal side-effects; to ensure compliance DOTS (directly observed treatment strategy) should be used
Paromomycin	15 mg/kg daily intramuscular injection	21 d; 93–95% cure rate	At 20 mg/kg/day for 21 d; 85% cure rate; used in combination with SSG at recommended doses for 17 d with 93% cure rate	Not used so far	

matching half-lives are important considerations in the design of drug combinations [44] as is the relationship between drug concentrations, drug resistance, and tolerance [45]. These questions have been explored in the field of anti-malarial drug combinations. The issues related to drug combinations for visceral leishmaniasis have recently been summarized [39]. Importantly, there are no drugs available for fixed-dose combinations for visceral leishmaniasis and the arsenal of drugs to chose from for coadministration or sequential administration is still limited. Hence, experimental studies [46] and clinical trials [23,24,47,48] are focused on coadministration of available drugs in a pragmatic approach. This approach in visceral leishmaniasis is not yet fully guided by a pharmacological or biological evidence base since our understanding of the pharmacokinetic/pharmacodynamic profiles of visceral leishmaniasis drugs, combination treatment regimes, and evolution of drug resistance in the field is still limited. Based on the different modes of action of current anti-leishmanial drugs [49,50] mutual protection against resistance may be achieved, but ultimately (experimental) proof-of-concept and integrated pharmacokinetic/pharmacodynamic studies are still to be carried out. The need for increased knowledge of parasite biology and pharmacology has been voiced [39]. Assessment of pharmacodynamic properties of three treatment arms (single-dose liposomal amphotericin B plus SSG, single-dose liposomal amphotericin B plus miltefosine, and miltefosine alone), pharmacokinetic properties of miltefosine alone and in combination with liposomal amphotericin B, and subsequent modeling of pharmacokinetic/pharmacodynamic relationships for miltefosine are planned for a phase II study for treatment of visceral leishmaniasis in East Africa [47]. This integrated approach will provide highly valuable information. The significant advantage that multidrug treatment regimens in visceral leishmaniasis have shown so far over monotherapy is reduction of treatment duration, cost, and frequency of adverse events [23]. This treatment approach is expected to be the strategy for visceral leishmaniasis in the future.

Recent clinical trials have been reviewed and summarized elsewhere [39,40]. Further information on ongoing trials may be found at www.dndi.org and www.clinicaltrials.gov.

Another combination approach discussed for PKDL [51] and HIV coinfected individuals is the combination of an immunotherapy or therapeutic vaccine with drug treatment. One rational for this approach is to decrease the parasite burden with an effective (preferentially fast killing) drug used at low dose or as a short-course treatment and boost the effector immune response with an immunostimulatory agent [52]. This approach has been tried with first-generation vaccines, recombinant proteins, adjuvant, and cytokines, but lacked availability of defined products suitable for registration. With defined vaccines and immunotherapies currently in development this approach is set to gain future attention.

Regional Differences in Clinical Drug Efficacy

Regional differences in clinical drug efficacy have been reported between and within countries. The most recent example is the finding for paromomycin in East Africa. A

dose of 15 mg/kg/day paromomycin sulfate for 21 days was efficacious in a phase III trial in India with a 94.6% cure rate, but gave an unacceptable low overall cure rate of 63.8% in East African countries [8]. The pharmacological and/or biological basis, patient or parasite factor, for this difference still needs to be established. Differences in treatment response to liposomal amphotericin B between Indian, Kenyan, and Brazilian patients have also been reported earlier in a phase II study [53]. A recent report addressed the question of differences in visceral leishmaniasis patient (demographic and nutritional) profiles in Brazil, East Africa, and South Asia [9], highlighting potential requirements of distinct strategies between geographical settings. These may be based on differences at the level of parasite, host or parasite host interactions.

PKDL

Pathology and Immunopathology of PKDL

PKDL, predominantly observed in the Indian subcontinent and East Africa, is a dermal manifestation in a fraction of treated visceral leishmaniasis patients caused by *L. donovani* [54]. Sporadic cases of PKDL have been reported due to *L. infantum*, especially in HIV–visceral leishmaniasis coinfection [55]. The clinical spectrum of disease ranges from hypopigmented macular lesions to infiltrated plaques to the chronic and most aggravated nodular form. Most patients present with a combination of lesions described as polymorphic form [54,56,57].

Dermal infiltration of lymphocytes, macrophages, and plasma cells in varying proportion is observed in granuloma of Indian PKDL in contrast to Sudanese PKDL, where plasma cells are virtually absent [54,57,58]. The parasites are mainly present in the superficial epidermis, and easily detected in nodular lesion compared to macular and papular lesions [59]. Neuritis in small cutaneous nerves indicating peripheral nerve involvement, mucosal lesions with destructive complication in oronasal regions, and ocular lesions have been described in Sudanese PKDL, but appear rare in Indian PKDL with a sole report of neuritis [54,57,60].

Immunosuppression, reactivation of residual parasite, or reinfection in a viscerally immune person is thought to be the underlying mechanism in the development of PKDL [54,58,61]. Mechanisms of parasite persistence in the host are not yet well established. However, increasing evidence indicates the involvement of host as well as parasite factors.

Predominantly, CD8$^+$ T cells are observed in dermal lesions as well as in lymph nodes of Indian PKDL, unlike in Sudanese PKDL patients where a preponderance of CD4$^+$ T cells is observed [57,62]. Increased production of interleukin (IL)-10 by keratinocytes and high levels of IL-10 in plasma and peripheral blood mononuclear cell cultures as well as elevated C-reactive proteins predicted the development of PKDL in Sudan [57]. Persistence of parasites in Indian PKDL despite high interferon (IFN)-γ and tumor necrosis factor (TNF)-α expression has been accounted to the presence of counteracting cytokines and minimal expression of IFN-γ receptor 1, and the TNF receptors TNFR1 and R2 [63,64]. Ample evidence is available to

conclude that an IL-10-rich milieu promotes *Leishmania* parasite persistence and reactivation in skin.

In the Sudanese population, PKDL susceptibility has been associated with polymorphism observed at promoter regions of IFN-γ receptor 1 and IL-10 genes. However, confirmatory experimental analysis to demonstrate a regulatory role of this polymorphism is awaited [65,66]. Such studies are yet to be performed in Indian PKDL.

In addition to immunological mechanisms, *Leishmania* genetic determinants also contribute to alterations of the host–parasite equilibrium in favor of the parasite, resulting in the persistence in the human host for up to 20 years in Indian PKDL. It is well established that parasites isolated from visceral leishmaniasis and PKDL patients are essentially the same [67], although polymorphism is observed at the 28S rRNA locus and β-tubulin locus [68,69]. Transient changes such as preferential expression of surface proteases in parasites isolated from PKDL lesions are suggestive of altered interaction with macrophages and may be responsible for the predilection of parasite to the dermis [69].

Current Treatment Options for PKDL

Treating PKDL patients is an important aspect of visceral leishmaniasis control programs as PKDL patients are deemed as the major reservoir in anthroponotic transmission settings. High incidence of refractoriness to antimony has been attributed to anthroponotic transmission via PKDL in India [70]. Three to four times longer treatment regimens than for visceral leishmaniasis, increasing antimony resistance, and poor patient compliance pose major challenges for treatment of PKDL. In Sudan, most of the PKDL patients self heal, and only severe and chronic patients are treated, while in Indian PKDL treatment is always required. In India, SSG is extensively used at a dose of 20 mg/kg/day for 120 days with cure rates of 64–92% [57,71]. As an alternative, various drugs including allopurinol, ketoconazole, and rifampicin alone or in combination with pentavalent antimonials have been tried in Indian PKDL with variable cure rates [72]. Amphotericin B and miltefosine, the frontline drugs for visceral leishmaniasis, have potential benefits for PKDL patients. Amphotericin B deoxycholate at a dose of 1 mg/kg/day by infusion for 60–80 doses over a period of 120 days was found 100% effective [73]. Miltefosine at a dose of 100 mg/day in divided doses for 12 weeks has been found effective in Indian PKDL [74]. However, a shorter regimen of 150 mg/day in three doses for 60 days was also found curative and advocated in patients capable of tolerating gastrointestinal symptoms caused by the drug [75].

Non-healing Sudanese PKDL patients with severe lesions are best treated with SSG at a dose of 20 mg/kg/day for 30 days, which may be prolonged to 2–3 months if necessary [57]. Liposomal amphotericin B at 2.5 mg/kg/day by infusion for 20 days has been effective with a cure rate of 83% without side-effects in Sudan [76]. Novel immunochemotherapy using alum-precipitated autoclaved *L. major* (Alum/ALM) vaccine plus Bacille Calmette-Guerin (BCG) and SSG was found safe and effective with a cure rate of 87% by day 60 in Sudanese PKDL [77].

However, systematic studies on larger sets of patients to evaluate drugs for treatment of PKDL are urgently needed to accomplish improved, effective, and shortened treatments.

Open Questions and Needs

Without doubt real progress has been made in the treatment of visceral leishmaniasis over the last decade. However, this progress was also driven by the repurposing of drugs initially developed for other indications and pragmatic approaches. A new chemical entity (NCE) dedicated to visceral leishmaniasis has yet to be identified along with candidates for fixed-dose combinations. Screening campaigns over the last 5 years have focused on high-throughput and high-content screening formats, yet the complexity of the *Leishmania* parasite and its lifestyle do not make it an easy candidate for this approach. Consideration has to be given to crucial aspects of parasite biology in order to identify compounds that hold potential to progress into treatments for visceral leishmaniasis [78]. There is also a strong need for refined models and approaches for preclinical candidate selection, optimal drug use, and dosing regimes with inclusion of pharmacokinetic/pharmacodynamic relationships in anti-leishmanial drug development.

Understanding of pathogenesis, host factors, host–parasite interactions, and regional differences in clinical treatment response require continued research efforts and are crucial to design optimal treatments for visceral leishmaniasis and PKDL. Lastly, the importance of access to treatment has to be emphasized for the effort of all disciplines involved in the drug discovery and development process to yield long-lasting fruits. Poor access to care for leishmaniasis remains a major barrier to control. Factors that determine access to drugs (drug affordability, drug availability, forecasting, distribution and storage, drug quality, drug legislation and pharmacovigilance, user-friendliness, etc.) have recently been reviewed and the importance of a concerted effort by all stakeholders emphasized [13].

Promising directions and strategies have been set and are ongoing. Now research efforts have to continue to support further progress in treatment of visceral leishmaniasis and PKDL.

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