Development of Drugs for the Treatment of Visceral Leishmaniasis: Pitfalls and Potential Drug Targets for Future Treatment and Elimination
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Background

Leishmaniasis is a parasitic disease caused by species of protozoa in the genus *Leishmania*. Three forms of the disease exist: cutaneous, mucocutaneous, and visceral leishmaniasis (VL), also known as kala-azar; the *Leishmania* protozoa are transmitted by the bite of the phlebotomine sandfly. Leishmaniasis is endemic in 88 countries worldwide, with an overall prevalence of 12 million people infected and over 350 million people at risk for infection.¹

Kala-azar is a lethal form of leishmaniasis in which the parasites are found throughout the body rather than simply confined to the reticuloendothelial system, and is characterized by fever, weight loss, hepatosplenomegaly, anemia and depression of the immune system; death occurs as a result of opportunistic infection resulting from the decreased activity of the immune system.² Kala-azar is endemic in 47 countries worldwide, with 60% of the cases occurring in India and 90% of India’s cases occurring in the Bihar State alone.³ VL is the second most common cause of death by parasite after malaria, with an estimated annual incidence of 500,000 cases and at least 50,000 deaths per year.¹³ In addition to significant mortality, VL causes extensive morbidity—in 2002, an estimated 2.1 million DALYs were lost due to VL.⁴ It is suggested that the actual mortality and morbidity burden of kala-azar is significantly higher than reported values, due to underreporting and difficulties in reporting due to deaths from
opportunistic infections.\textsuperscript{1} In addition to a high prevalence of VL in India, there is an ever-growing concern over coinfection of VL and HIV in areas of sub-Saharan Africa, particularly in Sudan and Ethiopia; coinfection rates of 34\% have been reported and HIV infection has been seen to increase the risk of developing VL by a factor of 100-1000 in endemic areas.\textsuperscript{5}

It has been shown that death can be avoided in 95\% of cases with timely access to treatment, even in basic field settings.\textsuperscript{3} However, as over 50,000 cases are dying each year, it is evident that there is an issue with access to effective interventions. To make matters more complicated, few efficacious treatments exist that are actually available to and utilized by the populations in greatest need. This report examines the current state of treatment options for VL and potential future drug candidates, while highlighting

\begin{figure}
\centering
\includegraphics[width=\textwidth]{leishmaniase_map.png}
\caption{Geographical distribution of visceral leishmaniasis. (Taken from den Boer et al. 2009)}
\end{figure}
the need for increased research and development of new efficacious and affordable drugs.

**Need for Treatments**

Due to a lack of commercial interest and a greater public health agenda, there has been an absence of research and development of novel drugs for treatment of neglected diseases. Between the years of 1975 and 2004, only two of the 1556 novel drug compounds that were developed were intended for the treatment of VL; furthermore, the two compounds, liposomal amphotericin B and miltefosine, were both developed primarily for the treatment for other diseases.\(^3\)\(^,\)\(^6\) Since the turn of the century, two non-profit organizations, the Drugs for Neglected Diseases initiative (DNDi) and the Institute for One World Health (iOWH), were founded and have been focusing resources on the development of novel treatments for neglected diseases, including kala-azar. Despite these new research efforts and the advances in drug development they have made, many of the new treatments for VL are unaffordable and still inaccessible to many in need. Our goal for a new drug target for VL is therefore a compound that will be short course, highly efficacious, deliverable in the community or out-patient setting, without resistance, affordable, and safe; unfortunately, no single treatment available today meets all of these criteria.\(^3\)
Existing Treatments

The first treatments developed for kala-azar were the pentavalent antimonial compounds, sodium stibogluconate and meglumine antimoniate, which were discovered in the 1940s and remain the mainstream treatments for all forms for leishmaniasis worldwide. Cure rates of 95% or higher have been obtained with the standard 30-day regimen; however drug resistance has been noted around the world, particularly in Bihar, India, where it is ineffective against VL in 60% of cases.\textsuperscript{3, 7} Although they are relatively inexpensive drugs, the antimonial compounds require intravenous or intramuscular administration, and include a high risk of serious side effects, including hepatotoxicity, nephrotoxicity, arrhythmias, reversible pancreatitis, and even death in African HIV/VL coinfection.\textsuperscript{3, 8} Their relative affordability, traditional usage, and efficacy in parts of the world allow the pentavalent antimonials to be continually used throughout the world, however they do not meet our needs for an idea drug target for kala-azar.

Pentamidine has been successfully used against many forms of leishmaniasis since the 1940s, however it has become a second-line drug due to the resistance that has developed in parts of the world. Its relatively low toxicity has led it to be used as a secondary prophylaxis for those coinfected with HIV/VL, however efficacy data are lacking on its use in coinfected individuals.\textsuperscript{3} Its potential as a first-line drug or potential ideal drug target has passed due to its resistance.
Paromomycin sulfate is a broad spectrum antibiotic compound that also demonstrates antiprotozoal properties; antileishmanial properties were identified in 1961 and it was first used against VL in the 1980s.\textsuperscript{3,9} Paromomycin has a low cost of $15 per patient per course and a low rate of side effects, making it a good drug target for kala-azar.\textsuperscript{3} Paromomycin, however, is administered over the course of 21 days in intramuscular injections, and has shown only a high efficacy against VL in India, making it a less desirable treatment option. Additionally, resistance has been easily demonstrated in the laboratory, making it a poor candidate for monotherapy.\textsuperscript{9} As the total treatment cost per patient is $15, it is the most affordable treatment option for VL.

Table I. Currently available treatments for VL. (Taken from den Boer et.al. 2009)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Pentavalent antimonials</th>
<th>Amphotericin B deoxychlorate</th>
<th>Liposomal amphotericin B (Ambisome)</th>
<th>Miltefosine</th>
<th>Paromomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>20 mg/kg daily for 30 days</td>
<td>1 mg/kg on alternate days x 15 doses in 30 days</td>
<td>Total dose of 20 mg/kg split over several doses (more required in Africa and in HIV+)</td>
<td>1.5 – 2.5 mg/kg daily over 28 days (India only)</td>
<td>15 mg/kg for 21 days (India only)</td>
</tr>
<tr>
<td>Administration</td>
<td>I.v. or i.m.</td>
<td>I.v.</td>
<td>I.v.</td>
<td>Oral</td>
<td>I.m.</td>
</tr>
<tr>
<td>Clinical efficacy</td>
<td>35 – 95% (depending on geographic region)</td>
<td>&gt; 97% all regions</td>
<td>Asia: Europe, Brazil: &gt; 97%; India: single dose 91%; Africa: not clearly established</td>
<td>Oral: India: 94 – 97%; Africa: 94% in immunocompetent VL</td>
<td>Oral: India: 94%</td>
</tr>
<tr>
<td>Resistance</td>
<td>As high as 60% (Bihar, India)</td>
<td>Not documented</td>
<td>Not documented</td>
<td>Lab isolates</td>
<td>Lab isolates</td>
</tr>
<tr>
<td>Toxicity</td>
<td>+++ Arhythmias, reversible pancreatitis, nephrotoxicity, hepatotoxicity, death especially in African HIV/ VL</td>
<td>+++ Nephrotoxicity (in-patient care needed), infusion-related fever</td>
<td>+ Minor/no nephrotoxicity, mild infusion-related fever</td>
<td>+ Gastrointestinal (20 – 50% of patients, usually mild), nephrotoxicity, hepatotoxicity, possible teratogenicity</td>
<td>+ Minor/no nephrotoxicity, reversible ototoxicity, hepatotoxicity (all relatively rare)</td>
</tr>
<tr>
<td>Issues</td>
<td>Prolonged treatment; painful injection; high toxicity, especially in HIV/ VL, resistance in India; recent drug quality problems</td>
<td>Prolonged treatment; need for slow I.V. infusion and nephrotoxicity requires hospitalization in relatively sophisticated setting; heat stability</td>
<td>Expensive; need for slow I.V. infusion requires hospitalization; heat stability (storage &lt; 25°C); not registered in all endemic countries</td>
<td>Prolonged treatment so that compliance in out-patients may be low; expensive; teratogenicity and long half-life require long contraceptive use; potential for resistance; not registered in Africa and Asia apart from India and not on WHO EML</td>
<td>Efficacy very low in Sudanese VL and unknown in South America; potential for resistance; relatively long treatment; only registered in India</td>
</tr>
<tr>
<td>Advantages</td>
<td>Registered in all endemic regions and included in WHO EML; can be given as ambulatory care (daily injections); relatively cheap</td>
<td>Registered in all endemic regions except Bangladesh and included in WHO EML; no resistance despite wide use</td>
<td>Relatively short treatment and included in WHO EML; extremely safe, also in HIV/VL; highest therapeutic index of all VL drugs</td>
<td>Oral treatment, can partly be given on outpatient basis; safe in HIV/VL; few side effects</td>
<td>The cheapest VL treatment; few side effects; included in WHO EML for the Indian subcontinent; can be given as ambulatory care (daily i.m. injections)</td>
</tr>
</tbody>
</table>

\textsuperscript{+}: Minor toxicity.
\textsuperscript{+++}: Major toxicity.
EML: Essential medicines list; I.m.: Intramuscular; I.v.: Intravenous; PM: Paromomycin; VL: Visceral leishmaniasis.
On its own, however, paromomycin is a poor drug target for VL due to its susceptibility for resistance and low efficacy in the field.

Amphotericin B deoxycholate has been shown to be a highly efficacious and relatively inexpensive treatment option for kala-azar in endemic regions around the world. It has been a second-line treatment for VL since the 1960s; however, the potential for serious side effects, such as nephrotoxicity, require in-patient administration over the 30-day treatment course, making it an undesirable course of treatment.

Lipid formulations of amphotericin B have been developed in recent years, and have been shown to improve the safety profile of the drug and improve its efficacy. These improvements have made this the most expensive treatment for VL, and therefore the most inaccessible to the most endemic countries; it is the drug of choice, however, against kala-azar in much of the developed world. An orally administered form is currently under development by DNDi and single-dose therapies have recently been shown to be incredibly efficacious against VL, making this a drug target of high potential in the future.

Miltefosine is another more recent drug development; it was developed in the 1980s as an anticancer agent and is the most recent drug to enter the market. Miltefosine is administered orally and lacks any serious side effects, making it a viable option for community or outpatient administration. In Phase IV trials in India, efficacy
rates of 82-95% were noted, which were slightly lower than other treatment options likely due to self-administration. Resistance has also been demonstrated in the laboratory, and will likely be higher in the field due to inconsistent self-administration and low adherence potential. Furthermore, it has been shown to be a potential teratogen, making contraceptive use in women a priority if miltefosine is administered. The oral administration of miltefosine gives it great potential as an ideal drug target for VL, however its lower efficacy and potential resistance make monotherapy impossible.

There are numerous drug treatment options for kala-azar currently available worldwide; none, however, meet all the requirements of an ideal drug target for VL.

**Developing Treatments**

In the next five years, there are no novel drug compounds that are expected to be approved for use against VL. Relatively few pipeline drugs are in development; as a result, efforts are currently focused on developing combination therapies using existing VL drugs as well as using known therapies for other diseases as treatment alternatives for VL.

Combination therapies offer the benefit of higher clinical efficacy, lower rates of toxicity and resistance, and greater cost-effectiveness by combining drug therapies in smaller doses. Currently, there are many continuing and planned combination VL treatment studies in process, mainly by the DNDi and other partner organizations. A majority of these studies in development are utilizing liposomal amphotericin B, oral
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Although many of these trials are ongoing, some have been completed and the data show that the combination therapies are more cost-effective than monotherapies and will likely prolong the life-span of the drug’s effective use. Until novel drug treatments are developed, combination therapies can be effective in decreasing the burden of VL in endemic areas using existing treatments.

There are also a number of “novel” drugs in development for VL—drugs that are approved for other uses but are being tested for use against kala-azar. In rodent models, the anticancer drug, tamoxifen, has been shown to treat and eliminate parasite burden for both cutaneous and visceral forms of leishmaniasis. Another existing drug that has found implications for treatment of VL in canines is domperidone, a dopamine D2 receptor antagonist. As canines are a reservoir of VL in parts of Latin America, this

Table II. Continuing and planned combination VL treatment studies. (Taken from den Boer et.al. 2009)

<table>
<thead>
<tr>
<th>Combination</th>
<th>Study location</th>
<th>Research performed by</th>
<th>Dose</th>
<th>Results expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambisome + MF</td>
<td>India, Bangladesh, Nepal</td>
<td>DNDI and partners</td>
<td>1 day Ambisome 5 MK + 7 days MF 1 day Ambisome 5 MK + 10 days PM 15 MK 10 days MF + 10 days PM 15 MKD</td>
<td>Study continuing, Phase III in India early 2010, Phase IV in all three countries in 2011</td>
</tr>
<tr>
<td>Ambisome + PM</td>
<td>East Africa</td>
<td>DNDI and partners</td>
<td>1 day Ambisome 10 MK + 10 days MF</td>
<td>Study planned, Registration of Ambisome and MF in 2011; results Phase III 2014</td>
</tr>
<tr>
<td>Ambisome + MF</td>
<td>India, planned for Bangladesh</td>
<td>WHO TDR/PaladinICMR</td>
<td>1 day Ambisome 5 MK + 14 days MF</td>
<td>March 2009 in India</td>
</tr>
<tr>
<td>Ambisome + SbY</td>
<td>East Africa</td>
<td>DNDI and partners</td>
<td>1 day Ambisome 10 MK +10 days SbY 20 MKD</td>
<td>Study planned; results Phase III 2014</td>
</tr>
<tr>
<td>SbY + PM</td>
<td>East Africa</td>
<td>DNDI and partners</td>
<td>17 days SbY 20 MKD + 17 days PM 15 MKD</td>
<td>Study continuing, PM registration 2010; results Phase IV 2012</td>
</tr>
</tbody>
</table>

DND: Drugs for Neglected Diseases initiative; ICIMR: Indian Council of Medical Research; MF: Miltefosine; MC: mg/kg; MKD: mg/kg/day; PM: Paromomycin; SbY: Pentavalent antimonials; TDR: WHO’s Special Programme for Research and Training in Tropical Diseases; VL: Visceral leishmaniasis.
drug could help eliminate the burden of kala-azar in parts of the world where it is endemic.\textsuperscript{1} Sitamaquine is a potential drug target that has undergone Phase II trials, however issues of safety and toxicity still remain under consideration, as well as issues of low efficacy (90\%).\textsuperscript{3}

One drug treatment of great potential is oral amphotericin B, which has been shown to be effective in mice against murine VL.\textsuperscript{11} This drug has overcome some of the physiochemical barriers to absorption in the digestive tract and shows promise for application in humans down the road. Oral administration of liposomal amphotericin B, an established highly efficacious treatment with low levels of serious side effects, shows incredible potential as an ideal drug target for VL. In the oral form, it would allow for community-based distribution and the increased demand would likely lower the high costs currently seen with the drug, which would make it the most practical option for VL treatment.

Current efforts to create a more robust drug pipeline for VL are in progress. With both the current sequencing of the *Leishmania* genome and the increased throughput of *in vivo* screening of compounds in macrophage amastagote models by DNDi, at least one new drug candidate is expected by 2012.\textsuperscript{3} Until that time, combination therapies and new applications of alternative drugs will need to be investigated.
Conclusions

Research into new drug treatments for VL is desperately needed to address the 500,000 incident cases of VL each year and the growing number of HIV/VL coinfections; however, these research initiatives are lacking considerably. With humans as the primary reservoir in India, where 60% of the world cases of VL occur, effective and accessible treatment could mean potential eradication, as there will no longer be a reservoir of disease. Development of non-profit organizations such as DNDi and iOWH have helped advocate for increased R&D and conduct new research into combination therapies and novel treatments, but the work of two non-profit initiatives is unlikely to develop a novel ideal treatment. The current status of drug availability for the treatment of VL is grim, with few, speculative opportunities on the horizon. In order to develop a novel, revolutionary treatment for VL, a treatment must be developed that will be highly efficacious, inexpensive, short course, available in the community or outpatient setting, safe and lacking resistance. Until we overcome the lack of commercial and political will, the situation will not improve, and visceral leishmaniasis will continue to be a neglected disease of extensive morbidity and mortality throughout the world.
References


