Sodium Stibogluconate: Therapeutic use in the Management of Leishmaniasis

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Received 30 July 2007; revised 17 December 2007

Leishmaniasis causes significant morbidity and mortality worldwide. The disease is endemic in developing countries of tropical regions, and in recent years economic globalization and increased travel has also spread to people in developed countries. In the absence of effective vaccines and vector-control measures, the main line of defense against the disease is chemotherapy. Organic pentavalent antimonials, including sodium stibogluconate have been the first-line drug for the treatment of leishmaniasis for the last several decades, and clinical resistance to these drugs has emerged as a primary obstacle to successful treatment and control. The present review describes the structure, activity, mode of action of sodium stibogluconate and mechanism of resistance towards this drug in leishmaniasis.

Keywords: Sodium stibogluconate, Visceral leishmaniasis, Resistance, Pentavalent antimonials

Introduction
Leishmaniasis is a parasitic infection caused by the obligate, intracellular protozoan of the genus *Leishmania* (family: Trypanosomatidae). Over 15 species of *Leishmania* capable of infecting humans have been classified into two main groups — Old World: *L. major*, *L. tropica*, *L. aethiopica* and the *L. donovani* complex (*L. donovani*, *L. infantum*, *L. chagasi*, *L. archibaldi*) and New World: *L. mexicana*, *L. amazonensis* and *Viannia* complex (e.g. *L. brasiliensis*, *L. guyanensis*). *Leishmania* has a digenetic life cycle with an extracellular developmental stage in the insect vector, a female phlebotomine sandfly, and an intracellular developmental stage in mammals. In sandflies, the parasites develop within the alimentary canal into flagellated and elongated form termed ‘promastigote’ that eventually matures in the insect midgut into an infective metacyclic promastigote. Inoculation into the mammalian host occurs when sandflies feed on blood meal. A typical inoculum contains around 100–1000 metacyclic promastigotes which quickly become engulfed by the leucocytes, particularly macrophages, neutrophils and dendritic cells. The parasites undergo a further transformation within these cells to form amastigotes.

Leishmaniasis is endemic in 88 countries with an estimated 12–15 million individuals infected and an annual incidence of around 2 million. The incidence of fatal visceral leishmaniasis (VL) is rising, largely due to urbanization and the human immunodeficiency virus (HIV) pandemic. Although most human cases occur as a result of transmission by sandfly bites, contaminated blood products and sharing of needles by intravenous drug users are other reported mechanisms of transmission.

Three major clinico-pathological categories have been recognized as cutaneous, muco-cutaneous and visceral form, each caused by distinct species. Following treatment of VL, a different clinical manifestation of infection with a condition termed ‘post-kala-azar dermal leishmaniasis’ might occur. The typical lesion of cutaneous leishmaniasis is a chronic ulcer with histological features of an intense lymphoid and monocytic infiltrate with granuloma formation. VL is characterized by dissemination of parasites throughout the patient’s reticuloendothelial system and after an incubation period ranging from 1 month to 2 years, patients typically develop pyrexia, wasting and hepatosplenomegaly.

First line drugs against visceral leishmaniasis

The pentavalent antimonial derivatives meglumine antimoniate and sodium stibogluconate (SSG) (Fig. 1) have been the main first line drugs for the treatment of all forms of leishmaniasis including VL since 1940. Meglumine antimoniate is marketed as Glucantime...
and Prostibanate. SSG is available commercially as Pentostam, Solustibosan, Stibanate, and generic sodium antimony gluconate. Amphotericin B (normal or liposomized) is used as an alternative, but has significant disadvantage of severe toxicity and high cost for a disease prevalent in third-world countries. The demonstration of the efficacy of miltefosine is a breakthrough, but single point mutations can lead to resistance, which suggests that resistance to this drug may occur rapidly. In this scenario, a review on the structure, activity and mechanism of resistance of SSG could help for efficient monitoring of pentavalent antimonial resistance at sites, where it is endemic.

Chemical structure and properties of sodium stibogluconate

SSG is chemically synthesized mixture formed by chemical reaction of stibonic and gluconic acids, rather than a single compound. Fractional analysis of SSG used against L. panamensis amastigotes demonstrated that the activity is associated with multiple chemical species. Pentostam is composed of multiple species having molecular weights ranging from 100-4000 Da and a low osmolarity of 789 milliOsmole for a 100 mg antimony/ml solution. In the dog, SSG is rapidly excreted from the body. Similar results have been reported for human patients given antimonial drugs. The main route of excretion for antimonial drugs is via the kidney.

Treatment against leishmaniasis

In areas with >94% response rates to antimonials, generic SSG remains the most effective option for VL treatment, mainly due to low cost. A comparative study between SSG and miltefosine treatment among Ethiopian patients coinfected with HIV demonstrated SSG to be more effective than miltefosine, an orally administered drug. During late 1970s and early 1980’s, different workers used different dose regimens of SSG to encounter the problem of unresponsiveness. Based on the pharmacokinetic study in Kenya, a prolonged dosage schedule of 10 mg/kg/day parenterally up to 60 days was recommended. It was also observed that children’s tolerance for higher and prolonged dosage of SSG was better than adults. In 1982, WHO recommended SSG in the dose of 20 mg/kg/day (maximum 850 mg) for 20-30 days in fresh cases and for double duration (40-60 days) in relapse cases (Report of the informal meeting on the chemotherapy of VL. WHO/TDR/Chem/Leish/VL/1982-83/Narobi); Subsequently, WHO (1990) recommended SSG in the dose of 20 mg/kg/day for 28 days. An interesting aspect of therapy with SSG is an apparent resistance to reinfection in previously treated mice. Resistance was observed in liver, but not in spleen or bone marrow and lasted for at least 6 days after the cessation of antimony treatment.

Species variation

Variation in the clinical response to SSG and meglumine antimoniate in VL has been a persistent problem in the treatment of leishmaniasis over the past 60 years. One explanation for this phenomenon is the intrinsic difference in species sensitivity to these drugs. In general, studies using the amastigote-macrophage model, L. donovani and L. brasilensis have been found to be 3- to 5-fold more sensitive to SSG than L. major, L. tropica, and L. mexicana.

Toxicity of sodium stibogluconate

Although pentavalent antimonial compounds are the most widely used drugs for the treatment of leishmaniasis, the side effects are frequent. Side effects include abdominal pain, vomiting, nausea, fatigue, headache, increase of liver enzymes, nephrotoxicity, arthralgia, fever, rash, cough, pneumonia, pancytopenia and reversible peripheral neuropathy. Recent studies suggest that elevation of amylase and lipase is common and that a subset of patients suffers clinically significant pancreatitis. Serious side effects, such as atrial arrhythmia and fibrillation, and ventricular arrhythmia, tachycardia and fibrillation are rare. It should be kept in mind that pentavalent antimony is contra-indicated in
patients with myocarditis, hepatitis and pancreatitis. Even the normal dose of SSG can lead to both cardiotoxicity and hematoxocity, because of its cumulative effect. Therefore, the patients with leishmaniasis being treated with antimony compounds should be observed cautiously for signs of cardiologic and hematologic changes. Neurological manifestations of SSG have been documented in Sudan, where exercise of Pentostam led to cases of cerebellar ataxia.

**Clinical resistance**

Although the selection of pentavalent antimonial resistant *Leishmania* has long been a part of laboratory studies, it is only in the past few years that acquired resistance has become a clinical threat. In most parts of the world, over 95% of previously untreated patients with VL respond to pentavalent antimonials, except the region endemic for VL in North Bihar (India) has the unique distinction of being the only region in the world, where widespread primary failure to SSG has been reported and so far uniquely contributed by *L. donovani* only. During the same period, only 2% of patients from the neighboring state of (Eastern) Uttar Pradesh failed treatment. There are reports of antimony resistance spreading to the Terai regions of Nepal, especially from the district adjoining hyperendemic areas of Bihar where up to 24% of patients seem to be unresponsive.

**Parasite resistance**

An *in vitro* amastigote-macrophage assay of *L. donovani* isolates from responders and non-responders has shown significant difference in amastigote sensitivity, suggesting acquired resistance in Bihar. In *L. infantum* isolates from immunodeficient and immunocompetent VL patients from France before and after meglumine antimonate treatment from 13 of 14 patients had shown decreased sensitivity in an amastigote-macrophage assay post-treatment. Although an amplicon has been observed in a few isolates from Sb-refractory patients, the significance of this observation has yet to be determined. Another concern is that increasing number of HIV/VL-coinfected patients will be a potential source for emergence of drug resistance. Furthermore, another route for spread of resistant parasites is the transmission of infection via needle sharing in HIV/VL-coinfected patients in Southern Europe.

**Mechanisms of action and resistance**

It is now generally accepted that all pentavalent antimonials [Sb (V)] are pro-drugs that require biological reduction to the trivalent form [Sb (III)] for antileishmanial activity. However, site (amastigotes or macrophage) and mechanism of reduction (enzymatic or non-enzymatic) remain controversial. Studies indicate that axenic amastigotes are susceptible to Sb (V) but not the promastigotes, suggesting that some stage-specific reduction occurs in this life cycle stage. But the mechanism by which amastigotes reduce Sb (V) is not clear. Both glutathione and trypanothione can non-enzymatically reduce Sb (V) to Sb (III), particularly under acidic conditions. However, promastigotes contain higher intracellular concentrations of trypanothione and glutathione than amastigotes and both stages maintain intracellular pH values close to neutral, independent of external pH. Thus, it is difficult to account for the selective action of Sb (V) against amastigotes stage by a non-enzymatic mechanism. As both stages can take up Sb (III) and Sb (V), the insensitivity of promastigotes to Sb (V) cannot be attributed to drug exclusion. Two possible candidates for the enzymatic reduction of Sb (V) to Sb (III) in amastigotes, a thiol-dependent reductase related to glutathione-S-transferases highly expressed in amastigotes and a homologue of a glutaredoxin-dependent yeast arsenate reductase have recently been identified. The level of expression of reductase has not been reported and the low specific activity of the recombinant enzyme with glutaredoxin raises questions as to the physiological nature of the electron donor in *Leishmania* spp.

Only a few studies on the mode of action of these drugs have been reported. Initial studies suggest that SSG inhibits macromolecular biosynthesis in amastigotes, possibly via inhibition of glycolysis and fatty acid oxidation, however, the specific targets in these pathways have not been identified. Recent studies have reported apoptosis in Sb (III)-treated amastigotes involving DNA fragmentation and externalization of phosphatidylserine on the outer surface of the plasma membrane. The mode of action of antimony in drug-sensitive *L. donovani* involves several effects on glutathione and trypanothione metabolism. Exposure to Sb (III) causes a rapid disappearance of trypanothione and glutathione from isolated amastigotes and promastigotes in *in vitro* culture. Significant portions of this thiol are effluxed from cells in approximately
equimolar amounts with the remainder being converted intracellularly to their respective disulfides (trypanothione and glutathione). Sb (III) has previously been shown to be a time-dependent reversible inhibitor of trypanothione reductase in \textit{in vitro} system. Since Sb (III) also inhibits recovery of intracellular thiols following oxidation with diamide, this possibly justifies inhibition of trypanothione reductase in \textit{in vivo} intact cells\textsuperscript{40}. The profound loss of these thiols (90\% in 4 h) coupled with the accumulation of disulfide (up to 50\% of the residual within 4 h) causes a marked decrease in cellular thiol redox potential. Similar effects on thiol levels and thiol redox potential have been observed when amastigotes are exposed to Sb (V), intrinsically linking the effects of the biologically active Sb (III) with the clinically prescribed Sb (V).

The mechanism by which \textit{Leishmania} acquire resistance to antimonials has been the subject of intensive research for several decades, often yielding apparently contradictory results. Diminished biological reduction of Sb (V) to Sb (III) has been demonstrated in \textit{L. donovani} amastigotes resistant to SSG\textsuperscript{44}. SSG resistant \textit{L. donovani} also shows cross-resistance to other Sb (V) drugs, but the same susceptibility to Sb (III) as the wild type, distinguishing it from the trypanothione pathway mutants described below. However, whether this mechanism also occurs in clinical isolates is not known\textsuperscript{45}.

The accumulation of Sb(V) and Sb(III) in promastigotes and amastigotes has been shown to be by different transport systems. Although Sb accumulation is lower in resistant forms than in sensitive forms, levels of accumulation could not be correlated to sensitivity in wild-type cells. Aquaglyceroporins have recently been demonstrated to mediate uptake of Sb (III) in \textit{Leishmania} and overexpression of aquaglyceropin 1 renders them hypersensitive to Sb (III)\textsuperscript{46}. Transfection of aquaglyceropin 1 in a Sb (V)-resistant field isolate also sensitizes it to SSG when in amastigote form in macrophages. Increased levels of trypanothione have been observed in some lines selected for resistance to Sb (III). This is due to increased levels of the rate-limiting enzymes involved in the synthesis of glutathione (γ-glutamylcysteine synthetase) and polymamines (ornithine decarboxylase), the two precursor metabolites to trypanothione\textsuperscript{47,48}.

Increased synthesis of glutathione and trypanothione from cysteine could help to replace thiols lost due to efflux as well as to restore thiol redox potential perturbed by accumulation of disulfides. Spontaneous formation of Sb (III) complexed with either glutathione, trypanothione or both has been demonstrated by proton nuclear magnetic resonance spectroscopy and mass spectrometry\textsuperscript{49}. Since glutathione-S-transferase (GST) is elevated in mammalian cells selected for resistance to arsenite, it has been proposed that formation of the metalloid-thiol pump substrates could be rate-limiting and GST could mediate this activity in \textit{Leishmania} spp. Surprisingly, GST is not detectable in \textit{Leishmania} spp., although there is an unusual trypanothione-s-transferase activity associated with the eukaryotic elongation factor 1B complex. The precise nature of the Sb-thiol complex remains uncertain but two routes of elimination of the complex can be envisaged. The first involves sequestration in an intracellular compartment and the second a direct efflux across the plasma membrane.

Earlier, it was observed that PgpA, a member of the ATP-binding cassette (ABC) transporters was amplified in some resistant lines\textsuperscript{50}. However, later it was found that this transporter was not responsible for drug efflux across the plasma membrane. First, overexpression of PgpA is reported to decrease influx of Sb rather than increase efflux, possibly due to a dominant-negative effect through interactions with other membrane proteins\textsuperscript{51}. Second, overexpression of PgpA does not mediate increased efflux of radioactive arsenite from cells or transport of arsenite across plasma membrane preparations\textsuperscript{52,53}. Finally, PgpA plays a relatively minor role in resistance and is localized in membranes that are close to the flagella pocket, the site of endocytosis and exocytosis in this parasite\textsuperscript{54,55}. Thus, the identity of the efflux pump in the plasma membrane and its role in resistance to antimonials remain to be determined. To further define the molecular mechanisms of resistance to antimonials in \textit{Leishmania} amastigotes, customized DNA microarrays have been used to screen for differentially expressed genes in an \textit{L. infantum} axenic amastigote cell line selected for Sb (III) resistance. This has indicated the ABC transporter MRPA as vital factor in conferring resistance to antimony in intracellular amastigote parasites\textsuperscript{56}.

However, the studies described above have identified PgpA as functioning to sequester Sb (III) in an in tracellular vacuolar compartment in \textit{Leishmania}. It is worth mentioning that resistance due to
intracellular sequestration of Sb (III) as a thiol conjugate would show higher rather than lower intracellular levels of Sb (III). Thus, either sequestration plays a minor role in resistance or the conjugates are rapidly exocytosed from the cell. The next important step is to relate mechanisms observed in laboratory studies to clinical resistance. In one study on field isolates amplification of a gene on chromosome 9, possibly involved in protein phosphorylation, has been identified. Unresponsiveness against SSG thus poses a serious problem in the management of patients with VL. In such cases, alternative drugs such as miltefosine or amphotericin B should be considered as second-line drugs.

Lipid-associated and liposomal formulations of sodium stibogluconate
To alleviate the problem of toxicity and to reduce the standard dose regimens, several laboratory studies have been done to formulate an efficacious vesicular SSG formulation. Reports suggest an enhanced antileishmanial activity of liposomal SSG than free form. But all these have been evaluated either based on their effectivity against liver parasites against experimental VL in resistant C57BL/6 mice or in acute infection models of VL. Neither SSG-encapsulated negatively charged liposomes nor neutral liposomes could successfully induce formidable protection against chronic infection. However, non-ionic vesicular entrapped SSG seems to be a promising formulation that can be used for clinical trials against leishmaniasis.

We have found that a single dose of 22 mg of total lipid/mouse of phosphatidylcholine-stearylamine cationic liposome (PC-SA) with 300 microgram of entrapped SSG (Sb) elicits a near complete protection against liver and splenic parasites in BALB/c mice infected with antimonial-sensitive AG83 strain of *Leishmania*. We also investigated the efficacy of PC-SA-Sb against patient isolated antimonial-resistant strain CK1R in *in vitro* infected macrophage model and *in vivo* infected murine model and found that treatment with 88 microgram of PC-SA/ml encapsulating 1.0 of microgram of Sb cleared-off more than 35% of resistant parasites *in vitro* cultured peritoneal exudates cells (Fig. 2). However, antileishmanial efficacy of PC-SA-Sb is more potent in *in vivo* infected mice, where 22 mg of total lipid/mouse of PC-SA-Sb demonstrates nearly 80% clearance of liver and spleen parasite load. Moreover, these formulations show promising activity even against deeply hidden bone marrow parasites (data not shown).

Immunological mechanism demonstrates that the PC-SA-Sb formulations induce dominant Th1 response, stimulating additional leishmanicidal mechanisms of macrophages by inducing nitric oxide production, with significant downregulation of immunosuppressive cytokines like interleukin-10 and transforming growth factor-beta. In addition, we observed that these SA-bearing cationic liposomes...
exhibit selective affinity towards phosphatidylserine enriched parasite membrane, resulting in specific disruption of membrane functionality of parasite. This dual mode of action of SA-bearing liposome based SSG therapy suggests that PC-SA-Sb could be a potential candidate for the future design of combinatorial therapy with SSG against VL.

Conclusion
Presently, there has been a changing response to pentavalent antimonials, and unresponsiveness to these drugs in certain part of Bihar is as high as 60 per cent. The advent of amphotericin B and its lipid formulations can be considered an important breakthrough with increased safety and shorter duration of treatment. Keeping the huge success of liposomal amphotericin in mind, the advent of combination therapy of cytotoxic liposome entrapped with SSG might provide an opportunity to clinicians to look at the combination chemotherapy of VL thus providing a safe and effective shorter course of treatment which would also be affordable.

Acknowledgement
We thank Siddhartha Roy, Director IICB, for supporting this work. The work was supported through grants by CSIR and UGC, Government of India.

References