Arsenite Resistance in Leishmania and Possible Drug Targets

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Abstract
Parasitic infections are of enormous public health importance. Leishmaniasis is currently regarded as the second-most dreaded parasitic disease after malaria (WHO). Visceral leishmaniasis or kala-azar, caused by *Leishmania donovani*, is the most fatal form of leishmaniasis afflicting millions of people worldwide. No vaccination is available against leishmaniasis and fast spreading drug resistance in these parasitic organisms is posing a major medical threat. All these emphasize the need for new drugs and molecular targets along with reappraisal of existing therapeutics. Identification and characterization of cellular targets and answering the problem of drug resistance in *Leishmania* has always been the main thrust of protozoal research worldwide. Model drug resistance phenotypes against drugs, viz. arsenite (an antimony related metal ion, the first line of treatment against leishmaniasis), have been widely used to address and understand mechanism of drug resistance. The present discussion is an attempt to understand the different factors associated with arsenite resistance in *Leishmania*.

Introduction
Leishmaniasis is a group of diseases caused by kinetoplastid protozoan parasite *Leishmania* sp. The causative organism of leishmaniasis are endemic in many parts of world and lead to three major clinical manifestation in human ranging from self-curing cutaneous lesions, noncuring disseminated mucocutaneous to life threatening visceral leishmaniasis. World Health Organization has shown concerns over the enormity of leishmaniasis that has been underrated for long chiefly due to nonreporting of cases in remote areas and the social stigma associated with the deformities. Impact of the leishmaniasis on public health can be judged from the expansion of endemic regions in last one decade, 12 million affected cases spread across 88 countries, 2 million new cases every year and annual mortality rate of more than 60,000 majority of which are children. Reports of coinfection of leishmaniasis with HIV in immuno-compromised host in more than 33 countries and its crossing the barrier of endemic regions has further aggravated the problem. Control of leishmaniasis continues to be elusive due to the absence of effective vaccines and efficient vector control measures; as a consequence chemotherapy remains the main weapon to combat the disease. Conversely, the lack of range of effective and nontoxic drugs; variation in efficacy as result of intrinsic variation in drug sensitivity; and the emergence of drug resistance limits the arsenal of antileishmanial drugs. It is the developing regions of the world, which are

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predominantly facing the wrath of drug resistance due to unavailable, expensive, newer chemotherapeutic agents. Steadily progressing chemoresistance to pentavalent antimonials (first line chemotherapy and arsenite related metal ion) and 3–5 fold difference in resistance observed between different *Leishmania* species against other alternative drugs like paramomycin, azoles and miltefosine urge to develop better drugs and identify new drug targets. Thus, unraveling the molecular mechanisms rendering the parasite chemoresistant is important to the discovery of new cellular targets and increasing our arsenal against this parasite.

**Arsenite Resistance in Leishmania**

Long clinical use of arsenicals and related antimony containing drugs in parasitic chemotherapy has resulted in undefined mechanism of resistance to these drugs in protozoans. Resistance to clinical drugs, the major impediment in the treatment of protozoal infection, has always counted on the ease to develop drug resistant in vitro cell lines that has been instrumental in understanding the mechanism of drug resistance. There have been number of reports for generation of an in vitro sodium arsenite (oxyanion) resistant cell lines in different *Leishmania* sp. to understand basic molecular mechanisms of drug resistance.

Drug resistance phenotype can result due to any of the following possibilities: (i) decrease in drug uptake; (ii) efflux of drug from parasite; (iii) loss of drug activation; and (iv) alteration of drug targets. Reports available till date suggests that the resistance to the oxyanion, arsenite, in the parasite *Leishmania* is multifactorial and involves events like xenobiotic conjugation and traffic, cytoskeleton phosphorylation, altered expression of different genes and enzyme systems. The following paragraphs detail the current concepts and advancements in this field.

DNA amplification has evolved as a very common means adopted by these parasites against drug pressure. Arsenic was found to be potent inducers of gene amplification in *Leishmania*. In most of the cases the gene amplified in response to drug is present as an extrachromosomal circular DNA molecule and code for enzyme or transporter system involved in detoxification process of drug. As found previously in methotrexate or tunicamycin resistant strains, duplicated parts of chromosomal region exist as extrachromosomal circular H-circle in arsenite drug variants of *Leishmania*. Direct involvement of 69kb H-circle was observed in resistance to arsenite as variants that revert to wild type after differentiation lack this extra DNA element. Two loci were found to be present in multiple copies in drug resistant variants. One is the H-locus, which harbors a P-glycoprotein related gene, ltpgpA, the first MDR homologue reported in *L. tarentolae*. Second locus is a 50kb linear amplicon of unknown function. ltpgpA and its homologue lmpgpA (reported from *L. major*) was reported to confer resistance to both trivalent arsenite As(III) and trivalent antimony Sb(III). Earlier P-glycoprotein related gene products of ltpgpA and lmpgpA were thought to be main players in mechanism for arsenite resistance. But it was later found that these amplicons could be attributed to low level of resistance to arsenite and antimonite and raise the possibility of the involvement of other mechanisms.

Arsenite resistant *L. tarentolae* has been reported to accumulate less arsenite than parental wild type cell line even when rate of arsenite accumulation was observed to be same in plasma membrane-enriched vesicle prepared from two strains. This suggested the involvement of some other drug resistance mechanism(s) being operative. Radioactive labeling and atomic absorption spectroscopic studies strongly suggested the involvement of an active drug extrusion system in arsenite resistance. Further the gene disruption studies of ltpgpA in *L. tarentolae* wild type and arsenite resistant strains showed that PgpA is not essential for resistance to oxyanions, although it might be required in the early steps of selection when resistance is being established. Eventually an ATP-coupled pump was identified to be involved in extrusion of metal-thiolates, i.e., thiolate derivatives of As(III). Low rate of transport was observed for free arsenite, but rapid accumulation was observed after reaction with reduced glutathione (GSH), conditions that favor the formation of As(GS)3. These reports suggest a novel mechanism in which pentavalent arsenite and antimony containing compounds are reduced to trivalent...