Scope

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Plan of the Study
Visceral leishmaniasis or kala-azar is the most fatal form of leishmaniasis which is caused by an obligatory intracellular protozoan parasite *Leishmania donovani*. With annual incidence of around 5,00,000 in 61 countries, India, Bangladesh, Nepal, Sudan and Brazil contribute more than 90% of total cases (Guerin et al. 2002). Since there is no vaccine available for prophylaxis (Brandonisio & Spinelli, 2002), the control of the disease relies solely on chemotherapy. The organic salt of pentavalent antimony [sodium antimony gluconate (SAG) or Sb(V)] has been the first-line drug for all forms of leishmaniasis since 1940 (Herwaldt, 1999). Unfortunately, the treatment is now eroded by large-scale increase in clinical resistance (Faraut-Gambbarelli et al., 1997; Lira et al., 1999) and becomes a major obstacle in the treatment. An increasing incidence of development of SAG resistance is a critical issue in India where over 50–65% of the estimated 2,50,000 annual cases are non-responsive to this first-line agent (Croft et al. 2006). With effective vaccines not yet in sight and the development of new drugs proceeding slowly, understanding the mechanism of antimony resistance becomes an urgent need. However, the progress of the work on understanding the drug resistance mechanism is probably not as rapid as parasites are becoming resistant (Ouellette, 2001).

The understanding of mechanism of antimony resistance mechanism will offer us: (i) to develop molecular probes or PCR-based diagnostics to monitor the development and spread of drug resistance in the field; (ii) design the chemotherapeutic strategy by development of tools to recognize resistance in very early infection which will prevent useless and often toxic chemotherapy; (iii) idea about more rational use of drugs and drug combinations to minimize development of resistance; (iv) to develop drug analogues effective against resistant parasite; (v) to find some novel drug targets; (vi) to reverse the resistance by inhibiting resistance mechanism. Moreover, we could also be able to stop the emergence of resistance to other parts of the world where pentavalent antimonials are still useful and their cheaper generic brands are available.

Drug resistance is a complex phenomenon which may be implicated by several metabolic pathways and membrane transporters. Differential gene expression study has become a standard tool for identifying the role of genes and studying wide range of biomedical and biological questions. DNA microarray offers analysis of expression of thousands of genes in a highly parallel, serialized and very rapid way. Once target genes are identified, characterization of the relationship of their biological functions to the process of drug resistance can be established. DNA microarrays has been proven a valuable tool for identification of methotrexate resistance mechanism in *Leishmania* (Guimond et al. 2003) therefore, it may be a powerful tool to study the multiplicity of the antimony clinical resistance mechanism.
Monitoring drug resistance in the field is also a big task as there is no molecular marker of SAG resistance available. *In vitro* amastigote macrophage model (Carter et al., 2001; Lira et al., 1999) involving manual counting of stained parasite is the only reliable method available for monitoring the resistance of isolates but it is laborious, time consuming and demand technical expertise. Therefore, method of drug-susceptibility tests is urgently required to set up feasible and efficient monitoring of regular drug use, drug response and the spread of resistance.

Present study aim to study the molecular mechanism of SAG resistance in field isolates of *L. donovani* by using differential gene expression analysis with the following objectives:

- Collection of unresponsive and responsive *L. donovani* clinical isolates and determination of their *in vitro* growth curve
- Development of luciferase reporter gene assay for monitoring resistance phenotype in field isolates.
- Sequencing of differentially expressed gene and its annotation and validation by real-time PCR.
- Cloning, over-expression and characterization of the selected gene.
- Establishment of the role of selected gene in clinical antimony resistance.

These objectives would help us in understanding the mechanism of drug resistance in the field. The expression of luciferase in field isolates will be useful for monitoring of resistance in field and development of *in vitro* assay for large scale screening of antileishmanials in HTS as well.