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Role of Serine *o*-acetyltransferase protein of *Leishmania donovani* in Amphotericin B drug resistance and oxidative stress tolerance

Afreen[1] and Vahab Ali[1]

1. Department of Biochemistry, Laboratory of Molecular Biochemistry and Cellular Biology, ICMR-Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, Bihar, India
*neerfarahzam@gmail.com

Background

Leishmaniasis, a neglected tropical disease caused by the parasite *Leishmania donovani*, manifests in various forms, the most severe of which is visceral leishmaniasis (VL). In humans in Bihar state of India, the prevalence of Post-KalaAzar Dermal Leishmaniasis, and HIV-VL coinfection is steadily rising, and the emerging cases of drug resistance make it difficult for the current therapeutics to eliminate VL. Investigating the essential metabolic pathway of *L. donovani* becomes crucial in the hunt for novel chemotherapeutics. Cysteine is important for parasite growth, motility, and oxidative stress defense [1]. The *de novo* cysteine biosynthetic pathway of *L. donovani*, involving two key proteins- serine *o*-acetyl transferase (SAT) and cysteine synthase (CS), is absent in humans and its product, cysteine regulates the downstream elements of trypanothione-based thiol metabolism, crucial for maintaining cellular redox homeostasis.

Materials and methods

The role of SAT protein in parasite infectivity and survival under drug pressure and oxidative stress was investigated by generating a SAT overexpressing *L. donovani* (LdSAT-OE) strain. SAT overexpression was confirmed by performing SAT enzymatic assay, GFP fluorescence, and immunoblotting. Cell viability and antioxidant enzyme assays were performed. J774A.1 macrophage infectivity was assessed.

Results

A possible involvement of SAT protein in the development of drug resistance was revealed by the observation that the LdSAT-OE survived better when subjected to drug pressure (e.g., Amp B). The expression and activity of the cysteine pathway proteins- LdSAT and LdCS as well as other downstream thiol pathway proteins was found to be elevated, similar to what was observed in drug-resistant promastigotes, which further confirmed the significance of LdSAT protein in drug resistance. In LdSAT-OE promastigotes, a reduction in intracellular reactive oxygen species (ROS) was seen along with an increase in thiol content. The reduced superoxide production in LdSAT-OE and the increased antioxidant activities, such as APx and SOD, further highlight the role of the SAT protein in stress tolerance. *In vitro* J774A.1 macrophage infectivity was assessed which revealed that LdSAT over-expression enhances the parasite infectivity (Figure 1).

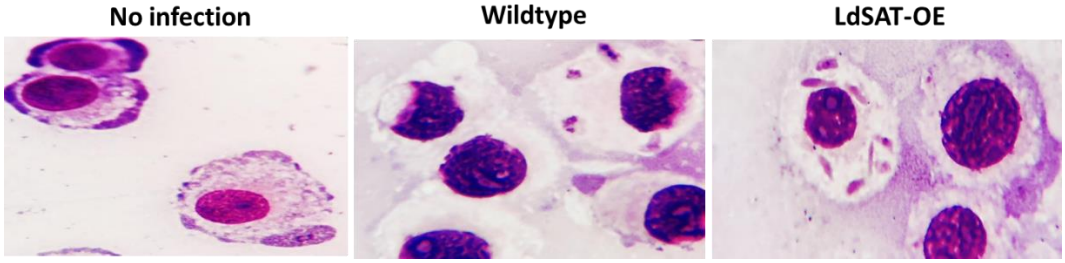

Figure 1: Macrophage infectivity. Giemsa stained J774A.1 macrophages without infection and with infection by wildtype and LdSAT-OE parasites.

Conclusions

Our work reveals the differences in drug sensitivity, oxidative stress susceptibility and percentage of infectivity between LdSAT-OE and wildtype, which indicates that SAT overexpression confers a survival advantage in *L. donovani* by regulating thiol-based antioxidant defense mechanism.

Funding: self-funded.

Conflict of interest: none declared.

References

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