Deciphering the interplay between cysteine synthase and thiol cascade proteins in the survival of L. donovani under oxidative stress

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Background: Leishmania possesses a unique trypanothione-dependent redox metabolism with pivotal role in protection from oxidative damage and drug resistance. The cascade of trypanothione biosynthesis depends on L-cysteine as the precursor, whereas, cysteine bioavailability is self-dependent on the cysteine biosynthesis pathway which includes enzyme cysteine synthase (CS). However, despite the apparent dependency of redox metabolism on cysteine biosynthesis pathway, the role of CS in drug resistance and redox homeostasis has remained unexplored. Herein, we have attempted to investigate the role of LdCS in Amphotericin B (Amp B) sensitive vs. resistant isolates of L. donovani.

Methods & Materials: LdCS was cloned in pXG-GFP+ vector to express LdCS as fusion proteins with a C-terminal GFP tag. The construct LdCS-GFP was transfected by electroporation in the L. donovani sensitive strain promastigotes and transformants selected up to final concentration of 200 μg/ml G418. MTT assay was performed to determine IC50 value of LdCS-GFP overexpressor and Amp B sensitive strains of L. donovani under different ROS inducers such as, \( \text{H}_2\text{O}_2 \), menadione and SNAP. Further, ROS levels, thiol content and enzymatic activities of LdCS, peroxidase and SOD were analyzed.

Results: Our results demonstrate stage-specific increase of LdCS expression and its enzymatic activity, accompanied by a higher thiol content, which implies that LdCS is upregulated in Amp B resistant isolates and during stationary stages of growth to meet the increased thiol demand of respective stages/isolates culminating into enhanced stress tolerance. In fact, overexpression of LdCS-GFP in sensitive strains imparted enhanced oxidative stress tolerance to the over-expressing parasites as compared to the wild type (WT) parasites. The IC50 values of LdCS-GFP toward \( \text{H}_2\text{O}_2 \), menadione and SNAP was found to be \( 217 \pm 8.7 \, \mu \text{M} \), \( 16.5 \pm 2.5 \, \mu \text{M} \) and \( 370 \pm 9.7 \, \mu \text{M} \), respectively, which was ~1.87, ~2.21 and ~1.34 fold higher than WT parasites. Furthermore, enzymatic assays, thiol content and immunoblot analysis showed that these oxidants induced LdCS-GFP, as well as endogenous CS and thiol cascade proteins expression in L. donovani suggesting a ROS regulated mechanism of LdCS expression and thiol pathway proteins.

Conclusion: The LdCS expression is modulated by ROS presumably to cater the metabolic demands of trypanothione and hence, alleviate oxidative stress.

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