Abstract

Title: Multi-Omics Profiling Reveals Sterol C22-Desaturase Loss as a Mechanism of Amphotericin B Resistance in *Leishmania infantum*

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The Leishmaniases are a spectrum of diseases afflicting millions in the tropical and sub-tropical world, where their sandfly vectors reside. Drugs are central to efforts to manage and control leishmaniasis although current therapies suffer a number of drawbacks. The increasing incidence of drug treatment failure with antimonial based drugs has led to amphotericin B, particularly in its liposomal formulation, becoming the drug of choice in many localities. The drug acts by binding ergosterol in the parasite membrane, leading to cell lysis. *Leishmania infantum* promastigotes were selected *in vitro* for resistance to Amphotericin B. Whole-genome sequencing identified a 21-bp deletion in one allele of the Sterol C-22 desaturase (SC22D) gene, that encodes a specialized P450-like enzyme identified as Cyp710C1. The enzyme is responsible for conversion of 5,7,24(28)-ergostatrienol to Ergostatetraenol a precursor to ergosterol. RNA sequencing revealed that only the allele carrying the deletion was present at the RNA level. Analysis of the sterol profile indicated that Cyp710C1's substrate accumulated while ergosterol was lost. A novel fluorescent probe specific for ergosterol also demonstrated loss of ergosterol in resistant parasites *in situ*. Over-expression of this mutant allele in WT parasites yielded amphotericin B resistance while over-expression of the WT

allele in the mutant cell line restored sensitivity. Resistant parasites were able to infect macrophages *in vitro*. Moreover, they retained virulence to mice and the resistant line was less susceptible to amphotericin B compared to WT in *in vivo* model. Protein structural modelling indicated that the mutation rendering the protein no longer able to function appeared in a predicted loop at the enzyme's surface and modelling possible protein-protein interactions implicated this loop in binding to one of the three cytochrome P450 oxidoreductase isoforms in the parasite.

Keywords: *Leishmania infantum* | Drug resistance |Sterol C22 desaturase | Deletion variant.