Abstract

Title: A mutation in sterol C22 desaturase leading to Amphotericin B resistance in *Leishmania infantum*

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Visceral Leishmaniasis is a neglected tropical disease, caused by parasitic protozoa of the *Leishmania* genus. In recent years, the polyene amphotericin B (AmB) has emerged as a treatment of choice against the disease. The drug acts by binding ergosterol in the parasite membrane, leading to cell lysis. *L. Infantum* promastigotes were selected for resistance to AmB. Sterol analysis of the resistant line identified a loss of ergosterol and an increase in 5,7, 24(28)-ergostatrienol. Genome sequence analysis revealed mutations in sterol C22 desaturase which converts 5,7, 24(28)-ergostatrienol to ergostatetraenol. The mutation comprises a 21 base pair deletion corresponding to a 7 amino-acid hydrophobic patch at the periphery of the enzyme. Over-expression of this mutant allele in WT parasites also yielded amphotericin B resistance while over-expression of the WT allele in the mutant cell line restored sensitivity. Moreover, the resistant parasites retained virulence in mice and the resistant line was not cleared by amphotericin B whereas WT were in treated mice. The data indicate that amphotericin B resistance in *Leishmania infantum* can come about through changes to the sterol pathway, as previously indicated in other amphotericin B resistance leishmania lines where mutations to different enzymes in the sterol pathway were noted. Interestingly, the *L. infantum* AmB resistant line described here is hypersensitise to nitric oxide inducing agents and also to pentamidine, as has been described for other AmB resistant lines, offering a potential route to treatment of resistant cases should their emergence become problematic in the field.