

Pentostam activation and uptake in *Leishmania major*: Role of the Sb(V) reductase LmAcr2 and the aquaglyceroporin LmAqp1.

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The pentavalent antimonial Pentostam, a first-line drug for leishmaniasis, is taken up by the macrophage and reduced to Sb(III), the active form of the drug. We propose that a portion of the drug is reduced in the macrophage, and Sb(III) is taken up by the amastigote and that another portion is reduced directly by the parasite. The relative contributions of these two pathways may depend on the relative rates and expression of their respective components in both the human host and parasite, which could lead to variability in drug response. We propose that Pentostam resistance can occur by mutation or downregulation of the uptake system or by loss of the Pentostam reductase. First, we identified and characterized the aquaglyceroporin LmAqp1 from *L. major* and demonstrated that it facilitates Sb(III) uptake. Transfection of *LmAQP1* into three strains produced Sb(III) hypersensitivity in all three. A variety of drug resistant parasites became hypersensitive after expression of *LmAQP1*, correlating with increased rates of Sb(III) uptake. Transfection of *LmAQP1* in a Pentostam resistant field isolate also sensitized the macrophage-associated amastigote form, which indicates that the macrophage reduces Sb(V) to Sb(III). Disruption of one allele of *LmAQP1* in *L. major* resulted in a 10-fold increase in resistance. This is the first report of uptake of a metalloloid drug by an aquaglyceroporin in *Leishmania* and suggests a strategy to reverse resistance in the field. Second, the sequence for ScAcr2p, the *S. cerevisiae* arsenate reductase, was used to clone the *LmACR2* gene, which complemented *ACR2* in *S. cerevisiae*. Transfection of *L. infantum* with *LmACR2* augmented Pentostam sensitivity in intracellular amastigotes. Purified LmAcr2 was shown to reduce Sb(V). This is the first report of an enzyme that confers Pentostam sensitivity in intracellular amastigotes of *Leishmania*, which indicates that the amastigote takes up both Sb(V) and Sb(III), and that the Sb(V) must be reduced to Sb(III) inside the parasite for drug action.

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