Activation of bicyclic nitro-drugs by a novel nitroreductase (NTR2) in *Leishmania*

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Recently, the bicyclic nitro-compounds (*R*)-PA-824, DNDI-VL-2098 and delamanid have been identified as potential candidates for the treatment of visceral *Leishmania*sis. Using a combination of quantitative proteomics and whole genome sequencing of susceptible and drug-resistant parasites we identified a putative NAD(P)H oxidase as the activating nitroreductase (NTR2). Whole genome sequencing revealed that deletion of a single cytosine in the gene for NTR2 resulted in expression of a non-functional truncated protein. Susceptibility of *Leishmania* was restored by reintroduction of the WT gene into the resistant line, which was accompanied by the ability to metabolise these compounds. Overexpression of NTR2 in WT parasites rendered cells hyper-sensitive to bicyclic nitro-compounds, but only marginally to the monocyclic nitro-drugs, nifurtimox and fexinidazole, known to be activated by a mitochondrial oxygen-insensitive nitroreductase (NTR1). Conversely, a double knockout NTR2 null cell line was completely resistant to bicyclic nitro-compounds. Recombinant NTR2 was capable of reducing bicyclic nitro-compounds in the same rank order as drug sensitivity *in vitro*. Thus, NTR2 is necessary and sufficient for activation of these bicyclic nitro-drugs. These findings may aid the future development of better, novel anti-*Leishmania*l drugs.