Benznidazole uptake by *Trypanosoma cruzi* is a determinant of variable drug efficacy and treatment failure

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Benznidazole (BZ) is the front-line treatment for Chagas disease. However, there is extensive variation in susceptibility within natural populations of the causative agent *T. cruzi*, and treatment failures are widely reported. The underlying reasons for this diverse efficacy are unknown. We used a range of genetic, cell biology and biochemical approaches to dissect the mechanisms of BZ resistance in *T. cruzi*. In combination with high resolution imaging and *in vivo* studies, this allowed us to identify BZ uptake as a major determinant of parasite susceptibility.

We show that BZ uptake by *T. cruzi* is mediated by endocytosis and that stage-specific and strain-specific differences in this process have important roles in drug efficacy. There is also considerable heterogeneity in drug accumulation by amastigotes, the replicative intracellular form of the parasite, even within the same infected host cell. Following uptake, BZ rapidly transits to the mitochondrial network, the site where it undergoes reductive activation. In the infectious, non-replicative trypomastigote life-cycle stage, low-level drug uptake is associated with reduced susceptibility. In addition, naturally resistant parasites have a reduced drug uptake capacity, a phenotype associated with treatment failure in experimental infections. To add further complexity, BZ uptake by mammalian cells, which is also endocytosis-mediated, varies between different host cell types. Our results therefore demonstrate that differences in BZ uptake, acting at several levels, provide a mechanism to explain the wide divergence in sensitivity within the *T. cruzi* population and highlight why sterile cure with this drug can be difficult to achieve.