Abstract

Leishmaniasis is an important disease for which existing therapies are inadequate. *Leishmania* parasites are auxotrophic for several amino acids and must acquire these from the host, presenting potential drug targets for parasite control. Exogenous phenylalanine is required for *Leishmania* growth in vitro, and we aimed to investigate its essentiality and uptake.

Using a defined growth medium, we observed that *Leishmania mexicana* promastigotes deprived of phenylalanine for a few hours could recover when phenylalanine became available. However, phenylalanine deprivation longer than 4 hours resulted in cell death, underscoring the potential of phenylalanine uptake as a drug target. We employed bioinformatic strategies to predict aromatic amino acid transporters in *L. mexicana* and reverse genetic approaches to identify phenylalanine transporters. Additionally, stable isotope-labelled phenylalanine was used for metabolomic analysis to track the fate of this amino acid in parasites.

Furthermore, phenylalanine deprivation impacted the cell cycle of *L. mexicana*. Flow cytometry showed normal cell cycle distribution at baseline (0 hours) with the majority in G1 phase. After 6 hours, there was a significant reduction in viable cells, with most arrested in G1, suggesting prolonged phenylalanine exposure may induce cell cycle arrest. Also, Transport assays were performed using radiolabelled

3H Phenylalanine, to determine the kinetics of uptake.

These findings enhance our understanding of phenylalanine uptake and its potential as a therapeutic target in leishmaniasis treatment.