Advancing sustainable drug development: comparative preclinical study of H80 and Miltefosine using imaging and proteomics

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Leishmaniasis treatment demands safer, orally available drugs with shorter treatment durations than current options. Although Miltefosine (MIL) is effective, it comes with severe side effects. Our research aims to surpass MIL's limitations by discovering compounds like H80, which has shown potent activity against various Leishmania strains with minimal drug resistance. Our study focuses on understanding H80's mechanism of action and molecular targets, crucial for rational drug design. Using fluorescence imaging and mass spectrometry-based proteomics, we analyzed the protein expression profiles of Leishmania parasites treated with H80 and MIL. Our findings revealed significant overlap in differentially expressed proteins (DEPs) between H80 and MIL treatment, particularly those involved in membrane transport and biosynthesis. This convergence suggests shared pathways impacted by both compounds, offering insights into their mechanism of action. Furthermore, fluorescence imaging shows H80's cellular uptake mechanism, indicating endocytosis-mediated internalization and cytoplasmic localization within parasites. Our research will investigate deeper into the biochemical pathways modulated by H80 compared to MIL, aiming to identify specific protein interactions impacted by H80 using proteomic techniques like LC-MS/MS analysis on promastigote *(L.infantum)*. We also compared two treatments of H80 (EC10 and EC50) to further explore the mechanisms of cell death in the parasite. These comparative studies of H80 treatments showed that Leishmania parasites responded in a dose-dependent manner, providing a more sophisticated understanding of how different H80 concentrations affect various cellular pathways and death mechanisms.

In conclusion, imaging studies demonstrated that THP-1 cells internalize H80 via endocytosis, leading to the colocalization of the compound and parasite in the cytoplasm of the macrophages. According to proteomics, H80 influences cytosolic proteins in L. infantum, while miltefosine modifies membrane components. Our results indicate that the mechanism of parasite death involves a decrease in vacuolar acidity, with pH playing a central role in the developmental switch between promastigote and amastigote forms, crucial for the parasite's cell cycle.

In the future, SeqAPASS (Sequence Alignment to Predict Across Species Susceptibility) will be utilized to analyse target proteins across diverse species, predicting ecotoxicological risks and identifying susceptible non-target organisms. This method aims to improve the safety of drug design by evaluating possible side effects and reducing environmental impact while maximizing therapeutic efficacy.

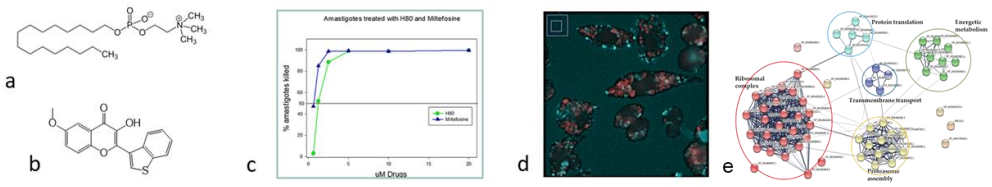

Figure 1. (a) Miltefosine structure. (b) H80 structure. (c) EC50 of Miltefosine and H80 in L *infantum* amastigotes. (d) fluorescence-based immunoassay for internalization study of H80 (e) MS samples were analysed with Progenesis (Waters) with a label free approach and the main pathways were studied with String software (STRING Consortium 2023)

References

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