Development of new drugs for leishmaniasis: what can we learn from the in vivo profiling of several promising chemical series

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Introduction

Drugs for Neglected Diseases Initiative (DNDi) is a collaborative, patients’ needs-driven, not-for-profit organization whose mission is to develop and make available new drugs for the most neglected tropical diseases, such as visceral leishmaniasis (VL). DNDi’s Lead Optimization programs aim to develop and improve chemical entities that have to fulfill the Target Product Profile (TPP) in terms of efficacy, safety and cost. To this end, building a robust and accurate screening cascade for identifying potential candidates from discovery screening to preclinical development is crucial. This is a dynamic, two-way process that depends on the results obtained through in vivo profiling and, retrospectively, from clinical outcomes.

In this analysis, efficacy data were obtained using the Syrian hamster model developed at LMPH (Laboratory of Microbiology, Parasitology and Hygiene). This hamster model, which aims to predict results in humans as much as possible, showed robust and highly reproducible results. Supplemented by PK data in the same species it allowed us to start building a preliminary assessment of the Pharmacokinetic/Pharmacodynamic (PK/PD) relationships for two new VL chemical series, oxaboroles (Anacor collaboration) and nitroimidazoles (TB Alliance/Auckland University collaboration).

Methods

In vitro efficacy determination for compound selection. In vitro IC50 against L. infantum are determined in an intramacrophage assay (LMPH). Following infection of primary peritoneal mouse macrophages by L. infantum (MHOM/MA/88/17) and 5 days incubation with compound, parasite burdens (number of amastigotes/macroage x number of cells counted) are microscopically examined after Giemsa staining. Only compounds that show (i) good in vitro anti-parasitic activity, (ii) an absence of cytotoxicity, and (iii) appropriate in vitro PK and physicochemical parameter progress to in vivo assessment.

In vivo PK in female golden Syrian hamster. PK analysis (profile over time, Tmax, Cmax, AUC0–t) is made after a 50 mg/kg single oral doses administration in fasting condition. Three animals and per group per site and plasma samples are collected at 1, 8 and 24 hours (oxaboroles) or 0.25, 0.5, 1, 2, 4, 8 and 24 hours (nitroimidazoles)

In vivo efficacy assessment. Determination of in vivo efficacy is performed at LMPH in the early curative model. Mifepristone (40 mg/kg, qd) is used as control.

PK/PD analysis. Analysis is made on 10 nitroimidazole and 9 oxaborole compounds as series representatives.

Results

For both nitroimidazoles and oxaboroles, percent reduction of amastigote burden is:

• Linear between the three organs (spleen, liver and bone-marrow).
• >Liver > spleen > bone-marrow.
• More variation-sensitive in bone-marrow that is a tissue, not weighed.

PK/PD parameters

Reduction of spleen weight - an indirect measurement of in vivo efficacy

• Relation between spleen weight reduction and percent reduction of amastigote burden is exponential.
• High reduction of spleen weight (>70%) is only measured for the best compounds: nitroimidazoles VL-2098, 32380, 35261 and oxaborole SCYX-5804 with respectively 100% and 99.3% reduction of amastigote burden in liver.

Conclusion

This model is being consolidated by:

• Continuing to gather data on oxaboroles and nitroimidazoles and performing this PK/PD analysis on other chemical series.
• Refining the analysis with various PK/PD sub-series of oxaboroles and nitroimidazoles.
• Including more parameters such as IC50 on other Leishmania strains, additional and more stringent PK parameters (spleen weight, sterile cure), efficacy in other animal models, in vivo ADME data and various PK parameters following IV administration.
• Better understanding the relevance of splenomegaly in hamster versus splenomegaly in humans that is used as diagnostic criterion for VL.
• Evaluating existing drugs or reformulated drugs to benchmark this model as a function of the different mechanisms of action (MoA) and, more importantly to reinforce it as a predictive model for human studies.

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In vitro versus in vivo efficacy

• An accurate triage of compounds before in vivo studies is crucial.
• Fine-tuning of selection criteria is possible by looking back at in vitro IC50 values of compounds that were tested in vivo.
• For Lead Optimization, an IC50 cut-off of 1µM against L. infantum seems to be appropriate for selecting excellent compounds only (percent reduction of amastigote burden in spleen >29%), with a few exceptions like 35500.

PK/PD parameter 1:

• For both chemical series, most compounds that show good in vivo efficacy have a ratio Cmax/IC50 > 5.
• Fixing a ratio of Cmax/IC50 > 5 as a predictive cut off for in vivo efficacy would have missed nitroimidazole 34317 and oxaborole SCYX-1103, that show 99.8% and 98.1% reduction of amastigote burden in spleen, respectively.

PK/PD parameter 2:

• For both chemical series, most compounds that show good in vivo efficacy have a ratio AUC0–24h/IC50 > 20.
• Fixing a ratio of AUC0–24h/IC50 > 20 as a predictive cut-off for in vivo efficacy would have missed only one compound: oxaborole SCYX-1103, that shows 98.1% and 99.4% reduction of amastigote burden in spleen and liver, respectively.

PK/PD parameter 3:

• For both chemical series, most compounds that show good in vivo efficacy have a duration of exposure above IC50 that is higher than 4 hours (Time above IC50 > 4h).
• Fixing a threshold of Time above IC50 > 4h as predictive for in vivo efficacy would have missed only one compound: oxaborole SCYX-1103, that shows 98.1% and 99.4% reduction of amastigote burden in spleen and liver, respectively.