Susceptibilty of *Leishmania* promastigotes to Amphotercin B

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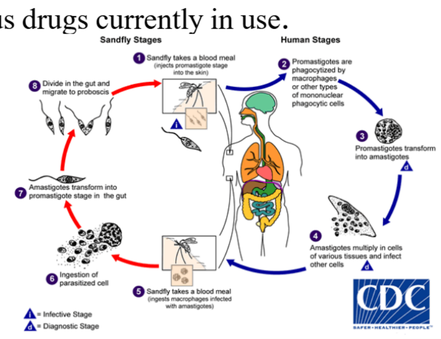
Introduction

Leishmaniasis is a protozoal infection causing significant morbidity and mortality worldwide, despite aggressive efforts to control the disease. Multiple pharmacologic agents have been shown to be effective in the treatment of this infection; these include pentavalent antimonials, various formulations of amphotericin B, miltefosine, paromomycin, pentamidine and sodium stibogluconate. (Burza et al., 2018).

However, significant issues still exist with the clinical use of these drugs, including adverse effects, costs, and routes of administration and the increasing rate of drug resistance in many *Leishmania* species(Ghorbani 2018). In addition, one of the challenges in addressing the clinical treatment of the infection is that the considerable variation in susceptibility among some *Leishmania* species to the various drugs currently in use.



Cutaneous Leishmanias (left)

Leishmania promastigotes (right)

Objectives

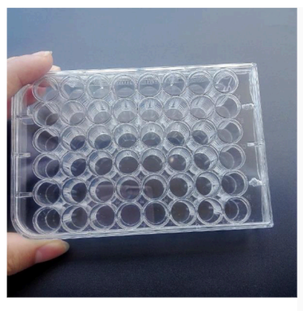
In this study, we compared the efficacy of one of the major anti-leishmanial drugs, amphotericin B, against two of the major strains of *Leishmania*, *L. donovani* and *L. major*, in dose-dependent lethality assays.

Methods/Project Design

Cell Culture:
Logarithmically growing *Leishmania donovani* and *Leishmania major* cells were seeded into a flask in supplemented complete M199 media. Cells were incubated at 25°C until cell concentration reached approximately 2.5 x 10^7 cells/ml. Cell concentrations were determined by hemocytometer counting.

Inhibition assay:
Cells were transferred to 48 well culture plates as 0.5mls per well and allowed to habituate for 24hrs. Amphotericin B was added to wells to the following final concentrations: 0, 0.1 µM, 0.5 µM, 1.0 µM, 5.0 µM, and 10 µM. After 24 hrs, 48, and 72hrs, 10 µL of cells were removed and cell concentrations were counted by hemocytometry.

48 well culture plate Hemocytometer



Results

Table 1 Hemocytometer results for *Leishmania major* cell numbers in cells/mL;

Concentration of drug (Amphotericin B) in *µM*	After 00 hours	After 24 hours	After 48 hours	After 72 hours
Control (0.0)	54.0	101.0	148.0	199.0
0.1	54.0	91.5	76.5	55.5
0.5	54.0	84.0	72.0	52.0
1.0	54.0	78.0	54.0	42.0
5.0	54.0	66.5	48.5	26.0
10.0	54.0	59.0	33.0	12.5

Fig. 1 Change in the *Leishmania major* cell numbers when treated with different concentration of Amphoteriin B drug at specified time intervals

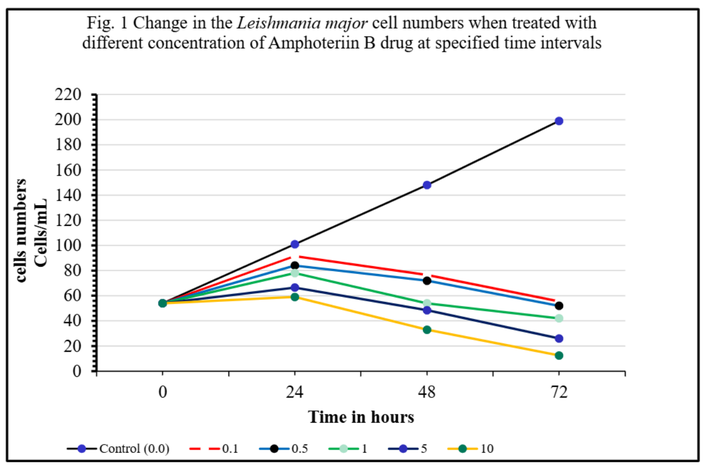


Fig. 2. *L.major* average number of cells with standard deviation at 72 hours of treatment with Amphotericin B drug concentrations

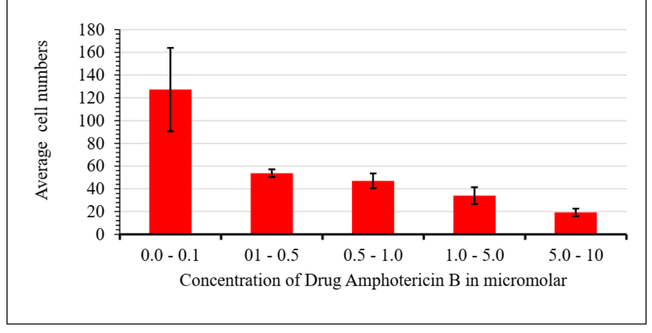


Table 2. Hemocytometer results for *Leishmania donovani* cell numbers counted;

Concentration of drug (Amphotericin B) in *µM*	After 00 hours	after 24 hours	after 48 hours	after 72 hours
Control (0.0)	60	108.5	131.0	194.0
0.1	60	95.0	80.0	71.5
0.5	60	84.5	73.5	57.5
1	60	74.0	56.0	42.0
5	60	61.5	42.0	26.5
10	60	50.0	29.5	11.0

Fig. 3 .Change in cell numbers of *Leishmania donovani* after treatment with different concentrations of Amphotericin B drug at specified time intervals

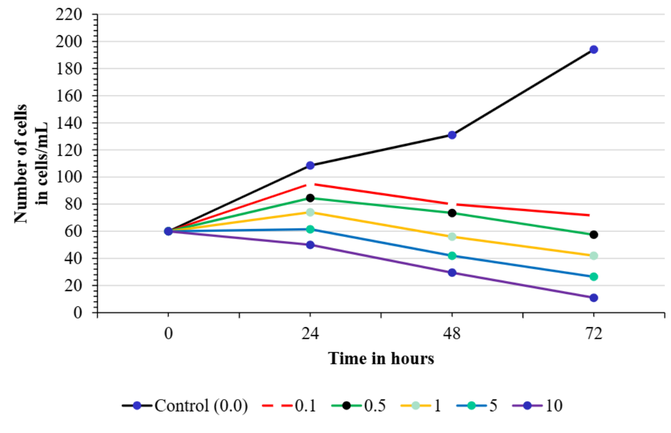
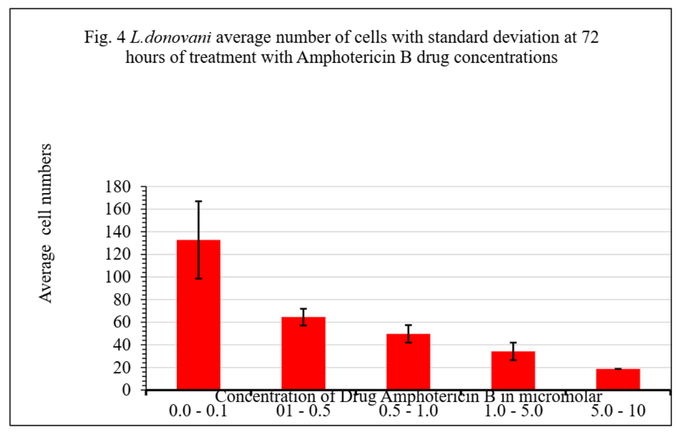


Fig. 4 *L.donovani* average number of cells with standard deviation at 72 hours of treatment with Amphotericin B drug concentrations



Discussion

Our results indicate that amphotericin B is effective for killing both *Leishmania donovani* and *Leishmania major* promastigotes in similar concentrations. This is evident in the graphical analysis where the line graphs used are showing a trend of decrease in the number of Leishmania cells for concentrations from 0.1 µM - 10 µM. In addition, non-treated control cells showed an increase, not a decrease, in number indicating that the cells were live and actively multiplying in the culture media..

Also evident from the results is that the death rates are estimated to likely to be equal from 24 hours-time period to 72 hours with presence of straight lines graph with negative gradients for the plates treated with Amphotericin B solutions, indicating a steady state lethality effect

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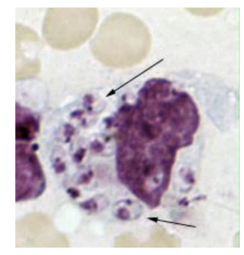
Future studies

Recommendations for future studies include the use of amastigotes as opposed to promastigotes. In contrast to promastigotes, amastigotes are not the free living form but rather live within a host cell and actively divide (see arrows, Fig below). This represents a model which more closely mimics what happens in an infected patient, and therefore the relative effectiveness of anti-leishmanial drugs may be different than on the free living promastigotes. Candidate cell lines for Leishmania infection includes the human RAW cell, HELA cells or primary human macrophage cells (Aulner et at. 2013).



Conclusions

Our results indicate that both *L. donovani* and *L. major* exhibit similar susceptibilities to soluble amphotericin B, with 50% lethality occuring within 48 hrs treatment in the micromolar range. These results confirm the usefulness of this treatment for leishmaniasis and suggest a potential clinical applicability of amphotericin B treatment in multiple infectious forms of the disease

References

Aulner et al. April 2013 PLOS Neglected Tropical Diseases volume 7 issue 4; p1-10 www.plosntds.org

Burza S, Croft SL, Boelaert M. Leishmaniasis Lancet. 2018 Sep 15;392(10151):951-970

Ghorbani and Farhoudi. Leishmaniasis in humans: Drug or vaccine therapy? Drug Des Devel Ther. 2018; 12;25-40

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