Divergent susceptibility to antimony and frequency of treatment failure in cutaneous leishmaniasis caused by *L. (V.) panamensis* subpopulations

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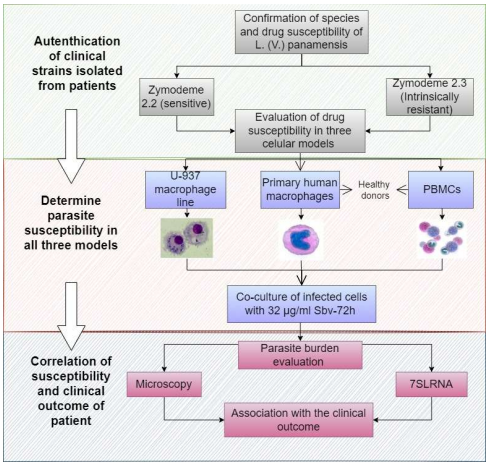
ABSTRACT

The relationship between *in vitro* parasite susceptibility to anti-leishmanials and the clinical response to treatment of cutaneous leishmaniasis remains unclear. To define the relationship between drug susceptibility and antimony treatment outcome, we determined antimony susceptibility of 50 *L. (V.) panamensis* strains from the corresponding patients with documented response to treatment and absence of known risk factors for treatment failure. To understand the contribution of host cells to drug susceptibility, we comparatively evaluated the anti-leishmanial activity of antimony (SbV) in various host cell models: *ex vivo* in primary human monocyte-derived macrophages isolated from healthy donors and peripheral blood mononuclear cells (PBMCs), and *in vitro* using the promonocytic U937 cell line. Cells were infected with promastigotes, and exposed to 32 µg SbV/ml for 72 hours at 34°C. Parasite burden was determined by microscopy or by RT-PCR of *Leishmania* 7SLRNA, and normalized with the human PPIB gene.

EXPERIMENTAL STRATEGY



Determine whether evaluation of drug susceptibility against antimony (SbV) in primary human host cells would more closely correlate with the clinical response to treatment, than the *in vitro* U-937 cell line.

RESULTS

There is a direct proportional correlation between the susceptibility for antimony determined by PCR and microscopy

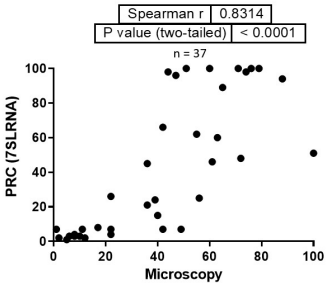


Figure 1. The correlation between the traditional method of microscopy and 7SLRNA was evaluated.

AKNOWLEDGMENTS

This work was financed by the NIH/NIAID Tropical Medicine Research Centers (TMRC) grant U19AI129910.

Drug susceptibility of *L. (V.) panamensis* to antimony is significantly lower in patients who fail treatment with meglumine antimoniate independently of the parasite burden readout method.

Conventional model using one addition of antimony

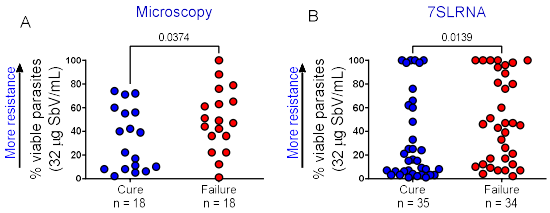
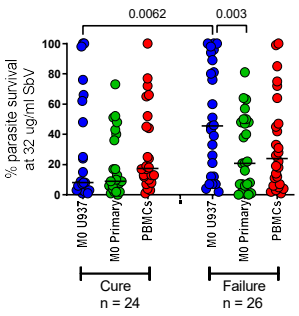


Figure 2. Comparision of level of drug susceptibility between cure and failure in U-937. Conventional model with one addition of antimony after hours post-infection.

Drug susceptibility determined in the U-937 cell line correlated with the clinical outcome, whereas the susceptibility result with primary cells did not



Model	Cure	Failure
U-937 macrophages	8%	45%
Human Primary macrophages	9%	21%
PBMCs	17%	24%

Table 1. Median percent parasite survival by clinical outcome

Figure 3. Parasite survival after 72 hours of 32 µg SbV/ml at 34°C in three models grouped by clinical outcome

There is a significant difference in the susceptibility profile of U-937 cell line and primary macrophages when compared by zymodeme.

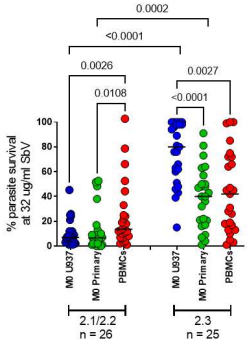


Figure 4. Parasite survival after 72 hours of 32 µg SbV/ml at 34°C in three models grouped by zymodeme.

Comparative analysis of drug susceptibility in different cellular models suggested that clinical strains of zymodeme 2.3 were resistant to SbV in the U-937 cell line, but displayed significantly greater susceptibility to SbV in primary macrophages and PBMCs

CONCLUSIONS

These results demonstrate that clinical strains isolated from patients who fail treatment with meglumine antimoniate have a higher level of tolerance/resistance to antimony treatment *in-vitro*.

These findings suggest that antimicrobial mechanisms of innate immunity of macrophages and PBMCs contribute to the control of infection, but confound the evaluation of susceptibility in primary cells.

The higher level of tolerance/resistant of *L (V) panamensis* strains to antimony in patients with treatment failure underscores the need for alternative therapies.