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Inherent paromomycin susceptibility and induction of resistance of SSG-sensitive and SSG-resistant Nepalese *Leishmania donovani* strains

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INTRODUCTION

An understanding of how drug resistance occurs may allow development of novel treatments where parasite insensitivity could be reversed by co-treating with an inhibitor of the drug resistance mediator. This understanding may also allow monitoring of field isolates, so that drug use could be managed more effectively in the clinic. Resistance to antimonial drugs is reducing the number of clinical options for the treatment of leishmaniasis, and paromomycin (PMM) is one of the alternative drugs that could be used.

In this study, the effect of exposure of promastigotes to increasing concentrations of PMM on the induction of PMM resistance was determined using strains with different sodium stibogluconate (SSG) susceptibilities, in order to determine if the background of SSG susceptibility could influence the development of PMM resistance. Three Nepalese *Leishmania donovani* strains were used: BPK 282/0 clone 4 (cl4): SSG-sensitive [S/S], BPK 087/0 clone 11 (cl11): SSG-intermediate resistant [S/R] and BPK 275/0 clone 18 (cl18): SSG resistant [R/R]

MATERIAL AND METHODS

Promastigotes: *In vitro* susceptibility to PMM (resazurin assay)

1. 96-well plates: Add 4 day-cultured promastigotes (5.10^5 / 10^6 parasites/well) + 0 to 2078 µM PMM
↓ 72h
2. Add 20 µl resazurin (1:10 ratio)
↓ 18h
3. Fluorescence reading (550-590nm)

Amastigotes: *In vitro* susceptibility to PMM (macrophage infection)

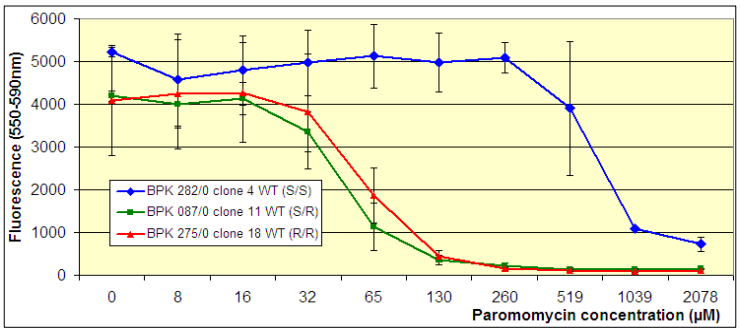
1. 24-well plates: Add peritoneal mouse macrophages (5.10^4/well)
↓ 24h
2. Infect with 7 day-cultured promastigotes (20:1 ratio)
↓ 24h
3. Replenish with 0 to 260 µM PMM
↓ 72h
4. Giemsa stain

26 weeks required to build resistance up to 97 µM of PMM

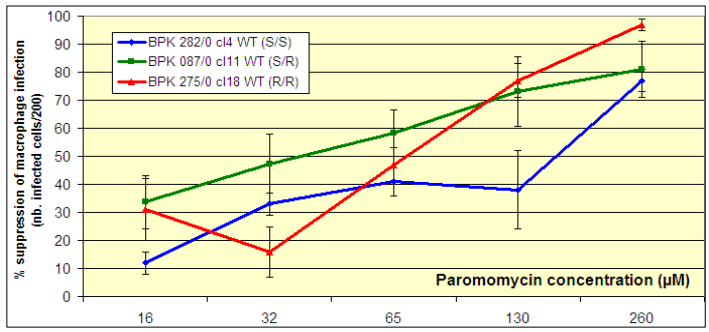
0 µM (Wild Type, WT) → 2 /4 / 8 / 16 /32 /65 µM PMM → 97 µM PMM-R

RESULTS AND DISCUSSION

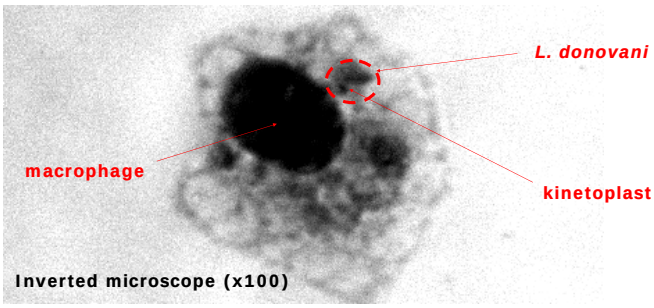
1. BPK 282/0 cl4 wild-type promastigotes are inherently resistant to PMM



2. BPK 282/0 clone 4 wild-type amastigotes express the same inherent resistance to PMM as promastigotes



***L. donovani* amastigotes inside peritoneal mouse macrophages**



3. Exposure of promastigotes to PMM did not increase the resistance of the naturally resistant strain to PMM paromomycin (µM)

Strain	Mean IC_{50} ± SD (µM)		
	PMM resistance	Promastigotes	Amastigotes
BPK 282/0 clone 4 [S/S]	Wild type	82 ± 22	166 ± 11
	97 µM PMM-R	N/A	147
BPK 087/0 clone 11 [S/R]	Wild type	50 ± 22	56 ± 18
	97 µM PMM-R	N/A	N/A
BPK 275/0 clone 18 [R/R]	Wild type	49 ± 13	67 ± 1
	97 µM PMM-R	N/A	132 ± 2

CONCLUSIONS

1. BPK282/0 clone 4 [S/S] is naturally naturally resistant to PMM, indicating that widespread use of the drug may result in the rapid spread of PMM resistance and limit the long-term clinical use of PMM.

2. *In vitro* induction of PMM resistance did not result in an increase in PMM resistance for BPK282/0 clone 4 [S/S].

3. BPK 087/0 clone 11 [S/R] and BPK 275/0 clone 18 [R/R] showed similar susceptibilities to PMM.

4. No apparent relationship between SSG resistance and PMM resistance was observed.

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