Experimental induction of paromomycin resistance in an antimony-resistant Nepalese strain of *L. donovani*

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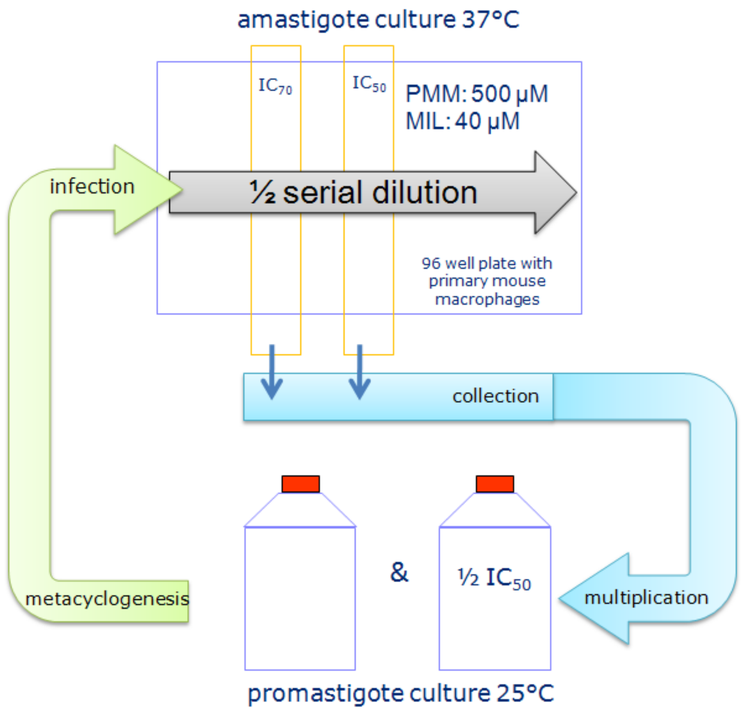
AIM OF THE STUDY

Due to increasing antimony (Sb) refractoriness, new treatment regimens and drugs need to be explored. Paromomycin (PMM) was recently licensed in India for the treatment of visceral leishmaniasis (Davidson 2009). In attempt to assess the longevity of future field use of PMM, the effect of drug pressure on resistance selection in strains with established Sb-resistance background was evaluated.

Although promastigotes are more commonly used for resistance induction protocols, amastigotes offer a significant advantage as the relevant stage in the vertebrate host. In the present experiment, artificial induction of PMM-resistance was performed using the *in vitro* amastigote model in primary mouse macrophages.

RESISTANCE INDUCTION PROTOCOL

1. Amastigote test: infection with promastigotes 10/1; drug exposure for 96h at 24h post infection,
2. Collection of surviving amastigotes at IC_{50} and IC_{70}
3. Multiplication as promastigotes in M199 medium at 25 °C



RESULTS

Original clone BPK 275/0 cl 18
Sb^V R - Sb^{III} R
IC_{50} = 45 ± 5.6 µM

BPK 275/0 cl 18 P1 PMM pressure
Loss of infectivity

BPK 275/0 cl 18
P1
IC_{50} = 131 ± 14 µM

BPK 275/0 cl 18
P2
IC_{50} = 199 ± 8.5 µM

Establishment of 10 clones
susceptibility of promastigotes and amastigotes

strain	*In vitro* susceptibility of promastigotes: PMM (µM) mean ± SEM	Sb^{III} (µg/ml eq.) mean ± SEM	MIL (µM) mean ± SEM
BPK 275/0 clone 18 original	19,7 ± 0,3	48,9 ± 2,2	2,3 ± 0,4
BPK 275/0 PMM induced	-	-	-
clone 1	19,2 ± 1,1	36,4 ± 3,2	7,9 ± 0,6
clone 2	23,5 ± 1,2	32,0 ± 1,6	3,7 ± 0,1
clone 3	20,9 ± 0,9	30,5 ± 1,7	4,0 ± 0,3
clone 4	20,9 ± 0,7	24,1 ± 1,5	5,2 ± 1,3
clone 7	17,6 ± 0,8	27,8 ± 1,1	3,1 ± 0,0
clone 8	14,5 ± 0,5	26,2 ± 0,7	3,3 ± 0,0
clone 11	12,4 ± 0,3	34,7 ± 0,7	7,4 ± 1,1
clone 12	11,8 ± 0,2	43,2 ± 3,2	10,4 ± 2,5
clone 13	11,9 ± 0,2	34,9 ± 0,7	3,4 ± 0,8
clone 14	10,5 ± 0,3	29,5 ± 1,6	6,9 ± 0,1

strain	*In vitro* susceptibiliy of intracellular amastigotes: PMM (µM) mean ± SEM	Sb^V (µg/ml eq.) mean ± SEM	Sb^{III} (µg/ml eq.) mean ± SEM	MIL (µM)
BPK 275/0 clone 18 original	45,0 ± 5,6	77 ± 0	51,1 ± 0,7	1,84
BPK 275/0 PMM induced	199,0 ± 8,5	77 ± 0	57,3 ± 0,9	2,08
clone 1	**448,4** ± 21,6	77 ± 0	53,8 ± 0,3	2,6 ± 0,1
clone 2	249,5 ± 8,5			
clone 3	223,7 ± 21,7			
clone 4	206,9 ± 6,5			
clone 7	221,4 ± 24,6			
clone 8	**379,5** ± 3,5			
clone 11	**364,6** ± 4,9			
clone 12	152,7 ± 14,6			
clone 13	366,9 ± 5,6			
clone 14	**84,9** ± 2,4			

CONCLUSIONS

1. PMM exposure of promastigotes affects infectivity → not appropriate for R-induction.
2. Rapid adaptation of the intracellular amastigotes to increased PMM pressure.
3. Development of resistance: >4-fold increase of IC_{50} after 2 passages !
4. Highly PMM resistant clones have been established.
5. Antimony resistant background remains unchanged!

Davidson RN, den Boer M, Ritmeijer K. Paromomycin. *Trans R Soc Trop Med Hyg*.2009 **(7):**653-60.

contact. www.ua.ac.be/lmph website: **www.leishrisk.net/kaladrug** funding: EC-FP7-222895