Experimental Induction of Paromomycin Resistance in an Antimony-resistant Nepalese strain of L. donovani.

Raquel Inocêncio, Sarah Hendrickx, Kristel Kuypers, Paul Cos and Louis Maes
University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium
raquel.daluz@ua.ac.be

First-line treatment failure for all clinical forms of leishmaniasis due to antimony (SbV) resistance is now well documented. Among new treatment regimens and drugs that are currently explored, paromomycin (PMM) is now actively promoted for treatment of visceral leishmaniasis (VL). However, it is pivotal to assess the effect of paromomycin drug pressure on strains with antimony-resistance background. The present laboratory study focused on the artificial induction of PMM-resistance using the in vitro amastigote model in primary mouse macrophages. Amastigotes are considered the relevant stage for such experiments and offer a significant advantage over the more commonly used promastigotes.

A cloned L. donovani field isolate (MHOM/NP/03/BPK275/0 CL18) known to be resistant to both SbV and SbIII was used for induction. The principle of the method was to maintain the highest possible PMM selection pressure during the alternate cycles of promastigotes used to infect macrophages and the intracellular amastigote. Amastigotes surviving the highest drug concentration were allowed to transform back to promastigotes to expand the population, either under continued drug pressure at half the IC50 or not. These next generation promastigotes was then used for infection of macrophages under increased drug pressure. The selection cycles were repeated until the maximum level of resistance was reached.

Parasites kept under constant drug pressure both at amastigote and promastigote level lost their ability to infect macrophages. However, the parasites under drug pressure at only the amastigote stage quickly developed decreased susceptibility. After one selection cycle, PMM-resistance showed a $>$2-fold increase of IC50 (from 44 μM to 138 μM). The second selection cycle resulted into a $>$4-fold increase (IC50 = 198μM) comparing to the original parent strain. Additional selection cycles did not result in a further increase but the IC50 value stabilized at about 198 μM. These data clearly show that resistance to PMM can be induced fairly rapidly at the level of intracellular amastigotes and that exposure of promastigotes to PMM results in loss of infectivity for macrophages. Cloning experiments are now ongoing to define PMM resistant lines.