Natural susceptibility of Leishmania donovani isolates from Indian subcontinent towards Miltefosine and impact of SSG resistance background on development of Miltefosine resistance

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With widespread antimonial resistance prevalent in anthroponotic VL in the Indian subcontinent, the oral drug Miltefosine (MIL) has been proposed as the first line drug. However, a long half-life of MIL coupled with a long treatment course indicate that resistance to this drug could develop quickly. In this situation, it becomes crucial to understand the natural susceptibility of prevailing parasite population to MIL and monitor the impact of background of SAG resistance on development of MIL resistance. We determined the in vitro MIL susceptibility of Indian and Nepalese clinical isolates of VL using a high throughput resazurin based bioassay for promastigotes. Indian field isolates (n=8), all resistant to SAG, exhibited MIL sensitivity ranging from 1.03±0.31 to 5.13±0.23 μg/ml (mean 2.40±1.41) at promastigote stage and 1.3±0.28 to 10.6±0.84 μg/ml (mean3.77±3.30) at amastigote stage. Nepalese strains (n=8) with different SSG-susceptibility background, showed variable MIL IC50 in promastigotes (range=0.87±0.14 to 2.66±0.11 μg/ml, mean=1.70±0.21 μg/ml) and a higher MIL susceptibility in amastigotes (range= 0.38±0.01 to 1.09±0.27 μg/ml , mean = 0.68 ± 0.09 μg/ml). Overall, KA isolates from the Indian subcontinent displayed mean IC50±SEM of 2.05±0.27 for promastigotes and 2.224±0.69 μg/ml for amastigotes, with a wide range of susceptibility to MIL (nearly 6 fold in promastigotes and 27 fold in amastigotes). The susceptibility at both parasite stages for 16 isolates correlated positively (r=0.598, p=0.014). Two SSG resistant Indian field isolates, (BHU573-Cl3 and BHU568-Cl1) were exposed stepwise to increasing MIL pressure up to 30μg/ml (in 29 weeks). 20 to 24.5 fold decrease in MIL susceptibility was evident in MIL adapted parasites at promastigote stage with mean IC50 of 33.34±7.22 μg/ml for BHU568Cl-1 and 49.17± 1.05 μg/ml for BHU573-Cl3. Similarly, in 3 Nepalese isolates with different SSG resistance background, MIL adaptation resulted in considerably higher resistance levels to MIL for promastigotes of all three strains (IC50 multiplied by 20 to 60 in comparison with the wild types). The level