Drug susceptibility of Indian field isolates of L.donovani and development of experimental resistance towards Miltefosine and Paromomycin

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Control of Visceral Leishmaniasis (VL) relies mainly on chemotherapy. The high toxicity and emergence of resistance towards Sodium antimony gluconate (SAG), has resulted in introduction of alternate drugs Miltefosine (MIL) as the first line drug in parts of Bihar. Anthroponotic VL transmission in India as well as long half-life of MIL poses threat of development of resistance. Paromomycin (PMM) is the new antileishmanial drug in phase IV trials which appears to be safe, affordable and effective. Here, we have investigated the antileishmanial activity of MIL and PMM in cloned field isolates (n=8) from VL patients in hyper endemic areas of Bihar, India. The isolates were initially characterized as Leishmania donovani by ITS -1 based PCR RFLP. Susceptibility of the isolates was evaluated at amastigote stage for both MIL and PMM. We observed a variable susceptibility to these drugs, with ED50 at amastigote stage ranging from 1.16- 10.76 mg/ml for MIL and 6.61-12.39 mg/ml for PMM. Based on the ED50 values, we selected two isolates for induction of resistance to MIL and PMM. The parasites were adapted via stepwise increase in concentration of MIL from 1.25-7.5mg/ml. The concentration of drug was increased gradually only when the adapted isolate showed growth comparable to wild type culture. The parasites adapted at 7.5mg/ml MIL concentration, showed decreased susceptibility towards MIL at both promastigote and amastigote stage, as compared to the wild type isolate. Likewise, induction of resistance to PMM from 5-25mg/ml was developed and parasites adapted at 25mg/ml PMM, showed 2 to 3 fold increase in ED50 for PMM at promastigote and amastigote levels. The adapted parasites showed similar growth and infectivity as compared to the wild type isolate. We propose to adapt these isolates to higher concentration of MIL upto 15mg/ml and PMM upto 50mg/ml. Once the proposed concentration of adapted parasites is achieved we will experimentally explore the mechanism of resistance towards these drugs. Simultaneously verification of experimental MIL resistance markers (LdMT, LdROS3) will be carried out in all field and MIL adapted isolates.