Abstract

The Metronidazole activation pathway in *E. histolytica* is still not clear. Involvement of different enzymes and low redox molecules like pyruvate-ferredoxin oxidoreductase (POR), Ferredoxin (FD), Superoxide dismutase (SOD), Ferredoxin nitroreductase (FDNtr) are reported to reduce the drug directly/indirectly. Search of the *Entamoeba* genome revealed three different oxygen insensitive nitroreductase genes (*ehntrs*), which is reported to be responsible for Mtz activation in *H. pylori*. The genes have been cloned, expressed in *E. coli* and biochemically characterized. The *ehntr1* of HM1:ISS strain of *Entamoeba* has inframe stop codon mutation, where as *ehntr1* of strain HK:9 has not. Overexpression of *ehntr1* from HK9 in the *Entamoeba,* strain HM1:ISS showed no effect on drug susceptibility. The Ehntrs were expressed as N-terminal His tag protein in *E. coli* and SDS-PAGE revealed expression of Ehntr1 and Ehntr2 in the soluble fraction whereas Ehntr3 was present in the pellet. Ehntr3 purification was further carried out by refolding strategy. Biochemical characterization of Ehntrs showed Mtz as a substrate utilizing NADH as cofactor. Complementation of *ehntr1* and *ehntr2* in Mtz^R and Mtz^S *E. coli* showed increased sensitivity towards Mtz.

Nitroimidazole resistant *nim* gene (*ehnim*) has also been identified in the parasite. The gene has been discovered recently in Mtz^R *Bacteroides* sp. and the expressed product of *nim* gene deactivates the drug, Mtz. The *ehnim* gene has been cloned, expressed and characterized in *E. coli*. Complementation of *ehnim* in Mtz^S *E. coli* revealed high-level of drug resistance towards Mtz with IC_{50} of 1000 μg/ml. Drug susceptibility pattern of *ehnim* towards tinidazole, ornidazole and dimetridazole also exhibited moderate level of drug resistance. Expression of *ntrs* and *nim* genes was confirmed at transcriptional level in the parasite by RT-PCR. As one of the prerequisite for studying the functioning of the Mtz activating and deactivating genes, the RNAi mechanism of the parasite was harnessed. *E. histolytica* specific both constitutive and inducible double stranded RNA expression vectors were constructed to silence the nitroductases, *nim* as well as any other target gene of the parasite to unravel the Mtz activation pathway. Computational identification of the parasite genome revealed putative microRNA candidates and their targets but no microRNA could be identified for nitroreducatse and *nim* genes.