Drug resistance in amoebiasis

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Amoebiasis caused by Entamoeba histolytica, is a major public health problem in developing countries. Morphologically similar E. dispar is non pathogenic. Because of the redefinition of E. histolytica and E. dispers, and the limited number of antiamoebic drugs available, a new approach to treat such individuals is necessary. The cost of treating asymptomatic individuals is highly exorbitant and not justifiable. The indiscriminate use of antiamoebic drugs can result in increased minimum inhibitory concentration (MIC) values against Entamoeba species, and treatment failure may emerge as an important public health problem. Development of new antiamoebic drugs is still in infancy and vaccine development appears to be distant dream. In future, the development of drug resistance may seriously affect the control of disease. This review discusses the factors involved in drug resistance mechanisms developed by the parasite.

Key words Amoebiasis - emetine - in vitro sensitivity - metronidazole - multidrug resistance mechanism

Entamoeba histolytica, associated with high morbidity and mortality continues to be a major public health problem throughout the world. Asymptomatic individuals account for almost 90 per cent of the infections. Poverty, ignorance, overcrowding, poor sanitation and malnutrition favour transmission and increased disease burden. Prevalence varies from country to country and within a country. In India, it was estimated to be 2-55 per cent in early nineties. Identification of two morphologically similar Entamoeba sp., pathogenic E. histolytica and non pathogenic E. dispers responsible for asymptomatic infection, have raised the question of treating all such cases. The recommendation is that E. histolytica should be specifically identified, treated with antiamoebic drugs. In individuals with only E. dispers, the treatment is unnecessary. Treating asymptomatic individuals indiscriminately may lead to drug resistance. Though it does not appear to be a serious problem now, reports of failed treatment with metronidazole and differences in drug susceptibilities do suggest that this could probably herald the development of drug resistance clinically. Therefore, understanding the mechanisms involved in multi drug resistance of different antiamoebic drugs is essential.

Antiamoebic drugs

Antiamoebic drugs are classified into three groups: luminal, tissue, and mixed amoebicides. Metronidazole is the major drug of choice and other nitroimidazole derived compounds like tinidazole, secnidazole and ornidazole are equally effective. Diloxanide furoate,
diiodohydroxyquin, paromomycin, emetine and chloroquine have also been used as alternate drugs. Diloxanide furoate is the mainstay for treating asymptomatic cyst carriers\(^14\). Chloroquine could be used along with metronidazole/emetine in cases of hepatic amoebiasis. However, emetine is rarely used on account of its toxicity. Metronidazole, tinidazole and other 5-nitroimidazole agents which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba are ineffective in treating cyst passers. Whereas, chloroquine acts on the vegetative forms of the parasite and kills it by inhibiting DNA synthesis, emetine kills the trophozoites mainly by inhibiting protein synthesis\(^15\).

**In vitro drug sensitivity**

To understand the magnitude of drug resistance, drug sensitivity of clinical isolates of *E. histolytica* is important. Recent studies have shown differences in drug sensitivity in *E. histolytica* isolates, indicating that there might be a small percentage of amoebae which are either resistant or may eventually become resistant due to indiscriminate use of anti-amoebic agents\(^10-13\). Upcroft and Upcroft\(^11\) have reported minimum inhibitory concentration (MIC) of metronidazole ranging from 12.5-25 µm. Adagu et al\(^12\) have shown the mean 50 per cent inhibitory concentration (IC\(_{50}\)) value to metronidazole as 18.47 µm for the most susceptible isolates of *E. histolytica* with a >30 µm value as the cut-off value for resistance. Burchard and Mirelman\(^10\), on examining the *in vitro* sensitivity to metronidazole and emetine of non-pathogenic zymodemes showed that all were equally sensitive to the both drugs (1-10 µg/ml). Recently, we have reported significantly higher IC\(_{50}\) value of all the four drugs (metronidazole, chloroquine, tinidazole and emetine) for the clinical isolates compared to the reference strain of *E. histolytica* (HM1: IMSS). Interestingly, *E. dispar* isolates showed higher IC\(_{50}\) value than the *E. histolytica* or the reference strain\(^13\).

**Drug resistance mechanisms in *E. histolytica***

Resistance in clinical isolates is less defined in biochemical terms because parasite populations are often heterogeneous. Parasite may evade drug action by hiding in sanctuaries. So far the mechanisms of drug resistance hypothesized in protozoan parasite are decrease of drug uptake because of loss of a transporter required for uptake, the efflux of drugs from the parasite either by the P-glycoproteins (Pgp) or by ATPases, the alteration of drug target, and loss of drug activation\(^16\).

Metronidazole and related nitroimidazole, tinidazole (which is not available in some countries), are the only effective drugs for the treatment of trichomoniasis and giardiasis. In the latter cases, clinical resistance to these drugs has been documented\(^17-19\). Laboratory induced metronidazole resistance in *E. histolytica* has been reported and metronidazole resistant *E. histolytica* strains have been maintained indefinitely in medium\(^20\).

The models available to study the mechanism of drug resistance in *E. histolytica* include *in vitro* induced metronidazole resistant cell lines of *E. histolytica*\(^20\) and emetine resistant mutant clones derived from virulent HM1: IMSS strain, which was selected after mutagenesis with alkylating agent, ethyl methanesulphonate\(^21\). Expression of iron containing superoxide dismutase (fe-SOD) and peroxiredoxin has been shown to be increased three to five fold in metronidazole resistant parasites and this has been confirmed at the gene expression level with decrease in the expression of ferredoxin and flavin reductase\(^22\). The critical involvement of fe-SOD and peroxiredoxin was confirmed by episomal transfection of these antioxidant enzymes into metronidazole susceptible isolates, which was associated with reduced drug susceptibility\(^22\).

Though the mechanisms of multi drug resistance (MDR) in *Entamoeba* emetine mutants have not been studied in detail, MDR in mammalian tumour cells is well characterized. Tumour cells become resistant to simple chemotherapeutic drugs *in vitro*\(^23\). The drug resistance is usually due to increased efflux of the drug from the tumour cells, which is energy dependent and is inhibited by calcium ion channel blockers\(^24\). The MDR gene encoding for the Pgp (170 kDa protein), has been isolated from drug resistant cancer cells\(^25\). This consists of two homologous halves containing six membrane spanning alpha-helices and a cytoplasmic ATP binding site for efflux of drugs\(^26,27\), with the ATP binding site showing an extensive amino acid sequence homologues with bacterial transport proteins\(^25,28\). P-glycoprotein also has a binding protein for chemotherapeutic agents such as vinblastin suggesting that it is directly involved in the drug transport mechanism in MDR cells\(^29\).
overexpression of surface Pgp also produces the MDR phenotype in protozoan parasites like Plasmodium, Trichomonas, Giardia, Leishmania, and Entamoeba.

E. histolytica has several features common with MDR phenotype described in mammalian tumour cells. These are cross-resistance to unrelated drugs, increased efflux and decreased accumulation of radiolabelled drugs, reversal of resistance by calcium channel blockers like verapamil, and overexpression of 4.5 kb long mRNAs homologous to the mammalian P-glycoprotein. So far, six Pgp-like genes (EhPgp1-EhPgp6) have been cloned and sequenced, but copy number has not been described in laboratory mutants. Of these, four were clearly expressed in drug resistant line (EhPgp1, EhPgp2, EhPgp5, EhPgp6), while the remaining two were pseudogenes (EhPgp3, EhPgp4). Studies on genetic relatedness suggest that Entamoeba P-glycoproteins are more related to the human and mouse P-glycoprotein than to the Plasmodium and Leishmania P-glycoprotein. The amino acid sequence of amoebic MDR like PCR products were 46 to 97 per cent identical with each other, 46 to 50 per cent identical to human MDR sequence and 30-35 per cent to the mammalian P-glycoprotein. So far, six Pgp like genes (EhPgp1-EhPgp6) have been cloned and sequenced, but copy number has not been described in laboratory mutants. Of these, four were clearly expressed in drug resistant line (EhPgp1, EhPgp2, EhPgp5, EhPgp6), while the remaining two were pseudogenes (EhPgp3, EhPgp4).

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References


