Effectiveness of some chemotherapeutic drugs on viability of Entamoeba histolytica trophozoites in vitro

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Abstract

Amoebic dysentery is an endemic disease in Iraq. Serious steps are needed in order to decline infection ratio and to prevent any complications resulted from this disease.

The study was carried out in vitro to evaluate the efficacy of some chemical drugs on the viability of Entamoeba histolytica isolated from human in culture.

Locke egg medium was used as growth medium for isolation of Entamoeba histolytica. Best results were obtained using this medium in isolation of trophozoites. After isolation, trophozoites were tested against a variety of chemical drugs using Pandroff tube which contain Jones broth medium. Various concentrations were used from chemical drugs which included metronidazole, albendazole, and mebendazole.

Entamoeba histolytica was diagnosed in 514 stool samples (41.12%) from a total of 1250 examined sample.

Assessment of the results was done by estimation of viable trophozoites in haemocytometer chamber. The viability of trophozoites was estimated using various concentrations of (100, 50 and 25 mg/ml) of metronidazole, albendazole and mebendazole.

Metronidazole was found to have a best inhibitory effect against Entamoeba histolytica, followed by albendazole and mebendazole which was less effective in comparison with metronidazole.

Key Words: In vitro, Chemotherapy, effectivness, Entamoeba histolytica

Introduction

Dysentery, both bacterial and amoebic has long been known as handmaident of war, often inflicting more causalities than bullets and bombs (1). E. histolytica have numerous virulence factors such as proteinases, lectin and amaebapore for digestion and penetration of intestinal mucosa leading to diarrhea as

well as malabsorption of nutrients (2 and 3) Amoebiasis is one of the major parasitic disease for which there is no means of effective immunoprophylaxis or chemoprophylaxis available. Therefore control of amoebiasis will be difficult at best, and a careful evaluation of risks and benefits should be made before the drug is used in pregnant or lactating women (4).

Eradication of Entamoeba histolytica may be difficult. Available treatments are imperfect at best and have toxic side effect such as gastric upset, optic atrophy, bitter taste, dermatitis and possibly carcinogenic (5). In addition, treatment failures in as many as 20% of cases that raises concern about possible resistance to the drugs used and especially during pregnancy (4). Treatment failure among amoebiasis patients often raises the possibility of drug resistance (6). As human is the only relevant host for this parasite, and effective treatment of luminal intestinal infection is necessary to interrupt transmission of the parasite therefore the search of new drugs with amebicidal activity is important (7).

Luminal drugs are poorly absorbed and reach high concentration in the bowel, but their activity is limited to cysts and trophozoites close to their mucosa. They are indicated in the eradication of dormant luminal cysts in patients with colitis or a liver abscess to prevent relapses and treatment of asymptomatic carriers (8).

Metronidazole is low in toxicity and is effective against both extraintestinal and colonic infections (9). It is the drug commonly used and recommended in the treatment for amoebiasis (10). Albendazole had been tested in vivo in giardiasis (11). Barwari (12) carried out a study in vitro and in vivo, to search for alternative new anti-amebic drugs, he found that metronidazole is the most effective antiamebic drug, and albendazole was less effective against E. histolytica in comparison to metronidazole. This study was planned to evaluate the efficacy of albendazole, mebendazole , and metronidazole on viability of E. histolytica trophozoites isolated from human in vitro.

Materials and Methods

Isolation of parasite

Entamoeba histolytica was isolated from stool samples collected from patients attended from Kirkuk Pediatric hospital, Kirkuk General Hospital, Primary Health Care Center. The samples were examined by wet mount technique using normal saline solution, when the samples showed active trophozoite motility they were cultured in Boeck and Dboholve's medium (13).

Viability tests: -

Various chemical drugs were tested on Entamoeba histolytica including metronidazole, albendazole and mebendazole. All medical drugs were used in various concentrations. At control tubes, trophozoites count was increased from 2000 trophozoites / 0.2 ml into 5184 trophozoite/ 0.2 ml after 48 hours of incubation then declined gradually until completely diminished at day 12.

After cultivation of Entamoeba histolytica for 48 hours in culture medium and successful production of large number of trophozoite, the trophozoites were prepared for use in the viability tests. Entamoeba histolytica trophozoite from cultures of different samples are used, was centrifuged for 2 minutes at 2000 rpm then transferred into sterile tube (serum tube), 0.2 is ml taken from this solution and trophozoite number was counted using haemocytometer chamber (14 and 15). It was then mixed with 0.3 ml of Jones medium and added into pandroff tube (small covered tapped tubes), incubated at 37°c for 12 days, being examined everyday by using a haemocytometer

chamber. The assessment of the effects of drugs was done according to the number of damaged amoebae, i.e. lysed trophozoite and none disintegrated trophozoite. The number of remaining trophozoite were counted from the original added trophozoites and reached 2000 trophozoite/ 0.2 ml of Locke solution from culture media. Controls were used without drugs to compare the growth of amoebae with the test groups.

Tested drugs:-

The following drugs were obtained from pharmacies, crashed and dissolved in distilled water and multiple dilutions were done to obtain concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and added to sensitivity tube (11). The drugs were:-
Mebendazole (Antiver USP 100 mg), (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt).
Metronidazole (USP, 500mg), Zimz Laboratories LTD. Group, India.
Albendazole (Albenol) tablets USP200 mg, Micro Labs Limited, India.

Statistical analysis:

Student t-test was performed to compare between control and patients groups at (0.05 and 0.01) levels, using the SPSS statistical analysis software version 17 (16).

Results

Table (1) shows the effect of 3 concentrations of metronidazole on Entamoeba histolytica during days of incubation, total reduction of viability at (100 mg /ml) occurred on day 3; on day 5 at 50 mg/ml and 25 mg/ml on day 6.
Albendazole showed complete lethal effect on Entamoeba histolytica on day 4 in 100mg/ ml concentration, while at 50 mg/ ml complete reduction of viability appear on day 5; and at day 6 on 25mg/ml as shown in Table (2).

The effect of mebendazole on Entamoeba histolytica was lower in comparison with Metronidazole and Albendazole. Total reduction of viability proceeded until day 7 at 100mg/ ml; on day 8 at 50mg/ml; and day 9 on concentration 25 mg/ ml, as seen in Table (3).

Discussion

Metronidazole treated tubes showed high declining in trophozoite count, total reducing of viability reached at 3rd day in 100 mg/ ml concentration, and at 5th day and 6th day in 50mg /ml and 25 mg/ ml respectively. In a relevant study metronidazole showed total reducing of viability after 48 hours at 100 µg/ ml (12), in another study MIC (Minimum Inhibitory Concentration, which is the lower concentration cause total reduction of viability while IC50 is the reduction of parasite count 50% compared to control) values of metronidazole ranging from 12.5 – 25 µg/ ml (17). Metronidazole activity was due to trapping electrons by virtue of its very low redox potential. The generation of H2 from pyruvate is halted and the organism soon becomes depleted of NADH and NADPH. The nitro ring of metronidazole becomes cleaved in the process producing toxic substances that hasten cell death (18). Metronidazole requires at least 10 days at high dosage to eradicate luminal amoebae (19). Resistance of Entamoeba histolytica/ Entamoeba dispar against metronidazole varies. In a study done to show the activity of antiamoebic drugs against Entamoeba histolytica and Entamoeba dispar, 45 clinical isolates (15 of which were Entamoeba histolytica, and 30 isolates were Entamoeba dispar) were maintained in polyxenic cultures followed by monoxenic cultures, the results showed

that all clinical isolates had a higher IC50 compared to reference strain in all four drugs. Entamoeba histolytica isolates appeared to be more susceptible {IC50 (μm) 13.2, 26.3, 31.2 and 12.4} in comparison to Entamoeba dispar {IC50 (μm) 9.5, 15.6, 28.9, 32.8 and 13.2} and the reference strain of Entamoeba histolytica {IC50(μm) 9.5, 15.5, 29.9 and 10.2} to the metronidazole, chloroquine, emetine and Tinidazole respectively. These results indicate that Entamoeba histolytica isolates do not seem to be resistant to the commonly used drugs (20).

Few studies showed the metronidazole resistance in Entamoeba histolytica the mechanism investigated using laboratory- induced resistant isolates instead of down regulation of the pyruvate ferrodoxin oxireductase and ferrodoxin pathways seen in Giardia lamblia and Trichomonas vaginalis, Entamoeba histolytica induced oxidative stress mechanism including super oxide dismutase and peroxiredoxin (17).

Total reduction of viability of trophozoite using 100mg/ ml 50 mg/ ml and 25mg/ ml of albendazole occurs at day 4, 5 and 7 respectively. This indicates a good inhibition of growth when compared to the control. These results are considered very close to the effect of metronidazole.

Albendazole, a benzimidazole carbamate commonly used for the treatment and control of intestinal helminthes infections, is also useful for the treatment of giardiasis. In a study to determine whether the drug has activity against other intestinal protozoa, such as E. histolytica, the results demonstrated that Albendazole inhibits the growth of E. histolytica trophozoites in axenic cultures and induces fine structural changes such as polyribosome aggregation and loss of cytoplasmic vacuoles at concentrations up to 10 micrograms/ml (21). In another study, percentage of viability was 42% using10μg/ ml albendazole after 48 hours, which is considered as (IC50), and complete inhibition occurs at 100μg/ ml (12). Experimental study (22) showed that albendazole is very effective anti-amebic drug as metronidazole in rabbits. A study done on giardiasis showed that albendazole (Zental) is an alternative drug for giardiasis, with 62 – 95% reported efficacy compared with 97% for metronidazole (15).

Mebendazole is considered a broad spectrum antihelminthic drug. Experimenting of mebendazole against Entamoeba histolytica has not been done in any previous study, so this can be considered as early stages in order to use mebendazole as an alternative drug for amoebic dysentery treatment. Using of mebendazole shows low inhibitory effect when compared to metronidazole, total reduction of viability occurs at day 7 at 100 mg/ ml concentration, while viability persists to the day 8 and 9 at 50mg/ ml and 25mg/ ml respectively.

The mode of action of mebendazole is by inhibiting uptake of glucose (18). In a comparative study between mebendazole and metronidazole to show their clinical trial in giardiasis of children, one hundred children of both sexes, ranging from 7 - 12 years old with Giardia intestinalis cysts or trophozoites in their stool samples, were randomly separated into two groups of 50 individuals each. Each group received mebendazole tablets 200 mg thrice daily for five days or metronidazole tablet 15 mg/kg/day in three divided doses, for seven days. The results were evaluated by microscopic examination of stools on two successive occasions: one week and two weeks after treatment. Each occasion consisted of three stool examinations on three successive days. The cure rate of mebendazole treated children was 43 out of 50 (86 per cent), and for metronidazole it was 45 out of 50 (90 per cent) with no

statistical difference between the two groups. No side-affects were observed in the mebendazole-treated group, whereas nausea, anorexia and metallic taste were observed in 4.9, 6 and 24% of metronidazole-treated children, respectively (23).

It is concluded that Metronidazole is the best drug used against Entamoeba histolytica in vitro studies. Albendazole can be used as an alternative drugs for amoebic dysentery treatment.

It is recommended to carry on In vivo studies on chemical drugs in order to determine the best concentration that can be used from this medicine against amoebic dysentery and using combination of drugs to eliminate the parasite from the intestine.

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Table 1: The effect of different concentrations of metronidazole on viability of Entamoeba histolytica in comparison to control.

Medical drug/ concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	T test
Metronidazole100mg/ ml	3.3	1.6	0	0	0	0	0	0	0	0	0	0	-3.54*
Metronidazole50mg/ml	5	3.35	1.65	1.65	0	0	0	0	0	0	0	0	-3.52*
Metronidazole25mg/ml	8.75	5	3.35	3.35	1.65	0	0	0	0	0	0	0	-3.48*
Control	184.2	259.2	239.4	153.8	85.1	63.3	56.6	50	23.6	11.6	5	0	

* P<0.05

Table 2: The effect of different concentrations of albendazole on viability of Entamoeba histolytica in comparison to control.

Medical drug/ concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	T test
Albendazole 100mg/ml	9.25	3.35	1.65	0	0	0	0	0	0	0	0	0	-3.51*
Albendazole 50 mg/ml	10.1	4.2	3.35	1.65	0	0	0	0	0	0	0	0	-3.49*
Albendazole 25 mg/ml	12.65	7.9	5	3.35	1.65	0	0	0	0	0	0	0	-3.44*
Control	184.2	259.2	239.4	153.8	85.1	63.3	56.6	50	23.6	11.6	5	0	

* P<0.05

Table 3: The effect of different concentration of mebendazole on viability of Entamoeba histolytica in comparison to control.

Medical drug/Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	T test
Mebendazole 100 mg/ml	15.8	11	7.05	4.2	1.65	1.65	0	0	0	0	0	0	-3.42*
Mebendazole 50 mg/ml	25.3	15.8	14.1	5.85	5	3.35	1.65	0	0	0	0	0	-3.32*
Mebendazole 25 mg/ml	30.7	17.45	13.5	9.55	6.6	5	3.35	1.65	0	0	0	0	-3.26*
Control	184.2	259.2	239.4	153.8	85.1	63.3	56.6	50	23.6	11.6	5	0	

* P<0.05