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Original Article

In Vitro Effects of Metronidazole and Albendazole on *Giardia lamblia* Isolated from Iranian Patients

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Abstract

Background: The aim of the present study was to evaluate the effects of metronidazole and albendazole against clinical isolates of *Giardia lamblia in vitro*.

Methods: From all human samples of containing cysts, 10 isolates were successfully excysted *in vitro*. Trophozites viability was assessed by eosine 0.1% and cultured axenically in TYI-S-33 modified medium supplemented with heat inactivated bovine serum 10%. All cultures were incubated in 37°C for 24-48 h. After this time trophozoites were exposed to different concentration (0.05, 0.1, 2, 10, 50 µg/ml) of drugs at 37º for 4 h. The IC_{50} estimated between 0.1 and 10µg/ml for metronidazole and 0.062 and 0.1 µg/ml for albendazole.

Results: Eight isolates were found susceptible to the metronidazole while all isolates were found susceptible to the albendazole. Statistical results indicated that there was significant difference (P<0.05) in the sensitivity to metronidazole and albendazole in all isolates.

Conclusion: The killing affects of albendazole on *G.lamblia* was greater than metronidazole.

Keywords: Giardia lamblia, *Metronidazole*, *Albendazole*, *Susceptibility*, In-Vitro, *Iran*.

Introduction

Giardia lamblia, is a protozoan parasite in the intestine that causes extensive morbidity in the worldwide. Giardiasis is an important cause of chronic diarrhea and malabsorbtion. *Giardia* infects approximately 2% of the adults and 6 to 8% of the children in developed countries (1).

Despite the recognition of clinical illness in the last 40 years, there have been few reviews of therapy for this infection and no definitive effective treatment protocols have been published. In addition, only a handful of agents which are available may have adverse effects or be contraindicated in certain clinical situation. Also, resistance may play a role in some infections (1).

In human giardiasis, therapeutic failure is occurring more and more frequently, due to low compliance with drug therapy, reinfestation or parasite resistance to metronidazole and/or the nitroimidazole-related compounds secnidazole, tinidazole plus ornidazole as well as quinacrine and furazolidone. Albendazole has been proposed as an alternative to metronidazole but is not always effective (2-3).

G.lamblia has been reported to be highly susceptible to albendazole *in vitro*, but the efficacy of drugs in clinical studies is controversial (4).

There is an obvious need for alternative antigiardial agents. The aim of the present study was to assess the effects of albendazole, metronidazole against *G.lamblia* trophozoites in *in vitro* condition.

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Materials and Methods

Isolation of G.lamblia cysts

G.lamblia cysts were isolated from fresh feces of patients with giardiasis in Tehran. The samples showed no contamination to the other intestinal parasites and fungi. The average number of cysts was more than 10/40 field.

G.lamblia cysts were washed, purified and concentrated from feces using sucrose flotation method with a simplified sucrose gradient method. The cysts after being washed twice in distilled water were exposed with antibiotics and fungicide at 4°C for a maximum of 4 days prior to use (5).

The excystation procedure was done using Bingham & Meyer technique (6). These procedures involved two steps: the induction of excystation performed in acid solution, and the culture and axenization in TYI-S-33 medium supplemented with bile and heat inactivated bovine serum 10% for 24-48 h (6).

In excystation procedure 1 volume of clean cysts was added to 9 volumes of HCl in pH=2 and 0.01N, and were incubated at 37 °C for 1h (5).

Culture and count

For this experiment we needed to prepare a large number of trophozoites. Hence the excysted parasites were added into the culture tubes containing 7 ml of TYI-S-33 (borosilicae glass Screw-capped vials) and were incubated at 37 °C for 24-48 h. Trophozoites were harvested by chilling the tubes in ice water for 10-15 min. Then counted by haemocytometer (Neubauer cell-counter chamber).The optimum trophozoite concentration used was 50, 000 cells/ml (6).

Evaluation of parasite viability

To assess trophozoites viability, eosine 0.1% staining method was used.

Drugs assessment

Metronidazole and albendazole were prepared from Daru Pakhsh Co. The chemotherapeutic agents used were metronidazole and albendazole. Stocks solution of metronidazole and albendazole were prepared in distilled water and dimethyl sulphoxide (DMSO) respectively. The final DMSO concentration in the culture tubes was always <0.5%. Different concentrations of each drug (0.05, 0.1, 2, 10, 50µg/ml) were prepared.

Trophozoites were exposed to different concentration (0.05, 0.1, 2, 10, 50 µg/ml) of the drugs at 37 °C for 4 h. After chilling in ice water, trophozoites were counted by haemocytometer. In control groups equivalent concentrations of distilled water and DMSO were used, in this regard we used water as the control for metronidazole group and DMSO (with the same concentration in stock solution) for albendazole group. The antiprotozoal activities of albendazole were compared with metronidazole within the same experiment.

Statistical analysis

The percentage of growth inhibition was calculated by comparison of growth rate of test group control group. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of the drug that inhibited growth by 50% as calculated by probit analysis. The 90% inhibitory concentration (IC_{90}) was similarly calculated (7).

Results

From 37 human faecal samples containing *G. lamblia* cyst, 10 samples were excysted in axenic cultured successfully.

The IC_{50} calculated for each isolate after 4 h drug exposure, is shown in Table1. For metronidazole IC_{50} varied from 0.7 to 10 µg/ml representing a range of variation of 14.28 fold while IC_{90} varied from 6 to 42.5 µg/ml representing a range of variation of 7.08 fold in susceptibility. For albendazole, the range of IC_{50} varied from 0.062 to 0.1µg/ml representing a range of variation of 1.61 fold while IC_{90} varied from 1.5 to3µg/ml representing a range of variation of 2 fold in susceptibility.

The results of IC_{50} in Table 1 showed that the mean for albendazole was 0.08 µg/ml and for

metronidazole was 3.32 µg/ml. In this regard, the ratio of drug concentration was 3.32/0.08 (41.5) and showed albendazole than to metronidazole 41.5 times more susceptible.

Table 2 shows the comparison of percentage of killed trophozoites of *G.lamblia* following 4h exposure to different concentrations of metronidazole and albendazole.

Table 1: Susceptibility of *G.lamblia* isolates to (metronidazole and albendazole) *in vitro* condition.

Isolate No.	IC_{50} Metronidazole (µg/ml)	IC_{50} Albendazole (µg/ml)	IC_{90} Metronidazole (µg/ml)	IC_{90} Albendazole (µg/ml)
1	2	0.065	13.5	1.55
2	4	0.095	20	2.3
3	1.5	0.065	10	1.5
4	9	0.1	42	2.4
5	1.4	0.08	14	1.6
6	1.8	0.078	20	2.4
7	0.7	0.063	6	1.5
8	2	0.1	20	3
9	10	0.1	42.5	2.4
10	0.8	0.062	7	1.47
Mean	3.32	0.08	19.5	2.01
S.D.	3.21	0.01	12.37	0.52
Variation(fold)	14.28	1.61	7.08	2

Table 2: Comparison of percentage of killed trophozoites of *G.lamblia* following 4h exposure to different concentration of metronidazole and albendazole

Drugs	Drug concentrations (µg/ml)	Percentage of killed trophozoites Following exposure to the drugs
Metronidazole	0.05	11
	0.1	32.3
	2	52.5
	10	75.8
	50	95.9
Albendazole	0.05	33
	0.1	60.4
	2	87
	10	100
	50	100

Discussion

The present study has demonstrated the superior potency of albendazole against *Giardia* trophozoites *in vitro* compared to metronidazole. Our finding was similar to the other reported data. Meloni *et al.* (1990) found that albendazole was 5-10 times more active than metronidazole or tinidazole against *G. lamblia* as judged the IC50 (8). Edlind *et al.* (1990) reported that albendazole was 50 times more active than metronidazole (9). Upcroft *et al*. (1999) reported that a great deal of variation in the antiprotozoal efficacies of the 13 compounds tested was revealed. Only one compound was less effective than metronidazole against all three species of protozoa examined. All other compounds were as effective or more effective than metronidazole against some or all organisms tested (10). According to Upcroft *et al*. (2001) study the MIC for metronidazole susceptible lines was 6.3 µM in those assays and that for the resistant lines was consistently higher (11). Majewska *et al.* (1991), found that all individual stocks were composed of parasite populations characterized by significantly (P<0.05) differing sensitivities to both ornidazole and metronidazole (12). Farbey *et al.* (1995) reported that dose-response curves were constructed for each isolate for metronidazole, the most common clinically used antigiardial agent, as well as for albendazole. Less than a 9-fold variation was found in the susceptibility of the isolates to albendazole, while for metronidazole there was well over a 16,000 -fold variation between the same groups of isolates (13). In 2003 clinical resistance against the drug has been reported by Wright *et al.*, including cases where patients failed both metronidazole and albendazole treatments. Maintaining the usefulness of the existing drugs is the most cost-effective measure to ensure the continued availability of antigiardial drugs (14).

We found difference in activity of the drugs against various isolates. These differences could be due to different strains of *G.lamblia*. The heterogeneity in drug sensitivity of parent *G.lamblia* populations may be one of the factors responsible for treatment failures of human giardiasis.

The results of this study are significant with respect to prospects for a new approach to the chemotherapy of giardiasis. Albendazole appears to be an ideal anti-giardial agent. It has lower toxicity than currently available chemotherapeutic agents and is relatively insoluble and poorly absorbed from the gut, thus maximizing contact with intestinal parasites and should not affect the intestinal flora.

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