Original Article

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Efficacy of some metronidazole derivatives against *Giardia lamblia*, *in vivo* study

Mohammad Hossein Motazedian[1], Nilofar Mohammadpour[2], Soghra Khabnadideh[3*]

[1]Basic Research in Infectious Diseases Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Parasitology, School of Medicine. Ahvaz University of Medical Sciences, Ahvaz Iran.
[3]School of Pharmacy, and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Giardiasis is a protozoal infection of small intestine caused by *Giardia lamblia*. This disease is usually asymptomatic, though it can present as acute or chronic diarrhea. Giardiasis, which is a major cause of intestinal infection, is endemic in Iran. Despite reports about drug resistance, long course treatment and various side effects of metronidazole, it is the drug of choice for giardiasis. In this study, we investigated the *in vivo* effects of five new derivatives (a-e) of metronidazole (MTZ) on the *Giardia lamblia* trophozoite in infected mice. *Giardia intestinalis* cysts were isolated from a patient and purified by sucrose gradient method. Fifty purified cysts were fed to the mice. After development of infection, the new metronidazole derivatives were given to the mice and the results were compared with metronidazole as the positive control group. Compounds *a* and *b* showed desirable antigiardiasis activity and could destroy the cyst and trophozoite of *Giardia lamblia* in mice after both two and four days, but the activity of the other compounds appeared only after 4 days.

Keywords: Giardiasis, Metronidazole, Treatment.

1. Introduction

Giardiasis is a protozoal infection of small intestine caused by *Giardia lamblia*. The disease is usually asymptomatic, or it can present as acute or chronic diarrhea (1, 2). Giardiasis has a worldwide distribution, and is a major cause of intestinal infection and diarrhea. Contaminated water seems to be the main source of infection (3, 4).

It can cause endemic and epidemic intestinal disease, and is more common in crowded areas and among homosexuals (5).

Manifestations of giardiasis range from asymptomatic carriage to fulminant diarrhea, malabsorption and weight loss. Symptoms usually develop after an incubation period of 5-25 days (6).

Infection follows the ingestion of cysts, which remain in the small intestine and release flagellated trophozoites that multiply by binary fusion (7).

Trophozoites attach to the mucosal epithelium by means of a ventral sucking disk. As a trophozoite encounters altered conditions, it forms a morphologically distinct cyst, which is the stage of parasite usually found in the feces. Cysts remain viable for several days in 5-24 °C, but do not tolerate heating or desiccation. They can be eradicated from water by boiling or filtration, but are resistant to routine chlorination methods (7).

Drugs such as quinacrine tinidazole, metronidazole, albendazole, paramomycin, and furazolidone are used for treatment of giardiasis. These drugs have various side effects ranging from nausea, vomiting, anorexia, dry tongue and urticaria to potential carcinogenic effects of albendazole (7). Besides, there have been reports about resistance to metronidazole, quinacrine, and fura-

Corresponding Author: Soghra Khabnadideh, School of Pharmacy, and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail: khabns@sums.ac.ir

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zolidone (8).

Efforts have focused on the development of new, less toxic and more efficacious antigiardiasis drugs with novel mechanism of action. However newer and less toxic antigiardiasis agents are available for clinical use, their efficacy in some invasive infections is not optimal (8).

In our previous works, we described the synthesis and also *in vitro* antigiardiasis activity of some new metronidazole compounds (8). As our new compounds showed desirable biological activity in higher concentration than metronidazole (8), in this study, we evaluated these new derivatives against infected mice *in vivo*.

2. Materials and methods

2.1. Cyst Purification

Giardia lamblia cysts were isolated from a patient with severe infection and with 2% sucrose gradient flotation technique cyst purification was done. The collected cysts were washed twice, and kept in a refrigerator until use (9).

For *in vivo* investigation, 100 white Syrian mice, aged of 3 to 6 months and weighed 23 to 27 g, were selected, and all were examined by formaldehyde ethyl acetate concentration method for Giardia infection. Forty cysts were inoculated to mice by gavage (9). After ten days, mice stools were again examined by formaldehyde ethyl acetate concentration method for Giardia infection (9). *Giardia lamblia* cysts were found in 70% of the samples.

The infected mice were separated and divided into 6 groups, each consisting of 10 mice. Five groups were considered for the activity of the synthesized compounds and one as the MTZ positive control (9).

2.2. Chemical Tested Compounds

2-(1H-1-imidazolyl)-1-phenyl-1-ethanol *(a)*, 2-(2–methyl–1 H-1-imidazolyl)–1-phenyl-1-ethanol *(b)*, 2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-phenyl-1-ethanol *(c)*, 2-(1H-1-imidazolyl)-1-cyclohexanol *(d)* and 1[bis-4-methoxyphenyl-phenylmethyl]-2-methyl-4-nitroimidazole *(e)* were used for *in vivo* tests.

These compounds were synthesized according to the previously described procedure (8). After inducing the infection in the mice, each mouse was orally treated with 0.0125 mg/gr of one of the new compounds for four days. Seven days after treatment, the treated mice were killed and their duodenum mucosal were searched for *Giardia trophozoite*.

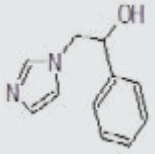
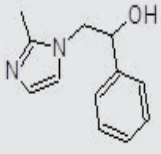
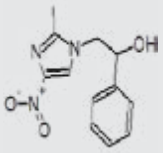
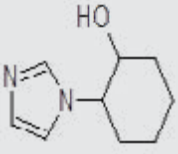
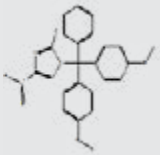
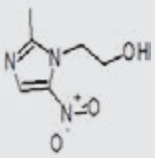
3. Results

After treating the infected mice with the synthesized compounds, compounds a and b showed efficacies similar to MTZ and eradicated the cysts and trophozoites after both 2 and 4 days. Compounds c, d and e were effective after 4 days, but the viable trophozoites were still detected in the intestines after 2 days (Table 1).

4. Discussion

Several studies have so far investigated the efficacy of different drugs on *Giardia lamblia* (10-13). These researchers tested the therapeutic effects of tinidazole, metronidazole, clotrimazole, miconazole, ketoconazole, and mebendazole on *Giardia lamblia trophozoites*. Several of these products have been approved for the treatment of

Table 1. Anti-giardiasis effects of the synthesized compounds.

Compds.	*a*	*b*	*c*	*d*	*e*	MTZ
Chemical Structures						
Efficacy 2 days after treatment	+	+	-	-	-	+
Efficacy 4 days after treatment	+	+	+	+	+	+

giardiasis. Metronidazol, quinacrine, furazolidone, paromomycin, nitazoxanide 5-nitroimidazole and benzimidazole derivatives, are the most commonly used drugs, and show the most inhibitory effects on growth and adhesion of the parasite (12, 14). Metronidazole and tinidazole are toxic for anaerobic microorganisms such as *Giardia lamblia*, and have shown significant in vitro effects on the parasite (15). Unfortunately, there are some reports about resistance to some of these compounds, including metronidazole both *in vitro* and in clinics (14, 16).

Failure to treatment with metronidazole, the drug of choice, has been reported, and its side effects were found to be more than albendazole and furazolidone. Alizadeh *et al* (17) and sadjjadi *et al* (18) reported 23% and 10% resistance against metronidazole, respectively. Research for finding new drugs to overcome resistance and side effect issues are needed for control and treatment of giardidsis.

In this study, we evaluated our 5 new synthesized derivatives against Giardia infection in mice *in vivo*. Our results showed compounds *a* and *b*, which contain imidazole and 2-methyl imidazole ring as their azole rings, respectively, and an aromatic ring in their side chain, are as effective as MTZ. According to our results, substitution of a nitro group at position 4 of imidazole ring decreased the activity; as compound c was not as effective as compounds *a* and *b*. Also substitution of a saturated ring or a bulk group in the side chain decreased the activity, because compounds *d* and *e* were not as effective as compounds *a* and *b*.

Although Kavousi (19) and Löfmark (15) showed that MTZ is the best choice for giardiasis, our compounds *a* and *b* could be good candidates as new anti-giardiasis agents, although more evaluations are needed about the toxicity and pharmacokinetic properties of these compounds.

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Conflict of interest

None declared.

6. References

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