Giardiasis treatment: An update with a focus on refractory disease

Kristine Mørch and Kurt Hanevik

Norwegian National Advisory Unit on Tropical Infectious Diseases, Department or Medicine, Haukeland University Hospital, Bergen, Norway and Department of Clinical Science and Faculty of Medicine, University of Bergen, Bergen, Norway.

Correspondence to Kristine Mørch, Department of Medicine, Haukeland University Hospital, N - 5021 Bergen, Norway. Tel: + 47 55975000. Email: Kristine.moerch@helse-bergen.no

Purpose of review

Giardiasis remains a common cause of diarrhea and intestinal enteropathy globally. Here we give an overview of clinical treatment studies and discuss potential mechanisms and molecular targets for in vitro testing of drug resistance.

Recent findings

Giardia is a cause of disease both in diarrheal and non-diarrheal cases. The prevalence of treatment refractory giardiasis is increasing. Recent studies reveal 5-nitroimidazole refractory infection occurs in up to 50% of cases. Mechanisms of drug resistance are not known.

Placebo controlled studies of drug efficacy, taking the self-limiting course of giardiasis into account, has not been reported. No randomized controlled trials of treatment of refractory infection have been performed the last 25 years.

Based on the clinical studies reported, combination treatment with a 5-nitroimidazole and a benzimidazole is more effective than repeated courses of 5-nitroimidazole or monotherapies in refractory cases. Quinacrine is effective in refractory cases, but potentially severe side effects limit its use.

Summary

A combination of a 5-nitroimidazole and albendazole or mebendazole, and quinacrine monotherapy, are rational choices in nitroimidazole refractory infections, but randomized controlled studies are needed.

Further research into more recent clinical isolates is necessary to uncover mechanisms for the increase in metronidazole refractory giardiasis observed during the last decade.

Keywords

Giardia, 5-nitroimidazoles, albendazole, paromomycin, quinacrine

Introduction

Giardiasis is a protozoan infection of the small bowel, frequently responsible for traveler' diarrhea [1, 2], and for complications from chronic or repeated infections [3, 4].

The infectious cysts can survive for months in the environment, explaining why the infection is closely associated with unsafe water. In low- and middle-income countries (LMIC), the incidence is very high and in some areas close to 100% of children have had one or more *Giardia* infections before the age of 2 years [3, 5, 6]. In high-income countries, *Giardia* is a frequent cause of waterborne outbreaks [7], as in Bergen, Norway in 2004 where approximately 2500 people were treated for giardiasis [8].

In a non-endemic area, clinical infection in all age groups range from asymptomatic carrier state to severe abdominal pain, diarrhea, vomiting, flatulence, malabsorption, anorexia and weight loss, with a fraction of patients developing post-infectious irritable bowel disease and chronic fatigue [8-11]. Histological findings in over 500 cases of giardiasis found normal appearance of the duodenal mucosa in the majority of cases; Only a minority of cases revealed duodenal inflammation and mild villous shortening on light microscopy [12]. However, in previously unexposed adults, protracted infection has been associated with duodenal inflammation [13, 14] and increased risk of post-infectious complications [15].

In low-resource settings, *Giardia* infection is common both in diarrheal and non-diarrheal cases, and is associated with chronic diarrhea [16]. It mainly occurs in children, and a serious manifestation is its contribution to malnutrition and stunting of growth following early or repeated infections [3, 17-20]. Two large multicenter studies of diarrhea etiology in LMIC reported that *Giardia* was more prevalent in controls than in diarrheal cases [21, 22], leading to speculations about a potential negative association between *Giardia* and acute infectious diarrhea. However, after adjusting for

metronidazole exposure in diarrhea cases in the MAL-ED study [3], a negative association between *Giardia* and diarrhea was not significant.

A small experimental study among adults challenged with *Giardia* revealed that establishment of infection was dose dependent and shedding was self-limited in 85% of individuals, but 15% of individuals developed chronic shedding [23]. The mean duration of infection in spontaneously cured cases was almost three weeks. It is rational to treat giardiasis both in endemic and non-endemic areas, in order to prevent complications, chronic disease and spread of the infection.

The first line treatment of giardiasis are the 5-nitroimidazoles, but there are reports of high incidence of nitroimidazole refractory cases of giardiasis [24, 25]. Although antimicrobial resistance is an increasing problem in giardiasis, in vitro susceptibility testing methods to help clinicians guide targeted treatment is not available. This review provide an overview of clinical studies of anti-*Giardia* treatment and suggest choice of treatment in refractory cases, and discuss potential mechanisms and molecular targets for future in vitro testing of drug resistance.

Treatment of giardiasis

Until a few decades ago, *Giardia lamblia* was considered an innocent bystander in the microbial gut flora, but after the pathogenicity of the parasite was recognized, different classes of drugs have been used [26]. Current therapies, with different availability across countries, include nitroimidazole derivatives (metronidazole, tinidazole, secnidazole and ornidazole), benzimidazoles (albendazole, mebendazole), nitazoxanide, furazolidone, quinacrine, chloroquine and paromomycin.

Current treatment options

Randomized controlled trials (RCT) of drugs used in first line treatment of giardiasis are listed in Table 1. Efficacy is evaluated by stool microscopy post-treatment in all the studies. Study designs are heterogeneous regarding study population, dose and duration of treatment, time until follow up and number of stool samples examined.

5-nitroimidazoles

5-nitroimidazoles are the most frequently used first line drugs. These drugs become activated after entering the parasite, and kills the microorganism by release of reactive, toxic, partially reduced intermediates [27]. Tinidazole, secnidazole and ornidazole have long half-lifes, and cure rates above 90% are reported when single dose (sd) regimens of 1-2 g in adults and 30 mg/kg (secnidazole), 20-40 mg/kg (ornidazole) and 50 mg/kg (tinidazole) in children, are given (Table 1). These nitroimidazoles are better tolerated than metronidazole. Metronidazole has a shorter half-life, and longer courses are needed to achieve similar efficacy; 250 mg three times daily (tid) for 7 days or 500 mg tid for 5 days in adults, and 5-7 mg/kg tid for 5-7 days in children. Reported side effects are gastrointestinal discomfort, anorexia, metallic taste, disulphiram-like effect, headache, vertigo, insomnia, irritability, neuropathy, seizures, rash, leukopenia, hepatitis and pancreatitis (references in Table 1).

Benzimidazoles

Albendazole and mebendazole are widely used anti-helminthic drugs, but treatment efficacy in giardiasis is variable (Table 1). The benzimidazoles exert their function by binding to, and preventing microtubule transport and assembly, and may also induce oxidative stress in the parasite [28]. Albendazole 400mg in adults, and 10 mg/kg in children, sd for 5 days, show 83-96% efficacy, while shorter duration is less effective. Efficacy for mebendazole vary greatly (14 - 95%), both for sd treatment and for dosage 200 mg tid up to 7 days. Benzimidazoles are usually well tolerated; reported side effects are nausea, vomiting, diarrhea and abdominal pain.

Aminoglycoside

The oral aminoglycoside paromomycin is the drug of choice for giardiasis in pregnant women, because it is poorly absorbed and has no systemic effect [29]. Paromomycin inhibits the protein synthesis in *Giardia* [30]. Although this is the only anti-*Giardia* drug considered completely safe during early pregnancy, few clinical studies of treatment efficacy has been reported (Table 1). In a controlled study from Cuba among 256 children, efficacy was 92% compared to 80% for metronidazole [31], while a small case series from 1962 using 15 mg/kg/d for 5 days reported 40% efficacy [32].

Acridine

Quinacrine is an effective anti-*Giardia* drug (Table 1), although its exact mechanism of action in *Giardia* is not known [33]. Two studies among children treated for 5 days report 100% efficacy. However, quinacrine has bothersome and potentially severe side effects. In a study reporting 77% efficacy, severe vomiting explained most of the treatment failures in small children [34]. In a study from Cuba reporting 84% efficacy, nausea, vomiting, discoloration of skin and headache was significantly more common than in the metronidazole group [35]. Yellow discoloration of skin is a common but harmless and self-limiting side effect [34-36], while neuropsychiatric disturbances ranging from restlessness, nightmares and insomnia to seizures and psychoses are rare but feared complications [37-39].

Furazolidone

Furazolidone has shown high efficacy in studies of first line therapy, although no RCTs are reported since 1980s (Table 1). It is a prodrug which release damaging intermediates when its nitro group is reduced [40]. The drug is contraindicated in glucose-6-phosphate-dehydrogenase (G6PD) deficiency and in neonates, due to risk of hemolytic anemia.

Nitazoxanide

Nitazoxanide is the only available drug for *Cryptosporidium,* and like metronidazole it is also activated by reduction of its nitro group and inhibits metabolic enzymes [41]. In controlled studies of giardiasis, it has shown efficacies between 44% and 91% (Table 1). It may cause some gastrointestinal discomfort but is usually well tolerated.

Chloroquine

Chloroquine is effective against non-falciparum malaria and rheumatic disorders. Its mechanism of action against giardiasis is not known, but it may limit the trophozoite adherence to the intestinal wall. Its effect against *Giardia* has been investigated in RCT's in Cuba; in a large study among children, efficacy was 86% compared to 91% for tinidazole and 62% for albendazole [42]. Reported side effects were bitter taste and gastrointestinal problems, similar to for tinidazole. Chloroquine may potentially cause prolonged Q-T time, and hemolytic anemia in G6PD deficiency [43].

Treatment of refractory giardiasis

Drug trials have usually shown efficacy above 90% for the nitroimidazoles (Table 1), however, treatment refractory disease is an increasing problem. In a study from England, nitroimidazole failure increased from 15% (n=8/53) in 2008 to 40% (n=35/87) in 2013, and among travelers from India treatment failure occurred in as much as 50% [24].

The association between location of contracting infection and nitroimidazole failure, in four clinical studies, is shown in Table 2. As much as 46% failure was reported after metronidazole 500 mg tid for 5 days in a large cohort from Cuba in 2018 [25]. Although there are few studies and number of cases are small, treatment failure among European travelers seemed to be more common after travel to Asia than to Africa and Latin America (Table 2).

A few clinical, mainly small, studies are available providing evidence for treatment efficacy in refractory cases (Table 3). Observational studies show that another class of drug, combination therapy, or repeated courses with increased dose/duration of the same drug is used with varying efficacy.

Quinacrine is effective in almost all cases, but due to availability problems and potentially severe side effects, the drug is normally preferred only when other treatment options fail. Both quinacrine in combination with a 5-nitroimidazole for up to three weeks, and quinacrine monotherapy for as short as three days, were effective in reported studies (Table 3).

Combination of drugs from different classes also seem to be an effective second line option. Albendazole in combination with metronidazole was effective in 90% in a small RCT in Italy [44], and 79% effective in a prospective treatment ladder study in Norway [45]. The combination of secnidazole and mebendazole was effective in 89% in a large treatment ladder study in Cuba, while a repeated course of 5-nitroimidazole after initial metronidazole treatment cured only 24% - 27% [25]. Albendazole monotherapy seem to be less effective, although number of cases reported are small (Table 3).

Drug resistance in giardiasis

Antimicrobial resistance is defined by WHO as "the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others" [46]. There are no defined molecular resistance mechanisms in *Giardia* yet, thus the term treatment refractory is better suited to describe treatment failure, as there is no doubt that also the hosts' combined immune defenses play a role in eradicating the parasite.

Treatment refractory clinical infections have mostly been observed against the 5-nitroimidazole drug metronidazole [24]. To exert its antibiotic function, metronidazole needs to be activated by

intracellular reduction of its nitro group, creating hyper-reactive intermediates, disrupting the microorganism by excessive oxidative stress [33]. Four enzymes have been identified in the metabolism of *Giardia* that seem capable of activating metronidazole by partial reduction into toxic hyper-reactive intermediates; nitroreductase 1 (NR) 1 [47], pyruvate:ferredoxin oxidoreductase (PFOR) 1 and 2 [48, 49] and the thiol-cycling associated enzyme thioredoxin reductase (TrxR) [48, 50]. This partial reduction only occurs under anaerobic or microaerophilic conditions.

Inducing resistance in vitro, by gradually increasing drug concentrations in growth media of a small fraction of *Giardia* strains that can be cultured, is quite easy. Most research on resistance mechanisms have been performed on such laboratory strains, isolated from infected humans decades ago [51]. Interestingly the drug resistance seen in laboratory induced strains is reduced or lost if the trophozoites pass through a cyst stage [52]. It is unknown how important this non-transmissible resistance is in clinical practice, but the rapidly increasing resistant infections seen, indicates the presence of new heritable traits conferring metronidazole resistance, or the ability to rapidly develop it. Typing of metronidazole resistant isolates in two studies show that several subgroups of assemblage A and B isolates are represented among resistant strains [53, 54].

Elucidating mechanisms of drug resistance

Methods for determining resistance in *Giardia* is not easily available. Only a small fraction of clinical isolates will grow in available liquid culture media, with assemblage A more often being successful than assemblage B parasites [55]. Strains will grow at different speeds making measurement of growth inhibition in drug dilutions very difficult to standardize, even for the few isolates that are culturable [56, 57].

Compared to bacteria, identifying genetic markers of resistance in an early eukaryote, binucleate, functionally tetraploid organism such as *Giardia* is challenging. A relatively high degree of genetic

diversity is seen in the metronidazole activating genes [48], both between isolates and in the up to four alleles of each gene. This offers *Giardia* substantial potential for variability to tweak its metabolism and for selection of well-adapted variants.

Studies analyzing both genes and gene expression in metronidazole resistant laboratory strains and their metronidazole-susceptible ancestor, find a quite broad and variable adaptive response in the resistant isotypes [58]. There seem to be both post-transcriptional and post-translational alterations [27, 52, 59], and *Giardia* thereby seem to have several ways it may develop tolerance to metronidazole.

A frequent finding in the few existing studies in metronidazole-resistant laboratory strains has been downregulation of NR1 [58-60]. One isolate has been shown to harbor a non-sense mutation in transcripts of this gene, inferring that it may have substantially lower levels of this metronidazole-activating enzyme [58]. Results from genetic analysis of the other metronidazole metabolizing enzymes have been less consistent, but indicates a role for NR2, PFOR, and TrxR and Flavin Mononucleotide-dependent oxidoreductases in resistance against metronidazole [27].

In a study examining protein expression in *Giardia* strains made gradually resistant *in vitro* against metronidazole and nitazoxanide, no specific trait or marker was identified [61]. Rather, it seemed that each of the three resistant strains had found their own strategy for tolerating high level of the drugs.

Thus, we can conclude that despite the carefully designed gene and protein expression studies in cultured isolates we still have very limited knowledge regarding the ways *Giardia* protects itself from drugs such as metronidazole. However, laboratory induced resistant strains gathered decades ago may be quite different from the metronidazole refractory isolates now circulating. The rapidly

increasing resistance reported [24, 25], suggest that there are genetic changes that helps the parasite overcome the toxic effects of metronidazole, but further research is needed in clinical isolates.

Conclusion

Nitroimidazole failure in up to 50% is reported in giardiasis, both among travelers and in high endemic countries. Repeated courses of nitroimidazole, and monotherapy with a drug with another mode of action, seem to be less effective than combination therapy. A combination of a 5-nitroimidazole and albendazole or mebendazole, and quinacrine as last option, are rational choices in nitroimidazole refractory infections.

Research in drug resistant *Giardia* laboratory strains have not yet succeeded in identifying mechanisms, or markers, of resistance but show that *Giardia* strains possess a varied armamentarium of adaptations. Further research into more recent clinical isolates seems necessary to uncover mechanisms for the emerging metronidazole refractory cases.

Acknowledgements

None

Financial support and sponsorship

This work was supported by the Norwegian National Advisory unit on Tropical Infectious Diseases, Haukeland University Hospital, Norway.

Conflicts of interest

None

Key points

- Giardiasis is an important cause of diarrhea and malabsorption.

- Clinical resistance against 5-nitroimidazoles is an increasing problem.
- A 5-nitroimidazole in combination with a benzimidazole, and quinacrine monotherapy, are effective second and third line treatment options.
- Mechanisms of clinical drug resistance are yet not known.

References

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest

•• of outstanding interest

1. Schlagenhauf P, Weld L, Goorhuis A, et al. Travel-associated infection presenting in Europe (2008-12): an analysis of EuroTravNet longitudinal, surveillance data, and evaluation of the effect of the pre-travel consultation. Lancet Infect Dis. 2015;15(1):55-64.
2. Pouletty M, De Pontual L, Lopez M, et al. Multiplex PCR reveals a high prevalence of multiple pathogens in traveller's diarrhoea in children. Arch Dis Child. 2019;104(2):141-6.
3. Rogawski ET, Bartelt LA, Platts-Mills JA, et al. Determinants and Impact of Giardia Infection in the First 2 Years of Life in the MAL-ED Birth Cohort. J Pediatric Infect Dis Soc. 2017;6(2):153-60.
4. Lengerich EJ, Addiss DG, Juranek DD. Severe giardiasis in the United States. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1994;18(5):760-3.
5. Gilman RH, Marquis GS, Miranda E, et al. Rapid reinfection by Giardia lamblia after treatment in a hyperendemic Third World community. Lancet. 1988;1(8581):343-5.
6. Roy M, Singha B, Dhar D, Roychoudhury S. Prevalence of Giardia intestinalis with other co-infecting parasites in Barak Valley, Assam, India: a molecular approach. J Parasit Dis. 2019;43(3):426-42.
7. Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health. 2007;5(1):1-38.
8. Hanevik K, Wensaas KA, Rortveit G, et al. Irritable bowel syndrome and chronic fatigue 6 years after giardia infection: a controlled prospective cohort study. Clin Infect Dis. 2014;59(10):1394-400.
9. Nygard K, Schimmer B, Sobstad O, et al. A large community outbreak of waterborne giardiasis-delayed detection in a non-endemic urban area. BMC Public Health. 2006;6:141.
10. Lopez CE, Dykes AC, Juranek DD, et al. Waterborne giardiasis: a communitywide outbreak of disease and a high rate of asymptomatic infection. Am J Epidemiol. 1980;112(4):495-507.
11. Morch K, Hanevik K, Rortveit G, et al. High rate of fatigue and abdominal symptoms 2 years after an outbreak of giardiasis. Trans R Soc Trop Med Hyg. 2009;103(5):530-2.
12. Oberhuber G, Kastner N, Stolte M. Giardiasis: a histologic analysis of 567 cases. Scand J Gastroenterol. 1997;32(1):48-51.
13. Hanevik K, Hausken T, Morken MH, et al. Persisting symptoms and duodenal inflammation related to Giardia duodenalis infection. J Infect. 2007;55(6):524-30.
14. Dizdar V, Hausken T, Laerum OD, et al. Prolonged Duodenal Mucosal Lymphocyte Alterations in Patients With and Without Postinfectious Functional Gastrointestinal Disorders After Giardia Infection. The Journal of infectious diseases. 2019;220(2):321-9.
15. Morch K, Hanevik K, Rortveit G, et al. Severity of Giardia infection associated with post-infectious fatigue and abdominal symptoms two years after. BMC Infect Dis. 2009;9:206.
16. Muhsen K, Levine MM. A systematic review and meta-analysis of the association between Giardia lamblia and endemic pediatric diarrhea in developing countries. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55 Suppl 4:S271-93.
17. Celiksoz A, Acioz M, Degerli S, et al. Effects of giardiasis on school success, weight and height indices of primary school children in Turkey. Pediatrics international : official journal of the Japan Pediatric Society. 2005;47(5):567-71.
18. Prado MS, Cairncross S, Strina A, et al. Asymptomatic giardiasis and growth in young children; a longitudinal study in Salvador, Brazil. Parasitology. 2005;131(Pt 1):51-6.
19. Nunez FA, Hernandez M, Finlay CM. Longitudinal study of giardiasis in three day care centres of Havana City. Acta tropica. 1999;73(3):237-42.

20. Donowitz JR, Alam M, Kabir M, et al. A Prospective Longitudinal Cohort to Investigate the Effects of Early Life Giardiasis on Growth and All Cause Diarrhea. Clin Infect Dis. 2016;63(6):792-7.
21. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). The Lancet Global health. 2015;3(9):e564-75.
22. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209-22.
23. Rendtorff RC. The experimental transmission of human intestinal protozoan parasites. II. Giardia lamblia cysts given in capsules. Am J Hyg. 1954;59(2):209-20.
24. Nabarro LE, Lever RA, Armstrong M, Chiodini PL. Increased incidence of nitroimidazole-refractory giardiasis at the Hospital for Tropical Diseases, London: 2008-2013. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2015;21(8):791-6.
25. Canete R, Noda AL, Rodriguez M, et al. 5-Nitroimidazole refractory giardiasis is common in Matanzas, Cuba and effectively treated by secnidazole plus high-dose mebendazole or quinacrine: a prospective observational cohort study. Clin Microbiol Infect. 2019.
••Good quality treatment ladder study from Cuba. Metronidazole resistance 46%, low efficacy of repeated courses of nitroimidazole, high cure rate after nitroimidazole in combination with mebendazole, and 100% efficacy after quinacrine as last step. The largest prospective study of treatment refractory giardiasis reported up to now.
26. Arguello-Garcia R, Leitsch D, Skinner-Adams T, Ortega-Pierres MG. Drug resistance in Giardia: Mechanisms and alternative treatments for Giardiasis. Adv Parasitol. 2020;107:201-82.
• Extensive review of drugs under investigation and potential targets for anti-Giardia agents.
27. Leitsch D. A review on metronidazole: an old warhorse in antimicrobial chemotherapy. Parasitology. 2019;146(9):1167-78.
28. Martinez-Espinosa R, Arguello-Garcia R, Saavedra E, Ortega-Pierres G. Albendazole induces oxidative stress and DNA damage in the parasitic protozoan Giardia duodenalis. Front Microbiol. 2015;6:800.
29. Kreutner AK, Del Bene VE, Amstey MS. Giardiasis in pregnancy. Am J Obstet Gynecol. 1981;140(8):895-901.
30. Edlind TD. Susceptibility of Giardia lamblia to aminoglycoside protein synthesis inhibitors: correlation with rRNA structure. Antimicrob Agents Chemother. 1989;33(4):484-8.
31. Nunez FA EA, Finlay CM. Eficacia de various esquemas de tratamiento para la infection por *Giardia lamblia* ninos. Rev Panam Infectol. 2004;6:17-20.
32. Carter CH, Bayles A, Thompson PE. Effects of paromomycin sulfate in man against Entamoeba histolytica and other intestinal protozoa. Am J Trop Med Hyg. 1962;11:448-51.
33. Leitsch D. Drug Resistance in the Microaerophilic Parasite Giardia lamblia. Curr Trop Med Rep. 2015;2(3):128-35.
34. Craft JC, Murphy T, Nelson JD. Furazolidone and quinacrine. Comparative study of therapy for giardiasis in children. Am J Dis Child. 1981;135(2):164-6.
35. Canete R, Escobedo AA, Gonzalez ME, Almirall P. Randomized clinical study of five days apostrophe therapy with mebendazole compared to quinacrine in the treatment of symptomatic giardiasis in children. World J Gastroenterol. 2006;12(39):6366-70.
36. Kavousi S. Giardiasis in infancy and childhood: a prospective study of 160 cases with comparison of quinacrine (Atabrine) and metronidazole (Flagyl). Am J Trop Med Hyg. 1979;28(1):19-23.
37. Genel F, Erermis S, Aksu G, et al. Quinacrine-induced psychiatric disturbances in a child with common variable immunodeficiency and chronic giardiasis. Hum Psychopharmacol. 2002;17(7):357-9.
38. Lindenmayer JP, Vargas P. Toxic psychosis following use of quinacrine. J Clin Psychiatry. 1981;42(4):162-4.

39. Weisholtz SJ, McBride PA, Murray HW, Shear MK. Quinacrine-induced psychiatric disturbances. South Med J. 1982;75(3):359-60.
40. Tatsumi K, Yamada H, Yoshimura H, Kawazoe Y. Metabolism of furazolidone by milk xanthine oxidase and rat liver 9000g supernatant: formation of a unique nitrofuran metabolite and an aminofuran derivative. Arch Biochem Biophys. 1981;208(1):167-74.
41. Muller J, Wastling J, Sanderson S, et al. A novel Giardia lamblia nitroreductase, GlNR1, interacts with nitazoxanide and other thiazolides. Antimicrob Agents Chemother. 2007;51(6):1979-86.
42. Escobedo AA, Nunez FA, Moreira I, et al. Comparison of chloroquine, albendazole and tinidazole in the treatment of children with giardiasis. Ann Trop Med Parasitol. 2003;97(4):367-71.
43. Borba MGS, Val FFA, Sampaio VS, et al. Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients Hospitalized With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection: A Randomized Clinical Trial. JAMA Netw Open. 2020;3(4):e208857.
44. Cacopardo B, Patamia I, Bonaccorso V, et al. [Synergic effect of albendazole plus metronidazole association in the treatment of metronidazole-resistant giardiasis]. Clin Ter. 1995;146(12):761-7.
45. Morch K, Hanevik K, Robertson LJ, et al. Treatment-ladder and genetic characterisation of parasites in refractory giardiasis after an outbreak in Norway. J Infect. 2008;56(4):268-73.
46. Hjelt K, Paerregaard A, Krasilnikoff PA. Giardiasis causing chronic diarrhoea in suburban Copenhagen: incidence, physical growth, clinical symptoms and small intestinal abnormality. Acta Paediatr. 1992;81(11):881-6.
47. Pal D, Banerjee S, Cui J, et al. Giardia, Entamoeba, and Trichomonas enzymes activate metronidazole (nitroreductases) and inactivate metronidazole (nitroimidazole reductases). Antimicrob Agents Chemother. 2009;53(2):458-64.
48. Saghaug CS, Klotz C, Kallio JP, et al. Genetic variation in metronidazole metabolism and oxidative stress pathways in clinical Giardia lamblia assemblage A and B isolates. Infect Drug Resist. 2019;12:1221-35.
49. Leitsch D, Burgess AG, Dunn LA, et al. Pyruvate:ferredoxin oxidoreductase and thioredoxin reductase are involved in 5-nitroimidazole activation while flavin metabolism is linked to 5-nitroimidazole resistance in Giardia lamblia. J Antimicrob Chemother. 2011;66(8):1756-65.
50. Leitsch D, Muller J, Muller N. Evaluation of Giardia lamblia thioredoxin reductase as drug activating enzyme and as drug target. Int J Parasitol Drugs Drug Resist. 2016;6(3):148-53.
51. Townson SM, Laqua H, Upcroft P, et al. Induction of metronidazole and furazolidone resistance in Giardia. Trans R Soc Trop Med Hyg. 1992;86(5):521-2.
52. Muller J, Ley S, Felger I, et al. Identification of differentially expressed genes in a Giardia lamblia WB C6 clone resistant to nitazoxanide and metronidazole. J Antimicrob Chemother. 2008;62(1):72-82.
53. Requena-Mendez A, Goni P, Rubio E, et al. The Use of Quinacrine in Nitroimidazole-resistant Giardia Duodenalis: An Old Drug for an Emerging Problem. J Infect Dis. 2017;215(6):946-53.
54. Lecova L, Weisz F, Tumova P, et al. The first multilocus genotype analysis of Giardia intestinalis in humans in the Czech Republic. Parasitology. 2018;145(12):1577-87.
55. Cruz A, Sousa MI, Azeredo Z, et al. Isolation, excystation and axenization of Giardia lamblia isolates: in vitro susceptibility to metronidazole and albendazole. J Antimicrob Chemother. 2003;51(4):1017-20.
56. Pearce DA, Reynoldson JA, Thompson RC. A comparison of two methods for assessing drug sensitivity in Giardia duodenalis. Appl Parasitol. 1996;37(2):111-6.
57. Leitsch D. Drug susceptibility testing in microaerophilic parasites: Cysteine strongly affects the effectivities of metronidazole and auranofin, a novel and promising antimicrobial. Int J Parasitol Drugs Drug Resist. 2017;7(3):321-7.
58. Ansell BR, Baker L, Emery SJ, et al. Transcriptomics Indicates Active and Passive Metronidazole Resistance Mechanisms in Three Seminal Giardia Lines. Front Microbiol. 2017;8:398.

59. Emery SJ, Baker L, Ansell BRE, et al. Differential protein expression and post-translational modifications in metronidazole-resistant Giardia duodenalis. Gigascience. 2018;7(4).
60. Muller J, Muller N. Nitroreductases of bacterial origin in Giardia lamblia: Potential role in detoxification of xenobiotics. Microbiologyopen. 2019;8(10):e904.
61. Muller J, Braga S, Heller M, Muller N. Resistance formation to nitro drugs in Giardia lamblia: No common markers identified by comparative proteomics. Int J Parasitol Drugs Drug Resist. 2019;9:112-9.
• This paper examines protein expression profiles in laboratory induced resistant strains and their susceptible ancestor strain. No distinct pattern was found. Rather, examination of enzymes involved in nitroreduction and detoxification of nitro radicals, NO or O2 suggest the use of different strategies for each drug and each strain.
62. Jokipii L, Jokipii AM. Comparison of four dosage schedules in the treatment of giardiasis with metronidazole. Infection. 1978;6(2):92-4.
63. Jokipii AM, Jokipii L. Comparative evaluation of two dosages of tinidazole in the treatment of giardiasis. Am J Trop Med Hyg. 1978;27(4):758-61.
64. Nigam P, Kapoor KK, Kumar A, et al. Clinical profile of giardiasis and comparison of its therapeutic response to metronidazole and tinidazole. J Assoc Physicians India. 1991;39(8):613-5.
65. Speelman P. Single-dose tinidazole for the treatment of giardiasis. Antimicrob Agents Chemother. 1985;27(2):227-9.
66. Jokipii L, Jokipii AM. Treatment of giardiasis: comparative evaluation of ornidazole and tinidazole as a single oral dose. Gastroenterology. 1982;83(2):399-404.
67. Bassily S, Farid Z, el-Masry NA, Mikhail EM. Treatment of intestinal E. histolytica and G. lamblia with metronidazole, tinidazole and ornidazole: a comparative study. J Trop Med Hyg. 1987;90(1):9-12.
68. Ozbilgin A, Ertan P, Yereli K, et al. Giardiasis treatment in Turkish children with a single dose of ornidazole. Scand J Infect Dis. 2002;34(12):918-20.
69. Oren B, Schgurensky E, Ephros M, et al. Single-dose ornidazole versus seven-day metronidazole therapy of giardiasis in Kibbutzim children in Israel. Eur J Clin Microbiol Infect Dis. 1991;10(11):963-5.
70. Rastegar-Lari A, Salek-Moghaddam A. Single-dose secnidazole versus 10-day metronidazole therapy of giardiasis in Iranian children. J Trop Pediatr. 1996;42(3):184-5.
71. Cimerman B, Camilo Coura L, JM CS, et al. Evaluation of Secnidazole Gel and Tinidazole Suspension in the Treatment of Giardiasis in Children. Braz J Infect Dis. 1997;1(5):241-7.
72. Hall A, Nahar Q. Albendazole as a treatment for infections with Giardia duodenalis in children in Bangladesh. Trans R Soc Trop Med Hyg. 1993;87(1):84-6.
73. Dutta AK, Phadke MA, Bagade AC, et al. A randomised multicentre study to compare the safety and efficacy of albendazole and metronidazole in the treatment of giardiasis in children. Indian J Pediatr. 1994;61(6):689-93.
74. Baqai R, Zuberi SJ, Qureshi H, et al. Efficacy of albendazole in giardiasis. East Mediterr Health J. 2001;7(4-5):787-90.
75. Yereli K, Balcioglu IC, Ertan P, et al. Albendazole as an alternative therapeutic agent for childhood giardiasis in Turkey. Clin Microbiol Infect. 2004;10(6):527-9.
76. Karabay O, Tamer A, Gunduz H, et al. Albendazole versus metronidazole treatment of adult giardiasis: An open randomized clinical study. World J Gastroenterol. 2004;10(8):1215-7.
77. Pengsaa K, Sirivichayakul C, Pojjaroen-anant C, et al. Albendazole treatment for Giardia intestinalis infections in school children. Southeast Asian J Trop Med Public Health. 1999;30(1):78-83.
78. Canete R, Rodriguez P, Mesa L, et al. Albendazole versus metronidazole in the treatment of adult giardiasis: a randomized, double-blind, clinical trial. Curr Med Res Opin. 2012;28(1):149-54.
79. Pengsaa K, Limkittikul K, Pojjaroen-anant C, et al. Single-dose therapy for giardiasis in school-age children. Southeast Asian J Trop Med Public Health. 2002;33(4):711-7.
80. Escobedo AA, Canete R, Gonzalez ME, et al. A randomized trial comparing mebendazole and secnidazole for the treatment of giardiasis. Ann Trop Med Parasitol. 2003;97(5):499-504.

81. Bulut BU, Gulnar SB, Aysev D. Alternative treatment protocols in giardiasis: a pilot study. Scand J Infect Dis. 1996;28(5):493-5.
82. Sadjjadi SM, Alborzi AW, Mostovfi H. Comparative clinical trial of mebendazole and metronidazole in giardiasis of children. J Trop Pediatr. 2001;47(3):176-8.
83. al-Waili NS, al-Waili BH, Saloom KY. Therapeutic use of mebendazole in giardial infections. Trans R Soc Trop Med Hyg. 1988;82(3):438.
84. Gascon J, Moreno A, Valls ME, et al. Failure of mebendazole treatment in Giardia lamblia infection. Trans R Soc Trop Med Hyg. 1989;83(5):647.
85. Canete R, Escobedo AA, Gonzalez ME, et al. A randomized, controlled, open-label trial of a single day of mebendazole versus a single dose of tinidazole in the treatment of giardiasis in children. Curr Med Res Opin. 2006;22(11):2131-6.
86. Almirall P, Escobedo AA, Ayala I, et al. Mebendazole compared with secnidazole in the treatment of adult giardiasis: a randomised, no-inferiority, open clinical trial. Journal of parasitology research. 2011;2011:636857.
87. Sabchareon A, Chongsuphajaisiddhi T, Attanath P. Treatment of giardiasis in children with quinacrine, metronidazole, tinidazole and ornidazole. Southeast Asian J Trop Med Public Health. 1980;11(2):280-4.
88. Levi GC, de Avila CA, Amato Neto V. Efficacy of various drugs for treatment of giardiasis. A comparative study. Am J Trop Med Hyg. 1977;26(3):564-5.
89. Murphy TV, Nelson JD. Five v ten days' therapy with furazolidone for giardiasis. Am J Dis Child. 1983;137(3):267-70.
90. Quiros-Buelna E. Furazolidone and metronidazole for treatment of giardiasis in children. Scand J Gastroenterol Suppl. 1989;169:65-9.
91. Rodriguez-Garcia R, Rodriguez-Guzman LM, Cruz del Castillo AH. [Effectiveness and safety of mebendazole compared to nitazoxanide in the treatment of Giardia lamblia in children]. Rev Gastroenterol Mex. 1999;64(3):122-6.
92. Escobedo AA, Alvarez G, Gonzalez ME, et al. The treatment of giardiasis in children: single-dose tinidazole compared with 3 days of nitazoxanide. Ann Trop Med Parasitol. 2008;102(3):199-207.
93. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by Giardia intestinalis and Entamoeba histolytica or E. dispar: a randomized, double-blind, placebo-controlled study of nitazoxanide. J Infect Dis. 2001;184(3):381-4.
94. Davila-Gutierrez CE, Vasquez C, Trujillo-Hernandez B, Huerta M. Nitazoxanide compared with quinfamide and mebendazole in the treatment of helminthic infections and intestinal protozoa in children. Am J Trop Med Hyg. 2002;66(3):251-4.
95. Ortiz JJ, Ayoub A, Gargala G, et al. Randomized clinical study of nitazoxanide compared to metronidazole in the treatment of symptomatic giardiasis in children from Northern Peru. Aliment Pharmacol Ther. 2001;15(9):1409-15.
96. Rossignol JF, Lopez-Chegne N, Julcamoro LM, et al. Nitazoxanide for the empiric treatment of pediatric infectious diarrhea. Trans R Soc Trop Med Hyg. 2012;106(3):167-73.
97. Munoz Gutierrez J, Aldasoro E, Requena A, et al. Refractory giardiasis in Spanish travellers. Travel medicine and infectious disease. 2013;11(2):126-9.
98. Nash TE, Ohl CA, Thomas E, et al. Treatment of patients with refractory giardiasis. Clin Infect Dis. 2001;33(1):22-8.
99. Lopez-Velez R, Batlle C, Jimenez C, et al. Short course combination therapy for giardiasis after nitroimidazole failure. Am J Trop Med Hyg. 2010;83(1):171-3.
100. Requena-Mendez A, Goni P, Lobez S, et al. A family cluster of giardiasis with variable treatment responses: refractory giardiasis in a family after a trip to India. Clin Microbiol Infect. 2014;20(2):O135-8.
101. Meltzer E, Lachish T, Schwartz E. Treatment of giardiasis after nonresponse to nitroimidazole. Emerg Infect Dis. 2014;20(10):1742-4.