Combination therapy in the management of giardiasis: what laboratory and clinical studies tell us, so far

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ABSTRACT

Treatment failures in patients suffering from giardiasis are not uncommon feature. The most frequent approach in these cases is to treat these patients with longer repeated courses and/or higher doses of the primary therapy, or using drugs from a different class to avoid potential cross-resistance. However, a higher rate of adverse events may limit this strategy. In this context, combination therapy (CT) is emerging as a valuable option against refractory giardiasis. In the attempt to evaluate the benefits of CT, a number of experimental studies, clinical series, and randomized clinical trials (RCTs), as well as several veterinary studies have been performed, with varying results. Here, we present a critical analysis of the available information regarding CT for the treatment of *Giardia* infection, as well as the authors' opinion with respect to its use. RCTs of combination therapy are limited and the optimal combinations and administration strategies need yet to be clarified. Analyses of the cost-effectiveness and RCTs of CTs for *Giardia* infection are required to assess the role of these drugs for the control of giardiasis, mainly in the case of treatment failures linked to suspected drug tolerance are the case.

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1. Overview on giardiasis and its treatment options

The flagellated protozoan *Giardia duodenalis* (syn. *G. lamblia* and *G. intestinalis,* from now referred as *Giardia)* is an intestinal parasite widespread globally, in temperate and tropical locations, especially where sanitary conditions are suboptimal. It affects mammals, including human, pets and livestock, by inhabiting the upper part of the small intestine. Eight morphologically homogeneous but genetically distinguishable groups, or assemblages (A–H), have been isolated from different mammalian species (Ryan and Cacciò, 2013). Assemblages A and B are found in human and other animals, being considered zoonotic, whereas the other assemblages display host specificity and do not infect humans (C and B in dog, F in cat, E in hoofed animal, G in rodents and H in sea mammals).

In human, *Giardia* is the most common intestinal parasitic protozoan diagnosed worldwide and it is the causative agent of giardiasis, an acute and chronic enteritis. Giardiasis may occur without clinical manifestations, although in many cases, it causes a self-limited and often persistent illness that is characterized by acute or chronic diarrhoea, abdominal pain, nausea, vomiting, flatulence and weight loss (Escobedo et al., 2010; Lalle, 2010). Although giardiasis can affect all age groups, children are at higher risk. *Giardia* infection in children has been associated with lower serum level of zinc, iron, and vitamins (A, B12 and folate), despite similar anthropometric indicators among infected and non-infected individuals (Olivares et al., 2002; Demirci et al., 2003; Quihui-Cota et al., 2008), and in early childhood, failure to thrive and poor cognitive function (Berkman et al., 2002; Celiksöz et al., 2005). Other long-term health consequences of giardiasis have been also documented, including chronic fatigue and post-infectious irritable bowel syndrome (Persson et al., 2015).

During the last 60 years of the past century, a number of anti-giardial drugs have been introduced and are still in use. The 5-nitroimidazole (5-NI) derivatives remain the most frequently prescribed drugs, including metronidazole (MTZ), tinidazole (TNZ) and secnidazole (SNZ). Other drugs include: the benzimidazole derivatives, albendazole (ABZ) and mebendazole (MBZ); the acridine derivative, quinacrine (QC); the nitrothiazolide, nitazoxanide (NTZ); the nitrofuran, furazolidone (FRZ); and the aminoglycoside, paromomycin (PRM). The advent of these drugs has been accompanied by a rise in the number of scientific publications concerning their pharmacology and clinical use (Escobedo et al., 2015). Despite their efficacy, treatment with these drugs is associated with several adverse effects, i.e., headache, metallic or bitter taste in mouth, nausea, vomiting, diarrhoea, dizziness, general body discomfort, loss of appetite, etc (Escobedo and Cimerman, 2007; Escobedo et al., 2010; Lalle, 2010). These bothersome side effects maybe hardly tolerated by a number of patients, whereas medical contraindications may limit the use of some of them in particular cases, as in pediatrics, where their dose requirements make difficult the administration of tablet formulations to children.

Follow-up of patients after treatment is paramount to evaluate the response to antigiardial drugs. Some individuals may experience treatment failure, despite having received successive courses of therapy, usually reported effective in curing giardiasis (Escobedo and Cimerman, 2007; Escobedo et al., 2010; Lalle, 2010). Potential reasons of non-effectivity may be associated to: poor patient compliance (Shepherd and Boreham, 1989), poor medicines quality, including spurious, falsely labeled, falsified, counterfeit medicines, chemical and/or physicochemical instability, inappropriate storage and transport, and poor quality control during manufacturing and importing medicines (Heyman and Williams, 2011), variation in the pharmacokinetics of drugs, inactivation of the drug by superinfection with other organisms, reinfection, inadequate drug levels, immunosuppression, resistance to the drug, sequestration in the gallbladder or pancreatic ducts (Boreham et al., 1987; Nash et al., 2001; Robertson et al., 2010), and unknown reasons (Nash et al., 2001; Robertson et al., 2010), including the possibility of invasive giardiasis (Martínez-Gordillo et al., 2014). Refractory giardiasis may be clinically challenging, especially when reinfection, noncompliance, poor quality of medicines and inadequate drug levels are not the causes.

One of the therapeutic strategies to overcome treatment failures with a single antigiardial drug regimen is to proceed with additional courses of the same drugs increasing the dose and/or duration of the treatment (Mørch et al., 2008; Yadav et al., 2014). Alternatively, since antigiardial drugs may vary in their modes of action as well as differences in resistance mechanisms may occur (Table 1), drugs combination might exert synergistic effects. Consequently, it is quite common to treat certain groups of patients with combinations of these medications to maximize therapeutic benefit. If antigiardial combination therapy (CT) provides an added benefit, an increased response rate is then expected. However, it should be noted that CT could also lead to antagonism for some drug combinations, enhanced toxicity, and significant additional costs.

CT has been successfully applied for the treatment of infectious and noninfectious diseases (i.e., tuberculosis, leprosy, malaria, cancer and asthma). A similar approach has been also introduced for management of giardiasis; when monotherapy has provided inadequate parasitological benefit, supplemental drugs have been added. CT in giardiasis may rely on drug repurposing and/or on combination of the already known antigiardial agents to provide a synergistic approach.

As CT has recently gained widespread acceptance for refractory cases of giardiasis, it is important to critically evaluate the current CT based on the evidences before endorsement.

2. Articles' search methods

A thorough PubMed search was conducted on CTs for *Giardia* infection. Journal articles spanning the time period between January 1966 and February 2016 were reviewed. Literature searches were restricted to the English, Italian, Spanish and Portuguese languages, and included combinations of the following terms: combination therapy, *Giardia* and giardiasis. All pertinent *in vitro*, randomized controlled trials (RCTs), and retrospective analyses were included. References from retrieved articles were also manually scanned for additional relevant publications.

Table 1
Mechanisms of resistance in *Giardia*.

Pharmacologic group	Drug	Main mode of action	Major resistance mechanism	Reference
Acridine derivatives	Quinacrine	Inhibition of oxygen consumption, binding to DNA, plasma membrane damage.	Decreased entry of drug.	Lalle (2010); Paget et al. (1989); Upcroft et al. (1996a,b)
5-Nitroimidazole compounds	Metronidazole (MTZ) Tinidazole Secnidazole Ornidazole	Reduction MTZ nitro group to nitroso radical highly reactive with DNA, free and protein cysteines, interfering with several biogical processes. Reduction is mediated by pyruvate: ferredoxin oxidoreductase (PFOR), thioredoxin reductase (TRxR) and NADPH oxidase. Similar mechanism proposed for the other drug.	MTZ resistance associate to inactivation of the PFOR/ferredoxin pathway, downregulation of nitroreductase 1 (NTR1) and upregulation of (NTR2).	Lalle (2010); Upcroft and Upcroft (2001); Leitsch et al. (2011); Leitsch et al. (2012); Müller et al. (2013); Müller et al. (2015); Uzlikova and Nohynkova (2014)
Nitrofuran derivatives	Furazolidone	Similar to 5-nitroimidazoles. Drug activation associated to NADH oxidase activity.	Decreased entry of drug or increased defend mechanisms against toxic radicals (increased level of thiol-cycling enzymes).	Lalle (2010); Leitsch (2015)
Aminoglycosides	Paromomycin	Inhibition of protein synthesis.	No resistant parasite as been yet isolated.	Lalle (2010); Katiyar et al. (1995)
Benzimidazole compounds	Albendazole Mebendazole	Binding to ß-tubulin and inhibition of cytoskeleton polymerization.	Not yet defined, but in resistant strain rearrangements of chromosomes and cytoskeleton, and alteration of gene expression (VSPs, β-giardin) has been reported.	Morgan et al. (1993); Upcroft et al. (1996a,b); MacDonald et al. (2004); Argüello-García et al. (2009)
5-nitrothiazolyl derivatives	Nitazoxanide	Noncompetitive inhibition of the PFOR and nitroreductases, alterations on the ventral disk and surface membrane.	Activation of a general stress response mechanism partially overlapping with MTZ resistance.	Müller et al. (2006); Hoffman et al. (2007); Müller et al. (2007); Müller et al. (2008)
Polypeptide antibiotic	Bacitracin Zinc	In bacteria, interferes with dephosphorylation step in cell membrane synthesis.	Not yet reported.	Andrews et al. (1995); Andrews et al. (1994); Gardner and Hill (2001)

PFOR: enzyme pyruvate:ferredoxin oxidoreductase.

3. Results

This review presents a detailed account of the published data on the use of CT in giardiasis. In the attempt to evaluate the benefits of antigiardial CT, a number of experimental studies, case reports, clinical series, and RCTs have been performed, with varying results. In this review, we summarize the available data. It should be taken into account that, covering a period of almost 60 years, the studies reviewed herein varied both in terms of accuracy and methodologies, especially in evaluating drug treatment outcomes. In this perspective, a rigorous comparison of experimental data was not feasible.

3.1. *Efficacy and safety of antigiardial combination therapy*

3.1.1. *Preclinical studies*

There are various experimental studies on combinations of different drugs for the treatment of giardiasis to evaluate antigiardial susceptibility and interactions between antigiardials. These include *in vitro* studies and studies in animals and animal models. All documented *in vitro* investigations have been performed with laboratory established cultures of *Giardia* trophozoites isolated from human patients and belonging to the zoonotic assemblage A. Experimental infections of animals were instead conducted either with the aforementioned human isolated or using cysts freshly isolated from naturally infected animal, often without any information on genotype (zoonotic or host-specific). Similarly, no assemblage information was available for naturally infected animal.

3.1.1.1. In vitro *studies. Due* to the wide prescription of MTZ as antigiardial chemotherapeutic, several *in vitro* evaluation of its effect in addition to known or potential antigiardial drugs were conducted. Two studies focused on the MTZ/QC combination (Gillin and Diamond, 1981; Smith et al., 1982). In the first one the *Giardia* Portland-1 trophozoites, isolated from an individual with diarrhoea, were used. The combined drugs had an additive antigiardial effect, being similar to the sums of their separate effects, and not synergic (Gillin and Diamond, 1981). In the other study, drug susceptibility assays were performed on *Giardia* trophozoites (WB) isolated from a patient who suffered of chronic symptomatic giardiasis and was cured by a combination of QC and MTZ, despite previous seven separate courses of either QC or MTZ alone. Compared to other isolates of *Giardia*, WB displayed a similar sensitivity to either drug alone but was more sensitive to QC/MTZ combination, suggesting that persistence of the infection was not ascribable to unique drug resistance properties of the WB isolate but to a lower cytotoxic phenotype (Smith et al., 1982).

The activity of MTZ associated to pyrantel pamoate (PP) was also investigated *in vitro*. PP is an inhibitor of cholinesterases commonly used as deworming drug in veterinary (Kopp et al., 2008). It has been proven to be effective also in the treatment of giardiasis in small animals (Barr et al., 1998), being able to inhibit the growth of *Giardia* Portland-1 trophozoites (Campanati et al., 2001). A significant synergic effect on *in vitro* growth inhibition of *Giardia* WB trophozoite was observed combining different concentration of MTZ and PP. The stronger effect was achieved with drug combination containing lower sub-optimal doses of both compounds all showing a fractional inhibitory concentration index (FICI) < 0.5 (Hausen et al., 2011). Furthermore, the combinations of MTZ and PP induced a reduction of parasite adhesion on rat intestinal epithelial cells (IEC-6 line) without affecting the viability of untreated cell line (Hausen et al., 2011). Tetrahydrolipstatin (known as orlistat), a lipase inhibitor approved for the treatment of obesity, has also been tested in combination with MTZ (Hahn et al., 2013). Orlistat alone has a giardiacidal effect *in vitro*, showing inhibition of *Giardia* replication and growth, with a IC50 lower than MTZ, on both WB and 14-03/F7 isolates, the latter obtained from a patient with MTZ refractory giardiasis. When orlistat and MTZ were used together, they showed an additive inhibitory effect. The analysis of the combination effects is in favor of an independent mode of action of the two drugs thus envisaging a beneficial effect for patients in the case of a combined therapeutic application (Hahn et al., 2013).

Also benzimidazoles were tested in combination with compounds belonging to different classes, but all showing structural and electronic similarities with the benzimidazole core. Two 4-R-ethyl-phenylcarbamate derivatives (IRE-6A and IRE-7B) have shown a modest antigiardial activity against *Giardia* Portland-I isolate (ABZ-susceptible) and its induced ABZ-resistant clone, although the drug potency was slightly reduced for the latter one (Jiménez-Cardoso et al., 2004). The combined treatment with ABZ, at its MIC50 concentration, and each compound at various concentration resulted in a synergistic lethal effect also on the ABZ-resistant clone. The drug combination was 5–9 times less toxic against CHO cell line (Jiménez-Cardoso et al., 2004). Similarly, synthetic derivatives of imidazo[1,2-a]pyridines and pyrimidines also exert growth inhibitory activity on *Giardia* WB trophozoite, without cytotoxicity on Vero cell line.[28] The combination of ABZ with each of the three most potent derivatives, all at their respective IC50, exerted a synergic effect increasing the overall antigiardial activity as the result of pharmacological interaction (Velázquez-Olvera et al., 2016).

In vitro cytotoxicity and adherence inhibition were evaluated for *Giardia* Portland-1 and BRIS/82/HEPU/41 isolates using dyadic combinations of azithromycin and doxycycline, two antibiotics known to inhibit bacterial protein synthesis, and two antigiardial nitro compound, TNZ and FRZ, and the antimalarial mefloquine (Crouch et al., 1990). All drug combinations exerted an additive inhibitory effect on the parasite growth, independent on the isolate. On the contrary, the combination of azithromycin-FRZ, doxycycline-mefloquine, doxycycline-TNZ and mefloquine-TNZ provided evidence of a synergistic activity that hampered parasite adherence (Crouch et al., 1990), in agreement with independent mechanisms of action of these compounds.

3.1.1.2. Treatment in animals and animal models. As previously pointed out, human infection is caused by the *Giardia* zoonotic assemblages A and B. Although epidemiological evidences suggest that *Giardia* infection in humans is often due to anthroponotic spread, animals, especially farm and companion animals, can be sources of human infection and vice-versa, being infected with both zoonotic or host-specific assemblages (Ryan and Cacciò, 2013; McDowall et al., 2011). Several studies reported on high prevalence of *Giardia* in stool samples of companion animals (i.e. cats and dogs) (Bouzid et al., 2015). Pets are prevalently infected with host-specific assemblages (C and D in dogs, F in cats), but the zoonotic A and B assemblages were also reported in several settings, and may prevail in environments commonly contaminated with cysts from the faeces of humans or other animals (Thompson 2004; Ballweber et al., 2010). Although their role as reservoir and the associated risk for their owners is unclear, their treatment to potentially reduce the risk of transfer to human beings cannot be ignored. To provide a complete overview of CT data from naturally infected animals or with animals experimentally infected with *Giardia* isolates, either from human or animal origin were included.

Several studies have been performed on naturally infected dogs to evaluate the efficacy of different dosages of the combination of three compounds (FPP combination): febantel, a prodrug *in vivo* metabolized to the benzimidazoles fenbendazole and oxyfenbedazole (Mc Kellar and Scott, 1990); pyrantel; and praziquantel (PZQ), a broad-spectrum anthelmintic against parasitic trematodes and cestodes (Andrews et al., 1983). In the first study, 3 groups of 5 beagles each, having natural infection with *Giardia* and a median age of 9 months, were administered with the United States (US) FPP formulation of combination tablets (68 mg of PZQ, 68 mg of PP, and 340.2 mg of febantel) daily for 1 or 3 consecutive days, whereas a control group received placebo tablets. Faecal samples were all negative for *Giardia* cysts only in the group treated for 3 days and no side effects were reported (e.g. vomit, diarrhoeic faeces) (Barr et al., 1998). Similar results were also reported in a subsequent study (Payne et al., 2002), in which 16 beagles, 7–10 months old, were treated daily up to 5 days and cyst shedding was evaluated for up to 24 days. Co-administration of a *Giardia* vaccine with the FPP combination tablets did not improve the treatment efficacy (Payne et al., 2002). The European formulation of FPP (50 mg PZQ, 144 mg pyrantel embonate, 150 mg febantel per tablet), registered for the treatment of several canine helminthiasis, was also evaluated on adult dogs infected with *Giardia*. The combination tablets was effective in removing *Giardia* cysts from the faeces when administered once at double the recommended dosage, or for two or three consecutive days, without adverse side effects (Giangaspero et al., 2002). A prolonged treatment up to 5 days was shown to not be statistically better than treatment for three consecutive days (Montoya et al., 2008). In a larger survey on adult dogs naturally infected with several parasite, including *Giardia*, a similar therapeutic efficacy was demonstrated comparing a treatment once daily for 3 days of febantel-pyrantel-PZQ (a dose of 15–5–5 mg/kg once) with MBZ (22 mg/kg) or fenbendazole (50 mg/kg) alone (Miró et al., 2007). Both the US and European formulations of FPP combination tablets were tested on kittens experimentally infected with *Giardia* cysts isolated from a naturally infected cat (Scorza et al., 2006). Kittens were treated daily for 5 consecutive days and stool samples analyzed daily for the presence of *Giardia* cysts. Treatment with the highest dose of FPP, a dose similar to that reported to be effective for the treatment of giardiasis in dogs, gave the best intra- and post treatment results. The dosage was well-tolerated with only transient salivation reported (Scorza et al., 2006).

The possible use of the combination of febantel and pyrantel embonate (160, 80, 40, 20 or 10 mg/kg of each drug) for the treatment of giardiasis in mammals, not only dogs and cat, was evaluated using gerbils, a well establish model of giardiasis (Buret et al., 1992), experimentally infected with the *Giardia* isolate WB (Olson and Heine, 2009). Drugs were administered daily for 3 consecutive days starting from day 5 post-infection and parasite load in the gut was evaluated at day 8. Whereas febantel alone was able to eliminate all trophozoites both at dosage of 160 and 80 mg/kg, combined therapy was effective also at 40 mg/kg indicating a synergistic activity of the two compounds. It is interesting to note that in this study the *Giardia* isolate was known and the authors reported the parasite clearance from the gut of tested animals and not the

faecal cyst shedding, clearly showing a giardiacidal effect of the treatment.

In search for alternative natural bio-interventions to ameliorate the treatment of giardiasis, the treatment with antigiardial compounds combined with natural products was assessed in different animal model of giardiasis.

MTZ in combination with silymarin (a mixture of a flavonoid and phenylpropanoid isolated from an extract of medicinal plant *Silybum marianum*) was tested in asymptomatic dogs naturally infected with *Giardia* (1–3 years of age) and treatment with silymarin alone, MTZ alone or placebo were compared. Even if no significant difference in efficacy between treatment with MTZ with or without silymarin could be reported, combined treatment with silymarin had a positive impact on dog well-being attenuating MTZ side effects (Chon and Kim, 2005).

Propolis, a bee glue preparation, exerts *in vitro* inhibitory activity on *Giardia* trophozoite growth (Torres et al., 1990), gaining popularity as alternative for treatment of human giardiasis (Núñez et al., 2004). The use of propolis and MTZ was evaluated in mice experimentally infected with *Giardia* trophozoites. The count of trophozoites in mice intestine was significantly reduced when the combined therapy was administered, especially after 6 days post infection, if compared to MTZ alone. Propolis alone proved to be a strong enhancer of the immune system, promoting an undesirable inflammatory response in the intestine. Nevertheless, the synergistic effect of both compounds resulted in an immunological balance, especially in terms of the T-lymphocyte profile, that protected the intestinal homeostasis and histological architecture (Abdel-Fattah and Nada, 2007).

Administration of *Lactobacillus casei* (*L. casei*) has been shown to hamper the adhesion of *Giardia* trophozoites in murine model of giardiasis (Shukla et al., 2008). Efficacy of the oral administration of the probiotic together with ABZ was also evaluated (Shukla et al., 2013). Using Portland-1 trophozoites to infect BALB/c mice, cyst excretion, intestinal trophozoite load, lactobacilli counts and histopathology were recorded throughout the experiment. Co-administration of the probiotic and ABZ exerted a synergistic effect reducing cyst excretion and intestinal trophozoite counts, inhibiting the parasite growth and adherence, likely as consequence of an improved colonization of lactobacilli. Furthermore, *Giardia*-induced damages to the gastrointestinal mucosa were counteracted in the combined therapy (Shukla et al., 2008).

Nanoparticles of silver, chitosan, and curcumin, and their combination, have been also considered as anti-giardial agents. Chitosan is a linear polycationic hetero polysaccharide copolymer obtained from deacetylation of chitin with broad-spectrum antibacterial activity (Martins et al., 2014). Curcumin is a natural polyphenolic compound extracted from the root of *Curcuma longa* with reported activity against different bacteria, viruses, fungi, and parasites (Moghadamtousi et al., 2014). Chitosan and silver nanoparticles were successfully applied as a system for pharmaceutical drug delivery (Marin et al., 2015; Ahmed and Aljaeid, 2016). In the work of Said et al., rats were experimentally infected with *Giardia* cysts, isolated from human stool samples, and treated daily for 8 days with 50 μg of each agent alone (chitosan, curcumin, chitosan nanoparticles, curcumin nanoparticles or silver nanoparticles) or in combination. Therapeutical efficacy was evaluated by faecal shedding of *Giardia* cysts and trophozoites loading in intestinal sections. A complete parasite eradication was reported only in rats administered with the three nanoparticles combination without any reported cytotoxicity and accumulation of silver at safe level in organs (Said et al., 2012). *In vitro* combination assays and the use of different animal models to explore the aforementioned treatment regimens envisage the concrete opportunity to develop, in the future, novel clinical protocols for efficient and safe application of CT against human giardiasis. However, the inherent problems associated with these kind of studies (e.g. difficult reproducibility due to use of undefined isolates of *Giardia,* not standardized protocols for the evaluation of drug efficacy, different pathophysiology of giardiasis between animals and humans) should be carefully evaluated before translating their results into clinical practice.

3.1.2. Clinical studies

3.1.2.1. Clinical series. The use of two or more drugs in combination has become the mainstay in the attempt to overcome resistance/drug tolerance traits in many infectious diseases, including giardiasis as well. Although theoretical advantages to CT have been shown by *in vitro* and animal studies, clinical data are sparse. Observational studies have shown that combining the 5-NI with partner drugs that have different mechanisms of action provides better outcomes. Some of the most common combinations using MTZ are: MTZ-QC (Gillin and Diamond, 1981; Smith et al., 1982; Taylor et al., 1987; Nash et al., 2001; Robertson et al., 2010; Lopez-Velez et al., 2010) MTZ-PRM (Lopez-Velez et al., 2010; Nash, 2013), and MTZ-ABZ (Mørch et al., 2008; Kampitak, 2010; Yadav et al., 2014). An infrequent combinations of MTZ-diloxanide (Mason and Patterson, 1987), MTZ-NTZ (Martínez-Gordillo et al., 2014), MTZ-ABZ-PRM (Lopez-Velez et al., 2010), and MTZ-propranolol have also been reported. For instance, for a patient with MTZ-refractory *Giardia* infection treatment with dl-propranolol, at a dose of 40 mg, and 400 mg of MTZ three times a day for 10 days ended up with the clearance of infection (Popovicí and Milovicí, 1990). The combination of TNZ-QC (Lopez-Velez et al., 2010), TNZ-PRM (Lopez-Velez et al., 2010), TNZ-Doxycycline (Mukku et al., 2015) and TNZ-ABZ-PRM-intravenous IgA (Lopez-Velez et al., 2010), as well as unusual combinations like MBZ-PP (Purnomo et al., 1980), PRM-FRZ, and Bacitracin-PRM (Nash et al., 2001), have proven successful. Noteworthy, in all these case series treatment schedules lasted longer than usually prescribed.

3.1.2.2. Randomized clinical trials. As just presented, some investigators have reported on the successful use of -and recommended- CT for treatment of non-responders. However, strong evidences, such as those provided by randomized clinical trials (RCTs), are still largely missing. For example, the combination of MTZ and QC has shown the most interesting results, supporting CT in clinical series, but RCTs on its use has not yet been performed. A few as six RCTs on CT have been published so far (Table 2) (Andrews et al., 1995; Cacopardo et al., 1995; Grant et al., 2001; Pengsaa et al., 2002; Pengsaa et al., 2004; Speich et al., 2013). In one trial bacitracin, bacitracin zinc and neomycin alone were compared to a combination of bacitracin zinc/neomycin (Andrews et al., 1995). Although a high cure rates was achieved, no synergistic activity was noted between bacitracin zinc and neomycin. In an Italian study, 20 patients, with long lasting symptomatic giardiasis (2–4 months) refractory up to five courses of MTZ, were randomized to receive ABZ (400 mg twice daily 7 days) and the same dose of ABZ plus MTZ (250 mg thrice daily 7 days). Nine out of 10 cases responded to a combination of ABZ-MTZ, while only 2 out of 10 responded to monotherapy with ABZ (Cacopardo et al., 1995), concluding that ABZ alone is not a therapeutic alternative for treatment MTZ refractory giardiasis. PZQ was found to cause unexpected and statistically significant reductions in the prevalence of *Giardia* infection, when given as a single dose of 5 mg/kg as a part of a *Taenia solium* cysticercosis chemotherapy intervention (Flisser et al., 1995). When PQ was co-administered with ABZ, it increases the serum level of albendazole sulphoxide, the active metabolite of ABZ (Homeida et al., 1994). Based on these evidences, two clinical trials were conducted (Pengsaa et al., 2002; 2004). In the first one, a combined single dose of ABZ 400 mg and PZQ 20 mg/kg achieved a parasitological cure rate of 74.2%; more than ABZ 800 mg as a single dose (50%), but less than TNZ 50 mg/kg as a single dose (92.6%)

Table 2
Comparative RCTs assessing the benefit of CT compared with monotherapy for *Giardia* infections.

Authors	Country	Combination therapy (dosage)	Number of cases treated	Parasitological cure rate	Comparators (dosage)	Number of cases treated	Efficacy of comparators	Comments on combination therapy
Andrews et al. (1995)	Romania	Bacitracin zinc with neomycin (60,000 U–60,000 U), two times daily for 10 days.	16	87.5%	Bacitracin (120 000 U)	16	87.5%	Few side effects involving gastrointestinal upset. No synergistic activity was noted between bacitracin zinc and neomycin.
					Bacitracin zinc (120 000 U)	19	94.7%	
					Neomycin (120 000 U)	22	86.4%	
					All, two times daily for 10 days.			
Cacopardo et al. (1995)	Italy	ABZ-MTZ (400 mg/day–250 mg thrice a day). Both for 7 days	10	90%	ABZ (400 mg) daily for 7 days	10	20%	All patients reported the disappearance of clinical symptoms, as well as no side effects. Diarrhoea was reduced already at day 2 post therapy start. Only 1 patient reported after 4 week symptoms relapse.
Grant et al. (2001)	Canada and Peru	Asymptomatic subjects (WG 2 g, 3 times a day) for 10 days, followed by MTZ (250 mg 3 times a day) for 7 days.).	11	63.6	Placebo (cornstarch, 2 g, 3 times a day) for 10 days, followed by MTZ (250 mg 3 times a day) for 7 days.).	13	76.9	There were no clinically important differences between those receiving supplemental WG or placebo. However, symptoms appear to have resolved more rapidly in the subjects taking WG in addition to MTZ.
		Symptomatic patients (MTZ, 250 mg 3 times a day, plus WG for 7 days.)	21	90.4	Symptomatic patients (MTZ, 250 mg 3 times a day, plus placebo for 7 days.)	17	94.1	

Table 2 (*Continued*)

Authors	Country	Combination therapy (dosage)	Number of cases treated	Parasitological cure rate	Comparators (dosage)	Number of cases treated	Efficacy of comparators	Comments on combination therapy
Pengsaa et al. (2002)	Thailand	ABZ-PZQ (400 mg–20 mg/kg) SD	31	74.2%	ABZ 800 mg SD	26	50%	Though several episodes of side-effects were observed, they were mild, except for 2 cases of abdominal pain and nausea/dizziness that needed medication.
					TNZ 50 mg/kg SD	27	92.6%	
Pengsaa et al. (2004)	Thailand	ABZ-PZQ (400 mg–20 mg/kg) SD	10	40%	ABZ (400 mg), SD	10	10%	No adverse effects were noted in any of the children investigated. No significant pharmacokinetic interaction between the ABZ and PZQ was demonstrated. The pharmacokinetics of albendazole sulphoxide, the active metabolite of ABZ, was similar whether ABZ was given alone or in combination with PZQ.
Speich et al. (2013)	Tanzania	NTZ-ABZ (1000 mg–400 mg), with each drug given separately on two consecutive days.	19	42.1%	ABZ (400 mg), SD	25	60%	The combination of the two drugs ABZ-NTZ has not got sufficient efficacy; in fact, curiously was less than placebo.
					NTZ (1000 mg), SD	21	57.1%	
					Placebo	25	52%	

(Pengsaa et al., 2002). In the second study, the same combined single dose of ABZ and PZQ was compared with a single dose of 400 mg and the parasitological cure was 60% and 10%, respectively (Pengsaa et al., 2004). Moreover, the pharmacokinetics of ABZ and albendazole sulphoxide was evaluated, although co-administered of ABZ and PZQ did not influence the compound pharmacokinetics (plasma-concentration–time profile) (Pengsaa et al., 2004).

In a RCT, more than 500 Tanzanian school-aged children (7–15 years old), half of them infected with at least *E. histolytica/E. dispar* and/or *Giardia*, were examined to evaluate the efficacy of treatment with single-dose ABZ (400 mg), single-dose NTZ (1000 mg) and NTZ-ABZ combination (1000 mg–400 mg), with each drug given separately on two consecutive days; or with placebo (Speich et al., 2013). Before treatment, the prevalence of children infected with *Giardia* was 16%, with more than 55% having a moderate/heavy infection. At three weeks post-treatment the prevalence of intestinal protozoa infection was even higher and no relevant differences in the cure rate of giardiasis could be reported for each treatment (NTZ-ABZ, 42.1%; single-dose ABZ, 60%; single-dose NTZ, 57.1%) compared to placebo (52%) (Speich et al., 2013). A reason for these inconsistent results could be found in the low sensitivity of ether-concentration technique and the analysis of only a single formalin fixed stool sample at the beginning of the trial.

A relatively recently recognized property of wheat germ agglutinin (WGA) is that, at modest concentrations (10–100 μg/mL), it may inhibit *in vitro Giardia* excystation as effectively as monoclonal antibodies directed against cyst wall antigens (Meng et al., 1996) and trophozoite growth in axenic cultures (Ortega-Barria et al., 1994). In addition, it is able to reduce cyst passage in mice infected with the protozoan *Giardia muris* (Ortega-Barria et al., 1994). According to these results, a study was performed and published in 2001, involving subjects presenting with giardiasis in Montreal and Lima, Canada and Peru, respectively. This double-masked, placebo-controlled study of dietary supplementation with wheat germ demonstrated that its components, possibly WGA, either alone or in combination with MTZ, may modify the course of giardiasis in humans (Grant et al., 2001).

4. The host factor

It has clearly shown that both parasite and host determinants play a role in disease outcomes and recovery (Cotton et al., 2011), making CT just a part in the host-parasite interplay. As stated by Elek in 1956, "*in a way all therapeutic treatments are combined therapy, since the drugs are effective only if body defense of the patient acts in synergy with these drugs*" (Elek, 1956). In this perspective, although treatment failures in *Giardia* infection may appear in patients without any underlying disease (Nash et al., 2001; Lopez-Velez et al., 2010), clinical situations like common variable immunodeficiency (Nash et al., 2001); renal transplant recipients, due to immunosuppressive therapy (Mukku et al., 2015); acquired immunodeficiency syndrome (AIDS) (Nash et al., 2001; Yadav et al., 2014) complex immunodeficiency (Nash et al., 2001); IgA deficiency (Lopez-Velez et al., 2010), acute lymphoid leukaemia (Yadav et al., 2014) and other conditions are not uncommon. In addition, the age, and the nutritional status, the host immune response, as well as a not yet defined genetic makeup, may impact in the host response to antigiardial drugs (Lopez-Romero et al., 2015).

5. Authors' considerations

Treatment of *Giardia* infections in particular concerning CT, still remains an area of ongoing investigation. In fact, RCTs using CT in individuals failing primary therapy are lacking. Hence, the selection of a combination therapy should be carefully evaluated based on extensive *in vitro* studies, existing animal and human data, meanwhile animal and human RCTs could run in parallel. Compound synergy reported *in vitro* may not necessarily been translated into a clinical benefit, as *in vitro* synergy studies are conducted in well-controlled environments where precise concentrations of multiple antibiotics are tested against known infectious doses of *Giardia*. This scenario can be quite different from clinical, where drug concentrations and *Giardia* trophozoite burdens in patients are unpredictable. Additionally, *in vitro* studies cannot take into account the added contribution of the host immune system. Although, CT is the common approach in clinical for the treatment of persistent refractory giardiasis, a rigorous evaluation to define the more appropriate therapeutical protocol for different CT is still missing and more properly designed RCTs are then required. Indeed, despite promising *in vitro* and case reports observations, no RCT has yet been conducted to evaluate MTZ and QC combination. All the studies reviewed herein represent hypothesis generating results on new treatment combinations. It is important to note that the available data are far to be completed. Incessant research efforts will help to shed light on safety and benefit of new or poorly explored drug combinations for symptomatic giardiasis in first-line monotherapies non-responder patients. In this perspective, *ad-hoc* CT studies should take into consideration several aspects, including the limits imposed by the tolerability and acceptability of multiple therapies and their cost.

It is understandable that physicians have already started to use CT in complicate clinical situations, often with different clinical results. However, lacking RCTs for most combinations, we urge clinicians to judiciously examine the available data before adopting CT as the standard of care of patients with treatment failures. In the field of CT for clinical giardiasis, still many questions await for an answer: *i*) do we understand the potential pharmacokinetic/pharmacodynamic interactions in CT?; *ii*) is CT really superior to monotherapy?; *iii*) when is CT best used?; *iv*) which patients most likely benefit from CT?; *v*) which combinations work better and which might even be harmful? *vi*) what do we know about CT cost-effectiveness? It is indispensable to clarify all these issues, as well as carry out properly designed RCTs in the near future.

6. Conclusions

To our understanding, CT in giardiasis should be reserved when single primary agents have failed to clear the infection. Despite of this, the efficacy of CT appears encouraging and likely represents the foundation for difficult-to-treat *Giardia* infections now and in the near future. However, it should also be considered that administration of two or three drugs may have more profound physiological consequences, alter the intestinal microbiota and increase drug-related adverse events and healthcare costs. Thus, to optimize the use of already available drugs in combination schedules against *Giardia*, any effort should be done to pursue adequately powered, well-designed, RCTs, which up to this moment are still scarce. Regardless of limitations, present data on CT provide a baseline for future studies in the field of refractory giardiasis management, but further research is required and expected.

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