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REVIEW

The old and new therapeutic approaches to the treatment of giardiasis: Where are we?

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Abstract: *Giardia lamblia* is the causative agent of giardiasis, one of the most common parasitic infections of the human intestinal tract. This disease most frequently affects children causing abdominal pain, nausea, vomiting, acute or chronic diarrhea, and malabsorption syndrome. In undernourished children, giardiasis is a determining factor in retarded physical and mental development. Antigiardial chemotherapy focuses on the trophozoite stage. Metronidazole and other nitroimidazoles have been used for decades as the therapy of choice against giardiasis. In recent years many other drugs have been proposed for the treatment of giardiasis. Therefore, several synthetic and natural substances have been tested in search of new giardicidal compounds. This study is a review of drugs used in *in vitro* and *in vivo* tests, and also drugs tested in clinical trials (nonrandomized and randomized).

Keywords: *Giardia lamblia*; treatment; new drugs

Introduction

Giardia lamblia (syn. *Giardia intestinalis*, *Giardia duodenalis*) is a flagellate protozoan which may be found infecting the human small intestine, causing a disease called giardiasis. The symptomatology of human giardiasis is extremely variable, many individuals have the asymptomatic form while some have abdominal pain, nausea, acute or chronic diarrhea – which may last several months, malabsorption and weight loss.[1–3] The clinical impact seems to be stronger in the first three years of life and in undernourished or immunodeficient individuals.[4] *G. lamblia* has often been pointed out as the cause of growth disorders among children,[3] also with the presence and frequency of diarrhea, for as long as the infection lasts, and the opportunity of reinfection, all constituting essential factors behind children's physical and mental debilitation.[5]

G. lamblia is found in mammals, including human beings, cats, dogs, beavers, and cattle. Giardiasis is transmitted by the ingestion of cysts present in food and water; water dissemination being easier due to cysts resistance to chlorination.[6,7] Cysts are highly infectious to men. Human volunteers have been experimentally infected with as few as 10 cysts.[8] These cysts may remain viable in the environment for up to three months under favorable conditions of temperature and humidity. Three aspects are important in the epidemiological context of the disease: the cysts' resistance to the environment, the amount of cysts eliminated by the patients, and the zoonotic aspect of the disease.[9]

Epidemics, in developed countries, have been attributed to an inappropriate water treatment, to its contamination with human or animal feces, particularly in surface

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water collections and lakes.[9] Direct transmission from person to person is another infection mechanism, particularly important in collective institutions, such as daycare centers and orphanages, among members of the same family, and between male homosexual partners.[10] In these populations, giardiasis reaches epidemic levels. *G. lamblia* has a cosmopolitan distribution with an estimated number of 2.8×10^8 cases of infections per year and is thus the most common intestinal parasite in humans in developed countries.[11] In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year.[12] In those countries this disease should be observed carefully, for it contributes substantially to generating mentally and physically impaired adults.

Thompson and colleagues[13] reviewed publications by several authors who reported genetic variations among *Giardia* samples isolated from human beings. Such differences are believed to significantly influence giardiasis epidemiology and control, particularly for host susceptibility, virulence, drug sensitivity, antigenicity, and *in vivo* and *in vitro* development.[5] Although some advances have been observed in isolating and characterizing *Giardia* samples, there are few studies regarding this parasite`s chemotherapy.[14] Resistance to different drugs used in the treatment of this disease has been reported and the number of cases is likely to increase.[15–17]

A variety of chemotherapeutic agents such as 5-nitroimidazole compounds, quinacrine, furazolidone, paromomycin, benzimidazole compounds, nitazoxanide have been used in the therapy for giardiasis. Nevertheless, therapeutic regimens and therapy reviews are little explored. Most drugs used have considerable adverse effects and, most of the time, they are contraindicated.[18–20] Furthermore, *Giardia* seems to have a great ability to resist these agents.[17,19,21,22]

In this context, the study of new chemotherapeutic agents plays a fundamental role – along with the reviews of the actually used drugs – in the rationale for treatment of giardiasis on the basis of more consistent data.

Many compounds have shown giardicidal activity in *in vivo* models or in animal models. In the present review, we have systematically addressed the main *in vitro* and *in vivo* studies and prospective trials in human population concerning the treatment of giardiasis.

Methodology

This is a review of giardiasis treatment in which we analyze the quality of the studies published in the Medline, PubMed, and EMBASE databases from 1966 to September, 2008.

Concentrating only on studies published in English, for each class of study (see below), we looked up the following key words in various combinations: giardia, giardiasis, treatment, therapeutic, therapy, drug, medication, phytotherapy, and chemotherapy. In those studies performed in humans, we did not have an age limit and searched for children and adult patients.

The studies were divided into four classes. Group I: *in vitro* studies; group II: *in vivo* studies; group III: clinical trials, nonrandomized, controlled or not; group IV: randomized control trials (RCT), blinded or not.

Inclusion criteria

We included the following studies: *In vitro* studies consisting of studies that tested the sensitivity and efficacy of the drugs against *Giardia*; *In vivo* studies consisting of studies that tested the efficacy of drugs against *Giardia* in experimental animals; Nonrandomized clinical trials consisting of studies that tested the efficacy of drugs against *Giardia* in humans; Randomized controlled clinical trials (RCT) consisting of studies designed to compare the efficacy between different drugs, between drugs and placebo, or to compare different schemes of the same drug in humans. These studies were necessarily randomized and controlled, but not necessarily blinded.

This review was made using two independent reviewers following the same inclusion criteria for searching the articles simultaneously. After they were finished, the reviews were analyzed. Those articles showing up in two reviews were automatically included in the final analysis. The remaining nonconsensual studies were analyzed by a third reviewer for a final decision as to include or exclude an article after the discussion between the first two reviewers was exhausted.

Statistical analysis

Data are presented as mean ± standard deviation (confidence interval [CI]), absolute numbers, or percentages. Comparisons between rates of cure of drugs were made using the chi-squared or the Student *t*-test methods. Only variables with $p < 0.05$ were considered significant.

Main results

In the initial search, 116 *in vitro* studies, 48 *in vivo* studies, 87 nonrandomized clinical trials, and 47 RCT were found. After selection for the inclusion criteria, 39 *in vitro* studies, nine *in vivo* studies, 23 nonrandomized clinical trials, and 34 RCTs remained (Tables 1–4).

Table 1 In vitro studies

Year	Drugs/Substances	Activity	Reference
1975	2,2-biimidazole	Yes	23
1983	Human milk	Yes	24
1984	Metronidazole	Yes	61
	Tinidazole	Yes (+ effective)	
	Furazolidone	Yes	
	Quinacrin	Yes (– effective)	
1985	Bithionol	Yes	25
	Dichlorophene	Yes	
	Hexachlorophene	Yes	
1985	Clomipramine	Yes	26
1986	Furazolidone	Yes	27
	Nitroimidazole	Yes	
1990	Azitromicin/Furazolidone	Yes	28
	Doxiciclin/Mefloquin	Yes	
	Doxiciclin/Tinidazole	Yes	
	Mefloquin/Tinidazole	Yes	
1991	Metronidazole	Yes	29
	Ornidazole	Yes	
1991	Azitromicin	Yes	30
1994	Serum immune specific	Yes	31
1994	Agglutinin of wheat germ	Yes	32
1994	Derivatives of allicin (diallyl trisulfide)	Yes	60
1995	Phytotherapics popular in Africa	Yes (+ effective)	33
	Methanolic extracts cathartics	Yes (– effective)	
	Methanolic extracts noncathartics		
1995	Albendazole	Yes (– effective)	34
	Metronidazole	Yes (+ effective)	
1999	Derivatives of flavonoid *Helianthenum glomeratum*	Yes	59
2001	Pyrantel pamoate	Yes	35
2001	Powder of Yucca schidigera	Yes	36
2001	Ciprofloxacin	Yes	37
2002	Nitazoxanide	Yes (+ effective)	38
	Albendazole	Yes (+ effective)	
	Metronidazole	Yes (– effective)	
2002	Mucin	Yes	39
2002	Derivatives of isoflavone	Yes	40
2003	Derivative etylphenylcarbamate	Yes (– effective)	41
	Albendazole	Yes (+ effective)	
2004	Gangliosides	Yes	42
2004	Derivate phenyl-carbamate	Yes (- effective)	43
	Albendazole	Yes (+ effective)	
2004	S-substituted 4,6-dibromo-mercaptobenzimidazole	Yes	58
	S-substituted 4,6-dichloro-2-mercaptobenzimidazole	Yes	
2005	Dodecanoic acid	Yes	44
	Metronidazole	Yes	
2005	Arsenic sodium	No	45
2005	Derivatives of *Artemisia ludoviciana*	Yes	57
2005	Derivatives of flavonoid glycosides	Yes	56
2006	Derivatives benzimidazoles	Yes (+ effective)	46
	Albendazole	Yes (++ effective)	
	Metronidazole	Yes (– effective)	

(*Continued*)

Table 1 (*Continued*)

Year	Drugs/Substances	Activity	Reference
2006	Nitrotiazol (Nitazoxanide)	Yes	47
	Metronidazole	Yes	
2006	Venom *Crotalus durissus terrificus*	Yes	48
	Venom *Bothrops jararaca*	Yes	
2006	Propolis	Yes	49
2006	Curcumin	Yes	50
2006	Metronidazole	Yes	55
	Furazolidone	Yes (+ effective)	
2006	*Dorstenia contrajerva*	Yes	54
	Senna villosa	Yes	
	Ruta chalepensis	Yes	
2007	Metronidazole	Yes (– effective)	51
	Analogous MTZ-Ms	Yes	
	Analogous MTZ-I	Yes	
	Analogous MTZ-Br	Yes	
	Analogous MTZ-N_3	Yes	
	Analogous MTZ-NH_3Cl	Yes	
2007	Extracts of blueberry	Yes	52
2007	Tiliroside	Yes (+ effective)	53
	Kaempferol-glucopyranoside	Yes	
	Astragalin	Yes	
	Quercitrin	Yes	
	Isoquercitrin	No	

In the 39 *in vitro* studies selected, 55 drugs were tested, 53 (96.4%) showed activity against giardia. Eighteen studies (46.2%) did not have comparative design with other drugs. Twenty-one studies (53.8%) compared activity between drugs: 11 (52.4%) compared activity between two drugs, and 10 (47.6%) compared activity between three or more drugs.

The most frequently tested drugs in *in vitro* studies were: metronidazole (nine studies, 16.4%), albendazole (five studies, 9.1%), furazolidone (four studies, 7.3%), azitromicyn, nitazoxanide, phenyl-carbamate derivatives, tinidazole, and kaempferol (two studies each, 3.6%). The other drugs had one study each (Table 5).

In the nine *in vivo* studies selected in which nine drugs were tested, eight (88.9%) showed activity against *Giardia*. One of them compared the efficacy between two drugs (11.1%), and the remaining study tested just one drug (Table 2).

Out of the 23 nonrandomized clinical trials, six studies (26.1%) had design to compare efficacy between drugs, three (13%) compared different schemes of the same drug, and three (13%) compared efficacy between one drug and placebo (nonrandomized). Eleven studies evaluated the effect of one drug without comparing either dosages or efficacy between drugs (see Table 3).

Twelve drugs have been tested in the 23 nonrandomized clinical trials, with an average sample size of 83.3 ± 53.3 patients per study (confidence interval [CI] = 57.2 to 109.4). The mean general rate of cure (RC) per drug was 85.5% ± 16.7 (CI = 80.0 to 91.0). The most frequently tested drugs were: metronidazole (nine studies, 39.1%), tinidazole (seven studies, 30.4%), ornidazole, and quinacrine (three studies each, 13%), secnidazole, furazolidone, and berberine (two studies each, 8.7%) (Table 7). In evaluating drug effectiveness, the following mean rates of cure were found: secnidazole (RC = 96% ± 2.8), ornidazole (RC = 93.6% ± 1.2), tinidazole (RC = 89.1% ± 8.8),

Table 2 *In vivo* studies

Year	Drugs/Substances	Activity	Reference
1991	Albendazole	Yes	62
1993	New oxadiazoles	Yes (+ effective)	63
	Metronidazole	Yes (– effective)	
1996	Ivermectin	Yes	64
1998	Disulfiram (Antabuse)	Yes	65
2000	Oxifendazole	Yes	66
2001	Ivermectin	Yes	67
2002	Inmunoglobulin (IgA)	Yes	68
2003	Vaccine against Giardia	No	69
2007	Antioxidant (Antox)	No	70

Table 3 Nonrandomized clinical trials

Year	Drugs/Substances	Activity	Reference
1972	Berberine	Yes	71
1975	Berberine	Yes	72
1977	Metronidazole	Yes	73
	Tinidazole	Yes	
	Nimorazol	Yes	
	Furazolidone	Yes (– effective)	
1978	Tiberal 1 g BID – G1	Yes	74
	Tiberal 50 mg/Kg/single dose – G2	Yes	
		SE > group G2	
1978	Metronidazole in four dosage schedules	Yes (+ effective in extended systems)	75
1978	Metronidazole		76
	Tinidazole	Yes (– effective)	
		Yes (+ effective)	
		SE > with metronidazole	
1978	Tinidazole		77
	Placebo	Yes (+ effective)	
1978	Tinidazole single dose highest		78
	Tinidazole seven days dose lower	Yes (+ effective)	
		Yes (– effective)	
1979	Metronidazole		79
	Quinacrine	Yes (+ effective)	
		Yes (– effective)	
1979	Ornidazole		80
		Yes	
1980	Metronidazole seven days		81
	Meronidazole single dose	Yes (– effective)	
	Quinacrine	Yes (– effective)	
	Tinidazole	Yes (+ effective)	
	Ornidazole	Yes (+ effective)	
		Yes (+ effective)	
		SE > with ornidazole	
1981	Furazolidone		82
	Quinacrine	Yes (+ effective)	
		Yes (– effective)	
		SE > with quinacrine	
1987	Metronidazole		83
	Tinidazole	Yes	
	Ornidazole	Yes	
		Yes	
		Similar	
1987	Tinidazole	efficiencies	84
1995	Metronidazole	Yes	85
1997	Metronidazole + diloxanide	Yes	86
1997	Pippali Rasayana	Yes	87
	Placebo		
		Yes	
1998	Albendazole		88
1999	Secnidazole	Yes	89
2000	Secnidazole	Yes	90
2008	Metronidazole	Yes	91
		Yes	

Abbreviations: BID, twice a day; SE, side effects.

Table 4 Randomized controlled clinical trials

Year	Drugs	Activity	Reference
1970	Mepacrine Metronidazole Furazolidone	–	92
1977	Tinidazole Metronidazole	Yes (+ effective and < SE) Yes (– effective and > SE)	93
1978	Tinidazole Metronidazole	–	94
1978	Tinidazole Placebo	Yes (+ effective)	95
1981	Tinidazole Metronidazole	Yes – Similar efficacy	96
1985	Tinidazole Metronidazole	Yes – Similar efficacy with appropriate doses	97
1989	Furazolidone Placebo	Yes	98
1989	Metronidazole Furazolidone	Yes Yes	99
1989	Menbedazole	No	100
1990	Metronidazole Menbendazole	Yes Yes	101
1991	Metronidazole Ornidazole	Yes – Similar efficacy	102
1992	Metronidazole Mebendazole	Yes Yes	103
1994	Metronidazole Albendazole	Effectiveness of cure similar SE > with metronidazole	104
1995	Metronidazole Albendazole	Yes Yes	105
1995	Bacitracin zinc Bacitracin Neomycin Neomycin + Bacitracin zinc	Yes Yes Yes Yes	106
1995	Metronidazole single dose Metronidazole for five days Albendazole for five days	Yes Yes Yes	107
1995	Metronidazole Ornidazole Mebendazole	Yes (effective) Yes (+ effective) Yes (– effective)	108
1999	Albendazole Tinidazole	Yes (+ effective) Yes (– effective)	109
2001	Metronidazole Mebendazole	Yes Yes	110
2001	Nitazoxanide Placebo	Yes (+ effective)	111
2001	Metronidazole Nitazoxanide	Yes – Similar efficacy	112
2001	Metronidazole + wheat germ Metronidazole + Placebo	Yes (+ effective) Yes (– effective)	113
2002	Albendazole Albendazole + Praziquantel Tinidazole	Yes (+ effective) Yes (– effective) Yes (+ effective) Albendazole and Tinidazole with similar effectiveness	114

(*Continued*)

Table 4 (*Continued*)

Year	Drugs	Activity	Reference
2002	Metronidazole	Yes (– effective)	115
	Ornidazole single dose	Yes (+ effective)	
	Ornidazole five days	Yes (+ effective)	
2003	Mebendazole	Yes	116
	Secnidazole	Yes	
2003	Albendazole	Yes (– effective)	117
	Tinidazole	Yes (+ effective)	
	Cloroquine	Yes (+ effective)	
		Tinidazole and Cloroquine with similar effectiveness and greater than Albendazole	
2004	Metronidazole	Yes	118
	Albendazole	Yes	
2004	Metronidazole	Yes (– effective)	119
	Albendazole	Yes (+ effective)	
2006	Metronidazole + saccharomyces boulardii	Yes (+ effective)	120
	Metronidazole +placebo	Yes (– effective)	
2006	Mebendazole	Yes	121
	Quinacrine	Yes	
2006	Mebendazole	Yes (– effective)	122
	Tinidazole	Yes (+ effective)	
2006	Metronidazole	Yes (– effective)	123
	Albendazole	Yes (+ effective)	
2007	Vitamin A	Yes	124
	Zinc	Yes	
	Vitamin + zinc	Yes (+ effective)	
	Placebo	No	
2008	Tinidazole	Yes (+ effective)	125
	Nitazoxanide	Yes (– effective)	

Abbreviation: SE, side effects.

quinacrine (RC = 85% ± 21.6), furazolidone (RC = 82% ± 14), and metronidazole (RC = 76.6% ± 20.6) (Table 8). The metronidazole was the most studied and tested drug for the giardiasis treatment. This drug had greater efficacy in larger doses and in more prolonged regimes (5 to 10 days), and achieved a cure rate of 87% to 100% in these schemes (Table 9).

Out of the 34 RCTs selected for analysis, 23 studies (67.6%) had design to compare efficacy between drugs, five (14.7%) compared different schemes of the same drug, and five (14.7%) compared efficacy between one drug and placebo (randomized). One study tested a drug without comparing it with any other drug or placebo. Eight studies (23.5%) were double-blind studies, five (62.5%) compared one drug with placebo, while three (37.5%) compared the efficacy between drugs.

Eighteen drugs were tested on the 34 RCTs. The average sample size was 98.9 ± 38.0 patients per study (CI = 83.7 to 114.1). The mean general rate of cure per drug was 83.0% ± 16.1 (CI = 78.4 to 87.6). Interestingly, the mean rate of cure of the placebo was 25%.

There was no significant difference either in the sample size/patient relationship or in the rate of cure observed between nonrandomized and RCTs studies (83.3 × 98.9 patients/study and 85.5% × 83.0%; $p > 0.05$).

The most frequently tested drugs in RCTs were: metronidazole (21 studies, 61.8%), tinidazole (10 studies, 29.4%), albendazole (nine studies, 26.5%), mebendazole (eight studies, 23.5%), ornidazole, furazolidone, and nitazoxanide (three studies each, 8.8%) (Table 10).

Among drugs showing greater effectiveness, the following mean rates of cure were found: ornidazole (RC = 97.6% ± 2.5), tinidazole (RC = 91.1% ± 6.3), metronidazole (RC = 81.5% ± 18.6), nitazoxanide (RC = 79.7% ± 1.8), and albendazole (RC = 73.4% ± 19.8) (Table 11). According to the nonrandomized clinical trials, metronidazole was the drug most frequently studied and

Table 5 *In vitro* studies: drugs more frequently tested

	Drugs/Substances tested	Number of studies	Observation
1	2,2-biimidazole	1	–
2	Human milk	1	–
3	Bithionol	1	–
4	Dichlorophene	1	–
5	Hexachlorophene	1	–
6	Clomipramine	1	–
7	Furazolidone	4	*
8	Nitroimidazole	1	–
9	Azitromicin	2	*
10	Doxiciclin	1	–
11	Mefloquin	1	–
12	Tinidazole	2	*
13	Metronidazole	9	*
14	Ornidazole	1	–
15	Serum immune specific	1	–
16	Agglutinin of wheat germ	1	–
17	Methanolic extracts cathartics	1	#
18	Methanolic extracts noncathartics	1	#
19	Albendazole	5	*
20	Pyrantel pamoate	1	–
21	Powder of *Yucca schidigera*	1	–
22	Ciprofloxacin	1	–
23	Nitazoxanide (Nitrotiazol)	2	*
24	Mucin	1	–
25	Derivatives of isoflavone	1	–
26	Derivative etylphenylcarbamate	2	*
27	Gangliosides	1	–
28	Dodecanoic acid	1	–
29	Arsenic sodium	1	–
30	Derivatives benzimidazoles	1	–
31	Venom *Crotalus durissus terrificus*	1	–
32	Venom *Bothrops jararaca*	1	–
33	Propolis	1	–
34	Curcumin	1	–
35	Analogous MTZ-Ms	1	–
36	Analogous MTZ-I	1	–
37	Analogous MTZ-Br	1	–
38	Analogous MTZ-N_3	1	–
39	Analogous MTZ-NH_3Cl	1	–
40	Extracts of blueberry	1	–
41	Tiliroside	1	–
42	Kaempferol-glucopyranoside	2	*
43	Astragalin	1	–
44	Quercitrin	1	–
45	Isoquercitrin	1	–
46	Dorstenia contrajerva	1	–
47	Senna villosa	1	–
48	Ruta chalepensis	1	–

(*Continued*)

Table 5 (*Continued*)

	Drugs/Substances tested	Number of studies	Observation
49	Derivatives of flavonoid glycosides	1	–
50	Derivatives of *Artemisia ludoviciana*	1	–
51	S-substituted 4,6-dibromo mercaptobenzimidazole	1	–
52	S-substituted 4,6-dichloro-2-mercaptobenzimidazole	1	–
53	Derivatives of flavonoid Helianthenum glomeratum	1	–
54	Derivatives of allicin (diallyl trisulfide)	1	–
55	Quinacrin	1	–

Notes: #phytotherapies are popular in Africa; *Drugs more frequently tested.

tested on the RCTs. Likewise, this drug had greater efficacy with larger doses and with more prolonged regimes (5 to 10 days), reaching cure rates of 89% to 97% with these schemes (Table 12).

On the RCTs, tinidazole and ornidazole were the drugs which showed good efficacy using a single-dose scheme. Albendazole shown great variability in efficacy, not only in a single dose (RC = 50% to 97%), but also in prolonged regimes (RC = 62% to 90%).

The side effects were poorly described in the majority of studies in the nonrandomized control trials, and they ranged from none to 59%, although they were mild and transient. As in nonrandomized clinical trials, the prevalence of side effects were poorly described in the majority of RCTs studies, ranging from few or absent to 70%, and were also mild and transient.

Discussion

In 1957, the Rhone-Poulenc laboratories synthesized 1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole) by manipulating the chemical structure of 2-nitroimidazole[126] and this proved to be a highly effective agent against *Trichomonas vaginalis* infections.[127] In 1962, Darbon and colleagues[128] reported that this could also be used in treatments against giardiasis. Thus, since it was discovered, metronidazole and other 5-nitroimidazoles – such as secnidazole, ornidazole, and tinidazole – are used by physicians to treat *G. lamblia* infections in addition to infections by other microorganisms. Nowadays, metronidazole is the most used drug to treat giardiasis worldwide; including in the USA.[129] However, the number of new drugs is increasing.

Doing this review, we found out that there were a high number of studies regarding the giardiasis treatment, even with the methodology used in the present study. However, the quality of them was very poor, mainly regarding their primary goal, their design, and sample size; in addition to a great heterogeneity detected between studies.

In all categories of studies, 298 were initially included (*in vitro*, *in vivo*, nonrandomized clinical trials, and RCTs), which, after selection, comprised 105 studies – representing 35.2% – that constituted the sample for the analysis. It is important to point out that we used relatively liberal criteria to select the articles, and the search was done only in the most important databases, comprising journals with more restricted and rigorous publication criteria.

One hundred and sixteen references to *in vitro* studies were found, which comprised 39 (33.6%) studies that constituted the data bank for analysis. Based on this, 50 drugs were evaluated, 48 (96%) of which showing activity against Giardia. Most of these studies had design to compare drugs among themselves (53 %): 52.4% to compare two drugs, and 47.6% to compare three or more drugs.

Many of the studies with two or more drugs did not necessarily compare the efficacy between drugs, but just analyzed and described the activity of the drugs without comparing their efficacy.

Although the number of known drugs tested was larger, we found out that the most widely tested drugs were metronidazole, albendazole, and furazolidone, and that the new drugs were larger in number, each with few studies (Table 5). In this context, several *in vitro* studies have been carried out in order to search for new substances with antigiardial activity. This way, many methods have been described aiming at determining the antigiardial activity of drugs *in vitro*.[4,19,64,130–133] However, some of these are laborious and require long and hard work; furthermore, they are very difficult to reproduce for they lack standardization.

In the initial search for new drugs with antigiardial activity, 48 *in vivo* studies were found but only nine (18.8%) constituted the data bank for analysis, according to the inclusion criteria. Ten drugs were tested in these studies, and eight (80%) were active against *Giardia*. The majority of studies did not compare drugs, but just tested the activity of one drug against *Giardia* (Tables 2 and 6).

Table 6 *In vivo* studies: drugs more frequently tested

	Drugs tested	Number of studies	Observation
1	Albendazole	1	–
2	News oxadiazoles	1	–
3	Metronidazole	1	–
4	Ivermectin	2	*
5	Disulfiram (Antabuse)	1	–
6	Oxifendazole	1	–
7	Inmunoglobulin (IgA)	1	–
8	Vaccine against Giardia	1	–
9	Antioxidant (Antox)	1	–

Note: *Drugs more frequently tested.

Again, the various models used and the absence of standardized design, besides the heterogeneity of these studies, make the comparative analysis difficult. In this context, several *in vivo* experimental models have been proposed. They are often beavers, young and adult rats,[134–137] rabbits,[138] dogs,[139] cats,[140] mice,[141,142] and gerbils.[143,144] However, the best results have only been obtained in gerbil experimental models. Gerbil (*Meriones unguiculatus*) is considered by several researchers the most appropriate experimental model for giardiasis due to its size, facility to handle, high susceptibility to infections, and large shedding of cysts in their feces.[143–148] Thus, we consider that the absence of standardized methods between studies limited the comparative analysis.

When we analyze the studies in human beings (nonrandomized trials and randomized control trials), we find great heterogeneity among them, besides the poor quality of their methodology.

Table 7 Drugs more frequently tested in nonrandomized clinical trials

	Drugs tested	Number of studies	Observation
1	Berberine	2	*
2	Metronidazole	9	*
3	Tinidazole	7	*
4	Nimorazole	1	–
5	Furazolidone	2	*
6	Tiberal	1	–
7	Quinacrin	3	*
8	Ornidazole	3	*
9	Diloxanide	1	–
10	Pippali Rasayana	1	–
11	Albendazole	1	–
12	Secnidazole	2	*

Note: *Drugs more frequently tested.

Table 8 Mean rate of cure of drugs more tested in nonrandomized clinical trials

	Drugs tested	Number of studies	Mean rate of cure % ± SD (CI)
1	Metronidazole	9	76.6 ± 20.6 (64.9–88.3)
2	Tinidazole	7	89.1 ± 8.8 (83–92.5)
3	Ornidazole	3	93.6 ± 1.2 (92.2–95)
4	Quinacrin	3	85 ± 21.6 (63.8–100)
5	Secnidazole	2	96 ± 2.8 (92.0–99.9)
6	Furazolidone	2	82 ± 14.0 (62.5–100)

Note: *Drugs more frequently tested.
Abbreviations: CI, confidence interval; SD, standard deviation.

No references selected were similar in design, dosages, duration of treatment, and results, which led to a great difficulty in grouping them according to the tested drug (required time or percentage of fecal cure, independent of duration of treatment). These findings agree with those by Zaat and colleagues.[149]

In the nonrandomized clinical trials, slightly more than a quarter of studies compared the efficacy between drugs, whereas 47.8% tested drugs without comparing them to a placebo or to another drug, just appraising their efficacy in treated patients versus untreated patients. Only 13% compared a drug to a placebo.

On the RCTs, we find that two thirds of the studies (67.6%) compared the efficacy between drugs; however, only 14.7% compared drugs to a placebo. Here, just one study did not include a comparison between drugs. About half the nonrandomized clinical trials tested different dosages of drugs (assessment of therapeutic schemes), whereas the RCTs were comparative studies of efficacy between drugs, with few studies using a placebo for comparing the efficacy of drugs (14.7%).

Regarding the number of drugs tested, we built an extensive list of them: 55 drugs in 39 *in vitro* studies, nine

Table 9 More effective doses of drugs tested in nonrandomized clinical trials

Drugs	Unit	Recommended doses
Metronidazole	mg/Kg/day	15–25 TID – 5 to 10 days
	mg	200–500 TID – 5 to 10 days
Tinidazole	mg	1–2 MID – One day
Ornidazole	mg	2 MID – One day
Quinacrine	mg	100 TID – 5 days
Secnidazole	mg/Kg	30 MID – One day

Abbreviations: TID, three times a day; MID, once a day.

Table 10 Drugs more frequently tested in randomized control clinical trials

	Drugs tested	Number of studies	Observation
1	Mepacrine	1	–
2	Metronidazole	21	*
3	Furazolidone	3	*
4	Tinidazole	10	*
5	Mebendazole	8	*
6	Ornidazole	3	*
7	Albendazole	9	*
8	Bacitracin zinc	1	–
9	Neomycin	1	–
10	Nitazoxanide	3	*
11	Wheat germ	1	–
12	Praziquantel	1	–
13	Cloroquine	1	–
14	Secnidazole	1	–
15	Saccharomyces boulardii	1	–
16	Quinacrin	1	–
17	Vitamin A	1	–
18	Zinc	2	–

Note: *Drugs more frequently tested.

drugs in nine *in vivo* studies, 12 drugs in 23 nonrandomized trials, and 18 drugs in 50 RCTs (Tables 1–4).

Regarding the sample size, in human studies, we found a comparatively small sample size in both nonrandomized and RCTs studies. We found a higher sample size in the RCTs as compared to the nonrandomized studies, though not statistically significant (98.9×83.3 patients/study; $p < 0.05$).

These findings show a great number of studies in which the external validation, and, consequently, the generalizability of the results is jeopardized. Numerous confounding factors make the analysis of these studies difficult, mainly due to problems in controlling some variables in the population studied.

The most frequently tested drugs in the present review are listed in Tables 5, 6, 7, and 10. We find that the most used drugs in human studies were all tested in *in vitro* studies, but not all drugs tested in *in vivo* studies were tested in human studies, although the number of drugs in the *in vivo* studies was as low as 10 drugs. Metronidazole was the most frequently tested drug. They were tested in 16.4% of *in vitro* studies, in 11.1% of *in vivo* studies, in 39.1% of nonrandomized studies, and in 61.8% of RCTs. Thus, this drug was the main drug in the available arsenal for giardiasis treatment, constituting a reference in relation to other drugs. This finding corroborates other reviews.[149,150]

Table 11 Mean rate of cure of drugs in randomized control clinical trials

	Drugs tested	Number of studies	Mean rate of cure % ± SD (CI)
1	Metronidazole	21	81.5 ± 18.6 (71.0–92.0)
2	Tinidazole	10	91.1 ± 6.3 (87.2–95.0)
3	Albendazole	9	73.4 ± 19.8 (58.7–88.1)
4	Mebendazole	8	65.6 ± 17.3 (50.4–80.8)
5	Ornidazole	3	97.6 ± 2.5 (95.4–99.8)
6	Nitazoxanide	3	79.7 ± 1.8 (77.2–82.2)

Note: *Drugs more frequently tested.
Abbreviations: CI, confidence interval; SD, standard deviation.

When only the nonrandomized and RCTs studies were analyzed, the two most tested drugs were metronidazole and tinidazole. However, mebendazole and albendazole were among the most tested in RCTs, and they were barely tested in nonrandomized studies.

We also noticed that the "new drugs" for giardiasis treatment were barely tested in all categories of studies reviewed in this work, either in *in vitro* studies or in RCTs. This demonstrates the difficulty in adequately testing one drug for giardiasis in order to have alternatives in case of resistance to one of the therapeutic schemes.

In spite of the large amount of drugs used in antigiardial therapy, some resistance has been reported regarding different therapeutic regimens, and this resistance has been mentioned by clinicians.[18,20,151] This characteristic makes *Giardia* a fearful microorganism, mainly among undernourished people, in whom the malabsorption syndrome is more common. In this scenario, developing and screening new antigiardial drugs seems to be a priority.

Table 12 More effective doses of drugs tested in randomized clinical trials

Drugs	Unit	Recommended doses
Metronidazole	mg/Kg/day	15–50 TID – 5 to 10 days
	mg	500–750 TID – 5 to 10 days
Tinidazole	mg	2 MID – One dose
	mg/Kg/day	50 MID – One dose
Albendazole	mg	400 MID – One day
	mg	400 MID – 5 days
	mg/Kg/day	10 MID – 5 days
Mebendazole	mg	200 TID – 5 days
Ornidazole	mg/Kg/day	20–40 MID – 1 to 5 days
Nitazoxanide	mg	500 MID – 3 days

Abbreviations: TID, three times a day; MID, once a day.

In order to analyze the optimal dosages for the most tested drugs, we evaluated the mean rate of cure for all (Tables 8 and 11). We found out that the most tested drugs and those with more efficacy in studies with human beings were tinidazole and metronidazole; though ornidazole had a great efficacy not only in nonrandomized but also in RCTs. However, ornidazole was tested in only six studies in the present review (three nonrandomized and three RCTs).

The optimal dosages found in this review for most drugs were those that achieved the best rate of cure for each drug separately. Tables 8, 9, 11, and 12 show the most widely used drugs and their mean rate of cure, along with the optimal dosages for each. Comparing the mean rate of cure between the most tested drugs, we detected a similar efficacy among them, none being better than the others, except for mebendazole in the RCTs.

The analyses of the side effects have been poorly appraised and documented in most studies. Apparently, they have been similar in all studies, and no drug was reported to be unsafe, causing only mild to moderate and transient side effects.

However, regarding the new drugs, only those tested in human beings had their side effects described, but we have few data about it at the moment.

In summary, in this review we found many studies on the giardiasis treatment; however, most of them presented various problems concerning the sample size, methodology, design, among others.

Moreover, the number of drugs tested was large, with a relative higher number of new drugs listed, mainly in the *in vitro* studies, and a lower number in the studies with humans. However, these new drugs were barely tested as compared to the old drugs, mainly in humans, increasing the need for new studies to provide standardization for the evaluation of antigiardial drugs. This can provide more accuracy and quickness for approval, as well as an adequate use not only for the new drugs but also the old ones.

Conclusion

In conclusion, this review raises some problems regarding the evidence for using old and new antigiardial drugs, in relation to the quality of previous and future studies. Yet, one must point out that the drugs in use nowadays are the most widely tested and that they are safe, although we must rethink and further study the problem of their increasing resistance.

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