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,5 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
water collections and lakes. 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. In these populations, giardiasis reaches epidemic levels. *G. lamblia* has a cosmopolitan distribution with an estimated number of $2.8 \times 10^6$ cases of infections per year and is thus the most common intestinal parasite in humans in developed countries. In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year. In those countries this disease should be observed carefully, for it contributes substantially to generating mentally and physically impaired adults.

Thompson and colleagues 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. Although some advances have been observed in isolating and characterizing *Giardia* samples, there are few studies regarding this parasite’s chemotherapy. Resistance to different drugs used in the treatment of this disease has been reported and the number of cases is likely to increase.

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. Furthermore, *Giardia* seems to have a great ability to resist these agents.

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-biimidazolo                                      | 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        |
| 1986 | Nitroimidazolo                                       | Yes      |           |
| 1990 | Azitromicin/Furazolidone                             | Yes      | 28        |
|      | Doxicilin/Mefloquin                                  | Yes      |           |
|      | Doxicilin/Tinidazole                                 | Yes      |           |
|      | Mefloquin/Tinidazole                                 | Yes      |           |
| 1991 | Metronidazole                                        | Yes      | 29        |
|      | Ornizadole                                           | 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 |
|      | Mechanolic extracts cathartics                       | Yes (- effective) | |
|      | Mechanolic extracts noncathartics                   | Yes (- effective) | |
| 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 | Ciprofloxacinc                                       | Yes      | 37        |
| 2002 | Nitrazoxanide                                        | Yes (+ effective) | 38 |
|      | Albendazole                                          | Yes (+ effective) | |
|      | Metronidazole                                        | Yes (- effective) | |
| 2002 | Mucin                                                | Yes      | 39        |
| 2002 | Derivatives of isoflavone                            | Yes      | 40        |
| 2003 | Derivative esthphenylcarbamate                        | 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-mercaptopbenzimidazole      | Yes      | 58        |
|      | S-substituted 4,6-dichloro-2-mercaptopbenzimidazole   | Yes      |           |
| 2005 | Dodecanonic 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)
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%), azitromycin, 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).

| Table 1 (Continued) |
|---------------------|
| Year | Drugs/Substances | Activity | Reference |
| 2006 | Nitrotiazol (Nitazoxanide) | Yes | 47 |
| 2006 | Metronidazole | Yes | |
| 2006 | Venom Crotalus durissus terrificus | Yes | 48 |
| 2006 | Venom Bothrops jararaca | Yes | |
| 2006 | Propolis | Yes | 49 |
| 2006 | Curcumin | Yes | 50 |
| 2006 | Metronidazole | Yes | 51 |
| 2006 | Furazolidone | Yes (+ effective) | |
| 2007 | Dorstenia contrajerva | Yes | 52 |
| 2007 | Senna villosa | Yes | |
| 2007 | Ruta chalepensis | Yes | |
| 2007 | Metronidazole | Yes (-- effective) | 51 |
| 2007 | Analogous MTZ-Ms | Yes | |
| 2007 | Analogous MTZ-I | Yes | |
| 2007 | Analogous MTZ-Br | Yes | |
| 2007 | Analogous MTZ-N | Yes | |
| 2007 | Analogous MTZ-NHCl | Yes | |
| 2007 | Extracts of blueberry | Yes | 53 |
| 2007 | Tilroside | Yes (+ effective) | |
| 2007 | Kaempferol-glucopyranoside | Yes | |
| 2007 | Astragalin | Yes | |
| 2007 | Quercitrin | Yes | |
| 2007 | Isoquercitrin | No | |

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).
| 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                    | Yes (– effective) | 76 |
|      | Tinidazole                       | Yes (+ effective) | SE > with metronidazole |
| 1978 | Tinidazole                       | Yes (+ effective) | 77 |
|      | Placebo                          | Yes (+ effective) | 78 |
| 1978 | Tinidazole single dose highest   | Yes (+ effective) | 79 |
|      | Tinidazole seven days dose lower | Yes (– effective) | 81 |
| 1979 | Metronidazole                    | Yes (+ effective) | 80 |
|      | Quinacrine                       | Yes (– effective) |           |
| 1979 | Ornidazole                       | Yes      |           |
| 1980 | Metronidazole seven days         | Yes (– effective) | 81 |
|      | Meronidazole single dose         | Yes (– effective) |           |
|      | Quinacrine                       | Yes (– effective) |           |
|      | Tinidazole                       | Yes (+ effective) |           |
|      | Ornidazole                       | Yes (+ effective) |           |
|      |                                   | Yes (+ effective) | SE > with ornidazole |
| 1981 | Furazolidone                     | Yes (+ effective) | 82 |
|      | Quinacrine                       | Yes (– effective) | SE > with quinacrine |
| 1987 | Metronidazole                    | Yes      | 83        |
|      | Tinidazole                       | Yes      | Similar   |
|      | Ornidazole                       | Yes      |           |
| 1987 | Tinidazole                       | efficiencies | 84 |
| 1995 | Metronidazole                    | Yes      | 85        |
| 1997 | Metronidazole + diloxanide       | Yes      | 86        |
| 1997 | Pippali Rasayana                 | Yes      | 87        |
|      | Placebo                          | Yes      |           |
| 1998 | Albendazole                      | Yes      | 88        |
| 1999 | Secnidazole                      | Yes      | 89        |
| 2000 | Secnidazole                      | Yes      | 90        |
| 2008 | Metronidazole                    | Yes      | 91        |

**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  
Placebo | Yes | 99 |
| 1989 | Menbendazole | No | 100 |
| 1990 | Metronidazole  
Menbendazole | Yes | 101 |
| 1991 | Metronidazole  
Ornidazole | Yes – Similar efficacy | 102 |
| 1992 | Metronidazole  
Menbendazole | Yes | 103 |
| 1994 | Metronidazole  
Albendazole | Effectiveness of cure similar SE > with metronidazole | 104 |
| 1995 | Metronidazole  
Albendazole | Yes | 105 |
| 1995 | Bacitracin zinc  
Bacitracin  
Neomycin  
Neomycin + Bacitracin zinc | Yes | 106 |
| 1995 | Metronidazole single dose  
Metronidazole for five days  
Albendazole for five days | Yes | 107 |
| 1995 | Metronidazole  
Ornidazole  
Mebendazole | Yes (effective)  
Yes (+ effective)  
Yes (– effective) | 108 |
| 1999 | Albendazole  
Tinidazole | Yes (+ effective)  
Yes (– effective) | 109 |
| 2001 | Metronidazole  
Mebendazole  
Nitazoxanide  
Placebo | Yes  
Yes (+ effective) | 110  
111 |
| 2001 | Metronidazole  
Nitazoxanide  
Nitazoxanide + Metronidazole + wheat germ  
Nitazoxanide + Placebo | Yes – Similar efficacy | 112  
113  
114 |
| 2002 | Albendazole  
Albendazole + Praziquantel  
Tinidazole | Yes (+ effective)  
Yes (+ effective)  
Albendazole and Tinidazole with similar effectiveness | 114 |
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 4 (Continued)

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

**Abbreviation:** SE, side effects.
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                         | 1                 | *           |
| 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 ethylphenylcarbamate     | 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$_{4}$Cl            | 1                 | –           |
| 40. Extracts of blueberry               | 1                 | –           |
| 41. Tiliroside                          | 1                 | –           |
| 42. Kaempferol-glucopyranoside          | 2                 | *           |
| 43. Astragalin                          | 1                 | –           |
| 44. Quercetin                           | 1                 | –           |
| 45. Isoquercetin                        | 1                 | –           |
| 46. Dorstenia contrajervia              | 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 and this proved to be a highly effective agent against Trichomonas vaginalis infections. In 1962, Darbon and colleagues 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. 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. 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).
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,\textsuperscript{134–137} rabbits,\textsuperscript{138} dogs,\textsuperscript{139} cats,\textsuperscript{140} mice,\textsuperscript{141,142} and gerbils.\textsuperscript{143,144} However, the best results have only been obtained in gerbil experimental models. Gerbil (\textit{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.\textsuperscript{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 6 | In vivo studies: drugs more frequently tested |
|----------|---------------------------------------------|
| Drugs tested | Number of studies | Observation |
| Albendazole | 1 | – |
| New oxadiazoles | 1 | – |
| Metronidazole | 1 | – |
| Ivermectin | 2 | * |
| Disulfiram (Antabuse) | 1 | – |
| Oxifendazole | 1 | – |
| Immunglobulin (IgA) | 1 | – |
| Vaccine against Giardia | 1 | – |
| Antioxidant (Antox) | 1 | – |

Note: *Drugs more frequently tested.

| Table 7 | Drugs more frequently tested in nonrandomized clinical trials |
|----------|---------------------------------------------------------------|
| Drugs tested | Number of studies | Observation |
| Berberine | 2 | * |
| Metronidazole | 9 | * |
| Tinidazole | 7 | * |
| Nimorazole | 1 | – |
| Furazolidone | 2 | * |
| Tiberal | 1 | – |
| Quinacrin | 3 | * |
| Ornidazole | 3 | * |
| Dioxanide | 1 | – |
| Pippali Rasayana | 1 | – |
| Albendazole | 1 | – |
| 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) |
| Metronidazole | 9 | 76.6 ± 20.6 (64.9–88.3) |
| Tinidazole | 7 | 89.1 ± 8.8 (83–92.5) |
| Ornidazole | 3 | 93.6 ± 1.2 (92.2–95) |
| Quinacrin | 3 | 85 ± 21.6 (63.8–100) |
| Secnidazole | 2 | 96 ± 2.8 (92.0–99.9) |
| Furazolidone | 2 | 82 ± 14.0 (62.5–100) |

Note: *Drugs more frequently tested.

Abbreviations: CI, confidence interval; SD, standard deviation.

| 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.
Giardiasis treatment

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

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 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 Sercnidazole | 1              | –           |
| 15 Saccharomyces boulardii | 1 | – |
| 16 Quinacrin | 1                | –           |
| 17 Vitamin A | 1                | –           |
| 18 Zinc | 2                | –           |

Note: *Drugs more frequently tested.

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.

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

| Drugs tested | Unit | Recommended doses |
|--------------|------|-------------------|
| Metronidazole | mg/Kg/day | 15–50 TID – 5 to 10 days |
|              | mg   | 500–750 TID – 5 to 10 days |
| Tinidazole | mg/Kg/day | 2 MID – One dose |
|             | mg   | 50 MID – One dose |
| Albendazole | mg/Kg/day | 400 MID – One day |
|             | mg   | 400 MID – 5 days |
|             | mg   | 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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