In-vitro susceptibility of *Giardia lamblia* to albendazole, mebendazole and other chemotherapeutic agents

R. CEDILLO-RIVERA* and O. MUÑOZ

Unidad de Investigación Clínica en Enfermedades Infecciosas y Parasitarias, Hospital de Pediatría,
Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F. 06725, México

Summary. The susceptibility of a strain of *Giardia lamblia* to benzimidazole carbamates, 5-nitroimidazoles, nitrofurans and other drugs was studied *in vitro*. Albendazole was the most active compound, with a 50% inhibitory concentration (IC₅₀) of 0.01 mg/L and a minimal lethal concentration (MLC) of < 0.04 mg/L; the IC₅₀ of mebendazole was 0.06 mg/L and the MLC < 0.5 mg/L. Among the 5-nitroimidazoles tested, ornidazole was the most effective (IC₅₀ 0.12 mg/L); tinidazole, metronidazole, secnidazole and hemezole were less active. Nifuroxazide, etofamide and nalidixic acid exhibited modest anti-giardial activity; quinifamide did not inhibit the growth of the parasite at a concentration of 200 mg/L. Albendazole and mebendazole are promising candidates for clinical use and should be further evaluated.

Introduction

The intestinal protozoon *Giardia lamblia* causes a range of infection from asymptomatic carriage to persistent and severe diarrhoea with malabsorption, and the "failure to thrive" syndrome in children.^{1,2} This parasite occurs worldwide, although it is more frequent in developing countries.³ Mepacrine, metronidazole or furazolidone are presently recommended for treatment.^{4–6} Strains of *G. lamblia* obtained from different areas of the world are susceptible to these compounds,^{7–9} but they occasionally provoke unacceptable side effects and treatment sometimes fails, partly because the patients do not always complete the course of therapy;^{4–6} moreover, these drugs are active against the normal intestinal flora.⁶ In the present study, we tested the susceptibility of *G. lamblia* to several compounds including the benzimidazole carbamates, albendazole and mebendazole, which are currently used for the treatment of helminthiasis and which may have less effect on the bacterial flora of the gut.^{10–12} *G. lamblia* has been reported to be highly susceptible to albendazole and mebendazole *in vitro*,^{13,14} but the efficacy of these drugs in clinical studies is controversial.^{15–17} Other drugs tested included etofamide and quinifamide, which are occasionally used in the treatment of intestinal amoebiasis,^{18,19} nifuroxazide, a nitrofuran used in some countries for the treatment of infectious diarrhoea,²⁰ nalidixic acid,

several 5-nitroimidazoles, and drugs presently used for the treatment of giardiasis.

Materials and methods

Strain

The *G. lamblia* strain Portland 1 (P1) was used in all experiments; it was maintained axenically at 37°C in Diamond's modified TYI-S-33 medium²¹ supplemented with heat inactivated bovine serum 10% and ceftriaxone 50 mg/L. Antibiotic was omitted during experimental assays.

Antimicrobial agents

Metronidazole, tinidazole, furazolidone and mepacrine were obtained from Sigma. Other drugs were obtained in Mexico City from the manufacturers or their subsidiaries: albendazole and nifuroxazide from SmithKline-Beecham, mebendazole from Janssen, etofamide from Carlo Erba-Farmitalia, quinifamide and nalidixic acid from Winthrop, hemezole from Silanes, ornidazole from Roche, and secnidazole from Rhône Poulenc. Metronidazole, tinidazole, mepacrine and ornidazole were dissolved in water, hemezole and nalidixic acid were dissolved in sodium bicarbonate solution (pH 7.2); other compounds were dissolved in dimethylformamide.

Standard growth curve

G. lamblia trophozoites were grown axenically in Diamond's modified culture medium as described

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* Correspondence should be sent to Dr R. Cedillo-Rivera, Rómulo Valdez 121, Colonia Presidentes Ejidales, Coyoacán, México, D.F. 04470, México.

above; when the logarithmic phase of growth was reached, the trophozoites were detached from the substrate by cooling on water-ice for 20 min. They were then distributed into 1.5-ml volumes of fresh culture medium in polypropylene microcentrifuge tubes to achieve inocula ranging from 1×10^3 to 5.5×10^5 /ml as determined by counting in a haemocytometer. After incubation for 48 h at 37°C, the number of trophozoites was again counted and a standard curve of cell numbers *versus* inoculum was constructed by linear regression analysis. A growth curve was also constructed by performing daily counts over a 9-day period starting with an inoculum of 2×10^4 parasites/ml.

Susceptibility assay

Susceptibility of *G. lamblia* to the antimicrobial agents was determined by the culture method of Hill *et al.*²² with some modifications: serial two-fold dilutions of drugs were prepared in 1.5-ml volumes of culture medium in microcentrifuge tubes. The tubes were then inoculated with *G. lamblia* P1 to achieve an inoculum of 5×10^4 trophozoites/ml. Controls, prepared by adding drug-free solvents to the culture media, were similarly inoculated. After incubation for 48 h at 37°C, trophozoites were detached by chilling and 50 μ l of each culture tube was subcultured into 1.5 ml of fresh culture medium without drug. After incubation for a further 48 h, the final number of parasites was determined by counting in a haemocytometer. The number of viable trophozoites remaining after exposure to drug for 48 h was deduced from the number of trophozoites attained in the subculture by reference to the linear regression plot of cell density *versus* inoculum. The percentage of growth inhibition was calculated by comparison with the growth in the drug-free control cultures. The 50% inhibitory concentration (IC₅₀) was defined as the concentration of the drug that inhibited growth by 50% as calculated by probit analysis.²³ The 90% inhibitory concentration (IC₉₀) was similarly calculated. The minimum lethal concentration (MLC) was defined as the lowest concentration of the drug at which no viable trophozoites were observed by subculture. Each experiment was done in triplicate and was repeated at least four times.

Results

Growth curve

The logarithmic phase of growth was reached within 48 h and the stationary phase at 5 days. The final cell density was $(1.5\text{--}2) \times 10^6$ trophozoites/ml (fig. 1).

Linear regression analysis of growth

Fig. 2 shows the standard curve, obtained by regression analysis, of the number of trophozoites present after incubation for 48 h of different inocula of *G. lamblia* P1.

Inhibitory and lethal concentrations of antimicrobial agents

The table shows the IC₅₀, IC₉₀ and MLC of each of the antimicrobial agents studied. Albendazole exhibited the greatest inhibitory and lethal activity against *G. lamblia* P1, with an IC₅₀ of 0.01 mg/L and an MLC of 0.03–0.04 mg/L. The IC₅₀ for mebendazole was 0.06 mg/L and the MLC was 0.2–0.4 mg/L. Ornidazole, tinidazole and metronidazole were the most active of the 5-nitroimidazoles; secnidazole showed an intermediate activity and hemeazole was the least active compound of this group. Among the antimicrobial agents currently used for the treatment of giardiasis, metronidazole was the most active, followed by mepacrine and furazolidone. Nifuroxazide, etophamide and nalidixic acid showed a modest

Fig. 1. Growth curve of *G. lamblia* P1 in Diamond's modified culture medium.

Fig. 2. Standard curve obtained by growth of different inocula of *G. lamblia* P1 for 48 h. The curve was constructed by linear regression analysis of the results of four separate experiments, each performed in triplicate. This standard curve was used to deduce the number of viable trophozoites remaining after exposure to antimicrobial agents (see Materials and methods).

Table. Susceptibility of *G. lamblia* to 13 antimicrobial agents

Antimicrobial agent	IC50 (mg/L)	SD	IC90 (mg/L)	SD	MLC (mg/L)
Albendazole	0.01	0.001	0.02	0.002	0.03–0.04
Mebendazole	0.06	0.020	0.13	0.030	0.20–0.50
Ornidazole	0.12	0.030	0.39	0.040	0.50–1.00
Tinidazole	0.14	0.027	0.44	0.090	0.50–1.00
Metronidazole	0.21	0.030	1.28	0.200	1.00–5.00
Mepacrine	0.39	0.050	1.72	0.350	1.50–5.00
Furazolidone	0.62	0.015	2.10	0.150	5.00–10.00
Secnidazole	0.62	0.140	2.10	0.300	5.00–10.00
Hemezole	1.55	0.440	3.80	0.500	10.00–15.00
Nifuroxazide	3.84	1.000	12.25	2.500	10.00–20.00
Etofamide	5.98	1.400	16.00	2.200	40.00–50.00
Nalidixic acid	12.13	1.860	30.20	5.500	45.00–50.00
Quinfamide	> 200	...	...	...	...

IC50, 50 % inhibitory concentration; IC90, 90 % inhibitory concentration; MLC, minimum lethal concentration; SD, standard deviation.

antiparasitic activity; quinfamide did not inhibit the growth of *G. lamblia* P1.

Discussion

These results indicate that the benzimidazole carbamates, albendazole and mebendazole are considerably more active against *G. lamblia* P1 than the other test drugs, including the 5-nitroimidazoles ornidazole, tinidazole and metronidazole. Meloni *et al.*¹³ found that albendazole was 5–10 times more active than metronidazole or tinidazole against *G. lamblia* as judged by the IC50. We found the difference in activity to be somewhat greater than this, although the difference in lethal activity of these compounds was similar to that reported by Meloni *et al.*¹³ In contrast to our results, Edlin *et al.*¹⁴ reported that albendazole was only 1.5 times as active as mebendazole but 50 times as active as metronidazole. The IC50 and MLC of albendazole observed in the present study were lower than those reported previously.^{13,14} These differences could be due to the use of different strains of *G. lamblia* and to the use of different methods to determine antimicrobial activity. The subculture method we used measures the capacity of cells to replicate (as the [³H]thymidine uptake method also does) and this provides a more sensitive assay.^{8,22} Moreover, dimethyl sulphoxide (DMSO) was used to dissolve the benzimidazole carbamates in previous studies.^{13,14} DMSO is known to stabilise microtubules and this could alter the results of the experiments since tubulin is the target of benzimidazoles.^{12,24}

Our results and those of others^{13,14} indicate that

albendazole and mebendazole might be useful alternative drugs in the treatment of giardiasis. These agents exhibit high activity against *G. lamblia* *in vitro*, elicit few side effects when used as anthelmintic agents and should not affect the intestinal flora. However, the results of clinical trials are conflicting. There have been three small, uncontrolled clinical studies of benzimidazoles in the treatment of giardiasis; in two of them albendazole and mebendazole were apparently curative,^{15,16} but in the other, mebendazole failed to eradicate the parasite.¹⁷ We are performing a controlled clinical trial to evaluate the efficacy of these drugs in giardiasis.

Among 5-nitroimidazoles, ornidazole was the most effective, followed by tinidazole and metronidazole. Secnidazole, which possesses a longer half-life,²⁵ was five-fold less active than ornidazole. Similar results were obtained by Boreham *et al.*²⁶ by use of a radiometric assay. Hemezole, a compound synthesised in Mexico, was the least active of the nitroimidazoles tested. Although nifuroxazide, etofamide and nalidixic acid showed only intermediate activity, it is possible that these drugs could reach inhibitory concentrations in the lumen of the intestine at therapeutic doses. Quinfamide did not inhibit the growth of *G. lamblia* even at a concentration of 200 mg/L.

Many antimicrobial agents inhibit the growth of *G. lamblia* *in vitro*; some of them, especially albendazole, mebendazole, but also etofamide and nifuroxazide, may be useful in treatment because of their activity and poor intestinal absorption.

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BOOKS RECEIVED

Medical Virology 10

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This book outlines the proceedings of the 10th International Symposium on Medical Virology, held in October, 1990 at Newport Beach, California. The text consists of 12 extensive reviews, presumably given as invited lectures during plenary sessions of the meeting. These are followed by 21 single-page abstracts. Although the reviews stand as individual articles, it is possible to discern some linking themes between them. Thus, the first four chapters cover various aspects of laboratory diagnostic virology, including the usefulness and applications of electronmicroscopy, the use of the polymerase chain reaction in detecting viral nucleic acid sequences, and the Q replicase amplification technique. The final four chapters are concerned with aspects of prevention and treatment of virus infections, specifically those transmitted by transfusion, a novel approach to influenza vaccination by use of a purified neuraminidase subunit vaccine, lessons learned from use of an attenuated varicella vaccine, and the potential of antisense oligonucleotides as antiviral agents. The intervening third of the book is more of a mixed bag, covering determinants of measles mortality, the epidemiology of HIV-1 infection,

human herpesvirus type 6, and papillomaviruses and human cancer. Perhaps a unique feature, for a book of this nature, is the absence of a chapter on hepatitis C virus, or, indeed, any of the hepatitis viruses. Paradoxically, this avoidance of such a high profile area of clinical virology may be seen as a strength, as it allows attention to be devoted to some of the "less glamorous" topics in clinical virology.

The reviews are authoritatively written and up-to-date, with several references from 1990. Individual preferences will no doubt vary, but for this reader the chapters on HHV6, and the influenza and varicella vaccines were of most interest. Each review is followed by a question-and-answer style discussion, written in the vernacular, of perhaps limited usefulness. Similarly, the abstracts at the end of the book, mostly concerned with comparisons of diagnostic techniques, will not be of general interest. Given the need to publish these reviews with sufficient speed to ensure that the information is still relevant, one can forgive the rather large number of typographical errors. Despite these limitations, overall there is much here to hold the attention of any medical virologist, or physician with an interest in virus infections, although at the price indicated, I suspect that most copies will be purchased for library collections rather than individual use.

W. L. IRVING