Heterogeneity in the sensitivity of stocks and clones of *Giardia* to metronidazole and ornidazole

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Abstract

The sensitivity *in vitro* to metronidazole and ornidazole of 7 stocks and of the cloned lines of 5 stocks of *Giardia* isolated from humans, rodents and monkeys was studied by the growth inhibition test. All 7 stocks of *Giardia*, irrespective of the host, differed in their sensitivity to these drugs, commonly used in therapy of human giardiasis. The differences were greater with ornidazole than with metronidazole. The 5 *Giardia* stocks from which clones were prepared were found to consist of populations with significantly ($P<0{\cdot}05$) differing sensitivities to ornidazole and metronidazole. There was a positive correlation between high resistance *in vitro* to both drugs of all clones of one parent stock and treatment failures of giardiasis in the patient from which the parasite stock had been isolated. The spectra of sensitivity of *Giardia* to anti-giardial drugs may have implications concerning the suspected zoonotic character of human giardiasis.

Introduction

The reasons for the failure in treatment of human giardiasis may be several; the most probable may be heterogeneity in the response of *Giardia* populations to antiprotozoal drugs. Recently BOREHAM *et al.* (1987) proved that cloned lines of 2 stocks of *G. intestinalis* were not homogeneous in their sensitivity to 4 anti-giardial drugs.

The object of the present study was to compare the sensitivity of stocks of *Giardia* isolated from humans and animals, and cloned lines prepared from them, to two 5-nitroimidazole compounds—metronidazole and ornidazole. We selected 2 human *G. intestinalis* isolates, one very sensitive to ornidazole and one highly resistant to both ornidazole and metronidazole. The *Giardia* isolates from animals were selected with regard to the suspected zoonotic character of giardiasis in humans; one of the animal *Giardia* isolates was infective to a human volunteer (unpublished observation by A. C. Majewska).

Materials and Methods

Parasite stocks

Seven stocks of *Giardia* were used. Two stocks of *G. intestinalis* originating from human giardiasis represented the extreme sensitivities of the parasite to anti-giardial drugs. In a preliminary study *in vitro* we found that stock HP-98, isolated from an asymptomatic case, was very sensitive, and stock HP-109, isolated from a symptomatic case of giardiasis with treatment failures, was highly resistant to anti-giardial drugs. The remaining 5 stocks (SLP-111, GGPRP-114, SP-115, LSLP-116, CP-117) were isolated from the following animals in a zoo—slow loris, Gambian giant pouched rat, siamang, lesser slow loris, and cuis, respectively (MAJEWSKA & KASPRZAK, 1990) with no symptoms of infection. However, one of the strains (CP-117) in experimental gerbils produced an infection characterized by intermittent cyst-passing, frequent watery diarrhoea, and marked weight loss (A. C. Majewska, unpublished observation).

Clones

The clones were derived from 5 stocks of *Giardia* by the method of GILLIN & DIAMOND (1980) for clonal growth in semisolid medium; 2 stocks of human *G. intestinalis* and 3 stocks of animal-derived *Giardia* (SLP-111, GGPRP-114, LSLP-116) were used. Five clones derived from each parent stock were established and examined.

Anti-giardial drugs

Metronidazole was a product of Polfa, Starogard, Poland (metronidazol, series no. 41169) and ornidazole was a product of F. Hoffmann-La Roche, Basel, Switzerland (Tiberal® Roche, series no. 5003).

Drug sensitivity test

The experiments were performed in 15 ml of medium in 16×125 mm screw-capped borosilicate tubes. Samples of *Giardia* stocks and clones prepared from log-phase cultures as described elsewhere (KASPRZAK & MAJEWSKA, 1985) were inoculated into tubes containing medium with appropriate drug concentrations. The medium without drugs served as a control.

The effect of the drugs was determined by growth inhibition in 3 d old cultures (log phase), assessed by the number of parasite cells in 1 ml of trophozoite suspension fixed in 1 ml of 2% formalin and counted in a Laborscale PSL-1 Counter. The number of cells in cultures without drug was taken to be 100% growth; the 50% inhibitory concentration (IC_{50}) was that which decreased the number of trophozoites to 50% of the control values. All experiments were duplicated, and mean IC_{50} values were calculated from 6 determinations.

The possibility that some of the differences between the *Giardia* isolates were due to their previous history since isolation can probably be ruled out; all the stocks and clones were maintained in the same axenic conditions, and the time of isolation of all stocks was the same. Furthermore, in a preliminary

Table 1. Activity of ornidazole and metronidazole *in vitro* against *Giardia* stocks

Stocks[a]	50% inhibitory concentration (μg/ml) Mean±SE[b]	95%CI[c]
Ornidazole		
CP-117	0·010±0·000	0·000–0·021
GGPRP-114	0·011±0·000	0·000–0·021
HP-98	0·030±0·000	0·019–0·041
SP-115	0·110±0·000	0·099–0·121
LSLP-116	0·162±0·002	0·150–0·173
SLP-111	0·225±0·002	0·214–0·236
HP-109	0·470±0·010	0·459–0·481
Metronidazole		
LSLP-116	0·115±0·002	0·103–0·127
CP-117	0·155±0·002	0·143–0·167
SP-115	0·215±0·008	0·203–0·227
SLP-111	0·233±0·002	0·221–0·246
GGPRP-114	0·242±0·002	0·231–0·256
HP-98	0·300±0·000	0·288–0·312
HP-109	1·118±0·010	1·106–1·131

[a]Arranged in order of increasing resistance.
[b]Mean of 6 determinations±standard error.
[c]95% confidence intervals for mean; brackets unite groups of stocks with similar drug sensitivity.

Table 2. Sensitivity to ornidazole *in vitro* of stocks of *Giardia* and clones derived from them

Stocks/clones[a]	50% inhibitory concentration (μg/ml) Mean±SE[b]	95% CI[c]
GGPRP-114	0·011±0·000	
GGPRP-114/6	<0·01[d]	
GGPRP-114/2	<0·01[d]	
GGPRP-114/3	<0·01[d]	
GGPRP-114/5	<0·01[d]	
GGPRP-114/4	<0·01[d]	
HP-98/5	<0·01[d]	
HP-98	0·030±0·000	0·021–0·039
HP-98/1	0·043±0·002	0·034–0·052
HP-98/3	0·053±0·002	0·044–0·63
HP-98/2	0·053±0·008	0·044–0·063
HP-98/4	0·065±0·005	0·056–0·074
LSLP-116/3	0·067±0·003	0·061–0·072
LSLP-116/1	0·108±0·002	0·103–0·114
LSLP-116/5	0·113±0·003	0·108–0·119
LSLP-116/2	0·138±0·003	0·133–0·144
LSLP-116/4	0·158±0·002	0·153–0·164
LSLP-116	0·162±0·002	0·156–0·167
SLP-111/4	0·193±0·002	0·189–0·198
SLP-111/2	0·212±0·002	0·207–0·216
SLP-111/1	0·225±0·002	0·221–0·229
SLP-111	0·225±0·002	0·221–0·229
SLP-111/7	0·227±0·002	0·222–0·231
SLP-111/6	0·245±0·002	0·241–0·249
HP-109/1	0·387±0·004	0·374–0·399
HP-109/3	0·398±0·002	0·386–0·411
HP-109/2	0·420±0·000	0·407–0·433
HP-109/4	0·422±0·002	0·409–0·434
HP-109/5	0·430±0·000	0·417–0·443
HP-109	0·470±0·014	0·457–0·483

[a]Arranged in order of increasing resistance within each group.
[b]Mean of 6 determinations±standard error.
[c]95% confidence interval for mean; brackets unite groups of stocks or clones with similar drug sensitivity.
[d]Minimum concentration used (0·01 μg/ml) caused >50% inhibition.

Table 3. Sensitivity to metronidazole *in vitro* of stocks of *Giardia* and clones derived from them

Stocks/clones[a]	50% inhibitory concentration (μg/ml) Mean±SE[b]	95% CI[c]
LSLP-116/5	0·107±0·012	0·095–0·118
LSLP-116	0·115±0·002	0·103–0·127
LSLP-116/4	0·127±0·005	0·115–0·138
LSLP-116/3	0·142±0·002	0·130–0·153
LSLP-116/2	0·237±0·003	0·225–0·248
LSLP-116/1	0·245±0·002	0·233–0·257
SLP-111	0·233±0·002	0·229–0·238
SLP-111/4	0·252±0·002	0·247–0·256
SLP-111/2	0·265±0·002	0·260–0·270
SLP-111/7	0·270±0·000	0·265–0·275
SLP-111/6	0·290±0·004	0·285–0·295
SLP-111/1	0·293±0·002	0·289–0·298
GGPRP-114/4	0·087±0·002	0·082–0·092
GGPRP-114/3	0·108±0·002	0·103–0·113
GGPRP-114/2	0·112±0·002	0·107–0·117
GGPRP-114/5	0·130±0·004	0·125–0·135
GGPRP-114/6	0·210±0·003	0·205–0·215
GGPRP-114	0·243±0·002	0·238–0·248
HP-98/3	0·245±0·005	0·240–0·250
HP-98/2	0·250±0·000	0·250–0·250
HP-98/5	0·260±0·000	0·260–0·260
HP-98/4	0·270±0·000	0·270–0·270
HP-98	0·300±0·000	0·300–0·300
HP-109/3	1·030±0·003	0·992–1·068
HP-109/5	1·042±0·006	1·004–1·080
HP-109/1	1·058±0·006	1·020–1·097
HP-109/2	1·073±0·003	1·035–1·112
HP-109/4	1·107±0·042	1·069–1·145
HP-109	1·118±0·014	1·080–1·157

[a]Arranged in order of increasing resistance within each group.
[b]Mean of 6 determinations±standard error.
[c]95% confidence interval for mean; brackets unite groups of stocks or clones with similar drug sensitivity.

study with a *Giardia* isolate from a human case (HP-98), we found that the sensitivity to ornidazole at the beginning of isolation (1984) was nearly the same as that after four years of serial passage (IC_{50}=0·03 and 0·031 respectively).

Statistical analysis

The significance of differences in the mean IC_{50} of metronidazole and ornidazole was determined by Student's *t* test. The Tukay range test was used to compare the differences in sensitivity between *Giardia* stocks and clones against metronidazole and ornidazole.

Results

All 7 *Giardia* stocks were tested against metronidazole and ornidazole. The results are given in Table 1. There were significant differences ($P<0·05$) in the sensitivity to metronidazole and ornidazole of all parent stocks. Both human and 4 of the animal stocks were more sensitive to ornidazole; the activity of this drug was 1·4–24 times higher than that of metronidazole. Only parent stock LSLP-116 was more sensitive to metronidazole.

The stocks could be arranged in 5 groups which differed significantly in sensitivity ($P<0·05$); the most

sensitive group was composed of 3 stocks, two isolated from rodents (GGPRP-114, CP-117) and one from a human case (HP-98). All the other groups consisted of one stock each.

The difference in the drug sensitivity to metronidazole between the parent stocks also showed the existence of 5 parasite groups (Table 1). Most stocks differed significantly ($P<0{\cdot}05$), but 3 stocks (SP-115, SLP-111, GGPRP-114) formed groups characterized by similar levels of drug sensitivity.

The arrangement of *Giardia* stocks according to their increasing drug resistance showed no association between ornidazole sensitivity and that of metronidazole for most stocks.

The results of testing the drug sensitivity of stocks and the clones derived from them are shown in Tables 2 and 3. All individual stocks were composed of parasite populations characterized by significantly ($P<0{\cdot}05$) differing sensitivities to both ornidazole and metronidazole. However, the heterogeneity in drug response of sets of clones was mostly less than that of parent stocks; the number of groups that differed significantly ranged between 3 and 5.

Discussion

Only a few studies *in vitro* have shown that different *Giardia* populations may have different drug susceptibility profiles (McINTYRE *et al.*, 1986; BOREHAM *et al.*, 1984, 1988). Our observations confirmed the general opinion that ornidazole was more active than metronidazole. Only one parent stock from a monkey (lesser slow loris) was more sensitive to metronidazole. Both parasite stocks isolated from rodents showed the highest sensitivity *in vitro* to ornidazole, which exceeded the response of the most sensitive human stock studied.

All seven stocks of *Giardia* studied, irrespective of the host (humans, rodents or monkeys), differed in their sensitivity to both nitroimidazole compounds. The differences were greater with ornidazole than with metronidazole. As in the previous study (GORDTS *et al.*, 1987), we found that the activity of ornidazole and metronidazole was not correlated.

The five *Giardia* stocks investigated were heterogeneous in their sensitivity to both anti-giardial drugs. Only in one set of clones did the parent stock show a middle value of sensitivity to ornidazole; in the remaining sets the parent stocks were either more sensitive or less sensitive than all their clones. According to BOREHAM *et al.* (1987), this may have been due to the fact that not all clones became established in culture. The heterogeneity in drug sensitivity of parent *Giardia* populations may be one of the factors responsible for treatment failures of human giardiasis (BOREHAM *et al.*, 1987). We found a positive correlation between high resistance *in vitro* to both nitroimidazoles of all clones of one parent stock and treatment failure in the patient from whom the parasite stock had been isolated.

The spectra of sensitivity to anti-giardial drugs which we have found in *Giardia* isolated from humans and animals may have some important implications with respect to the suspected zoonotic character of human giardiasis. On the one hand the isolates from rodents had the highest sensitivity to ornidazole, and all the animal-derived stocks were more sensitive to metronidazole than were the human stocks. On the other hand, only the parasites from monkeys and humans had similar spectra of sensitivity to ornidazole. This may indicate that the infection is more commonly transmitted between hosts of the same species (in this case human hosts, in which the *Giardia* are subjected to drug pressure resulting from treatment) than between different host species, that is between animals and humans.

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References

Boreham, P. F. L., Phillips, R. E. & Shepherd, R. W. (1984). The sensitivity of *Giardia intestinalis* to drugs *in vitro*. *Journal of Antimicrobial Chemotherapy*, **14**, 449–461.

Boreham, P. F. L., Phillips, R. E. & Shepherd, R. W. (1987). Heterogenity in the responses of clones of *Giardia intestinalis* to anti-giardial drugs. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 406–407.

Boreham, P. F. L., Phillips, R. E. & Shepherd, R. W. (1988). Altered uptake of metronidazole *in vitro* by stocks of *Giardia intestinalis* with different drug sensitivities. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 104–106.

Gillin, F. D. & Diamond, L. S. (1980). Clonal growth of *Giardia lamblia* trophozoites in a semisolid agarose medium. *Journal of Parasitology*, **66**, 350–352.

Gordts, B., De Jonckheere, J., Kasprzak, W., Majewska, A. C. & Butzler, J.-P. (1987). *In vitro* activity of antiprotozoal drugs against *Giardia intestinalis* of human origin. *Antimicrobial Agents and Chemotherapy*, **31**, 672–673.

Kasprzak, W. & Majewska, A. C. (1985). Further improvements in isolation and axenic growth of *Giardia intestinalis* strains. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 551–557.

Majewska, A. C. & Kasprzak, W. (1990). Axenic *Giardia* isolates from primates and rodents. *Veterinary Parasitology*, **35**, 169–174.

McIntyre, P., Boreham, P. F. L., Phillips, R. E. & Shepherd, R. W. (1986). Chemotherapy in giardiasis—clinical responses and *in vitro* drug sensitivity of human isolates in axenic culture. *Journal of Pediatrics*, **108**, 1005–1010.

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