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Short Report

Induction of metronidazole and furazolidone resistance in *Giardia*

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The nitroimidazoles are accepted as the drugs of choice for treating giardiasis in most countries (DAVIDSON, 1984). Furazolidone, a nitrofuran, which is especially useful in young children, is a recommended drug but is no longer available in Australia. Treatment failures with both classes of drugs are not uncommon and drug resistance has been indicated as one cause. Cross resistance between the 5-nitroimidazoles is a further complication to successful treatment (BOREHAM *et al.*, 1991).

Metronidazole is the most commonly used 5-nitroimidazole with potent antiprotozoal activity as well as activity against many obligate anaerobic organisms. However, resistance to metronidazole has been demonstrated in trichomonads and in *Bacteroides fragilis*, both in natural populations and induced in the laboratory under drug pressure (MEINGASSNER *et al.*, 1978; BRITZ & WILKINSON, 1979). Resistance to furazolidone has also been demonstrated in strains of *Salmonella enteritidis* isolated from poultry following prophylactic administration of nitrofurantoin (RAMPLING *et al.*, 1990).

Previously we reported a laboratory-induced metronidazole-resistant line of *Giardia intestinalis* which grows in low, sub-lethal concentrations of the drug (BOREHAM *et al.*, 1988). We report here on the laboratory induction of both metronidazole and furazolidone resistant lines of *Giardia* which grow in concentrations of drug lethal to the parent stock.

Drug-resistant lines were derived from 4 strains, BAC2, OAS1, WB1B and BRIS/87/HEPU/713, which differed in karyotype and in geographical and host origin (CAPON *et al.*, 1989) (Table 1). *Giardia* trophozoites were grown and harvested as previously described (BOREHAM *et al.*, 1984). Resistance to both metronidazole and furazolidone was induced in all 4 strains by the following methods:

(i) *Intermittent drug exposure.* Trophozoites were subjected to drug for 48 h *in vitro* and allowed to recover before further drug exposure. The initial concentration of drug was the respective ID_{50} value for each strain, i.e. the concentration of drug required to inhibit the uptake of [^{3}H]thymidine by 50% (BOREHAM *et al.*, 1984) (Table 1). At successive treatments the level of drug was increased to allow survival of 10–20% of the trophozoites. After 13 weeks, the exposure time was reduced to 24 h for each treatment and this regimen was continued for a further 17 weeks. Lines derived from intermittent metronidazole and furazolidone exposure are designated M1 and F1 respectively (Table 2).

(ii) *Constant drug exposure.* The 8 drug-resistant lines (4 metronidazole- and 4 furazolidone-resistant lines derived after intermittent drug exposure was completed) were

Table 1. Characteristics of *Giardia* strains used in this study

Strain	Host	Geographical origin	ID_{50} (μM)[a] Metronidazole	Furazolidone
BAC2	Cat	Perth, Western Australia	0·93	0·90
OAS1	Sheep	Calgary, Canada	0·75	0·90
WB1B	Human	Afghanistan	1·15	0·50
BRIS/87/HEPU/713	Human	Brisbane, Queensland Australia	0·85	1·40

[a]The concentrations of metronidazole and furazolidone which inhibit the uptake of [^{3}H]thymidine by 50% (BOREHAM *et al.*, 1984).

Table 2. The final concentration of metronidazole and furazolidone used in the induction of drug-resistant *Giardia* lines in the laboratory

Drug-resistant lines[a]	Final drug concentration (μM)
BAC2-M1	397
BAC2-M2	11·7
OAS1-M1	397
OAS1-M2	11·7
WB1B-M1	397
WB1B-M2	11·7
WB1B-M3	115
BRIS/87/HEPU/713-M1	397
BRIS/87/HEPU/713-M2	11·7
BRIS/87/HEPU/713-M3	85
BAC2-F1	45
BAC2-F2	8
BAC2-F3	34
OAS1-F1	40
OAS1-F2	8
OAS1-F3	56
WB1B-F1	40
BRIS/87/HEPU/713-F1	38
BRIS/87/HEPU/713-F2	8

[a]M1, M2 and M3 designate metronidazole-resistant *Giardia* lines induced by intermittent drug treatment, constant drug treatment and ultraviolet light mutagenesis, respectively. Similarly F1, F2 and F3 designate furazolidone-resistant lines.

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grown continuously in concentrations of drug which supported growth of the parasite. The initial concentrations of drug used were 5 times the ID_{50} level of the parent strain. This level was maintained for 13 weeks and increased finally to 11·7 μM for metronidazole and 8 μM for furazolidone. The lines grown in metronidazole and furazolidone are designated M2 and F2 respectively.

(iii) *Ultraviolet light-induced mutagenesis.* Trophozoites were harvested, resuspended in phosphate-buffered saline, pH 7·2, placed in a 5 cm diameter Petri dish, exposed to ultraviolet (UV) light (254 nm) at an intensity of 3·5 J/m^2 for 10 min and allowed to recover for 48 h. The trophozoites were then exposed to furazolidone at 5 and 10 times the respective ID_{50} level of the parent strain and to metronidazole at 100 times that of the parent strain ID_{50}. Lines which survived metronidazole and furazolidone following UV mutagenesis are designated M3 and F3 respectively.

Ten metronidazole- and 9 furazolidone-resistant lines of *Giardia* were induced using the above methods. Table 2 lists those lines which survived selection pressure and the final concentration of drug used in each case. Intermittent drug treatment allowed the parasite to survive short exposures of the highest final concentration of drug. UV mutagenesis allowed the induction of higher levels of resistance than constant drug exposure.

Two of the 4 strains which were exposed to metronidazole survived to establish lines following UV mutagenesis (WB1B-M3, BRIS/87/HEPU/713-M3). Similarly, only 2 lines were established from the 4 furazolidone-treated strains (OAS1-F3 and BAC2-F3). Lines WB1B-M3 and BRIS/87/HEPU/713-M3 grew in metronidazole at 100 times the ID_{50} of the parent strain. However, this was reduced to about 50 times the ID_{50} (62 and 42 μM metronidazole respectively) because of slow growth. At these concentrations of drug the doubling time was approximately 10-fold slower than the parent strain. These 2 lines have now been growing in this concentration of drug for 2 years. Lines OAS1-F3 and BAC2-F3, which were selected in 10 and 5 times the ID_{50} of the parent strains respectively, are currently growing in 20 times the ID_{50} value of the parent strain. This is greater than the maximum solubility of the drug in aqueous solution. Thus the drug was prepared as a suspension in TYI-S-33 medium and potential bacterial and fungal contamination of the lines was controlled by addition of antibiotics (benzylpenicillin, 200 μg/ml; garamycin 40 μg/ml; ticarcillin, 100 μg/ml; clindamycin, 50 μg/ml and amphotericin, 1 μg/ml).

Giardia grown *in vitro* is normally killed by metronidazole and furazolidone at approximately 1–2 μM for most stocks. We have induced resistance by several mechanisms to both these drugs and have shown that some lines can survive in several hundred times the ID_{50} value for that strain. This is the first report of laboratory-induced resistance to furazolidone in *Giardia*.

It appears that prophylactic use of nitroheterocyclic drugs in animal husbandry can induce high levels of drug resistance (RAMPLING *et al.*, 1990). Considering the global problem with drug resistance in malaria, and reports of resistance in *Entamoeba* and *Trichomonas*, the emergence of drug-resistant strains of *Giardia* in natural populations needs to be carefully monitored.

We have reported that chromosome rearrangements were associated with the induction of metronidazole resistance in strain BRIS/83/HEPU/106 (UPCROFT *et al.*, 1992) and are currently investigating the molecular mechanisms of induced resistance in the lines described in this report.

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