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THE SIGNIFICANCE OF VARIATION IN THE SUSCEPTIBILITY OF *SCHISTOSOMA MANSONI* TO THE ANTISCHISTOSOMAL DRUG OXAMNIQUINE

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Clinical and laboratory evidence is reviewed which shows that there is a great deal of variation in the susceptibility of Schistosoma mansoni *to oxamniquine. This variation occurs both among endemic regions and whithin endemic regions in Brazil and Kenya. It is genetically controlled. It is suggested that the parasite possesses a large capacity for developing resistance to the drug and that resistance will develop where sufficient drug pressure is maintained.*

This is probably the most exciting period in the history of man's search for effective means of controlling schistosomiasis. Three drugs – oxamniquine, praziquantel and metrifonate – that are effective, safe and orally administered are now available. At least one good molluscicide is available and a search for others is in progress. Much progress has been made in recent years in the immunology of schistosomiasis and a vaccine may become available in the foreseeable future (UNDP/World Bank/WHO Special Programme, 1987). With these and other developments the strategy of morbidity control advocated by WHO (1985) seems to be a realistic goal.

The current situation reminds one of the late 1950s and early 1960s when the availability of safe and effective anti-malarial drugs and potent insecticides led the experts to believe that malaria would soon be eradicated from the major endemic areas of the world. But in a relatively short time both the malaria parasites and their mosquito vectors developed resistance to the chemicals used against them and it is now widely accepted eradication is not a goal that can be attained in the foreseeable future (WHO, 1984). Acquired drug resistance is a common occurrence in bacterial infections (Franklin & Snow, 1981), trypanosomiasis (Williamson, 1970), coccidiosis (Ruff & Reid, 1977) and nematode infections of livestock (Pritchard et al., 1980).

There is evidence that *Schistosoma mansoni* has the capacity to develop resistance to oxamniquine. First, it has been shown by Jansma et al. (1977) that resistance to oxamniquine (and to hycanthone) can be induced experimentally. Secondly, there is marked variation in the susceptibility of *S. mansoni* to oxamniquine both between and within endemic areas. The purpose of this paper is to review the evidence for naturally occurring variation in susceptibility. But before I do so it may be useful to state briefly the biological basis of drug resistance.

THE BIOLOGICAL BASIS OF DRUG RESISTANCE

Drug resistance is a natural consequence of a fundamental property of every normal species of living organisms, namely genetic variability. Four features of genetic variation are especially relevant to a consideration of drug resistance. First, within every normal species there is a vast amount of genetic variation. This is expressed phenotypically as variation in physiology, morphology, behaviour, etc. Secondly, genetic variation gives a species the capacity to cope with changes in its environment, enabling it to survive, reproduce and adapt to new circumstances. Thirdly, genetic variation is the raw material upon which natural selection acts and produces new populations and eventually new forms. Finally, genetic variation is the result of random mutations that take place spontaneously. In a large population many of its genes are likely to experience a mutation in each generation.

When an obnoxious substance, such as an antischistosomal agent, is introduced into a population one or more genes may already be present which will enable those individuals thus equipped to survive the harmful effects of the substance and to reproduce. If the gene frequency is high and the selection pressure is maintained the population may, in the absence

of "diluting" factors such as migration and back mutation, quite quickly come to consist mostly of individuals resistant to the substance. If either the gene frequency or the drug pressure is low resistance may be slow in appearing. If there is no gene for resistance when the substance is introduced, there is still a chance that in the course of time the appropriate mutation will occur, especially if the substance is mutagenic, and therefore sooner or later the population will become resistant to the substance.

GEOGRAPHICAL VARIATION IN SUSCEPTIBILITY

In free-living species geographical isolation results in populations that differ significantly among themselves in one or more characteristic. Many animal species consist of more or less distinct geographical races. This is also true of parasitic species. There is both laboratory and clinical evidence for geographical variation in the susceptibility of *S. mansoni* to oxamniquine.

Laboratory Evidence – During the development of oxamniquine Foster and Cheetham (1973) found that an East African isolate was much more tolerant to the drug than a Puerto Rican isolate. The curative dose (ED_{99}) by the oral route was 50% higher and by intramuscular injection 200-250% higher. Foster (1973) confirmed that the East African isolate was more resistant to oxamniquine than the Puerto Rican isolate.

Presumably using a Puerto Rican isolate in *Cercopithecus* monkeys, Foster (1973) found that a minimum oral dose of 100 mg/kg was required before there was any activity against the parasite. This was about three times the dose effective against a South African isolate in *Cercopithecus* monkeys (Fripp, 1973).

Clinical Evidence – In a review of clinical experience with oxamniquine, Foster (1987) notes that the effective dose varies from one geographical region to another. In South America and the Caribbean islands 15-20 mg/kg can usually be expected to give a cure rate of over 80% and a reduction in faecal egg output of more than 90%. In Africa the dosage that will produce comparable results varies from 15 mg/kg in West Africa, through 30 mg/kg in East and Central Africa and 40 mg/kg in Zaire and Ethiopia, to 60 mg/kg in Egypt, Sudan and South Africa.

Kaye (quoted by Foster, 1987) has provided evidence suggesting that this geographical variation in the effective dosage is related to variation in the susceptibility of the parasite to oxaminiquine, not to differences in the pharmacokinetics of the drug in people from different geographical areas. He found that when given the same dose patients from Tanzania, South Africa and Brazil attained similar drug concentrations in their blood. Kaye reported anomalous results for Sudanese patients: they had lower serum drug concentrations than Brazilian patients. However, Daneshmend & Homeida (1987) have found the maximum serum drug concentration in a group of Sudanese patients to be four times that found by Kaye and they suggest that the higher dosage required in the Sudan may be due to a lower susceptibility of the parasites rather than to altered drug pharmacokinetics in the Sudanese.

VARIATION IN SUSCEPTIBILITY WITHIN ENDEMIC REGIONS

Considerable variation in the susceptibility of *S. mansoni* to oxamniquine has been found within endemic areas in Brazil and Kenya. Isolates which had not been exposed to the drug previously have been found to be highly tolerant to oxamniquine.

Brazil – Araujo et al. (1980) compared the responses of seven isolates of *S. mansoni* in mice to oxamniquine – and also to niridazole and hycanthone. They found a great deal of variation among the isolates, all of which were from people infected in the State of Minas Gerais, whether the criterion used was death of worms, oogram changes or worm shift to the liver. Variation occurred whether the dose used was 50 mg/kg or 100 mg/kg. With the latter dose, for example, the percentage of dead worms found in the liver ranged from 1.1 to 67.5 and oogram changes from 10% to 100%. These observations confirmed the results of less extensive studies by Katz et al. (1973) and Dias et al. (1978).

Dias et al. (1982) compared the responses of 4 or 5 generations in mice of two isolates of *S. mansoni* from patients that had been unsuccessfully treated with oxamniquine and one susceptible isolate. A dose of 100 mg/kg produced oogram changes in the susceptible isolates but not in those from uncured patients. They found that all three isolates, as well as other isolates from patients treated with

hycanthone or niridazole but not cured, were completely susceptible to praziquantel.

Meang et al. (1987) studied two Brazilian among other isolates in mice and found that one of them was more than 10 times as resistant to oxamniquine as the other. Some worms from the resistant isolate survived treatment with 1000 mg/kg and their progeny were completely resistant to this dosage.

Kenya – In Kenya we have recently begun work which aims to find out if *S. mansoni* worms naturally tolerant to the oxamniquine dose in clinical use occur in worm populations of endemic areas. The results of two studies, which show that there is considerable variation in the susceptibility of the parasite to oxamniquine, are summarized below.

The first study involved infected children at four primary schools in central Kenya who, as far as we could ascertain, had never been treated previously. Two of the schools are located in a highly endemic area (Mwea rice irrigation scheme) in Kirinyaga district, about 100 kilometers northeast of Nairobi. The other two are located in another highly endemic area in Machakos district about 70 kilometers south east of Nairobi. The two areas are separated from each other by a region of low endemicity and there seems to be little human movement between them.

The children weregiven two doses of 15 mg/kg of body weight, a day apart. Those still passing ova, as detected in a single stool sample by the Kato-Katz technique, a month later were treated again with the same dose. When cure rates and reduction in egg out-put are considered together the second treatment gave a much poorer response than the first, particularly in the Kirinyaga schools (Table I). We believe that this indicates that the retreated group harboured a higher proportion of *S. mansoni* worms tolerant to the dose of oxamniquine used than did the original group. Some isolates from uncured and from untreated patients were passaged into albino mice via *Biomphalaria pfeifferi* and the susceptibility to various doses of oxamniquine was determined (see below).

The study was repeated in the Mwea area. Again there was a much poorer response to the second treatment both in the original two schools and in a new school (Table II).

Table III summarizes the results for isolates from the Mwea irrigation scheme. N2 and N50 were isolated from untreated children. The two children were subsequently treated and had no ova in their faeces 4 weeks after treatment but ova re-appeared 9 weeks after treatment. G43 and G110 were from 2 children who continued to pass ova after two courses of treatment. Differences in susceptibility are evident. While a dose of 100 mg/kg killed 57% of the worms of the N2 and N50 isolates, a dose of 300 mg/kg killed only 48% of the G43 worms and 500 mg/kg reduced the number of the G110 worms by only 72%.

Table IV summarizes the data for 6 isolates from Machakos district. S4, S5, SK and K26 were isolated from untreated children who were subsequently treated and cured. A5, B18 and B51 were isolated from children who were not cured after two courses of treatment. Considerable differences in susceptibility are evident. Whereas a dose of 150 mg/kg killed 88% of the worms of the K26 isolate, it only killed 30% of the B51 worms. While 100 mg/kg eliminated 51% of the S4 worms, 200 mg/kg only eliminated 58% of the B51 worms, and 250 mg/kg only eliminated 64% of the B18 worms.

If the two areas are considered together then differences in the response of isolates from central Kenya are even more striking. Thus while a dose of 150 mg/kg eliminated 88% of the worms of one isolate (K26) it only killed 30% of the worms of another isolate (B51). And whereas a dose of 50 mg/kg killed 36% of the worms of one isolate (N50) it only eliminated 10% of those of another isolate (S4).

The response to the drug in mice reflected the response in children. The isolates that were taken from untreated children who were later cured with 30 mg/kg were more susceptible than those taken from children who were uncured after two courses of treatment.

CONCLUSIONS

From the available information on the response of *S. mansoni* to oxamniquine three conclusions may be drawn.

TABLE I

Response of *Schistosoma mansoni* infection in Kenyan children treated once or twice with Oxamniquine (30 mg/kg)*

District	School	*First treatment* No. treated	Cure rate (%)	Reduction in egg output (%)+	*Second treatment* No. treated	Cure rate (%)	Reduction in egg output (%)+/**
Kirinyaga	Gathigiriri	91	54	99	41	42	0
Kirinyaga	Nguka	95	62	85.5	31	29	69
Machakos	Kaani	97	65	98.5	27	89	48
Machakos	Kyanguli	107	83	99.5	16	75	33

* Based on Coles et al., 1987.
+ Based on geometric mean.
** Calculated from egg counts before and after the second treatment.

TABLE II

Response of *Schistosoma mansoni* infection in Kenyan children at Mwea, Kirinyaga District, treated once or twice with Oxamniquine (30 mg/kg)*

School	*First treatment* No. treated	Cure rate (%)	Reduction in egg output (%)+	*Second treatment* No. treated	Cure rate (%)	Reduction in egg output (%)+/**
Gathigiriri	56	54	98	22	36	82
Nguka	52	56	98	14	21	47
Rurii	61	77	99	16	38	38

* Based on Kinoti et al., 1987.
+ Based on geometric mean.
** Calculated from egg counts before and after the second treatment.

TABLE III

Response of *Schistosoma mansoni* isolates from Kirinyaga district, Kenya to various doses of Oxamniquine in mice

Experimental mice					Control mice	
Dose (mg/kg)	Isolate	No. mice	Mean no. worms recovered*	Reduction in worm nos. (%)	No. Mice	Mean no. worms recovered*
50	N2	7	6.9 ± 1.4	32	18	10.2 ± 4.2
50	N50	9	7.6 ± 5.2	36	24	11.9 ± 5.2
100	N2	8	4.4 ± 2.7	57	18	10.2 ± 4.2
100	N50	10	5.1 ± 4.7	57	24	11.9 ± 5.2
200	N2	9	2.8 ± 1.9	73	18	10.2 ± 4.2
200	N50	10	4.4 ± 2.0	63	24	11.9 ± 5.2
300	G43	9	17.0 ± 7.5	48	4	32.8 ± 7.1
300	G110	3	6.7 ± 1.5	69	3	21.7 ± 9.3
500	G110	5+	6.0 ± 1.5	72	3	21.7 ± 9.3

* ± Standard deviation.
+ 5 out of 10 mice died of acute oxamniquine toxicity.

TABLE IV

Response of *Schistosoma mansoni* isolates from Machakos district, Kenya to various doses of Oxamniquine in mice

Experimental mice					Control mice	
Dose (mg/kg)	Isolate	No. mice	Mean no. worms recovered*	Reduction in worm burden (%)	No. Mice	Mean no. worms recovered*
50	S5	9	14.4 ± 7.5	10	7	15.9 ± 5.4
100	S4	10	8.4 ± 6.1	51	6	17.3 ± 7.1
100	SK	6	13.7 ± 7.2	46	6	25.5 ± 6.4
150	B51	8	9.3 ± 5.2	30	8	13.3 ± 6.3
150	K26	7	4.6 ± 2.3	88	7	36.4 ± 10.5
200	B51	8	9.3 ± 3.9	58	6	22.0 ± 5.4
200	A5	7	7.1 ± 3.5	60	8	17.8 ± 9.0
250	B18	6	8.8 ± 8.5	64	6	24.3 ± 3.0
250	A5	9	8.6 ± 4.2	72	7	30.7 ± 5.6

* ± Standard deviation.

1. As a species, *S. mansoni* possesses an enormous range of susceptibility to the drug. In the mouse the known curative doses (ED90 – ED99) range from 44 mg/kg to 1000 mg/kg of body weight (Foster & Cheetham, 1973; Meang et al., 1987). This variation is continuous, not discontinuous.

2. Variation in susceptibility to the drug is genetically controlled. This is evident from geographical variation, which is clearly demonstrated by the need for different clinical doses for different geographical regions. It is also evident from the fact that an isolate in mice will maintain its level of susceptibility from one generation to another (e.g. Dias et al., 1982; Meang et al., 1987).

3. Although there are differences in susceptibility among geographical regions, the differences are, as is true of other types of geographical variation, only statistical and there is a great deal of variation within geographical regions. This is true at least for the Brazilian and Kenyan worm populations that have been studied.

4. The enormous variation in susceptibility found in the parasite indicates that *S. mansoni* possesses a large capacity for developing resistance to the current therapeutic doses of oxamniquine. It can be expected that when and where it is continuously subjected to high drug pressures the parasite will become drug resistant. It would be wise therefore to prepare for this eventuality, for instance by increasing research on the mode of action of the drug with a view to making modifications when it becomes necessary, establishing the genetics of susceptibility/resistance, and maintaining a flow of new antischistosomal agents.

REFERENCES

ARAUJO, N.; KATZ, N.; DIAS, E. P. & SOUZA, C. P. 1980. Susceptibility to chemotherapeutic agents of strains of *Schistosoma mansoni* isolated from treated and untreated patients. *Am. J. Trop. Med. Hyg., 29:* 890-894.

COLES, G. C.; MUTAHI, W. T.; KINOTI, G. K.; BRUCE, J. T. & KATZ, N., 1987. Tolerance of Kenya *Schistostoma mansoni* to oxamniquine. *Trans. R. Soc. Trop. Med. Hyg., 81:* 782-785.

DANESHMEND, T. K. & HOMEIDA, M. A., 1987. Oxamniquine pharma-cokinetics in hepatosplenic schistosomiasis in the Sudan. *J. Antimicrob. Chemother., 19:* 87-93.

DIAS, L. C. S; PEDRO, R. J.; RIGO, E.; GOTO, M. F. & MAFRA, G. K., 1978. Linhagem humana de *Schistosoma mansoni* resistance equistosomacidas. *Rev. Saúde Pública, 12:* 110.

DIAS, L. C. S.; PEDRO, R. J. & DEBERALDINI, E. R., 1982. Use of praziquantel in patients with schistosomiasis mansoni previously treated with oxamniquine and/or hycanthone: resistance of *Schistosoma mansoni* to schistosomicidal agents. Trans. R. Soc. Trop. Med. Hyg., 76: 652-659.

FOSTER, R., 1973. The preclinical development of oxamniquine. *Rev. Inst. Med. Trop. S. Paulo, 15* (Supl. 1) 1-9.

FOSTER, R., 1987. A review of clinical experience with oxamniquine. *Trans. R. Soc. Trop. Med. Hyg., 81:* 55-59.

FOSTER, R. & CHEETHAM, B. L., 1973. Studies with the schistosomicide oxamniquine (UK-4271) I. Activity in rodents and *in vitro*. *Trans. R. Soc. Trop. Med. Hyg., 67:* 674-684.

FRANKLIN, T. J. & SNOW, G. A., 1981. Biochemistry of antimicrobial action, pp 172-203. Third edition. Chapman & Hall, London, New York.

FRIPP, P. J., 1973. Laboratory trials on oxamniquine (U. K.-4271), a candidate schistosomicide. *J. Trop. Med. Hyg., 76:* 316-320.

JANSMA, W. B.; ROGERS, S. H.; LIU, C. L. & BUEDING, E., 1977. Experimentally produced resistance of *Schistosoma mansoni* to hycanthone. *Am. J. Trop. Med. Hyg., 26:* 926-936.

KATZ, N.; DIAS, E. P.; ARAUJO, N. & DE SOUZA, C. P., 1973. Estudo de uma capa humana de Schistosoma mansoni resistente aos agentes cquistosomicidas. *Rev. Soc. Bras. Med. Trop., 6:* 381-382.

KINOTI, G. K.; MUTAHI, W. T.; COLES, G. C.; BRUCE, J. J. & KATZ, N., 1987. Further observations on the tolerance of Kenyan *Schistosoma mansoni* to oxamniquine and praziquantel (Manuscript submitted for publication).

MEANG, F. S. W.; MARSHALL, I. & HIGGINS, M., 1987. Oxamniquine resistance in *Schistosoma mansoni:* fact or fiction? *Ann. Trop. Med. Parasitol., 81:* 337-339.

PRITCHARD, R. K.; HALL, C. A.; KELLY, J. D.; MARTIN, I. C. A. & DONALD, A. D., 1980. The problem of antihelminthic resistance in nematodes. *Aust. Vet. J., 56:* 239-251.

RUFF, M. D. & REID, W. M., 1977. Avian coccidia. In J. P. KREIER *Parasitic protozoa* Vol. III, pp. 33-69. Academic Press, New York.

UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1987. Prospects for immunological intervention in human schistosomiasis. *Acta Trop., 44* (Supl. 12): 1-120.

WILLIAMSON, J., 1970. Review of chemotherapeutic and chemoprophylactic agents. p. 125-221. In H. W. MULLIGAN, *The African Trypanosomiases*, George Allen and Unwin, London.

WORLD HEALTH ORGANIZATION, 1984. *Advances in malaria chemotherapy: Report of a WHO Scientific Group.* Technical Report Series, No. 711.

WORLD HEALTH ORGANIZATION, 1985. *The control of Schistosomiasis: Report of a WHO Expert Committee.* Technical Report Series No. 728.