ANTI-NEMATODE ACTIVITY OF SIXTEEN COMPOUNDS AGAINST *TRICHINELLA SPIRALIS* IN MICE - A POSSIBLE NEW SCREEN FOR MACROFILARICIDES

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Abstract. There are few small animal models for filariasis, even more so for onchocerciasis. Therefore it is difficult to test under drug screening conditions large numbers of potentially macrofilaricidal compounds. One way around this difficulty is to use mice infected with *Trichinella spiralis* which by reason of anatomical location in the host would show some correlation in antinematode activity between the test and target organisms. This study investigated the activity of 16 compounds against the immature larval stage of *T. spiralis*. All the nine benzimidazole compounds (albendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, 780118, 780120, 790163, and 790392) were active, the most potent being oxfendazole. The benzothiazoles (CGP21306, CGP20376, CGP21833 and CGP24588A) also indicated some anti-nematode activity together with 35vr, an imidazopyridine, but not as marked as the benzimidazole group. However, the organic arsenical compounds (Mel Ga and Mel Ni) showed little activity and this was at a rather highly toxic level. The prospects of using the *Trichinella*-mouse model as a primary screen to test for potential macrofilaricides are discussed.

INTRODUCTION

Few widely applicable and effective drugs are available that act against the adult stages of the filarial parasites responsible for lymphatic filariasis or onchocerciasis. The Macrofil Chemotherapy Project (Macrofil), funded by the WHO Special Program for Research and Training in Tropical Diseases (TDR) and the Onchocerciasis Control Program (OCP) was set up to fill this need for "adulticidal" or "macrofilaricidal" drugs.

Macrofil has suggested the use of mice infected with *T. spiralis,* a nematode whose newborn larvae, like adult filariae, live outside the gastrointestinal tract (in muscle tissue) of their host. The chemotherapeutic response of filarial parasites it is reasoned, is quite similar to that of the *Trichinella* worm even though the phylogenetic relationship is remote for both groups of parasites. This prediction could be supported by the fact that problem of bioavailability of compounds against tissue and lymphatic filarial worms (target organisms) *in vivo* might well resemble those against the encysted muscle stage of *T. spiralis* (test organism). Being sequestered within the host tissue, compounds causing reversible paralysis of worms are unlikely to kill either the muscle larvae of *T. spiralis* or filarial parasites.

The *in vitro* test with the *Trichinella* screen run by Jenkins and Carrington (1981), indicated that there was some correlation between activity of compounds against the test organism and *Brugia pahangi in vivo.* Campbell and Denham (1983) noted that compounds which are active against migrating and encysted tissue phase *Trichinella* larvae generally also showed similar activity against the immature enteral stage worms.

The objective of this experiment was to evaluate the efficacy of the *Trichinella*-mice model as a tool for primary screening of macrofilaricides.

MATERIALS AND METHODS

The strain of *Trichinella spiralis* used in this experiment was the 'London strain' (Nelson *et al,* 1963) maintained by passage through laboratory rats and mice. Female TO mice (Tizer Orginal, Tuck and Company) which usually harbored between 50,000 to 100,000 muscle larvae each (Larsh, 1963) were used as stock.

To circumvent the problem of waiting for the migration and development of larvae in the muscle, the effect of test compounds was determined against the immature enteral stage of *T. spiralis.* Six

to seven weeks old female albino mice of the Tizer Original strain were used in the experiment. Mice were each infected with 250 infective larvae orally. The methods of infecting mice and counting larval forms were described by Denham (1968) and Surin (1984).

The anthelmintics, all of which were known filaricides, were donated by their manufacturers. The compounds and their donors are shown in Table 1. All the compounds were dissolved or suspended in 1% Tween 80 in distilled water and administered orally. Each compound was evaluated in groups of five mice at different dose levels four hours after infection. This is to ensure that treatment was unambigously against immature larval *Trichinella* in the intestine. For each level of compound tested in the group, there were five untreated control mice.

Worms were recovered and counted at autopsy five days post treatment. Activity of each compound was assessed from the number of immature worms killed (*ie* 100% minus percentage worm recovery at a particular dose level = percentage suppresion rate).

RESULTS

The results of the experiments are shown in Tables 2-4. In these tables the mean recoveries of immature larvae from each treated group are given as a percentage of the recoveries in the appropriate control untreated mice. Thus, 100% means no anthelmintic activity. The chemotherapeutic efficacy of the 16 compounds is summarized in Table 5.

Table 1

Compounds used in the experiments and their source. Also shown is their chemical class.

Compounds	Manufacturer	Chemical class
Mel Ga	Dr EAH Friedheim	Arsenical (melaminylthioarsenite)
Mel Ni	Dr EAH Friedheim	Arsenical (melaminylthicarsenite)
35vr	Wellcome Foundation	Imidazopyridine
Mebendazole	Janssen Pharmaceutica	Benzimidazole carbamate
Oxfendazole	Syntex	Benzimidazole carbamate
Flubendazole	Janssen Pharmaceutica	Benzimidazole carbamate
Albendazole	Smith, Kline and French	Benzimidazole carbamate
Oxibendazole	Smith, Kline and French	Benzimidazole carbamate
790163	Janssen Pharmaceutica	Benzimidazole carbamate
790392	Janssen Pharmaceutica	Benzimidazole carbamate
790118	Imperial Chemical Company	Benzimidazole carbamate
780120	Imperial Chemical Company	Benzimidazole carbamate
CGP21306	Ciba Geigy	Benzothiazole isothiocynate
CGP20376	Ciba Geigy	Benzothiazole
CGP21833	Ciba Geigy	Benzothiazole isothiocynate
CGP24588A	Ciba Geigy	Benzothiazole "ester"

DISCUSSION

It is evident from the results that the level of activity is dependent upon the class of compounds being tested against the immature worms, the benzimidazole carbamate group being the most potent and the arsenicals the least effective against *Trichinella.*

Almost all the benzimidazole carbamates titrated were 100% effective at a dose of 12.5 mg/kg (compound 780118 displayed a suppressive rate of 84%). The demonstration of a high potency against immature larvae of *T. spiralis* at a relatively low dose is in concordance with the widely held view that benzimidazoles are very effective against the intestinal stage of immature nematodes (Campbell and Denham, 1983). The most outstanding compound

Table 2

The recovery of immature entral stage larvae of *T. spiralis* from mice treated orally 4 hours *pi* at different levels with compounds compared with recovery from untreated mice.

Compounds	Larval recovery (standard deviation) and % of controls at titrated dose (mg/kg)									
	400	Untreated	200	Untreated	100	Untreated	50	Untreated	25	Untreated
Mel GA	30.2 (16.4) 25%	116.7 (28.0)	99.2 (27.3) 81%	122.5 (30.8)	105.8 (14.9) 91%	116.7 (28.0)	-	-	-	-
Mel Ni	3.6 (3.8) 3%	105.0 (10.1)	56.0 (22.4) 58%	96.5 (37.4)	101.5 (5.8) 100%	101.0 (27.2)	-	-	-	-
35vr	-	-	-	-	0.8 (1.6) 1%	105.0 (10.1)	0	105.0 (10.1)	44.0 (11.9) 46%	96.0 (31.8)
790392	-	-	-	-	-	-	0	95.6 (37.4)	1.6 (1.8) 2%	105 (10.1)
780118	-	-	-	-	-	-	0	96.0 (31.8)	0	95.4 (13.2)
780120	-	-	-	-	-	-	-	-	0	95.2 (15.9)
CGP21306	-	-	26.6 (6.8) 28%	95.6 (15.5)	68.8 (12.9) 72%	95.6 (15.5)	104.4 (19.2) 108%	96.5 (37.4)	-	-
CGP20376	-	-	-	-	8.6 (4.8) 9%	96.4 (15.6)	24.0 (9.5) 25%	95.6 (37.4)	52.8 (11.2) 55%	96.4 (15.6)
CGP21833	-	-	-	-	12.0 (3.2) 12%	104.6 (12.6)	27.5 (8.5) 29%	95.0 (37.4)	-	-
CGP24588A	-	-	0	96.5 (37.4)	7.0 (3.7) 6%	107.2 (10.7)	48.8 (10.1) 47%	103.2 (14.8)	90.4 (10.0) 88%	103.2 (14.8)

- = Not done

Table 3

The recovery of immature enteral stage larvae of *T. spiralis* from mice treated orally 4 hours *pi* at different levels with compounds compared with recovery from untreated mice.

Compounds	Larval recovery (standard deviation) and % of controls at titrated dose (mg/kg)							
	12.5	Untreated	6.3	Untreated	3.2	Untreated	1.6	Untreated
35vr	53.0 (11.2) 43%	122.8 (30.8)	87.4 (7.1) 82%	106.4 (6.8)	-	-	-	-
Mebendazole	0	105.0 (10.1)	0	104.0 (16.2)	0	104.0 (16.2)	20.8 (6.6) 20%	104.0 (16.2)
Oxfendazole	0	97.6 (11.6)	0	97.6 (11.6)	0	97.6 (11.6)	0.8 (0.8) 1%	97.6 (11.6)
Albendazole	0	104.4 (11.7)	7.0 (3.9%)	104.4 (11.7)	34.0 (7.7) 33%	104.4 (11.7)	-	-
Oxibendazole	0	99.2 (12.6)	0	99.2 (12.6)	8.0 (5.1) 8%	99.2 (12.6)	56.0 (6.3) 57%	99.2 (12.6)
970163	0	92.6 (9.7)	1.4 (3.1) 2%	92.6 (9.7)	7.2 (6.1) 8%	92.6 (9.7)	60.0 (16.3) 65%	92.6 (9.7)
790392	0	93.2 (11.3)	4.2 (4.4%) 5%	93.2 (11.3)	15.6 (9.9) 17%	93.2 (11.3)	47.2 (10.1) 51%	93.2 (11.3)
780118	15.0 (5.1) 16%	95.4 (13.2)	45.8 (9.0) 48%	95.4 (13.2)	88.0 (8.9) 92%	95.4 (13.2)	-	-
780120	0	95.2 (15.9)	13.4 (4.8) 14%	95.2 (15.9)	65.0 (12.1) 68%	95.2 (15.9)	-	-
CGP20376	85.0 (32.2) 78%	109.1 (31.7)	112.0 (45.8) 102%	109.1 (31.7)	99.0 (30.0) 91%	109.1 (31.7)	-	-
CGP21833	69.0 (11.8) 64%	109.1 (31.7)	97.5 (24.3) 89%	109.1 (31.7)	-	-	-	-

- = Not done

Table 4

The recovery of immature enteral stage larvae of *T. spiralis* from mice treated orally 4 hours *pi* at different levels with compounds compared with recovery from untreated mice.

Compounds	Larval recovery (standard deviation) and % of controls at titrated dose (mg/kg)									
	10.0	Untreated	5.0	Untreated	2.5	Untreated	1.3	Untreated	0.8	Untreated
Mebendazole	0	105.0 (10.1)	0	96.0 (31.8)	2.8 (5.2) 2%	122.5 (30.8)	-	-	56.6 (12.1) 54%	104.0 (16.2)
Flubendazole	0	96.0 (31.8)	0	93.4 (12.3)	17.4 (8.1) 19%	93.4 (12.3)	32.0 (6.0) 34%	93.4 (12.3)	-	-
Oxfendazole	-	-	-	-	-	-	-	-	35.2 (8.8) 36%	97.6 (11.6)
Albendazole	0	105.0 (10.1)	0	96.0 (31.8)	21.8 (5.6) 18%	122.5 (30.8)	-	-	-	-
780120	0	122.5 (30.8)	-	-	-	-	-	-	-	-

- = Not done

Table 5

Chemotherapeutic efficacy (% reduction rate) of compounds against immature enteral phase of *T. spiralis* in mice.

Compounds	% worm reduction rates at titrated dose (mg/kg)									
	400	200	100	50	25	12.5	6.3	3.2	1.6	0.8
Mel Ga	74	19	9	-	-	-	-	-	-	-
Mel Ni	97	42	0	-	-	-	-	-	-	-
35vr (B2)	-	-	99	100	54	57	18	-	-	-
Mebendazole	-	-	-	-	-	100	100	100	80	46
Oxfendazole	-	-	-	-	-	100	100	100	99	64
Albendazole	-	-	-	-	-	100	93	67	-	-
Oxibendazole	-	-	-	-	-	100	100	92	43	-
790163	-	-	-	-	-	100	98	92	35	-
790392	-	-	-	100	98	100	95	83	49	-
780118	-	-	-	100	100	84	52	8	-	-
780120	-	-	-	-	100	100	86	32	-	-
CGP21306	-	72	28	0	-	-	-	-	-	-
CGP20376	-	-	91	75	45	22	0	9	-	-
CGP21833	-	-	88	71	-	36	11	-	-	-
CGP24588A	-	100	94	53	12	-	-	-	-	-

- = Not done

from this group was oxfendazole which displayed virtually a complete curative rate at a dose of 1.6 mg/kg body weight.

Imidazopyridine demonstrated a moderate activity in suppressing the immature intestinal stage of *T. spiralis* in comparison to the benzimidazoles. 35 vr achieved a complete cure rate at a single dose of 50 mg/kg body weight.

The group of heavy metal compounds which demonstrated relatively little activity at a physiologically acceptable dose were the organic arsenicals of Friedheim. The present findings clearly confirm some reports that arsenical compounds are inactive against *T. spiralis* (Campbell and Blair, 1974). As illustrated in Table 2. Mel Ga and Mel Ni only showed some activity at 400 mg/kg.

The efficacy of the isothiocynates against immature *T. spiralis* were varied, CGP21306 indicated activity at a comparatively high dose of 200

Table 6

Chemotherapeutic efficacy (% reduction rate) of compounds against immature enteral phase of *T. spiralis* in mice.

Compounds	% Worm reduction rates at titrated dose (mg/kg)			
	10	5	2.5	1.3
Mebendazole	100	100	98	-
Flubendazole	100	100	81	66
Albendazole	100	100	-	-
780120	100	-	-	-

- = Not done

mg/kg while CGP21833 registered an equivalent activity at 50 mg/kg body weight.

The benzthiazole (CGP20376) and the "ester" (CGP24588A) showed an almost equivalent activity at 100 mg/kg, eliminating more than 90% of the immature worms in the intestine.

It is conceivable that a drug could remain in mice and start to act on the worms only after some period of time. Therefore this compound is likely to exert its chemotherapeutic effect after maturation of the worms. Nevertheless this test is still regarded as a mean of detecting activity against immature intestinal worm as the first moult occurs around 8 hours pi (Ali Khan, 1966). Since the objective was to demonstrate both efficacy and the comparison of potency, drugs were tested at various doses starting at about the expected minimal curative doses. However, one has to consider the fact that there is a change in efficacy of a given dosage of compound with increasing maturation of the worm indicating that the different response to drug treatment by different intestinal stage may be one of relative sensitivity only. For example, Karunakaran and Denham (1980) have shown, in mice, oxfendazole at 1.6 mg/kg eliminated 99% of 6-hour worms, whereas 800 mg/kg was only 91% effective against 5-day worms. Similarly with oxibendazole, 6.25 mg/kg was 99% effective against 6-hour worms, while 3,200 mg/kg was only 69% effective against 5-day worms.

There is no reason to believe that the pharmacodynamics of these compounds in mice with enteral larval stages is different from those in mice with intestinal adult worms so that when compounds are more active against the larval stage, it is possible that there is some kind of synergism between activity of the anthelmintics and the immune response.

The immunological status of mice enterally infected with *T. spiralis* is complicated. It is established that host immune response to this parasite is protective and stage specific in mice (Denham, 1966, 1968). The immune response of the mice used in this experiment was likely to be minimal as infection occured only four days before treatment.

As a generalization, compounds that affect the immature enteral stages are also active against developing and even encysted parenteral stages. To circumvent the problem of differential susceptibility, compounds could be screened for activity against *Trichinella* by infecting mice with known number of larvae and then treating them for the next 35 days. Activity against any stage of the parasite would then be indicated by a reduction in the number of larvae found in muscle digests on subsequent necropsy. However, this regime would be lengthy and very prodigal of labor and compound. Alternative strategies could be adopted depending on the objective of the screen. If the activity against extraintestinal parasites were of secondary interest a reasonable compromise would be to treat from time of infection until the 5th day and then count muscle larvae on day 28 or day 35. This system would allow detection of lethal effects on all the preadult and adult stages, as well as chemosterilant effects on the adults and is likely to select compounds that affect the extraintestinal stages, since compounds that kill

juvenile intestinal worms are often active against the muscle stages. In this experiment, worms were recovered at necropsy five days after treatment, allowing the possibility that early larvipositing by adults and then migrating into the tissue might be missed. It would be interesting to test the validity of this assumption by doing a muscle larval count 28 days after infection.

If activity against extraintestinal parasite is the primary objective, ideally, test compounds should be given orally starting from day 5 to day 16 to test effects against migrating developing larvae. The host musculature is digested for larval count on day 28.

As a regular primary screen to test macrofilaricides empirically, this model has no previous record of success, either good or bad. Until a large and wide range of compounds have been tested, then only it is possible to check the correlation between anti-*Trichinella* and antifilarial activity.

Arguments for introducing *Trichinella* as a regular marcofilaricide screen include host metabolic function, as do other *in vivo* assays; is efficient as an *in vivo* assay in that it uses a small host and relatively small amount of test compounds; requires no complicated technology; the test organism is a parasitic nematode, with parenteral location and little sensitivity to known drugs. The limitation of a *Trichinella* assay is that the parasite is phylogenetically remote from filariae, therefore metabolism may differ.

This experiment demonstrated that many compounds which were macrofilaricidal also possess anti-*Trichinella* properties, especially against the immature stages. Results obtained from the implanted *Brugia pahangi*-jird screen (Surin and Denham, 1990), when compared with those obtained in the present study showed some similarity in anti-nematocidal activity against both parasites, for most of the compounds.

However, the contrast in the activity of the arsenicals (Mel Ga and Mel Ni) against implanted *B. pahangi* in jirds (Surin and Denham, 1990) and immature *T. spiralis* in mice is evident. Campbell and Blair (1974) also showed equivocal and mostly negative results with arsenicals and other organometallic compounds against *T. spiralis* at tolerated dose. This suggests a difference in the reaction mechanism on the parasites by this group of compound from, for example, the benzimidazole carbamates on the parasites.

Ideally, to make a comparison of the activity of the compounds against *B. pahangi* and *T. spiralis,* they should be tested in the same host species, adminstered by the same route with the same regime of treatment against both worms. In this case, compounds were given to mice orally against immature enteric stage rather than the vascular or muscular-dwelling larval stage which might be regarded as analogous to the lymphatic or cutaneous-dwelling filarial worms. While one might question the benefits of screening organic compounds bearing heavy metals with the *Trichinella* screen, it is evidently valuable for the detection of potential macrofilaricides among the benzimidazoles class and their derivatives.

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