Veterinary Parasitology, 32 (1989) 325–340
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Factors Affecting the Efficacy of Ivermectin against *Heligmosomoides polygyrus (Nematospiroides dubius)* in mice

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(Accepted for publication 3 October 1988)

ABSTRACT

Wahid, F.N., Behnke, J.M. and Conway, D.J., 1989. Factors affecting the efficacy of ivermectin against *Heligmosomoides polygyrus (Nematospiroides dubius)* in mice. *Vet. Parasitol.*, 32: 325–340.

The efficacy of ivermectin against *Heligmosomoides polygyrus* was investigated in 5 mouse strains (CFLP, NIH, $C_{57}Bl_{10}$, BALB/c and CBA) using a variety of dose levels, and subcutaneous and oral administration. *Heligmosomoides polygyrus* were not completely eliminated when 5 mg kg^{-1} of ivermectin was given 6 days after infection, but 10 mg kg^{-1} was totally effective. Significant mouse-strain variation in drug efficacy was detected, NIH mice requiring treatment with higher doses than CFLP mice in order to bring about a comparable level of larvicidal activity. Ivermectin was more effective when given subcutaneously than when given orally, regardless of the dose administered or the strain of mouse tested. The anthelmintic effect of the treatment was more persistent in CFLP mice given 20 mg kg^{-1} subcutaneously than in NIH mice or in mice treated orally, and a 20-day interval between administration and infection was insufficient to prevent inhibition of parasite survival. Ivermectin was shown to be totally effective at 20 mg kg^{-1} given subcutaneously in killing immune arrested larvae of *H. polygyrus*.

INTRODUCTION

The murine trichostrongyle *Heligmosomoides polygyrus (Nematospiroides dubius)* has been used extensively as a laboratory model of chronic gastrointestinal nematode infection because in most available mouse strains primary infections last 8–10 months (Ehrenford, 1954; Williams and Behnke, 1983; Keymer and Hiorns, 1986). The parasite has been exploited by pharmaceutical firms in anthelmintic screens for many years because it is easy and inexpensive to maintain and has a life cycle which is very similar to that of parasites with

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0304-4017/89/$03.50

medical and veterinary importance. The adult stages of *H. polygyrus* can be readily removed from the mouse intestine by a single treatment with pyrantel, an anthelmintic which has been widely employed to abbreviate infections for experimental purposes (Behnke and Wakelin, 1977; Jacobson et al., 1982). The tissue stages of *H. polygyrus* (1–8 days post infection) are hardly affected by pyrantel and the drug cannot be used to terminate infections earlier than 10–14 days post infection (Behnke and Wakelin, 1977; Behnke and Robinson, 1985). Nevertheless, there is evidence that the earlier stages are immunogenic, particularly L_4 and hence there is a requirement for drugs that show high efficacy against the tissue-dwelling larval stages of *H. polygyrus* (Jacobson et al., 1982; Cayzer and Dobson, 1983; Pritchard et al., 1984) in order to allow experimental manipulation during this phase of infection.

Ivermectin, the 22, 23 dihydro derivative of avermectin B1, an important anti-parasitic agent derived from naturally occurring fermentation products, exhibits a broad spectrum of activity at extremely low dosages against a wide variety of nematode and arthropod parasites of livestock (Campbell and Benz, 1984; Campbell, 1985). Ivermectin is also effective against *H. polygyrus* and we have used the drug at a variety of dose levels to abbreviate infections developing through the immunogenic L_4 stage. During the course of these experiments we became aware that ivermectin was not totally effective in eradicating larval parasites at the doses reported previously to be completely larvicidal (5 mg kg^{-1} orally; Sayles and Jacobson, 1983). This prompted a reinvestigation of the drug doses and other variables influencing efficacy against *H. polygyrus* and our findings are presented in this paper.

MATERIALS AND METHODS

Animals

Random-bred male and female CFLP and syngeneic NIH, BALB/c, $C_{57}Bl_{10}$ and CBA mice were bred and maintained under conventional animal-house conditions in the Zoology Department of Nottingham University. The mice were used when about 6–8 weeks old.

Parasite

The strain of *H. polygyrus* used in this work was obtained in 1975 from the Wellcome Research Laboratories (Beckenham, Kent) and has been maintained since, in outbred CFLP mice. The maintenance of the parasite and the methods used for infection and recovery have already been described (Behnke and Wakelin, 1977; Jenkins and Behnke, 1977). In order to assess drug efficacy against arrested larvae, NIH mice were immunized by a divided primary infection schedule with 120 larvae being given on Days 0, 2, 4, 7, 9 and 12. The mice

were then treated with pyrantel (Strongid-P paste, Pfizer) on Days 15, 21, 28 and 35. The challenge infection was administered on Day 56. Further details of this immunization procedure were reported by Behnke and Wakelin (1977).

Faecal egg counts

One gram of faeces was taken from the pooled faeces of all the mice in each group, deposited over the preceding 24 h, and was dispersed in 10 ml of 50% saturated saline. This suspension was washed through a sieve (aperture size, 800 μm) with 35% zinc sulphate solution and the eggs were counted after flotation in standard McMaster counting slides, as described by Gordon and Whitlock (1939). The counts were expressed as the number of eggs g^{-1} of whole faeces.

Anthelmintic treatment

Ivermectin was available as a commercial preparation, Ivomec which contains 1% w/v of the anthelmintic (MSD AGVET). The required concentrations of ivermectin were obtained by appropriate dilution of this preparation with sterile distilled water. The resulting fine suspension was injected in volumes 0.1–0.3 ml within minutes of preparation.

Pyrantel embonate (Strongid-P paste, Pfizer) was administered orally to some mice in Experiment 12 at a dose of 100 mg kg^{-1}, which is known to be adequate for removing all adult worms from the intestinal lumen (Behnke and Wakelin, 1977).

Cortisone

Cortisone acetate (Cortistab, Boots) was given by subcutaneous injection every second day from Day 10 after challenge infection in Experiment 12. The first two injections were at 2.5 mg/mouse and the remainder at 1.25 mg/mouse. All the control and treated mice in Experiment 12 were given oxytetracycline hydrochloride (Terramycin, Pfizer) in their drinking water at a concentration of 3 g l^{-1} (Behnke and Parish, 1979).

Analysis of results

All the results are presented as the mean number of worms recovered (MWR) $\pm$ SEM and the percentage reduction in mean worm recovery was calculated in relation to the combined MWR of non-treated and saline or water-treated control groups within a particular experiment. The data were analysed for significance using a nonparametric Mann–Whitney U-test and a value of $P < 0.05$ was considered to be significant (Siegel, 1956).

RESULTS

Dose response

Several experiments were carried out in which the dose of ivermectin used was varied. Table 1 presents the results of one such experiment in female NIH mice. Ivermectin was administered orally at 7 dose levels, 6 days following infection with 250 larvae.

At 10 and 20 mg kg^{-1}, ivermectin prevented all the parasites from surviving until autopsy. At these dose levels we could not detect any adverse effect of the drug on our mice. Ivermectin, at 5 mg kg^{-1}, did not clear the entire worm burden, although the MWR was reduced by 98.2%. Lower doses were proportionally less effective and at 0.3125 mg kg^{-1} (6.1% reduction) the drug failed to affect parasite survival. This experiment was repeated using the same strain of mice and covering comparable dose levels of ivermectin. The results were almost identical. At 5.63 mg kg^{-1} the MWR on Day 21 was reduced by 98.7%. At 0.35 mg kg^{-1} the worm burden was reduced by only 15.7%.

Comparison of oral and subcutaneous routes of administration of ivermectin

Ivermectin is known to be effective against nematode parasites whether given orally or subcutaneously. In Experiment 2, four dose levels were compared in

TABLE 1

Experiment 1. The effect of varying the dose of ivermectin administered orally

Dose[1]	Group[2]	No. mice	Mean worm recovery ± SEM	Reduction[3] (%)
20.0	A	3	0	100
10.0	B	3	0	100
5.0	C	5	3.8 ± 2.0	98.2
2.5	D	6	45.8 ± 6.3	78.7
1.25	E	6	72.3 ± 15.7	66.4
0.625	F	5	179.2 ± 2.0	16.0
0.3125	G	5	202.4 ± 4.3	6.1
None	H	6	207.5 ± 4.6	
Saline	I	6	222.5 ± 6.3	

[1]Ivermectin was administered orally on Day 6 of infection at the dose shown in mg kg^{-1}.
[2]The mice used were all female NIH and were killed on Day 21 of infection for worm counts.
[3]The percentage reduction in worm recovery was calculated in relation to the mean worm recovery from Groups H and I combined (215.0 ± 4.1). Groups H and I did not differ significantly.
Statistical analysis of results: Groups A, B, C, D, E and F were all significantly different from Groups H and I, combined ($P < 0.001$); Group G was not significantly lower.

TABLE 2

Experiment 2. Comparison of the efficacy of ivermectin after oral or subcutaneous administration on Day 6 post-infection

Dose[1]	Mean worm recovery ± SEM[2] (% reduction)		*P*
	Oral treatment	Subcutaneous treatment	
5.0	45.6 ± 13.3 (77.6)	34.2 ± 11.3 (83.2)	0.155
2.5	69.5 ± 6.6 (65.9)	114.2 ± 9.1 (43.9)	0.04
1.25	110.0 ± 10.5 (46.0)	164.8 ± 5.5 (19.1)	0.02
0.3125	191.8 ± 4.8 (5.8)	198.0 ± 5.5 (2.8)	0.12
Water	204.2 ± 4.4	197.8 ± 2.5	NS
None	209.0 ± 8.1		

[1]The dose of ivermectin is given in g kg^{-1}. All the mice were infected with 250 L_3 of *H. polygyrus*, treated with ivermectin or sterile water on Day 6 and were killed for worm counts on Day 21 post-infection.

[2]All groups comprised 6 female NIH mice. The percentage reduction in MWR was calculated in relation to the MWR from the mice receiving sterile water and no treatment. These groups did not differ significantly from one another. The overall MWR from the combined control groups was 203.7 ± 3.2 ($n = 17$).

groups of 6 NIH mice treated either orally or subcutaneously. Again the animals were infected with 250 larvae and were dosed on Day 6.

The results are presented in Table 2 and it can be seen that no dose was completely effective in eradicating the parasites, although three of the doses reduced the MWR significantly. At intermediate dose levels 1.25–2.5 mg kg^{-1}, the orally dosed mice showed better protection than those treated subcutaneously but at higher doses the reverse was true. Other experiments reported in this paper show greater and more consistent reductions in worm burden following subcutaneous treatment (see Tables 4 and 5).

The efficacy of ivermectin given at different times during infection

It was important to establish whether ivermectin would be equally efficacious when given on different days following infection with *H. polygyrus*. NIH mice were therefore treated orally with ivermectin, on alternate days following the larval inoculum and at 2 dose levels which were calculated to give high but not complete protection. The results of Experiment 3 are presented in Fig. 1. The data show that on Day 6 ivermectin was less effective compared with earlier or later stages of infection. At 5 mg kg^{-1}, 82.4% of the worms were cleared when ivermectin was given on Day 6, compared with 96.8% on Day 2 or 95.1%

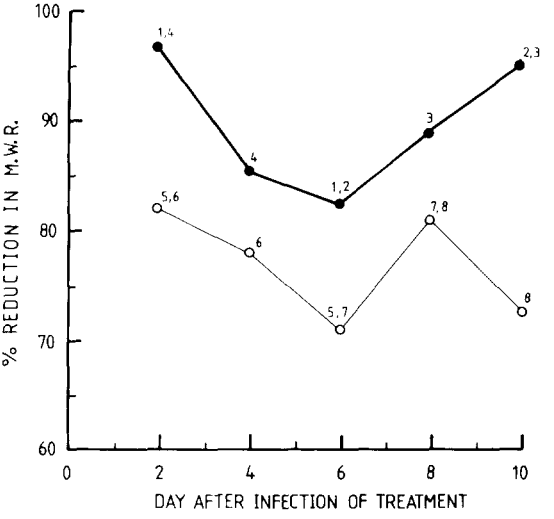

Fig. 1. Experiment 3. The effect of ivermectin given at 2.5 and 5.0 mg kg^{-1} at different times during infection. Groups of 5 female NIH mice were treated with 2.5 (○) or 5.0 (●) mg kg^{-1} ivermectin on the days shown. All the mice were infected with 250 larvae and were killed for worm counts on Day 21. The results are expressed in terms of percentage reduction in worm recovery in relation to the combined worm burden (180.0 ± 2.2, $n=10$) of a non-treated control group (181.2 ± 2.9, $n=5$) and a control group treated with sterile water (178.8 ± 3.4, $n=5$). Statistical analysis of results: the points with the same superscript were compared and have the following P values: 1, $P=0.08$; 2, $P=0.08$; 3, $P=0.56$ (NS); 4, $P=0.08$; 5, $P=0.04$; 6, $P=0.111$ (NS); 7, $P=0.04$; 8, $P=0.155$ (NS).

on Day 10. A dose of 2.5 mg kg^{-1} administered on Day 6, resulted in 70.9% protection whereas treatment on Day 2 gave 82.1%.

This experiment was repeated using CFLP mice (Experiment 4; 5 female mice per group) and again 2 dose levels were employed. Mice were treated orally on Days 1, 3, 5, 7 and 9. Although some variation in MWR was obtained, there was no indication that the efficacy of ivermectin varied with time of injection. Thus, when mice were treated at 5 mg kg^{-1}, the percentage reduction in MWR ranged from 99.2% (Day 7) to 100% (Days 7 and 9). At 2.5 mg kg^{-1} the worm burdens were reduced from 97.0% (Day 3) to 99.0% (Day 7). It was also apparent that both doses were considerably more effective in CFLP mice (Experiment 4) than in NIH mice (Experiment 3).

The effect of host strain and sex

Experiments 3 and 4 suggested that ivermectin was not equally effective in different mouse strains. Male mice of 4 different syngeneic strains were therefore compared and female mice of two of the strains were also investigated.

TABLE 3

Experiment 5. Comparison of the efficacy of oral treatment with ivermectin at 2 dose levels in 4 strains of mice

Group of mice[1]		Mean worm recovery ± SEM[2] (% reduction)		
Strain	Sex	No treatment	2.5 mg kg^{-1} [3]	10 mg kg^{-1}
BALB/c	Male	149.5 ± 5.7	24.0 ± 7.1 (83.9)[4]	0.8 ± 0.4 (99.5)
BALB/c	Female	133.7 ± 3.8	4.0 ± 1.8 (97.0)[4]	0 (100)
$C_{57}Bl_{10}$	Male	142.7 ± 4.2	10.8 ± 2.9 (92.4)[5,6,7]	0 (100)
$C_{57}Bl_{10}$	Female	143.3 ± 5.0	12.1 ± 3.0 (91.5)[5]	0 (100)
NIH	Male	145.3 ± 4.1	38.9 ± 4.8 (73.3)[6,8]	7.6 ± 3.2 (94.8)
CBA	Male	152.2 ± 6.4	33.2 ± 5.6 (78.2)[7,8]	0 (100)

[1]All the mice were infected with 200 *H. polygyrus* L_3 and were killed on Day 21 post-infection.
[2]The percentage reduction in MWR was calculated by comparison with the control (non-treated) group of the same strain and sex.
[3]Ivermectin was administered orally on Day 6 post-infection at the doses shown in mg kg^{-1}.
Statistical analysis of results. The worm burdens in groups with the same superscript no. were compared and have the following P values: 4 and 5, $P = 0.15$; 6 and 7, $P = 0.01$; 8, $P = 0.7$.

Ivermectin was given orally at 2 dose levels on Day 6 following infection with 200 larvae. The results of Experiment 5 are shown in Table 3.

The worms in male NIH mice were least affected by ivermectin at both dose levels. Worms in CBA mice were also poorly affected in comparison with those in $C_{57}Bl_{10}$ mice. The sex of the host had no influence on the efficacy of ivermectin in $C_{57}Bl_{10}$ and BALB/c, although in the latter strain female mice had lower worms burdens than males, but the difference was not statistically significant.

The relationship between efficacy of ivermectin and mouse body weight

It was evident from Experiment 5 that when ivermectin was given at a particular dose level to mice of different strains, the drug's efficacy in removing worms was not identical. The results from Experiments 3 and 4 implied that NIH mice would have to be given a higher dose of ivermectin than CFLP mice in order to achieve the same level of protection. CFLP mice are significantly heavier than NIH mice (40–60 g compared with 20–30 g for NIH). It was possible therefore that the main factor determining drug efficacy was host weight, possibly determined by fat content, as the drug is highly lipophilic. An exper-

iment was devised to explore the relationship between drug efficacy and host weight using NIH and CFLP mice. Male mice of both strains were allocated to 5 groups. Two groups of each strains were given ivermectin at 2.5 mg kg^{-1} orally or subcutaneously and it is clear from Table 4 that this dose level was again considerably more effective in CFLP than in NIH mice irrespective of the route of administration. Two further groups of mice of each strain were given a uniform dose of ivermectin calculated from the mean weight of the heterologous mouse strain to be required in order to give the latter strain a dose of 2.5 mg kg^{-1}. Thus the NIH mice in Groups D and E received almost double the dose given to Groups B and C, whereas the CFLP mice in Groups I and J received almost half the dose given to Groups G and H. Despite these adjustments for body weight of the heterologous strain, ivermectin was still more effective in CFLP than in NIH mice. Even at the overall lower dose given to mice in Groups I and J the percentage protection was greater than in NIH mice in Groups D and E, given the highest dose (97.1 vs. 89.6, 99.8 vs. 96.4). It is also apparent that subcutaneous administration of ivermectin was more effective than the oral route.

TABLE 4

Experiment 6. The relationship between the efficacy of ivermectin and mouse body weight

Group	Strain[1]	Treatment		Mean worm recovery ± SEM	Reduction (%)
		Dose	Route		
A	NIH	None		160.1 ± 5.3	
B	NIH	2.5	Oral	43.2 ± 2.8	73[4,8]
C	NIH	2.5	SC[4]	22.4 ± 2.9	86[6,9]
D	NIH	4.5[2]	Oral	16.6 ± 3.7	89.6[5,10]
E	NIH	4.5[2]	SC	5.7 ± 2.0	96.4[7,11]
F	CFLP	None		155.7 ± 5.1	
G	CFLP	2.5	Oral	3.6 ± 1.5	97.7[4,5]
H	CFLP	2.5	SC	0	100[6,7]
I	CFLP	1.4[3]	Oral	4.8 ± 1.9	97.1[8,10]
J	CFLP	1.4[3]	SC	0.3 ± 0.3	99.8[9,11]

[1]All groups comprised 7 male mice infected with 250 larvae, treated with ivermectin on Day 6 and killed for worm counts on Day 21.

[2]The dose of ivermectin given to Groups D and E was calculated using the mean body weight of mice in Groups G and H and the dose if given to the CFLP mice in Groups G and H would have resulted in a mean dose of 2.5 mg kg^{-1}.

[3]The dose of ivermectin given to Groups I and J was calculated using the mean body weight of mice in Groups B and C and if given to the NIH mice in Groups B and C would have resulted in a mean dose of 2.5 mg kg^{-1}. Statistical analysis of results. Groups with the same superscript were compared and have the following P values: 4, 6, 7, 8 and 9, $P=0.001$; 5, $P=0.02$; 11, $P=0.01$; 10, $P=0.05$.

[4]SC, subcutaneous.

The effect of intensity of infection on the efficacy of ivermectin

Groups of 6 female CFLP mice were infected with either 50, 100 or 250 larvae of *H. polygyrus*. Ivermectin was given at 2.5 mg kg^{-1} or 10 mg kg^{-1}, orally or subcutaneously on Day 6 following infection. In total, the experiment comprised 15 groups of mice. At 10 mg kg^{-1} all the parasites were killed irrespective of the infection intensity or route of drug administration. At 2.5 mg kg^{-1} the percentage reduction in MWR relative to the control untreated group at the same infection intensity ranged from 92.7% (orally treated mice infected with 50 larvae) to 100% (subcutaneously treated mice infected with 50 larvae). No significant relationship was observed between infection intensity and drug efficacy.

Persistence of the anthelmintic effect following treatment with ivermectin

Five experiments were carried out, in which we investigated the duration of the anthelmintic effect following treatment of mice with ivermectin. The drug was administered at 2.5 or 20 mg kg^{-1} either orally or subcutaneously to CFLP and NIH mice, on various days up to 3 weeks before infection. The results are summarised in Table 5. Orally administered ivermectin did not persist long. The only significant difference observed was in Experiment 8 where treatment at 20 mg kg^{-1}, 2 days before infection, gave a significant reduction in parasite burden. Subcutaneously administered ivermectin was more persistent particularly at 20 mg kg^{-1} in CFLP mice, worm burdens being reduced when mice were treated up to 20 days before infection. NIH mice treated subcutaneously with 20 mg kg^{-1} showed residual activity for 7–14 days.

Experiment 11 (Table 6) compared the residual activity of ivermectin, 1 week following treatment at 5 dose levels and by both oral and subcutaneous routes in CFLP mice. Oral treatment at 20 mg kg^{-1} resulted in 22.6% reduction in worm burden, but the lower dose levels did not significantly affect parasite survival. Administration of the drug subcutaneously, however, resulted in marked activity at 10 and 20 mg kg^{-1}, and a weak effect at 5 mg kg^{-1}, but no significant effect at the lowest dose level.

The effect of ivermectin on immune-arrested larvae

When a challenge inoculum is administered to NIH mice which have been made immune by the divided immunizing regime, the majority of larvae fail to develop and become arrested in development. These arrested larvae will begin to develop when mice are treated with the immunosuppressive drug cortisone (Behnke and Parish, 1979). This model can be used to determine whether a particular anthelmintic has efficacy against larval stages of *H. polygyrus*

TABLE 5

Persistence of the anthelmintic effect of ivermectin in mice treated orally or subcutaneously

Experiment	Mouse	No. of mice per group	Ivermectin		Percentage reduction in mean no. worms recovered in mice treated on days:								Mean worm recovery from control group ±SEM
			Dose (mg kg^{-1})	Route of administration	−20,21	−15,14	−11,10	−7	−5	−4	−2	+6	
7	Male CFLP	6	20	Subcutaneous	56.6	69.5*	73.4*		100*			100*	190.6±7.0
9	Female NIH	4	20	Subcutaneous		28.1*	14.4	70*		91.2*	99.7*		96.7±1.9
10	Female NIH	4–5	20	Subcutaneous	11	14.1		78.6*				100*	165.8±7.0
8	Female CFLP	5	20	Oral			3.5	0	13.3		95.1*	100*	168.9±6.7
10	Female NIH	4–5	20	Oral	9.8	11.6		8.7				100*	165.8±7.0
8	Female CFLP	5	2.5	Oral			1.6	0	8.4		0.3	97.0*	168.9±6.7
9	Female NIH	4	2.5	Subcutaneous			0			10.8	21.9		96.7±1.9

Statistical analysis of results: *denotes a significant reduction in relation to the control group.

TABLE 6

Experiment 11. Persistence of ivermectin in CFLP mice given a range of doses 1 week before infection

Dose[1]	Mean worm recovery ± SEM[2] (% reduction)	
	Oral treatment	Subcutaneous treatment
Water	158.0 ± 8.7	
20	122.3 ± 8.9	3.6 ± 3.6[3]
	(22.6)	(97.7)
10	144.0 ± 14.7	63.2 ± 16.1[4]
	(8.0)	(60.0)
5	146.6 ± 5.9	116.8 ± 9.2[5]
	(7.6)	(26.1)
2.5	152.8 ± 7.1	146.4 ± 11.8
	(3.3)	(7.3)
1.25	129.3 ± 8.4	164.8 ± 8.4
	(18.2)	(0)

[1]Ivermectin was administered 7 days prior to infection. The doses given are shown in mg kg^{-1}.
[2]All groups comprised 5 female CFLP mice infected with 250 larvae and killed on Day 21 for worm counts.
Statistical analysis of results. All the groups were compared with the group treated with water and those with a superscript no. have the following P values: 3 and 4, $P=0.02$; 5, $P=0.9$. All the remaining groups had higher values for P.

TABLE 7

Experiment 12. The effect of ivermectin on immune arrested larvae

Group	Treatment				Mean worm recovery ± SEM	
	Immunizing infection[1]	Challenge infection[2]	Anthelmintic[3] Day 10	Cortisone[4] Day 10+	Day 10	Day 26
A	–	+	-	–	70.0 ± 3.6	94.0 ± 4.1
B	–	+	Pyrantel	–	Nd[5]	15.9 ± 1.4
C	–	+	Ivermectin		Nd	0
D	+	+	-	–	2.3 ± 1.5	2.2 ± 0.6
E	+	+	-	+	Nd	42.5 ± 4.8
F	+	+	Pyrantel	+	Nd	43.5 ± 4.5
G	+	+	Ivermectin	+	Nd	0

[1]Groups of female NIH mice were immunized by the divided primary infection.
[2]All the groups were challenged on Day 0 with 100 larvae and were killed for worm counts 10 or 26 days after infection.
[3]Pyrantel was given orally at 100 mg kg^{-1}. Ivermectin was administered subcutaneously at 20 mg kg^{-1}.
[4]Cortisone was administered every 2 days from Day 10 onwards.
[5]Nd = not done.

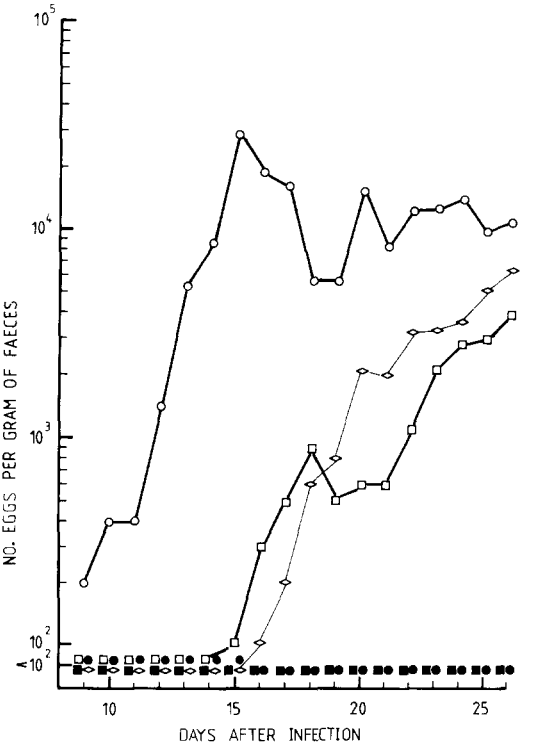

Fig. 2. Experiment 12. The effect of ivermectin on immune arrested larvae of *H. polygyrus*. Faecal egg counts were carried out on the 5 groups in this experiment. See Table 7 for full explanation: Group A, ○; Group D, ■; Group E, □; Group F, ◇; Group G, ●.

arrested in the intestinal tissues. Table 7 presents the results of one such experiment and Fig. 2 summarises the faecal egg counts which were monitored throughout the challenge infection.

As can be seen from Table 7, pyrantel had a significant effect against the adult stages of the parasite when administered 10 days after infection, but some worms survived (Group B), presumably larvae which had not yet returned to the gut lumen. Ivermectin completely cleared all adult worms and any persisting larvae (Group C). Mice which had been immunized were resistant to infection and very few worms were recovered by incubation on Days 10 and 26 (Groups D and A). However, these mice contained larvae which failed to emerge on incubation because when cortisone was administered from Day 10 onwards the worm burden on Day 26 was 42.5 ± 4.8 (Group E). Figure 2 shows that the worms in mice in Group E began to produce eggs within 5 days of cortisone treatment, confirming that development was triggered by cortisone. No eggs were observed in the faeces of the mice from Group D.

When immune-challenged mice were treated with pyrantel on Day 10 and

subsequently with cortisone, adult worms developed. Egg output in Group F, was indistinguishable from that in Group E and comparable worm burdens were recovered on Day 26. Pyrantel was therefore ineffective against the arrested larvae in immune mice 10 days after challenge infection. In contrast, ivermectin was 100% effective. No eggs were observed in the faeces of mice from Group G and no worms were recovered on Day 26 post challenge, even though cortisone treatment was maintained from Day 10 to Day 26.

DISCUSSION

Ivermectin (Ivomec) is an anthelmintic drug, with relatively wide spectrum activity against a variety of parasitic organisms. It is a member of the parent family of chemicals, the avermectins, originally discovered as having anthelmintic properties in a screen involving *H. polygyrus* (Campbell et al., 1984). Despite this, information on the effects of ivermectin on *H. polygyrus* in vivo, is not readily available to support the design of experiments exploiting the drug's potent larvicidal effects, in manipulating the course of infection for immunological analyses of the host–parasite relationship. The study reported in this paper was necessary to provide us with data on optimal doses of ivermectin. The adult stages of *H. polygyrus* are immunosuppressive in the mouse (Behnke, 1987) whilst the larval stages provide the antigenic stimulus, which in responder strains results in the development of acquired immunity (Jacobson et al., 1982; Behnke and Robinson, 1985). The larvae of *H. polygyrus* develop for 8–10 days in the muscularis externa and undergo a moult during this period. In order to identify the larval stages which provide the immunogenic signal in mice it was necessary to be confident that the anthelmintic drug used to abbreviate primary infections was 100% effective; even a single worm escaping the effects of treatment would complicate the interpretation of such experiments. Our results are in broad agreement with those published earlier by Sayles and Jacobson (1983) but differ significantly from those obtained by Rajasekariah et al. (1986).

It is apparent from the results we have presented, that the dose of ivermectin reported by Sayles and Jacobson (1983) to be totally effective in killing L_4 stages of *H. polygyrus*, 6 days following infection, was not completely effective in our system. Although 5 mg kg^{-1} gave over 95% reduction in parasite burdens in CFLP mice, NIH mice, treated at this dose level by subcutaneous injection, were less effectively cleared of worms (range, 82.4% reduction in Experiment 3 to 98.2% in Experiment 1). A dose of 5 mg kg^{-1}, considered by Sayles and Jacobson (1983) to be totally effective, was therefore an inappropriate dose to use in our system for achieving a complete clearance of all parasites. The lowest dose at which we could be confident that the entire worm burden would be eradicated was 10 mg kg^{-1} but a dose of 20 mg kg^{-1} would guarantee total

protection. At this dose we could not detect any harmful side effects on our mice.

Overall our dose response data appear to show higher values necessary in order to achieve the drug efficacies reported by Sayles and Jacobson (1983), but these differences are marginal in comparison to the results reported by Rajasekariah et al. (1986). The latter authors found that 0.3 mg kg^{-1} was totally effective; over an order of magnitude difference in dose level. Rajasekariah et al. (1986) used MAG mice in their study and it is possible that host strain differences influenced drug efficacy. Indeed we found that there were marked differences between mouse strains but we consider that the magnitude of the difference between our two sets of results to be too large to be explained solely in this way.

In comparison with the optimal doses of ivermectin used in treating ruminants, dogs and man (0.1–0.3 mg kg^{-1}; see reviews by Campbell and Benz, 1984; Campbell, 1985), the doses required to ensure total eradication of *H. polygyrus* from mice were significantly higher. This may reflect relatively greater resistance to ivermectin by *H. polygyrus*, a possibility which is supported by the observation that in vitro cultured L_4 stages of *H. polygyrus* responded to treatment with ivermectin in a distinctive way (Jenkins and Ibarra, 1984). The worms were not killed by relatively high doses of the drug although paralysis was induced at lower concentrations. It is also conceivable that physiological characteristics of the mouse necessitated higher dosage regimes in order to achieve toxic levels in the intestinal environment. In support, Ostlind et al. (1985) found that 2 mg kg^{-1} of ivermectin were required to ensure total clearance of *Syphacia obvelata* from mice.

The efficacy of ivermectin against *H. polygyrus* was markedly affected by the mouse strain. NIH mice required a significantly higher dose than comparably infected CFLP mice to achieve total clearance of worms and this difference was not solely explicable in terms of the total dose administered. CFLP mice, at almost twice the weight of NIH mice, received a higher total dose, based on a per kg body weight calculation. To compensate for this we gave NIH mice an identical total dose to that used in treating CFLP mice, but *H. polygyrus* were still not cleared as effectively as in the latter strain. Furthermore, we demonstrated that the anthelmintic effect of ivermectin was more persistent in CFLP than in NIH mice, especially when administered subcutaneously. This is consistent with observations made by McKellar and Marriner (1987) on trichostrongyle infections in sheep and by Barth (1983) in cattle. The explanation for the persistence of ivermectin after subcutaneous administration may be slow drug release from the site of injection (Lo et al., 1985) leading eventually to higher and long-lasting plasma levels (Campbell, 1985; McKellar and Marriner, 1987). In our experiments, mice treated orally at 20 mg kg^{-1} did not show a residual effect for longer than 2–4 days. In contrast CFLP mice given

this dose subcutaneously, 20 days prior to infection developed lower worm burdens than controls. Moreover, this persistent effect was more marked in CFLP than in NIH mice, indicating again a degree of strain variation. These results have important implications for the design of experiments involving the administration of challenge infections after drug-abbreviated primary exposure. Oral dosing will not entail a long interval between drug administration and challenge, but subcutaneous application of ivermectin will require an interval of at least 3, and possibly 4–5, weeks to ensure that residual activity of the drug is totally abolished. The situation is further compounded by strain variation in drug efficacy and persistence and these aspects would have to be evaluated for each strain employed.

The final aspect of ivermectin efficacy which we investigated was the drug's activity against arrested larvae. Experiment 12 exploited a system described earlier for inducing immune arrested development of *H. polygyrus* in mice (Behnke and Parish, 1979). At 20 mg kg^{-1} ivermectin was totally effective in killing all arrested worms, whereas pyrantel failed to affect such larvae. This result is consistent with studies on arrested trichostrongyles in sheep (McKellar and Marriner, 1987), pigs (Murrell, 1981) and cattle (Barth and Preston, 1987).

In conclusion, our study was initiated because of the paucity of information on the optimum use of ivermectin in terminating larval infections with *H. polygyrus*. This report has provided pertinent information and moreover has identified aspects of the drug's effect which warrant further investigation. Our observations on mouse strain variation in efficacy and persistence have implications for the use of ivermectin in different breeds of ruminants in agriculture.

ACKNOWLEDGEMENTS

We would like to thank Professors D. Wakelin and P.N.R. Usherwood for the provision of facilities for this study in the Zoology Department at Nottingham University. We are grateful to W.G. Ryan of MSD Agvet, Hertfordshire, Gt. Britain, for the provision of Ivomec, and to K. Cosgrove for supervision over the maintenance of our experimental animals. J.M.B. would like to acknowledge support from the M.R.C. through project grant G8328675/T, and F.N.W. would like to acknowledge support from the Iraqi government through the provision of a postgraduate studentship.

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