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Trichuris muris: The Anthelmintic Effect of Mebendazole in Mice is Dependent upon the Pre-treatment with Steroids

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Abstract

During an investigation to assess the effectiveness of anthelmintic drugs against *Trichuris muris (T. muris)*, rodent whipworm, we observed that the susceptibility of mice to *T. muris*, in both resistant and susceptible stains, was highly dependent on the type of steroids used to establish murine trichuriasis. Prednisolone butylacetate (PB) was more effective than hydrocortisone acetate (HA) for establishing murine trichuriasis. In addition, most of the adult worms were expelled from the HA-treated resistant strain of mice (BALB/c), but not from the susceptible (ICR strain) or athymic nude mice. The efficacy of mebendazole against *T. muris* was apparently depressed in PB-treated mice. These results indicate that efficacy of anthelmintics on murine trichuriasis are dependent upon the type of steroids that is required to establish *T. muris* infection, and suggest that murine trichuriasis should be used with caution for the assessment of anthelmintics as an animal model of human trichuriasis.

Key words: *Trichuris muris*; trichuriasis; anthelmintics; mebendazole; steroid therapy.

Introduction

Mebendazole is a broad spectrum anthelmintic, especially for soil transmitted helminthiasis, such as *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*. Several anthelmintics are available for ascariasis or hookworm infection, but few are effective against trichuriasis, except mebendazole. Mebendazole is more expensive than other anthelmintics, such as pyrantel pamoate, and its efficacy is unsatisfactory. As such, a drug that is inexpensive and more effective against *Trichuris* infection is desired. In mice, it is known that steroid treatment is required to establish *T. muris* infection (Ito and Hosaka, 1972; Kagei and Kihara, 1973). In the present study, we observed that the efficacy of mebendazole in treating murine trichuriasis is remarkably reduced by pre-treatment with prednisolone butylacetate (PB), but only slightly reduced with hydrocortisone acetate (HA).

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Materials and Methods

Animals

We used three different mouse strains: BALB/c (resistant strain), ICR (susceptible strain), and congenitally athymic KSN nude mice. The mice (6 weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and fed at the Department of Parasitology, School of Medicine, Kanazawa University according to the guidelines of the Experimental Animal Center of Kanazawa University. KSN nude mice were obtained from Drs. O. Nikaido

and Y. Ishigaki (Faculty of Pharmaceutical Science, Kanazawa University).

Infection and treatment

T. muris eggs were obtained from Dr. Y. Ito, Department of Parasitology, School of Medicine, Kitasato University, Japan and Dr. M. Niimura, Department of Parasitology, School of Medicine, Chiba University, Japan. The eggs were kept in 4°C until use.

To determine the intensity of the infection, a total of 134 mice were inoculated with 100 mature eggs of *T. muris*, using a gastric tube on day 0. On days 0, 1, 9 and 10, HA (Fuji Pharmaceutical Co. Ltd., Tokyo, 2.5 mg/head, subcutaneously) or PB (Banyu Pharmaceutical Co. Ltd., 2.0 mg/head, intramuscularly) was injected into each mouse, except for the KSN nude mice (Ito, 1991). Each mouse was then caged separately throughout the experiment.

On the 40th day of infection, the mice were examined for *Trichuris* eggs in the feces. When the eggs were found, the number of eggs per gram (EPG) and the number of adult worms in the feces were counted for an additional 14 days. In the same experiment, we observed the natural expulsion of the worms from BALB/c and ICR mice for 9 weeks after the eggs were appeared in feces. After the experiment, mice were sacrificed, then adult *T. muris* worms were recovered from the coecum and colon to evaluate the rates and intensities of infection.

To determine the efficacy of mebendazole against *Trichuris* infection, 33 *Trichuris*-infected mice were each given 2 mg of mebendazole for 3 consecutive days at least 50 days after infection. The number of adult worms in the feces was then counted everyday for 2 weeks. Ivermectin (0.3 mg/head) was administered to 9 mice in the same way. At the end of the experiment all the mice were sacrificed, and the retained adult worms in the digestive canal were counted.

Results

We observed that *Trichuris* infection in mice was dependent on the type of steroid administered (Table 1). The infection rates of *T. muris* in BALB/c and ICR mice, both treated with PB, were almost the same as in athymic nude mice that were not treated with a steroid. The infection rate of HA-treated BALB/c mice, however, was lower than that of HA-treated ICR mice. The average percent intensity of the infection, that is, the mean number of worms recovered from mice, in both strains of HA-treated mice was less than half that in PB-treated mice. These results suggest that the infection is highly dependent upon the type of steroid administered in the resistant strain of mice, and that PB is more effective for establishing the infection than is HA.

The number of worms naturally expelled in BALB/c mice within 2 weeks was much higher than that in ICR mice. In addition, the number of worms was extremely high in HA-treated BALB/c mice compared to that in ICR mice with either HA or PB treatment. Furthermore, we found that in HA-treated BALB/c mice, most of the worms were expelled within the first week of observation. In PB-treated mice, however, the expulsion of adult worms was more gradual than that in HA-treated mice (Table 2). In addition, adult worms were expelled slowly over 9 weeks in both mouse strains. In contrast, HA- or PB-treated ICR mice expelled very few worms within 2 weeks (Data not shown). No worms were found in the feces of KSN nude mice during the observation period (16 wk post-infection). These results indicate that although the mice are treated with steroids, natural expulsion of the adult worms must occur, and that the intensity and duration of the expulsion depend on the type of steroid used. Therefore, the efficacy of anthelmintics can be estimated in ICR and KSN nude mice when *T. muris* worms appear in feces within 2 weeks after administration of the drug; however, care must be taken when evaluating drug efficacy in BALB/c mice.

We then evaluated the anthelmintic effects of mebendazole and ivermectin, both known anthelmintics for *Trichuris* infection, on murine trichuriasis that had been established by steroid treatment. PB treatment significantly reduced the anthelmintic effect of mebendazole in both mouse strains. The cure rate was 2% in BALB/c mice and 6% in ICR mice. In ICR mice, HA treatment increased the anthelmintic effect of mebendazole two-fold, as compared to the PB treatment. The effect was extremely low, however, in comparison with ivermectin. The administration of HA seemed to

Table 1 Infectivity and anthelmintic effects of mebendazole or ivermectin on mice treated with steroids

	BALB/c (resistance)		ICR (susceptible)		KSN nude
	Hydrocortisone	Prednisolone	Hydrocortisone	Prednisolone	None
	2.5 mg × 4 sc*	2.0 mg × 4 im†	2.5 mg × 4 sc	2.0 mg × 4 im	None
Infection rate‡ (%)	50 (44)§	100 (12)	96.5 (29)	97.6 (44)	100 (5)
Ave. % intensity‖	10.4	24.9	3.9	24.3	21.5
% of worms expelled naturally¶	81.7 (2)	30.1±34.7 (11)	2.2±6.7 (9)	1.0±2.8 (9)	0 (2)
Mebendazole	89±28.3** (7)	2 (2)	26±14.7 (5)	6 (2)	100 (2)
Ivermectin	89 (1)	100 (2)	80 (3)	53 (2)	87 (1)

*sc: subcutaneously injection.

†im: intramusculary injection.

‡Infection rate (%) = $\frac{\text{No. of mice positive for eggs}}{\text{No. of mice inoculated}} \times 100$.

§Parenthesis indicates the number of mice examined.

‖% intensity = $\frac{\text{No. of adult worms recovered per mouse}}{\text{No. of eggs administered in mouse}} \times 100$.

¶ $\frac{\text{No. of naturally expelled worms within 2 wks after establishment of infection}}{\text{No. of naturally expelled worms within 2 wks after establishment of infection + remained worms in caecum}} \times 100 \pm SD$.

**Worm reduction rate (%) = $\frac{\text{No. of adult worms expelled within 2 wks after treatment}}{\text{No. of adult worms expelled within 2 wks after treatment + remained worms in caecum}} \times 100 \pm SD$.

Table 2 Natural expulsion of adults worms from BALB/c mice treated with prednisolone butylacetate (PB) or hydrocortisone acetate (HA)

BALB/c mice		EPG (×1,000) at 40 days of infection	No. of adult works appeared in feces						Adult worms remained in intestine
			1st wk*	2nd wk	3rd wk	4th wk	5th wk	6th wk	
PB-treated	1	3	0	1	0	1	2	3	0
	2	10	2	1	1	0	0		4
	3	16	0	0	3	1	0		0
	4	17	0	0	9	0	1		5
	5	24	0	0	0	5	0		1
	6	27	4	5	0	1	0		0
	7	27	2	10	1	0	0		0
HA-treated	1	1	4	0	0	0	1		0
	2	72	10	0	1	ND	ND		1

ND: not done.

*Worms in feces were counted from Day 41 to Day 47 of infection.

have little influence on the efficacy of mebendazole in BALB/c mice, as the number of expelled worms was approximately equal to that of naturally expelled worms. Therefore, the efficacy of mebendazole against *T. muris* in HA-treated BALB/c mice is not clear. Whereas the infected ICR mice treated

with HA failed to respond to mebendazole, ivermectin was effective in both strains of mice treated with any of the steroids we employed.

Discussion

It has been demonstrated that BALB/c mice are difficult to infect with *T. muris* without steroid treatment (Ito and Hosaka, 1972; Kagei and Kihara, 1973; Rajasekariah *et al.*, 1991). Susceptible mice, such as ICR mice, however, can be successfully infected, although the infection rate is low (Hosaka and Ito, 1973). In the present study, the infection rate of BALB/c mice was clearly lower than that of ICR mice. These findings are consistent with an earlier report suggesting that *Trichuris* infection in mice is genetically restricted (Else and Grencis, 1991). Because the cellular immune response is important in the establishment of murine trichuriasis, steroid treatment may also impair the cellular immune response in mice. We observed that more worms were retained in PB-treated than in HA-treated BALB/c mice. It is possible that since PB has a longer-lasting and stronger action than HA, its immuno-suppressive effect is prolonged in the PB-treated host. This may explain higher infection rate and greater intensity of infection in mice treated with PB than in mice treated with HA.

The anthelmintic effect of mebendazole is apparently reduced in resistant (BALB/c) mice, except HA-treated BALB/c mice, and also in susceptible (ICR) mice in an immuno-suppressed condition. Furthermore, the number of naturally expelled worms and of mebendazole induced worms in HA-treated-BALB/c mice was almost the same. These results suggest that *Trichuris*-infected BALB/c mice, established by HA treatment, are not suitable for the evaluation of drug therapy.

We suggest that the anthelmintic effect of mebendazole is affected by the host's immune status, because athymic nude mice respond well to mebendazole. Even with the administration of ivermectin, the anthelmintic effect was decreased in PB-treated mice. These results indicated that PB pre-treatment is much preferable to HA pre-treatment for the establishment of murine trichuriasis and for anthelmintic effects on mebendazole or ivermectin.

Immunosuppression leads to the poor efficacy of treatment in several parasitic infection such as schistosomiasis (Doenhoff and Bain, 1978), murine malaria (Lwin *et al.*, 1987). In nematode infection, Dwork *et al.* (1975) reported that, in strongyloidiasis, the immuno-suppressive patients responded poorly to anthelmintic therapy as compared to the immunologically-stable patients. Additionally, Sato *et al.* (1992) observed that concurrent HTLV-1 infection markedly reduced the chemotherapic effect of strongyloidiasis patients. In the present study, we observed immunosuppressive treatment resulted in the reduction of anthelmintic effects of mebendazole in murine trichuriasis. Although the mechanisms are unknown, results of the present study suggest that mebendazole may be ineffective in the expulsion of *Trichuris* worms, when administered to an immuno-suppressed patient simultaneously being treated with prednisolone.

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