EFFECT OF ALBENDAZOLE AND MEBENDAZOLE ON SOIL-TRANSMITTED HELMINTH EGGS

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Abstract. Primary school children from Nakhon Si Thammarat Province, Thailand, on endemic area of soil-transmitted helminths, were selected for study. The infected children were divided into two groups and pair-matched according to intensity of infections : group I were given albendazole (400mg) single dose and group II were given mebendazole (100mg) twice daily for 3 days. On the day following treatment, the number of *Trichuris* eggs in the stool markedly increased and the egg shape was also altered. These phenomena did not occur in *Ascaris* infections since 100% cure rate were obtained using both drugs. Incomplete ovicidal effect of the drugs to *Trichuris* and *Ascaris* eggs were demonstrated, embryos were observed to develop within the treated eggs and they hatched after feeding them to experimental animals. In hookworm infection, albendazole stimulated the females to release more eggs after medication, but both drugs showed complete ovicidal effect upon examining the eggs from the second bowel movement.

INTRODUCTION

The prevalence of soil-transmitted helminths in endemic areas in Thailand was reported to be 87% (Muennoo *et al*, 1992) even though a control program of mass treatment with mebendazole (100 mg) twice daily for 3 consecutive days has been conducted twice yearly for the last 15 years (Jongsuksantigul *et al*, 1995). In 1994, treatment with a single dose of albendazole (400 mg) was started in the north and northeastern part of Thailand, although after 1 year the prevalence of hookworm has not differed from the begining of the study (Jongsuksantigul *et al*, 1995). Albendazole and mebendazole were reported to be highly effective against nematodes and apparently caused no severe complications (Chavarria *et al*, 1973). Moreover, both drugs were shown to have ovicidal effects against soil-transmitted helminths (Maisonneuve *et al*, 1985; Wagner and Chavarria, 1974). Albendazole acts by inhibiting glucose metabolism in the worms, causing them to become weak and dislodged, and eventually expelled from the host's body (Cline *et al*, 1984; Kraivichian *et al*, 1992). It also inhibited the hatching of eggs (Maisonneuve *et al*, 1985). Similarly, mebendazole acts by blocking glucose uptake by the nematodes (Brugmans *et al*, 1971) and ceasing the development of the larvae (Wagner and Chavarria, 1974).

The objective of the present study was to observe the effect of the two drugs on the development of eggs recovered from stools after treatment, and the viability of the infective stage was also examined. This project was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand.

MATERIALS AND METHODS

Stool examination was performed on specimens from children at primary schools in Muang District, Nakhon Si Thammarat Province, southern Thailand by the modified thick smeared method (Kato-Katz). Twenty-six children whose fecal samples exhibited *Ascaris* and *Trichuris* eggs and 24 children with hookworm infection were selected for this study with the consent of the teachers and parents, and were divided into two groups having similar egg counts. They received the recommended dose of albendazole and mebendazole for intestinal nematodes as follows : Group I, single dose of 400 mg of albendazole; Group II, mebendazole 100 mg twice daily for 3 consecutive days. All the children were requested to submit their "whole day" fecal materials when they came for medication, and continued for seven (*Ascaris* and *Trichuris*) or ten (hookworm) consecutive days after they have taken the first dose of allocated anthelmintic drugs.

Each stool sample in the *Ascaris* and *Trichuris* study was then processed using the brine floatation method for separating eggs from fecal materials. It

was also modified to quantitatively determine the number of eggs per 5 grams of feces. After the eggs floated they were washed 5 times with tap water by simple sedimentation method. The last sediment was collected by centrifugation. Finally, they were kept in a small flat-bottomed wide-mouthed plastic jar (5 cm in diameter) with filtered water. The jars were kept at room temperature (25-32°C), manual aeration was carried out with pipette several times a day. One hundred eggs were then observed microscopically every other day for their development and survival. The fully developed eggs were fed to mice, rats and kittens. Fecal samples of these experimental animals were examined 12, 24, and 36 hours after feeding.

Fecal samples of hookworm cases were examined for the presence of eggs by modified thick smeared method and for the development of larvae by polyethylene tube cultivation method. Examination for larvae in culture tubes was done on days 7, 9 and 10. The number of larvae on the particular day were recorded.

RESULTS

Ascaris lumbricoides and *Trichuris trichiura*

All *Ascaris* eggs were in normal form after treatment with albendazole and mebendazole.

Some *Trichuris* eggs were morphologically altered as shown by distortion of the eggshells or abnormal size or both, immediately after drug administration. They were grouped as "typical" and "atypical" eggs. Typical eggs measure an average of 52 × 22.5 μm and is barrel-shaped with mucoid plug prominences at each end. Atypical forms (1) appear as "typical" but smaller (45.9 × 25.5 μm) or larger (81.6 × 25.5 μm) or, (2) have eggshells that were deformed and without or with 1, 2 or 3 plugs, or (3) have a membrane like structure found on the eggshells of a few eggs. In both groups, the number of atypical eggs increased rapidly from the second day and was still present in some cases on the last day (D7) of observation (Table 1).

The count of *Trichuris* eggs on the first day after medication was greater than at the beginning of the study, and decreased rapidly on the second day and gradually dropped on day 3 and day 4. Egg counts on day 5 were much lower than those obtained on the first 4 days (Table 2). On the seventh day, the average egg counts from the albendazole-treated group dropped to 55.3% and from the mebendazole-treated group dropped to 11.5% of the pre-treatment level.

Eggs of *Ascaris* and *Trichuris* before and after treatment were collected and cultured. Development from the unsegmented stage to the larval form

Table 1

Percentage of atypical eggs of *Trichuris* in feces before treatment (D0) and 7 days after treatment (D1-D7).

Drugs	Percent of atypical eggs							
	D0	D1	D2	D3	D4	D5	D6	D7
Albendazole	0	0.2	26.3	51.7	37.1	55.9	32.7	7.1
Mebendazole	0	0.3	3.5	34.7	31.5	64.7	91.3	62.5

Table 2

Egg count before treatment (D0) and 7 days after treatment (D1-D7) in *Trichuris* infection determined by brine floatation method.

Drugs	Average egg count/5 g feces							
	D0	D1	D2	D3	D4	D5	D6	D7
Albendazole	165	556	150	149	118	73	30	91
Mebendazole	156	264	184	208	135	51	16	18

was not retarded by the drugs. After treatment, *Trichuris* eggs developed to the larval stage in 32 - 33 days. *Ascaris* eggs were embryonated on day 32 and day 38 in mebendazole- and albendazole-treated groups, respectively (Table 3).

Table 3

Development of *Ascaris* and *Trichuris* eggs in culture after treatment (U-untreated, A-albendazole, M-mebendazole).

Parasites	Days before 1st embryonated egg appeared		
	U	A	M
Ascaris	37	38	32
Trichuris	43	32	33

Dead eggs have also been observed after cultivation, and counts of viable eggs per 100 eggs was performed. In the albendazole-treated group, 33.9 - 47.4% of *Trichuris* eggs had degenerated (Table 4).

Embryonated eggs of *Trichuris* needed 9 - 12 days to develop into the non-mobile larva or the infective stage, and *Ascaris* eggs required 14 - 15 days to reach the same stage. The infectivity of these *Ascaris* eggs was checked by feeding them to mice, white rats and kittens, and a few larvae were found in the intestine of mice and rats, and in the feces of kittens. A few empty eggshells of *Trichuris* were also found in the feces of the infected kittens (Table 5). Three months later, examination of the infected kittens' stools did not reveal any *Trichuris trichiura* eggs.

Table 4

Percentage of viable eggs in cultures of *Ascaris lumbricoides* and *Trichuris trichiura* collected after treatment (100 eggs/observation).

Parasites	Cultivation (days)	Untreated	Albendazole	Mebendazole
Trichuris trichiura	10	100	55.6	100
	20	99.2	66.1	100
	30	99.1	52.6	100
	37	100	65.4	99.7
Ascaris lumbricoides	10	99.7	96.5	100
	20	100	97.3	100
	30	100	100	100

Table 5

Percentage of *Ascaris* larvae and *Trichuris* eggs recovered from infected animals (U-untreated, A-albendazole, M-mebendazole).

Laboratory animals	Cultivation (days)	*Ascaris* larvae			*Trichuris* eggs		
		U	A	M	U	A	M
Mice	45	2.4	1.0	1.8	[a]	[a]	[a]
	100	1.5	2.0	0.8	[a]	[a]	[a]
White rat	110	3.9	1.0	5.6	[a]	[a]	[a]
Kitten	130	[c]	[c]	[c]	[b]	[b]	[b]

[a] embryonated eggs were seen in intestinal contents
[b] a few empty eggshells were found in first fecal movement after infection
[c] larvae were found in feces

Table 6

Number of negative fecal cultures of hookworm cases collected 10 days (D1-D10) after treatment with albendazole and mebendazole.

Drugs	No. of cases	No. of negative in cultivation									
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Albendazole	11	9	11	10	9	8	8	8	7	6	6
Mebendazole	13	13	13	12	11	11	10	8	6	6	6

Table 7

Hookworm larval counts in fecal culture collected 10 days (D1-D10) after treatment with albendazole and mebendazole.

Drugs	Total no. of larvae in culture tube (%)										
	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Albendazole	1,534 (100)	1,986	0 (0)	10 (0.7)	42 (2.7)	20 (1.3)	82 (5.3)	5 (0.3)	13 (0.8)	1 (0.1)	4 (0.3)
Mebendazole	4,726 (100)	0 (0)	0 (0)	6 (0.1)	3 (0.1)	0 (0)	2 (0.1)	90 (1.9)	147 (3.1)	41 (0.9)	38 (0.8)

Hookworm *(Necator americanus)*

The examination of fecal samples from the two methods collected 10 days after treatment revealed similar results. All hookworm cases previously confirmed by modified Kato thick smear method also gave positive results after cultivation. In the albendazole-treated group, incomplete ovicidal effects (9/11 = 81.8%) were observed in samples from day 1. The two drugs had complete ovicidal effects on day 2 samples since no larvae were seen from the culture tubes (Table 6). Fecal cultures from day 3 to day 10 samples were positive with a few larvae (Table 7).

Upon examination of fecal samples obtained on day 30 after treatment, the two drugs effected 100% cure rates in *Ascaris* infections. The lowest cure rate was 12.5% in *Trichuris* infections treated with albendazole. Egg reduction rates were higher than 90% using either drug for all three nematodes (Table 8).

Table 8

Cure rates and egg reduction rates effected by albendazole (400 mg single dose) and mebendazole (100 mg × 2 × 3 days) in soil-transmitted helminthic infections.

Drugs	Cure rate			Egg reduction rate		
	A.l	*T.t*	Hw	*A.l*	*T.t*	Hw
Albendazole	100	12.5	54.5	-	[a]	92.1
Mebendazole	100	50.0	30.8	-	99.1	95.6

[a] not done.

DISCUSSION

Previous reports indicated that albendazole and mebendazole might affect the ovaries and uteri of female *Trichuris* worms causing rapid released of eggs immediately after treatment (Wagner and Chavarria, 1974). This study showed also that female whipworms and hookworms released more eggs (*Trichuris*–3.3 times when exposed to

albendazole and 1.7 times when exposed to mebendazole, and hookworm–1.3 times when exposed to albendazole) based on examination of feces from the first bowel movement after treatment. Both drugs altered the size and shape of *Trichuris* eggs which were demonstrated in stool samples until day 6, on day 7 majority of the eggs appeared to be typical eggs found in normal untreated cases.

One albendazole-treated case showed negative stool findings on day 8 and in the mebendazole-treated group, negative findings occured at day 5, 6 or 7.

Wagner and Chavarria (1974) also concluded that *Trichuris* eggs become sterile as a direct effect of the drug. In this study however, treated *Trichuris* eggs exhibited normal growth as in the control group and the development occurred in both typical and atypical eggs.

Maisonneuve (1985) reported that albendazole is highly effective in suppressing the hatching of *Ascaris* and *Trichuris* eggs. The present study demonstrated hatching of eggs in the intestine of experimental animals, but unfortunately they were not susceptible hosts.

In hookworm infections, effect of the drugs remained for 2 days after treatment as reported by Hainan (1994) then followed by the reappearance of eggs with typical development as in non-cured cases.

In this study cure rates for trichuriasis and hookworm infections were low since atypical eggs of *Trichuris* were always detected by flotation method and hookworm cases were only considered fully cured when the fecal examination showed negative results in both Kato-Katz's and cultivation methods. However, the two drugs gave 100% cure rate for ascariasis.

Cline (1984) indicated that albendazole has a larvicidal effect and is active against pre-intestinal stages of *N. americanus* in human infections. A single dose (400 mg) of the drug could reduce egg production of the female worms to four-fold of the untreated group. It is probable that a higher dose of albendazole will be recommended as the drug of choice in hookworm control programs since it yielded good results in both prevention of infection and reduction of the intensity of infection.

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