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Albendazole Stimulates the Excretion of *Strongyloides stercoralis* Larvae in Stool Specimens and Enhances Sensitivity for Diagnosis of Strongyloidiasis[▽]

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We succeeded in stimulation of excretion of *Strongyloides stercoralis* larvae in stool by oral administration of a single dose of 400 mg albendazole to strongyloidiasis patients. This result overcame the false-negative results of stool examination due to low larval numbers. Stool samples were collected from 152 asymptomatic strongyloidiasis patients in the morning, prior to eating. After breakfast, they were given a dose of 400 mg albendazole, and stool samples were collected the following morning. Agar plate culture (APC), modified formalin-ether concentration technique (MFECT), and direct-smear (DS) methods were used to examine stool specimens within 3 h after defecation. The results before and after albendazole was taken were compared. All APCs that were positive became negative after albendazole administration, while MFECT showed a 1.4- to 18.0-fold increase in larval numbers in 97.4% (148/152) of the samples. The DSs were positive in 3 out of 3 smears at a larval number of ≥45 larvae per g (lpg) of stool, and in 1or 2 out of 3 smears at a larval number between 35 and 44 lpg. At a larval number of <35 lpg, the DS became negative. Interestingly 90.5% (19/21) of the samples that were negative by all methods before albendazole administration became positive by MFECT after the treatment. Thus, MFECT can be effectively used for diagnosis of strongyloidiasis with prior administration of albendazole to the subject.

Strongyloidiasis is a helminthic infection caused by *Strongyloides stercoralis*, a worm that is particularly dangerous for immunosuppressed patients. Although many methods are presently being used to diagnose this disease, a gold standard for the detection of larvae in the stool is still needed (14). Parasitological methods include agar plate culture (APC) (10), the Baermann method (4), the formalin-ether concentration technique (FECT) (13), the quantitative formalin ethyl acetate concentration technique (QFECT) (9), and the newly modified FECT (MFECT) (1). However, each method has its own disadvantages, resulting in false negativity. In addition, female *S. stercoralis* parasites, unlike other intestinal roundworms, embed in the mucosa of the small intestine, where they lay embryonated eggs that immediately hatch (8). Thus, the larvae are often scanty and irregularly excreted (11, 15). Repeated stool examination is therefore recommended for the diagnosis of strongyloidiasis (6). During an intestinal-helminth survey by direct fecal smear (DS) examination, some participants tested negative for *Strongyloides* larvae. After albendazole treatment for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, the stools were reexamined. Surprisingly, stools that were previously negative for *S. stercoralis* larvae became positive (data not shown). It was then considered possible that albendazole treatment could increase the sensitivity of stool examination methods for diagnosis of strongyloidiasis. Thus, this research aimed to enhance the sensitivity for the diagnosis of strongyloidiasis by using a single 400-mg oral dose of albendazole to stimulate the excretion of larvae into the stool for easier detection by MFECT and/or DS, particularly in cases where strongyloidiasis was strongly suspected, such as in patients with unexplained chronic diarrhea and patients returning from areas where strongyloidiasis is endemic.

MATERIALS AND METHODS

Subjects and stool samples. One hundred fifty-two strongyloidiasis patients referred from other research projects were invited to join our study. All patients had previously been diagnosed with asymptomatic strongyloidiasis for a period of 1 month to 2 years. These patients had not received any anthelmintic drugs and were diagnosed by APC, together with either FECT or MFECT. The ages of the patients ranged from 7 to 70 years. All came from the Moklan subdistrict (7 km from the laboratory unit of Walailak University) in the Thasala district of Nakhon Si Thammarat, a province in southern Thailand. The patients were asked to collect the entire amount of their morning stool in a plastic container at about 6:00 a.m., before breakfast. The samples were labeled as "stool before albendazole administration." A single dose of 400 mg albendazole was then given orally after breakfast, at about 9:00 a.m. The whole morning stool was then collected again from each patient within 21 h after albendazole administration; these samples were labeled "stool after albendazole administration." Subsequently another single dose of 400 mg albendazole was given after breakfast for two consecutive days to complete the treatment. All stool samples were sent to the laboratory without preservatives and tested within 3 h after defecation.

All 152 subjects were willing to collect their stools two times, before and after taking albendazole, to compare APC, MFECT, and DS for the detection of larvae before and after albendazole administration (see below). However, only 17 of 152 agreed to collect their stools another six times on six different days prior to and after taking the drug to compare the fluctuations of larval excretion versus larval excretion after albendazole administration (see below). Stool samples were collected again 14 days later for determination of the correlation between incomplete cure with albendazole treatment and the amount of larval excretion (3).

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Agar plate culture. APC was performed as described previously (10). Briefly, 3 g of each stool sample was placed at the centers of nutrient agar plates and incubated at room temperature (28°C to 33°C) for up to 5 days. Worm motility tracks, larvae, and free-living adult worms were monitored by stereomicroscope on day 3 (after 48 h). In case of a negative finding, observations were continued for another 2 days. Ten milliliters of 10% formalin was added to the agar surface of each microscopically positive dish to collect worms for species identification using a compound microscope (40×). The APC results were qualitatively read as positive or negative.

Modified formalin-ether concentration technique. MFECT was performed as described previously (1). Briefly, 2 g of fresh stool sample was suspended and stirred well in a tube containing 10 ml of 0.85% saline. The fecal suspension was strained through two pieces of 4- by 4-cm wire mesh into a plastic centrifuge tube. The first mesh (1.2- by 1.2-mm pore size) was placed on top of the funnel, while the second mesh (2- by 2-mm pore size) was used by hand. Trapped fecal materials on both meshes were washed with 3 ml of 0.85% saline. The resulting suspension was centrifuged at 700 × *g* for 5 min. The supernatant was then decanted, and the volume was adjusted to 7 ml with 10% formalin without mixing; 3 ml of diethyl ether was immediately added. The centrifuge tube was closed and shaken vigorously by hand for 1 min and immediately centrifuged at 700 × *g* for 5 min. The debris plug was loosened, and the top three layers were poured off. Approximately 1 ml of 10% formalin was added to the sediment. Then, the larvae in the sediment were counted and presented as larvae per g (lpg) of stool (10% formalin was used for the wet preparations for MFECT).

Direct smear. DS was performed as described previously (2, 7), with some modifications. A drop of 0.85% saline was placed on a slide. Stool was randomly stuck to a wooden stick by dipping the tip approximately six times into the sample. Stool was dispersed in the drop of 0.85% saline using the tip of the wooden stick coated with the stool. A 22- by 22-mm coverslip was applied, and the entire smear was scanned with a microscope (magnification, ×100). A positive DS meant that at least 1 larva per smear was found. Three smears were examined, both before and after albendazole was taken. For confirmation of *S. stercoralis* larvae, a drop of 10% formalin was added to an edge of the coverslip and allowed to diffuse to the saline smear. Direct examination was performed if the larvae were not actively motile.

Comparison of APC, MFECT, and DS for the detection of larvae before and after albendazole administration. Upon receiving the samples, the weights of stool specimens were determined, using preweighed containers, and recorded. APC, MFECT, and DS were performed both before and after albendazole administration, as described above. For DS, three smears were performed.

According to the results of the three different methods before albendazole administration, we divided the subjects into four categories: (i) category 1 was composed of 13 samples that were APC positive, MFECT positive, and DS positive; (ii) subcategories 2a and 2b were composed of 79 and 33 samples, respectively, which were APC positive, MFECT positive, and DS negative (after albendazole was taken, all samples in subcategory 2a were positive by DS, whereas those in subcategory 2b were negative by DS); (iii) category 3 was composed of 6 samples that were APC positive, MFECT negative, and DS negative; (iv) category 4 was composed of 21 samples that were APC negative, MFECT negative, and DS negative (Table 1). The category 4 patients had positive APC results with a low number of larvae and/or adult worms 1 to 2 years previously during another research project but were negative on the day before albendazole was administered in our study.

Fluctuation of larval excretion versus larval excretion after albendazole administration. Stool samples were collected from each subject on days 1, 2, 4, 7, 14, 28, 42, and 43. Days 42 and 43 were the days before and after albendazole administration, respectively. All stool samples were tested by MFECT only, except that on days 42 and 43 APC, MFECT, and DS were used.

Statistical analysis. SPSS 13.0 for Windows was used to perform all statistical analysis. Descriptive statistics, including mean, standard deviation (SD), and range, were generated. Nonparametric Wilcoxon signed-rank tests were used to determine the differences in larval numbers by MFECT before and after albendazole administration in 13, 79, 33, 6, and 21 pairs of categories 1, 2a, 2b, 3, and 4, respectively. A *P* value of <0.05 was considered statistically significant.

RESULTS

After 400 mg albendazole intake, APC results became negative in all cultured stool samples (Tables 1 and 2). However, MFECT results showed an increase in the number of larvae, by 1.4 to 18 times over those before albendazole intake, in 97.4% (148/152) of the stool samples. On the other hand, a decline in the larval numbers in two samples from subcategory 2b was noted, and no larvae were detected in two samples from category 4 (Table 1). Likewise, the DS became positive in 56.8% of samples (79/139; 13 samples in category 1 were not tested) using three smears: 48.3% (67/139) of previously negative DS samples were positive in all three smears, 5.8% (8/139) of previously negative DS samples were positive in two smears, and 2.9% (4/139) of previously negative DS samples were positive in only one smear (Table 1). The DS was positive in all three smears when the larval number by MFECT was ≥45 lpg and positive in only one or two smears when the larval number was between 35 and 44 lpg.

Cases that were positive only by APC, as in category 3, became positive by MFECT after albendazole stimulation. Interestingly, albendazole could dramatically and effectively stimulate the excretion of larvae in 90.5% (19/21) of the samples from category 4, which represented cases that were clinically suspicious only and could not be diagnosed by APC, MFECT, or DS until they became MFECT positive after albendazole intake.

Thirty-six of 152 subjects (23.0%) showed an incomplete cure by albendazole treatment, defined as the recovery of larvae 14 days after drug administration of three doses of 400 mg for three consecutive days; however, the incomplete cure had no relationship to the increase in the number of larvae. The larval number after the first dose of 400 mg albendazole was still 2 to 7 times higher than that before albendazole administration in all 36 subjects (data not shown).

S. stercoralis infections show an irregular and fluctuating pattern of larval excretion (Table 3). However, when albendazole was administrated orally, larval numbers increased to the maximum larval excretion point, where most larvae were driven out of the mucosa into the stool (Table 3). Albendazole had no effect on the excretion of *A. lumbricoides*, *T. trichiura*, and hookworm eggs (data not shown).

DISCUSSION

Albendazole, a broad-spectrum anthelmintic drug, is an effective treatment for uncomplicated strongyloidiasis (12). However, so far, the drug has not been used for the diagnosis of strongyloidiasis. The mechanism of action of albendazole, after being metabolized in the liver into albendazole sulfoxide, is to inhibit tubulin polymerization and subsequently decrease glucose uptake, thus exhibiting larvicidal, ovicidal, and adulticidal activities (5). This suggests that the larvae are dead and thus unable to grow on APC. There are no data on how long after albendazole treatment the cultivation of larvae in stool samples by APC can become positive again. Also, even without treatment, some larvae will gradually die within 4 h after defecation, which negatively influences the sensitivity of the FECT or MFECT (unpublished data). Thus, after albendazole intake, MFECT should be performed on fresh stool within 4 h after defecation. In addition, to avoid dilution in a large amount of stool, albendazole should be administered after as much stool as possible has been excreted. Furthermore, patients should limit food intake after oral administration of albendazole, and meals should have a high fiber content in order to decrease

TABLE 1. Comparison of APC, MFECT, and DS results before and after one dose of 400 mg albendazole in the four categories of samples

Category	MFECT: No. of positive samples	MFECT: No. of larvae/g of stool: Range	MFECT: No. of larvae/g of stool: Mean ± SD	P^a	DS (no. of positive samples[b]): 3[c] smears	DS: 2[d] smears	DS: 1[e] smear
1 (APC$^+$, MFECT$^+$, DS$^+$) (13 samples)							
Before albendazole administration	13	56–2,400	272.2 ± 640.9	0.001	13	0	0
After albendazole administration (APC negative, 13 samples)	13	168–30,000	2772.3 ± 8195.1		13	0	0
Fold increase		1.4–18.0	6.3 ± 5.0				
2a (APC$^+$, MFECT$^+$, DS$^-$) (79 samples)							
Before albendazole administration	79	2–32	14.3 ± 9.1	<0.001	0	0	0
After albendazole administration (APC negative, 79 samples)	79	35–208	63.4 ± 30.8		67	8	4
Fold increase		2.0–17.5	6.6 ± 4.4				
2b (APC$^+$, MFECT$^+$, DS$^-$) (33 samples)							
b1							
Before albendazole administration	31	1–10	5.1 ± 3.4	<0.001	0	0	0
After albendazole administration (APC negative, 31 samples)	31	2–32	13.7 ± 9.5		0	0	0
Fold increase		1.5–10.7	3.3 ± 2.2				
b2							
Before albendazole administration	2	4–16	10 ± 8.5		0	0	0
After albendazole administration (APC negative, 2 samples)	2	2–10	6 ± 5.7		0	0	0
Fold increase		0.5–0.6	0.6 ± 0.1				
3 (APC$^+$, MFECT$^-$, DS$^-$) (6 samples)							
Before albendazole administration	0	0	0	0.028	0	0	0
After albendazole administration (APC negative, 6 samples)	6	4–20	10.5 ± 6.0		0	0	0
Fold increase		NA[f]	NA				
4 (APC$^-$, MFECT$^-$, DS$^-$) (21 samples)							
Before albendazole administration	0	0	0	<0.001	0	0	0
After albendazole administration (APC negative, 21 samples)	19	2–20	7.5 ± 6.1		0	0	0
Fold increase		NA	NA				

[a] P values comparing the difference in the number of larvae per gram of stool by MFECT before and after albendazole administration in the four categories.
[b] At least 1 larva per smear was found.
[c] Three smears were examined; 3 smears were DS positive when the larval number by MFECT was ≥45 lpg.
[d] Three smears were examined; 2 smears were DS positive when the larval number by MFECT was between 35 and 44 lpg.
[e] Three smears were examined; only 1 smear was DS positive when the larval number by MFECT was between 35 and 44 lpg.
[f] NA, not applicable.

loose bowel movements and increase large particles in the stool. Our observations of a 9-year-old child (not included in this study) showed that 2 g of "hard to formed" stool contained 210 lpg (420 larvae per defecation), contrasting with another stool collection of 50 g mushy stool containing 10 lpg (500 larvae per defecation) 2 days later. In other words, the decline in the amount of stool caused by water absorption in the large bowel might have the effect of increasing the number of excreted larvae. We did not show MFECT data for larvae per defecation in this study because the amounts of stool before and after albendazole intake were the same. It was also noted that stool passed later than 24 h after albendazole intake should be avoided, because in 5 cases in which the stool was collected 48 h after albendazole intake, we found no larvae or

TABLE 2. Qualitative comparison of APC, MFECT, and DS results before and after one dose of 400 mg albendazole

Time	Parameter	Value: APC	Value: MFECT	Value: DS
Before albendazole administration	No. (%) of positive samples	131 (86.2)	125 (82.2)	13 (8.6)
	No. (%) of negative samples	21 (13.8)	27 (17.8)	139 (91.4)
After albendazole administration	No. (%) of positive samples	0 (0)	150 (98.7)	92 (60.5)
	No. (%) of negative samples	152 (100)	2 (1.3)	60 (39.5)

TABLE 3. Comparison of the number of irregularly excreted *S. stercoralis* larvae per gram of stool by MFECT on seven different days before albendazole administration and on the day after albendazole administration in 17 subjects

Subject no.	No. of larvae on day of stool collection:									
	1	2	4	7	14	28	42[a]	Mean[b]	Median[c]	43[d]
1	430	610	586	260	203	160	74	332	260	545
2	130	200	190	182	96	85	68	136	130	420
3	506	216	546	312	120	103	56	266	216	434
4	750	385	344	350	130	150	72	312	344	775
5	46	32	20	34	22	20	15	27	22	62
6	31	46	50	48	52	36	65	47	48	198
7	10	8	5	7	4	1	3	5	5	44
8	30	18	35	14	34	40	30	29	30	83
9	13	20	28	49	100	80	120	59	49	480
10	16	12	20	28	38	89	26	33	26	208
11	35	46	55	30	13	5	5	27	30	53
12	4	6	10	23	8	7	12	10	8	35
13	3	0	1	0	1	1	2	1	1	7
14	0	0	4	0	0	0	1	1	0	5
15	0	0	0	1	0	0	0	0	0	6
16	0	0	1	0	0	0	0	0	0	5
17	1	0	0	0	0	0	0	0	0	4

[a] The day before 400 mg albendazole administration.
[b] Mean number of larvae per gram of stool on seven different days before albendazole administration.
[c] Median number of larvae per gram of stool on seven different days before albendazole administration.
[d] The day after 400 mg albendazole administration.

a low number of larvae with deteriorated bodies (data not shown). The cause might have been that the excreted larvae were dead and had already been decomposed by intestinal enzymes and the bacterial flora and thus became undetectable.

Our results showed clearly that a single dose of 400 mg albendazole was able to stimulate the excretion of larvae into stool samples, which could then be detected by MFECT; larvae could also be detected by DS when the larval number was greater than 35 lpg. There were two samples in category 2b with a lower number of larvae excreted after albendazole intake. This might be explained by the larval number having already reached the maximum larval excretion point prior to albendazole intake, thus rendering the production of larvae by parasitic females insufficient and impeding the stimulating effect of albendazole. Similarly, two patients with uncomplicated strongyloidiasis, with episodes of diarrhea showing loose stools with larval numbers of 28 and 16 lpg by MFECT on the day before albendazole administration, produced only 23 and 13 lpg, respectively, after the first dose of 400 mg albendazole. After taking two more doses, the patients recovered from diarrhea. The larval number was possibly decreased, because during the episodes of diarrhea, larval excretion had already reached the maximum point, the point at which most larvae are driven out of the mucosa into the stool, because the diarrhea stimulated the excretion of larvae into the stool. Two samples from category 4 showed no larvae by any of the three testing methods before and after albendazole intake, possibly for one of the following reasons: the patients were cured naturally and the parasitic females died, or the number of parasitic females was too low, resulting a production of larvae too low to be detected, although excretion occurred after albendazole intake.

Therefore, albendazole administration plus MFECT should be employed in cases where larvae are scant or irregularly excreted and where more than one collection of stool is required (6). The sensitivity of MFECT for the detection of larvae is 18 lpg if 1 larva is found per smear (approximately 30 μl per smear) and 9, 6, 4 or 5, 3, 2, and 1 lpg if 1 larva is found per 2, 3, 4, 6, 9, and 18 smears, respectively. Thus, if the larval number is less than 4 lpg, more than 4 smears must be examined.

Although albendazole could increase the number of larvae by 1.4 to 18.0 times, the use of DS was limited by the number of larvae produced and excreted. In cases of light infection, the number of parasitic females was too low to shed enough larvae for detection by DS. This study revealed that approximately 50% of samples that were negative by DS before albendazole intake could be detected by DS after albendazole intake. The administration of one dose of 400 mg albendazole to stimulate the excretion of larvae can be employed as a supportive method for the diagnosis of strongyloidiasis in areas where only DS is available.

In conclusion, the application of albendazole plus MFECT for enhancing the diagnosis of human strongyloidiasis should be used in patients suspected of asymptomatic strongyloidiasis. This includes patients with unexplained chronic diarrhea, patients returning from areas where strongyloidiasis is endemic, and patients with negative results by other parasitological tests. The technique could also be applied in areas where APC and MFECT are not available but DS is routinely used.

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