Morphological observation of *Enterobius vermicularis* expelled by various anthelmintics

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INTRODUCTION

When a prevalent population of enterobiasis is treated with anthelmintic for one time, the positive rate of anal swab returns to pretreatment level within 18~30 days. And the interval of positive conversion is variable by different anthelmintics used. After pyrantel treatment, for example, the rate returns to previous level as early as 18~21 days, whereas after mebendazole or pyrvinium treatments, this phenomenon is delayed up to 27~30 days(Cho *et al.*, 1977).

The impact of the above results was laid on determining the proper interval of repeated chemotherapies to prevent recurrence: The chemotherapy should be repeated before gravid females migrate out again, and the interval should be long enough to minimize the unnecessary repetition. Based on these facts, Hong *et al.* (1980) tried to achieve a complete control of enterobiasis among institutionalized children by giving mebendazole every 20 days and by repeating 4 times.

The results of Cho *et al.* (1977) suggest that the currently available antipinworm drugs show

a different range of anthelmintic efficacy especially on the early stage of worm development. According to the drug used, unexpelled young pinworms continue to grow into gravid stage and migrate out the anus at different follow-up periods. In these connections, there have been several reports on the evaluation of anthelmintic activity of piperazine salts, pyrantel and pyrvinium in animal models. Brown *et al.* (1954) screened 32 piperazine salts in rats infected with *Syphacia obvelata*. And they found that the potent salt, piperazine hydrochloride expelled immature worms only in 56% and 6%, for the worms grown up to 5 days or up to 3 days respectively, at the dose of 200mg/kg/2 days. Kagei and Kihata(1971) reported that pyrantel did not show the anthelmintic action against immature *Syphacia obvelata* and *Aspiculuris tetraptera* shorter than 2mm in length.

This study was undertaken to study the anthelmintic efficacy of various antipinworm drugs, especially their efficacy on its early stage of development in *Enterobius vermicularis* by the morphological observation of expelled worms.

MATERIALS AND METHODS

1. Subjected population:

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A total of 142 children in 3 orphanages in Anyang City, Kyunggi-Do, Korea was subjected for this study. It consisted of 106 males and 36 females, ranging the ages of 3~12 years (Tables 1 & 2). The general sanitary conditions including annual budget, the number of nursing staffs, the status of water supply and linen change, and the number of children living in a room were comparable among these orphanages. The children had often been suffered from contact-borne dermatitis and pinworm infection. These contact-borne diseases were prevalent throughout the year due to the infected newcomers.

2. Anal swabs:

During the first 6 days of this study, scotch tape anal swabs were done preliminarily for 4 times every other day. Only one swab was taken each time from each child. The bathing was avoided from 2 days before the scheduled swabs.

3. Grouping, used drugs and follow-up:

Out of 142 examined children, 131 had done all of 4 preliminary swabs. They were categorized into 5 groups considering the following factors: The rate and consecutiveness of positive anal swabs, the orphanage, the sex and age of children examined (Table 2). After the grouping, all of the study processes were done in double blind way. Each group was treated with placebo (vitamin B complex), mebendazole (100 mg, single dose), pyrantel pamoate (10 mg/kg, single dose), pyrvinium pamoate (5 mg/kg, single dose) and piperazine citrate (70 mg/kg, *single dose*) respectively.

After the treatment, two kinds of follow-up were done; anal swabs and worm collection. Anal swabs were taken on 5, 10, 14 and 21 days after the treatment respectively. For the purpose of morphologic study of expelled worms, 3-day stool were collected from every case.

Table 1. Anal swab positive rates of 4 preliminary examinations by orphanage

Orphanage	No. examined	Positive rate on the day(%)				Total(%)
		D-6	D-4	D-2	D-0	
A	48	72	52	83	75	71
K	33	84	71	83	79	80
P	61	79	80	84	93	84
Total	142	78	68	83	84	78.4

Table 2. The rates and consecutiveness of positive anal swabs, the orphanage, sex and age of children in randomized 5 group

Group	No. of case	Anal swab positive rate (%)	No. of cases of			Orphanage			Sex		Age(year) Mean(Range)
			CP*	AP*	CN*	A	K	P	M	F	
Control	26	81	15	10	1	9	6	11	19	7	7.4(5~10)
Mebendazole	26	79	15	9	2	10	4	12	20	6	7.8(5~12)
Pyrantel	27	78	15	10	2	9	6	12	22	5	8.0(4~11)
Pyrvinium	26	82	15	10	1	9	5	12	20	6	8.3(3~12)
Piperazine	26	79	14	11	1	9	5	12	18	8	7.5(4~12)
Total	131	80	74	50	7	46	26	59	99	32	7.8(3~12)

* CP: Positive for 4 times of anal swabs, AP: One to three positive during 4 anal swabs, CN: Negative for 4 times

The stool were fixed first with 10% formalin, and then filtered through a chemical mesh, 100 μ m pore size. The unfiltered parts were examined both with naked eye and with a dissecting microscope.

The sex was differentiated in every pinworm. Using micrometer, worm length was measured individually. The total number of collected pinworms, the number of male worms, and the frequency distribution of females by length were compared among 5 groups.

Twenty-one days after the first treatment, the subjected children in 5 groups were concomitantly treated with mebendazole for the second time. Once more the stool were collected for 3 days as in the first try. And expelled pinworms were discriminated by sex and were measured.

RESULTS

1. Follow-up anal swabs:

The results of follow-up swabs were presented in Fig. 1. The control group showed a slight variation in the positive rate (73.1~88.5% by follow-up days). In mebendazole group, however, the rate gradually dropped down to 0% on the 21st day. The positive rate in pyrantel group dropped to its lowest level (7.4%) on the 14th day. But it was rapidly elevated to 37.0% on the 21st day. In pyrvinium group, the positive rates dropped to 15.4%, 11.5% and 3.8% on the 10th, 14th and 21st day respectively. The

Fig. 1. The results of anal swab positive rate before and after treatment (↓) in 5 groups.

anal swab result in the piperazine group showed dropping to 53.8% and 42.3% on the 10th and 14th days respectively. However, it was raised again to 56.0% on the 21st day.

2. Results of the first treatment:

1. Follow-up case number for worm collection:

All the treated children were subjected for the 3-day stool collection including the control group. Inevitably there were some deletes in all groups. However the follow-up rates were higher than expected, and showed the minimal variation among different groups; 83.3% in control group, 91.0% in mebendazole group, 86.4% in pyrantel group, 85.9% in pyrvinium and in piperazine group. By day the rate gradually went down during 3 days.

2. Daily expulsion of pinworms in each group:

From each group, the cases who were cooperative to collect 3 days stool were selected (Table 3). The relative percentage of the number of pinworms in those cases were counted by the

Table 3. Total number of collected *Enterobius* by day in cases followed-up for 3 days

Group	No. of cases followed	Total No. of pinworms	No. on the day(%)		
			D+1	D+2	D+3
Control	16	225	7	202	16
Mebendazole	20	762	32(4.2%)	316(41.5%)	414(54.3%)
Pyrantel	19	1,415	631(44.6%)	687(48.6%)	97(6.8%)
Pyrvinium	18	1,889	303(16.0%)	962(50.9%)	624(33.1%)
Piperazine	19	1,036	302(29.1%)	436(42.1%)	298(28.8%)

collection day.

In control group, a number of both male and female pinworms was naturally expelled. Among them, 30.8% discharged no worms at all during 3 days, however, remaining cases discharged pinworms of various developmental stages in the daily stool, mostly 1~4 worms. There was an extraordinary case that discharged 63 male and 81 female pinworms in a day.

In mebendazole group, the largest number was collected at the 3rd day after treatment. However in remaining 3 groups, the largest number was collected on the 2nd day.

Though the number of harvested pinworms differed by collection day, the comparative number of the male and female pinworms, and their developmental stage were not different by day. In Table 4, the sex and length of *E. ver-*

micularis collected after mebendazole treatment were shown by the collection day. By χ^2 -test the numbers in each day did not show significant difference in the sex, and length distribution in females ($\chi^2=31.36$, $.05 < p < .1$).

3. Total number of *E. vermicularis* collected:

A total of 6,165 worms was collected after the treatment. The number of collected worms by group and the frequency distribution of cases in each worm burden were presented in Table 5.

Except for the control group, the remaining 4 groups showed no statistically different frequency distribution of cases ($\chi^2=5.63$, $.1 < p < .5$) in each worm burden category.

4. Length distribution of collected females:

Not all the collected worms retain the complete morphology; some of them was broken

Table 4. The number of pinworms by sex and by length during 3 days of follow-up after mebendazole treatment*

Day after treatment	No. of male worms	No. of females in the range of length(mm)										
		Total	Up to 4.00	4.01~5.00	5.01~6.00	6.01~7.00	7.01~8.00	8.01~8.50	8.51~9.00	9.01~9.50	9.51~10.00	Over 10.01
First day	2	27	4	3	2	7	8	1	1	0	1	0
Second day	43	252	20	32	39	82	50	8	12	5	3	1
Third day	55	340	43	29	47	71	90	25	16	11	2	6

*Only unbroken worms were presented

$\chi^2=31.36$, $.10 > p > .05$

Table 5. Total number and frequency distribution of cases in each category of worm burden of *Enterobius* in the first treatment

Group	No. of cases	Total No. collected(mean)	No. of cases in worm burden of				
			0	1~10	11~40	41~100	Over 100
Control	26	262(4.0)*	8	16	1	0	1
Mebendazole	25**	912(36.5)	4	8	7	3	3
Pyrantel	26**	1,526(58.7)	4	6	6	3	7
Pyrrvinium	26	2,122(81.6)	3	6	8	2	7
Piperazine	26	1,343(51.7)	3	6	6	6	5
Total	129	6,165	22	42	28	14	23

* Mean No. per day per case

** One case each in mebendazole and pyrantel groups were failed to be followed-up

Table 6. The number of *Enterobius* by sex and by length in each group*

Group	No. of male worms	No. of females in the range of length(mm)										
		Total	Up to 4.00	4.01~5.00	5.01~6.00	6.01~7.00	7.01~8.00	8.01~8.50	8.51~9.00	9.51~9.50	9.51~10.00	Over 10.01
Control	84	132	2	5	8	20	32	29	12	16	4	4
Mebendazole	143	703	71	68	96	173	163	48	38	25	14	7
Pyrantel	99	1,228	24	45	81	235	336	181	123	107	57	36
Pyrvinium	333	1,604	34	46	87	220	478	272	201	156	77	33
Piperazine	195	998	14	42	90	222	304	131	90	79	19	7

* Out of 6,165 *Enterobius*, 5,519 unbroken worms were presented here

during fixation and washing. The frequency distribution of unbroken females by length were shown in Table 6 by group.

The ranges of length of females in respective group were as follows; 2.01~11.26mm in mebendazole group, 1.43~11.02mm in pyrantel group, 2.13~10.45mm in pyrvinium group, and 1.84~10.77mm in piperazine group. The cases in control group discharged not only gravid females longer than 8mm but also immature or mature females of various size (3.86~11.23mm in length).

When compared with control group, the male worm number was about 2~3 times larger in mebendazole, pyrvinium and piperazine groups. Statistically significant difference was observed in the frequency distribution of females by length between treated 4 groups ($\chi^2=408.1$, $p<.005$). Especially the number of short females in mebendazole group was larger than those of remaining groups.

3. Results in the second mebendazole treatment:

The follow up rate of stool in the second treatment was lower than that of the first. By group there were no differences in the collection rate (64.1~67.9%).

Table 7 showed the total number of pinworms collected in each group. The result of worm collection in control group was very similar to that of mebendazole group of the first treatment

Table 7. Total number of *Enterobius* collected in each group after the second treatment

Group	No. of cases	Total No. of collected worms	No. per case
Control	22	1,125	51.1
Mebendazole	23	156	6.8
Pyrantel	23	227	9.9
Pyrvinium	19	123	6.5
Piperazine	22	222	10.1
Total	109	1,853	—

because they were treated with mebendazole for the first time in this study. When compared with control group, only one fifth to one tenth of pinworms were collected in the remaining 4 groups. The numbers in pyrantel and piperazine groups were about two-times larger than those in mebendazole and pyrvinium groups.

In Table 8, the frequency distribution of pinworms by sex and by length were presented. In control group, the whole range of female length, 2.10~10.67mm, were collected. Between treated groups, clear differences were found in the number of longer worms in the second treatment. In mebendazole group the largest worm was 8.74mm in length. In pyrvinium group, there was no female longer than 8.31mm. However, in remaining 2 groups, the females of whole range of length (2.57~10.46 mm) were collected though the numbers were smaller than those of control group.

Table 8. Number of *Enterobius* by sex and by length in each group collected in the second treatment*

Group	No. of male worms	No. of females in range of length(mm)										
		Total	Up to 4.00	4.01~5.00	5.01~6.00	6.01~7.00	7.01~8.00	8.01~8.50	8.51~9.00	9.01~9.50	9.51~10.00	Over 10.01
Control	169	611	15	34	16	119	183	73	63	38	14	6
Mebendazole	3	125	7	15	29	45	23	4	2	0	0	0
Pyrantel	4	193	4	18	30	49	52	16	10	6	6	2
Pyrrvinium	21	70	8	13	17	21	8	3	0	0	0	0
Piperazine	4	183	8	22	21	38	50	13	17	8	4	2

* Out of 1,853 *Enterobius*, 1,393 unbroken worms were presented here

DISCUSSION

Cho *et al.* (1981) reported that in female *Enterobius*, the length from anterior end to esophagus and the length of tail showed the minimal growth during processes of gravidation (5~11mm). However, the length from esophagus to anus grew almost proportionately with the degree of gravidation. As a result, the total length of the worm represented the development of *Enterobius*. Therefore we used the female length as an indicator of development throughout this study.

At the first treatment there were no difference at all in the range of female in length between groups. Females of 2~11mm in length were found in all groups. The number of collected young females were significantly larger in mebendazole group than in other groups. However a sizable number of short females were also found in other groups. Thus this difference afforded only meager support to the superior efficacy of mebendazole on the short, underdeveloped pinworms. Therefore, we instituted the second treatment to confirm above findings again. The non-expelled females at the first treatment were allowed to grow up for 21 days, and were treated with mebendazole again. At the second collection, the maximum growth was clearly different among the drugs used.

Whether the grown-up females at the second

collection were really treatment-resisted or reinfected, depended on the life span of *E. vermicularis*. If the life span is 15~27 days as reported by Cram (1943), the interpretation of our result became ambiguous. If the span is shorter than 21 days, the worms at the second collection would be all reinfected. In this case, we could not explain how the used drugs showed the different prophylactic actions.

If the span is longer than 21 days, preferably 35~42 days as reported by Schüffner (1947) and Akagi (1973), the longer worms found in the specimens of second collection would be the young worms that resisted the first treatment. This in turn means that pyrantel and piperazine are less effective in worm reduction on the early developmental stages of pinworm when compared with mebendazole and pyrrvinium. The latter two drugs truncated a certain early stages as well as older worms completely, when the worm collection data and anal swab results are considered.

The gravid females which are migrating out the anus were known to be 8~13 mm in length. However, not all of the females longer than 8mm exclusively migrate but only a portion of them individually and irregularly begin to migrate. In this respect, worm measurement at the second collection were fairly well coincided with the results of follow-up anal swabs. At the 21st day, the egg positive rates were 37.0% and

56.0% in pyrantel and piperazine groups respectively. From these two groups we collected the whole range of females 2~11mm in length. However, in mebendazole and pyrvinium groups, the egg positive rates of anal swab were 0% and 3.8% in respect. In these two groups, females longer than 8.74mm and 8.31mm were not found. In this connection, the large number of 7~8mm long females at the second collection made an explanation possible how the positive rate of anal swabs return to the pretreatment level within 30 days after any kinds of drug treatment (Cho *et al.*, 1977).

Mebendazole initiates expulsion of pinworms between 24~48 hours after treatment, whereas piperazine and levamisole do as early as a few hours (Comley, 1980). In this connection, van den Bossche (1972) noted that the mebendazole-induced expulsion of *Syphacia muris*, *Ascaridia galli* and *Syngamus trachea* took almost 3 days. However, Muttalib *et al.* (1980) reported that fecal expulsion of *Ascaris lumbricoides* was the highest on the 3rd day and may be delayed up to the 6th day. In present study, the largest number of pinworms was collected on the 3rd day in mebendazole group. For the completeness of worm collection it is definitely recommended to get 6 days stool collection. However, for the purpose of present study, *i.e.*, the determination of relative length among expelled worms, we believe 3 days stool collection is good enough.

SUMMARY

When enterobiasis cases were treated with anthelmintics only for one time, the interval to recurrence was variable by different drugs used. And this phenomenon is supposedly connected with biological or developmental cycle of the worm and the consequent efficacy of the different anthelmintics. This study was undertaken to confirm this fact by studying the expelled

worms morphologically to correlate the anthelmintics efficacy and stage of worm development in *Enterobius vermicularis*.

A total of 131 children in 3 orphanages was examined by 4 anal swabs (mean positive rate, 80%). They were randomized into 5 experimental groups. Each group was treated with placebo, mebendazole, pyrantel, pyrvinium, and piperazine (70mg/kg, single dose) respectively. After treatment, all stool were collected for 3 days to get the expelled *Enterobius*. A total of 6,165 pinworms was studied under the microscope. The sex was discriminated and the length was individually measured.

A number of male pinworms was collected in all groups. Females of 2~11mm in length were also collected in 5 groups. However, significantly larger number of short females was observed in mebendazole group compared with other groups.

Twenty-one days after the first treatment, all children were again treated with mebendazole. Once more stool were examined. A total of 1,853 worms was collected. In mebendazole group, there were no females longer than 8.74 mm in the second treatment. In pyrvinium group, 8.31mm in length was the longest for female. However in control, pyrantel and piperazine groups, females of 2~11 mm in length were collected.

From above results, one could conclude that the removing ability of mebendazole and pyrvinium was satisfactory for the worms in the early stage of development in *Enterobius*. Pyrantel and single dose of piperazine showed less effective in worm reduction ability especially on those at the early stages.

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＝국문초록＝

각종구충제로 배출된 요충의 형태학적 관찰

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송 천 용

요충구충제를 투약하고 4~5일 간격으로 항문주위 도말을 40일까지 실시할 경우 음전(陰轉)되었던 웅란양성율은 다시 치료전 수준으로 되돌아 간다. 그런데 구충제의 종류에 따라 다시 치료전 수준으로 되돌아 가는 시간이 다르다는 사실을 Cho등(1977)이 보고한 바 있다. 이와같은 차이가 생기는 것은 각 구충제가 구충하는 요충 발육단계의 범위 특히 어린 요충에 대한 구충능력이 다르기 때문이라고 생각하였다. 이러한 생각을 형태학적으로 증명하기 위하여 내가지 구충제로 배출된 충체를 수집하고 계속하였다.

경기도 안양시에 있는 3개 보육원 원아 142명을 대상으로 하루 걸러 네번의 항문도말을 실시하고 그 결과에 따라 131명을 26~27명씩 5개군으로 나누었다. 각군은 대조군(對照群), 메벤다졸, 피란텔, 피르비닐 및 피라라진 투여군으로 하였다. 투약후 3일간 배출된 충체를 수집하고 10% 포르말린에 고정하였다.

첫 투약후 21일째 되는 날 대상 어린이 모두에게 메멘다졸을 다시 투여하고 3일간 총체들 수집하였다. 수집된 요충을 우선 압늪과 솟늪으로 구별하고, 각 총체의 길이를 측정하여 총체발육의 정도로 하였다. 그 결과를 종합하던 다음과 같다.

1. 대상자 142명의 치료전 항문도말 배변의 평균 총탄 양성율은 78.4% 이어서 이들 보육원아 요충의 높은 유행집단임을 확인하였다.

2. 첫번 투약후 5, 10, 14, 21일째에 실시한 항문도말 양성율은 대조군에서 73.1~88.4%의 범위에 있었다. 메멘다졸군에서는 30.8%, 3.8%, 3.8% 및 0%이었고, 피란덴군에서는 29.4%, 14.8%, 7.4% 및 37.0%로서 14일과 21일 사이에 양성자가 갑자기 늘었으며, 피르비눔군에서는 38.5%, 15.4% 및 3.8%로 11.5% 차차 낮아졌다. 피페라진을 한번 투여한 군에서는 50.0%, 53.8%, 42.3%로 떨어졌다가 21일째에는 56.0%로 올라갔다.

3. 날짜를 달리하여 수집된 총체에서 성별 길이별 분포의 차이는 인정할 수 없었다.

4. 첫번 투약후 3일간, 대조군에서 262마리, 메멘다졸군에서 912마리, 피란덴군에서 1,526마리, 피르비눔군에서 2,122마리, 피페라진군에서 1,343마리를 각각 수집하였다. 이들 총체에서 투약군 사이에 솟늪의 솟자, 압늪 길이면 솟자에서 명백한 차이가 없었다.

5. 두번째 투약에서 얻은 1,853마리중 1,393마리를 계측한 결과 메멘다졸군에서는 8.74mm의 압늪이 제일 긴 것이었고, 피르비눔군에서는 8.31mm의 압늪이 제일 성숙한 것이었는데 비하여 피란덴 및 피페라진 군에서는 10.5mm까지의 다 자란 요충이 배출되었다.

위의 결과에서 메멘다졸 및 피르비눔은 어린 요충의 어떤 시기에서부터 성숙요충까지를 거의 완전히 구충하는데 비하여 피란덴 및 피페라진은 이에 상응(相應)하는 어린시기 요충에 대하여 구충능력이 낮은 것으로 판단할 수 있었다.