ANTHELMINTIC EFFECTS OF ALBENDAZOLE, MEBENDAZOLE AND DIETHYLCARBAMAZINE ON *TRICHINELLA SPIRALIS* IN MICE

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ABSTRACT

The efficacy of albendazole, mebendazole and diethylcarbamazine on Trichinella spiralis *infection in mice was determined. Four groups of animals were treated with drugs, each at a dose of 50 mg/kg body weight for 3 consecutive days on days 2-4, 10-12, 21-23, and 28-30 post inoculation. After 35-42 days, infected mice were examined for trichinella larvae. Mebendazole and albendazole were effective in eliminating, respectively, 99.77% and 99.95% of worms in the intestines of infected mice, while diethylcarbamazine could not eliminate worms.*

During the invasive phase, the efficacy of mebendazole and diethylcarbamazine were nearly the same (76.21% and 72.85% respectively) while albendazole showed a 36.22% decrease in larva. Mebendazole not only prevented 97.91% of the larvae in muscle from entering the musculature phase, but it also reduced the larvae by 96.70% during the encapsulated phase. Moreover, albendazole and diethylcarbamazine decreased the amount of larvae in muscle from entering the musculature phase by 50.59% and 16.05% respectively. For the musculature phase we observed that albendazole reduced 63.76% of the encapsulated larvae in muscle but diethylcarbamazine could not eliminate the encapsulated larvae.

Mebendazole proved to be more effective than albendazole and diethylcarbamazine. Side effects such as conjunctivitis and ruffled hair were transiently observed after treatment with mebendazole and albendazole during the musculature phase.

INTRODUCTION

The efficacy of albendazole, mebendazole, oxfendazole and fenbendazole in the treatment of infections with immature and mature worms of *Trichinella spiralis* in the guts of mice and rats.[1,2,3,6,7] has been demonstrated. However, there have been only a few published studies on the efficacy of these drugs in the treatment of larvae entering the muscular stage and encapsulated larvae in the musculature phase with a once-daily dosage for a long period (5-7 days)[1,2] or a twice-daily dosage for three consecutive days.[8] Diethylcarbamazine has also been recommended for the treatment of trichinosis.[4]

The purpose of this study was to compare the efficacy of albendazole, mebendazole and diethylcarbamazine in the treatment of various phases of the larvae, comprising enteral, invasive, entering musculature and the encapsulated phases.

MATERIALS AND METHODS

Male albino mice 5-6 weeks old and weighing 18-30 grams were purchased from the National Laboratory Animal Center, Mahidol University. All mice were maintained under conventional laboratory conditions. The Thai strain of *T. spiralis,* originally obtained from a naturally infected pig in northern Thailand, was kindly provided by Dr.Rumpueng Dissamarn, Department of Livestock Development, Ministry of Agriculture and Co-operative. This strain has been maintained in mice for many years in the laboratory of the Parasitology Department, Faculty of Public Health, Mahidol University.

Each experimental mouse was infected with 300 larvae of *T. spiralis* by stomach tube and no mice died after infection. After 35-42 days the infected mice were anaesthetised with chloroform, skinned and eviscerated. The carcases were finely minced and digested with artificial gastric juice (1% pepsin w/v and 0.7% concentrated HCl v/v) while stirring for 3-4 hours at 37°C. The digested solution was filtered and the supernatant was estimated by dilution count using 2″×3″ glass slide (sampling 3 times per sample).[5]

Mebendazole (MBZ; 20 mg/ml; Jansen Pharmaceutica Ltd.) albendazole (ABZ; 20 mg/ml; Smith Kline & French Co. Ltd.) and diethylcarbamazine (DEC; 50 mg/tab diluted with distilled water; Halewood Chemical Ltd.) were given to mice by stomach tube. The treatment schedule was performed by giving 50 mg/kg body weight for 3 consecutive days on days 2-4, 10-12, 21-33, and 28-30 post inoculation. The efficacy of drug was assessed by comparing the larvae recovered from individually treated animal with untreated controls. ANOVA test was used for the statistic analysis. A probability greater than 0.05 was not considered significant.

RESULTS

Efficacy of the drugs on the enteral phase

Three consecutive days of 50 mg/kg body weight of MBZ was found to eradicate the total infection in 7 mice. As for the 3 remaining mice only 4, 5 and 263 larvae were recovered respectively (99.77% effective). ABZ eliminated 99.95% of the worms which result was not significantly different from that of the MBZ-treated group ($P > 0.05$). The mean numbers of larvae recovered from the MBZ - and ABZ-treated mice were significantly lower than of those from the controls and the DEC-treated mice ($P < 0.005$). DEC had no effect on the worms in the intestines of mice (Table 1).

Efficacy of the drugs on the invasive phase

MBZ and DEC were 76.21% and 72.85% effective against the invasive phase of the worms respectively. ABZ partially diminished the larvae by only 36.22% which was significantly lower than those of MBZ and DEC ($P < 0.005$).

Efficacy of the drugs on the entering musculature phase

MBZ was very effective against the developing muscle larvae (97.91%). The mean worm burden in the MBZ-treated group was significantly lower than in the controls and the DEC-treated group ($P<0.005$) as well as in the ABZ-treated group ($0.01<P<0.025$).

Compared with the controls, ABZ was effective in worm elimination (50.59%) ($P < 0.05$), but this effectiveness was not statistically different from that of DEC ($P > 0.05$). In contrast, the effectiveness of DEC (16.05%) was not statistically different from that of the controls. It should be noted that only 10-40% of the entering musculature larvae recovered from the digested muscle were still alive in the treated groups. Conjunctival blood congestion was observed in mice after administration of MBZ (5 from 10 mice) and ABZ (2 from 10 mice) in 24 hours and all mice treated with these two drugs showed ruffled hair. These adverse reactions were resolved within 72 hours after drug administration.

Efficacy of the drugs on the encapsulated larvae in the musculature

MBZ was also effective on the encapsulated larvae (96.73%) which was significantly different from the control ($P < 0.005$). ABZ was somewhat less effective (63.76%) and there was no significant difference from that of MBZ-treated group ($P > 0.05$), but compared with the controls, this difference was still significant ($P < 0.005$). DEC had no effect on the encapsulated larvae at all (0.60% effective) and this effect was not significantly different from the control ($P > 0.05$) (Table 1). Mice treated with MBZ were found to have a few digested larvae still alive. Nine out of 10 mice treated with MBZ had conjunctival blood congestion. All mice treated with MBZ and ABZ has ruffled hair. These side effects diminished 48 hours after the first treatment and disappeared after 72 hours.

DISCUSSION

The efficacy of mebendazole, albendazole, febendazole and oxfendazole in the treatment of *T. spiralis* in experimental animal varied according to the time lapse after infection and the frequencies of drugs administered. [1,2,4,6-8] MBZ was shown to be 100% effective even with a single oral dosage of 50 mg/kg, when it was given within 2-7 hours after inoculation of the infective larvae.[1,2] With this dosage schedule, the efficacy was reduced to 45-85% when the drug was given 72 hours after larval inoculation.[1,2,6,7] The reports also indicated that MBZ and ABZ administration for 3 consecutive days could totally eliminate worms in the enteral phase. McCracken, *et al.*,[7] succeeded in eliminating only 78% of the larvae in the enteral phase by treating mice with 50 mg/kg of MBZ for 3 consecutive days. However, at 72 hours post inoculum, 62% were eliminated with a single dose of 150 mg/kg, yet, MBZ at a dosage of 7.5 mg/kg twice daily for 3 consecutive days (on day 3-5) decreased 93% of the worm burden in the intestines, which figure is still less than ours (99.77%).

Moreover, McCracken and Taylor[8] illustrated the effectiveness of MBZ in the treatment of *T. spiralis,* during the invasive and encapsulated larvae phases of infection with a dosage of $\leqslant$ 12.5 mg/kg twice daily for 3 consecutive days at 75-90% and 84-92% respectively. The results of our study indicated that MBZ decreased the larvae from the musculature and invasive phases by 96.73% - 97.91% and 76.21% respectively. Thus, the efficacy of MBZ may be influenced by the frequency of drug administration and division of the daily oral dose.

TABLE 1 Number of *T. spiralis* larvae recovered from the musculature of control mice and mice treated orally with mebendazole, albendazole and diethylcarbamazine at a dosage of 50 mg/kg/day for 3 consecutive days on the various days after infection

Day after infection	Parameter	Control	MBZ	ABZ	DEC
2-3-4	Mean ± SD*	11620 ± 4675.1	27 ± 78.6	6.1 ± 6.5	13559 ± 9622.9
(enteral	Efficacy (%)		99.77	99.95	−16.69
phase)	No. of mice	10	10	10	10
10-11-12	Mean± SD	30254.4 ± 12776.4	7197.9 ± 5982	19297 ± 10903	8214 ± 4361
(invasive	Efficacy (%)		76.21	36.22	72.85
phase)	No. of mice	9	10	10	10
21-22-23	Mean ± SD	18565 ±11953	391.6 ± 337.5	9172.4± 5449.6	15585.8 ± 9993.5
(entering	Efficacy (%)		97.91	50.59	16.05
musculature	No. of mice	9	10	10	10
28-29-30	Mean ± SD	12906.7 ± 6646.6	421.6 ± 731.5	4677.5 ± 3118.2	12829.5 ± 6158
(Encapsulated	Efficacy (%)		96.73	63.76	0.60
phase)	No. of mice	9	10	10	10

* Mean number of recovered larvae per mouse ± standard deviation.

In the treatment of gastro-intestinal helminths in children and adults, MBZ is usually given in oral doses of 100 mg twice daily for 3 consecutive days. The actual dose for a patient weighing 65 kg is 1.5 mg/kg. During the recent outbreak of trichinosis at Kanchanaburi Province, the patients came to see the physicians at the time of the parenteral phase,[9] and physicians from the local community hospital used MBZ in oral doses of 100 mg twice daily for 1 week in the treatment of light infected cases, and 3 weeks for the severe cases. All patients gradually recovered better health condition. Shortly thereafter, several biopsies of gastrocnemeus muscle from treated patients were performed and revealed that *T. spiralis* (musculature phase), was still found in some of the patients (Dr.Krisda Chongsakul; the Tong-Phapoom local community hospital, Tong-Phapoom District, Kanchanaburi Province, personal communication). The survival of larvae in the muscle was probably due to inadequate doses of MBZ. However, the MBZ caused abatement clinical signs and symptoms. Further study on the effect of MBZ on *T. spiralis* should be carried out in man regarding doses, contraindications, and the efficacy in eliminating worm in the enteral and the parenteral phases.

Fernando and Denham[2] studied the efficacy of MBZ against entering musculature and encapsulated larvae at the high dose of 50 mg/kg once daily for 7 days, with no mention of side effects. McCracken[1,8] found evidence for the lethal effect of MBZ on *T. spiralis* during the invasive phase in mice. The minimum dose of 1 mg/kg and 3.25 mg/kg twice daily for 3 consecutive days (day 14-16) for the treatment of clinical trichinosis showed lethal effects in some mice, but again there was no mention of side effects during treatment of the encapsulated phase. We have demonstrated blood congestion in conjuctiva of mice. It is recommended that a detailed study of side effects of MBZ and ABZ during entering musculature and encapsulated larvae phase in mice be carried out

It is possible that higher doses of drugs for treatment of *T. spiralis* in man might be required than the dose currently used for the treatment of gastro-intestinal helminths. However, total doses and the series of divided daily oral doses as well as the side effects should be reconsidered in man.

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บทคัดย่อ

การศึกษาประสิทธิภาพของยาอาลเบนดาโซล มีเบนดาโซล และไดเอทิลคาร์บามาซีน ที่มีผลต่อพยาธิ ทริคิเนลล่า สไปราลิส ในหนูไมซ์ โดยการให้ยาขนาด 50 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม เป็นเวลา 3 วัน ต่อเนื่องกัน โดยเริ่มให้ยาวันที่ 2 วันที่ 10 วันที่ 21 และวันที่ 28 หลังจากได้รับเชื้อพยาธิแล้ว หลังจากนั้น 35-42 วัน ได้ทำการตรวจหนู พบว่ามีเบนดาโซลและอาลเบนดาโซลสามารถกำจัดพยาธิในระยะที่อยู่ในลำไส้ของหนูได้ 99.8% และ 99.95% ตามลำดับ ส่วนไดเอทิลคาร์บามาซีนไม่สามารถกำจัดพยาธิระยะนี้ได้ ยามีเบนดาโซลและไดเอทิลคาร์บามาซีนให้ผลใกล้เคียงกัน (76% และ 73% ตามลำดับ) ในการกำจัดพยาธิที่อยู่ในระยะเคลื่อนที่เข้าสู่กล้ามเนื้อในขณะที่อาลเบนดาโซลให้ผลเพียง 36% การทดลองนี้พบว่ายามีเบนดาโซลสามารถลดจำนวนตัวอ่อนที่เข้าไปอยู่ในกล้ามเนื้อได้ 97.8% และระยะเป็นแคปซูลได้ 96.7% ส่วนอาลเบนดาโซลและไดเอทิลคาร์บามาซีนให้ผลในการลดตัวอ่อนระยะเข้ากล้ามเนื้อ 50.6% และ 16% ตามลำดับ สำหรับระยะแคปซูลยาอาลเบนดาโซลลดจำนวนตัวอ่อนได้ 63.8% แต่ไดเอทิลคาร์บามาซีนจะไม่ได้ผลเลย อย่างไรก็ตามยามีเบนดาโซลและอาลเบนดาโซลทำให้มีอาการข้างเคียงเล็กน้อย เมื่อให้ยาในระยะที่ตัวอ่อนเข้าอยู่ในกล้ามเนื้อ ซึ่งอาการข้างเคียงนี้จะปรากฏอยู่เพียง 2-3 วันที่ให้ยา