Efficacy of Albendazole Against Early and Late Stage of *Trichinella spiralis* Infection in Mice

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Abstract

Efficacy of albendazole against early and late stage of *Trichinella spiralis* infection in mice was determined. To determine the efficacy of albendazole against the early stage (enteral phase) of trichinosis, mice experimentally infected with *T. spiralis* were treated with albendazole 20 mg/kg at 7 days post infection for 15 days. Larvae were recovered from the infected mice 7 days after the treatment. The reduction rate of the larvae was 100 per cent. Efficacy of albendazole against the late stage (parenteral phase) of infection was determined at 30 days post infection. Mice were treated with albendazole at 20 mg/kg for 30 days. Larvae were recovered from the infected mice 7 days after the treatment. The reduction rate of the larvae was 71 per cent compared to the control group. In conclusion, albendazole was more effective in the early stage of infection than the late stage, the reduction was 100 per cent and 71 per cent with respect to the control group respectively.

Key word : *Trichinella spiralis*, Trichinosis, Albendazole

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Trichinosis is a parasitic zoonosis caused by *Trichinella* species that presents throughout the world. Transmission to humans occurs through the ingestion of raw meat containing infective larvae. The larvae develop into adults in the gut of the host 10-28 hours after being ingested(1). Mating occurs by the second day of infection and females begin to deposit motile larvae 5 days later. Larvae are shed for approxi-

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mately 4 to 16 weeks, the number of newborn larvae produced depends on the immune status of the host (2,3). Each female produces approximately 1,500 larvae in the nonimmune host. The newborn larvae travel through the bloodstream and reach striated muscle cells. Invasion of the striated muscle cells stimulates the development of the nurse cells and the larvae become infective. In the nurse cells, the larvae begin to coil and the nurse cells complete the formation of the cysts within 2 to 3 weeks. Larvae can invade almost any tissue but can develop to the infective stage only in striated muscle cells. Symptoms of trichinosis are divided into 3 clinical phases corresponding to the periods, intestinal invasion by adult worms in the enteral phase, migration of the newborn larvae in the migratory phase and encystment of larvae in the parenteral phase(1,4).

Specific treatment of trichinosis has not yet been standardized. Benzimidazole derivatives such as mebendazole and albendazole are used for treating human trichinosis(5). However, studies using these drugs in mice infected with *Trichinella spiralis* demonstrated that they are unable to kill the encysted larvae (5-8). Treatment of human trichinosis caused by *Trichinella spiralis* in the stage of encapsulating with mebendazole is also ineffective(5). There are limited data concerning the effect of albendazole on *T. spiralis*.

The objective of this study was to evaluate the efficacy of albendazole among mice infected with *Trichinella spiralis* in the enteral (early) phase and parenteral (late) phase.

MATERIAL AND METHOD

The authors used 6 week old mice purchased from the National Laboratory Animal Centre, Mahidol University, Bangkok, Thailand. *T. spiralis* larvae were obtained from an infected rat at the Department of Parasitology, Faculty of Medicine, Chulalongkorn University.

Isolation of *T. spiralis* larvae from the infected rat for infection

T. spiralis larvae were isolated from an infected rat by digestion and isolation with modified Baermann's technique(9). Briefly, an infected rat was killed and the encysted larvae were detected from the rat's diaphragm using the press preparation technique. To isolate the encysted larvae from the infected rat, skin and internal organs (except the heart) of the infected rat were removed. Each organ was digested with artificial digestive fluid (Pepsin 3 g, HCl 7 ml and ddH_2O to 1,000 ml) at 1 : 10 (W/V) at 37°C for 2.5 hours. *T. spiralis* larvae were then isolated from the mixture by modified Baermann's technique, the mixture was filtered through 6-layer gauze and the *T. spiralis* larvae were activated to move by pouring 100 ml warm water (37°C) to the mixture. The larvae density was determined by dilution count technique.

Infection of mice with *T. spiralis* larvae

Fifty-four mice were infected with *T. spiralis* larvae (isolated from the infected rat) at 10 larvae/g of mouse by force feeding (approximately 300 larvae/mouse). Mice anesthetized with diethyl ether were fed by using polyethylene tube connected to a syringe containing *T. spiralis* larvae. After being infected, mice were then reared with mouse food until used.

Detection of *T. spiralis* larvae and adults from the infected mice

To determine the success of infection, adults of *T. spiralis* were detected from the intestine of the infected mice by digestion method and modified Baermann's technique. *T. spiralis* larvae were also detected from the infected mice by the press preparation technique and isolated by the digestion method and modified Baermann's technique as described previously.

Treatment of infected mice with albendazole suspension

Albendazole was mixed with sterile water to the concentration of 1 mg/ml. Mice were treated with albendazole 10 mg/kg twice a day. The infected mice were divided into 2 groups; group A, A1: ten infected mice without treatment and A2 fifteen infected mice treated with albendazole 7 days after infection for 15 days. In group B, B1: ten mice without treatment and B2: fifteen mice treated with albendazole at 30 days after infection for 30 days (Fig. 1).

Data analysis

The data were recorded and analyzed by using the Excel 6 software program. Differences between the control group and treatment group were compared by the *t*-test. Statistical analysis was performed with the level of significance at p-values < 0.001.

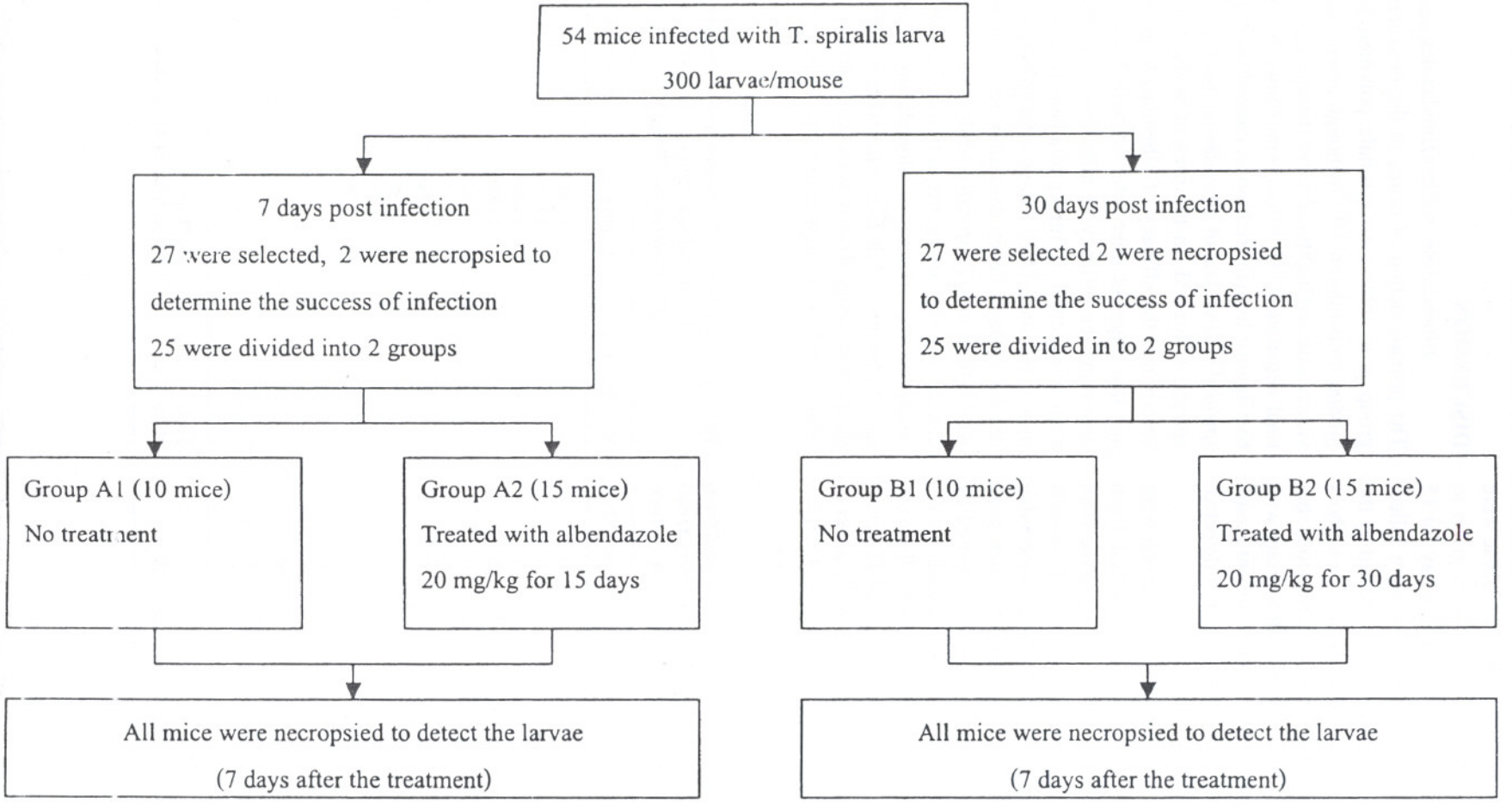

Fig. 1. Illustrates the experimental design of the efficacy of albendazole from early to late stage of trichinosis.

RESULTS

To determine the success of *T. spiralis* infection, 2 infected mice were killed on day 7 after infection. *T. spiralis* adults were identified in the mice (43 and 47 adult worms were found) but the larvae were not detected. Albendazole suspension was given to the infected mice (A2) 10 mg/kg twice a day for 15 days. 7 days after the treatment, mice were killed, and *T. spiralis* larvae and adults were detected by the press preparation and digestion method. The authors did not find *T. spiralis* adults in the control group, but the larvae were detected from the control group with the total larvae being 207,977. In the treatment group, both *T. spiralis* adults and larvae were not detected. The results are shown in Table 1.

At 30 days after infection, two mice were necropsied and the larvae were identified from necropsied mice (21,378 and 20,308) all larvae were encysted. In group B2, mice infected with *T. spiralis* were treated with albendazole 10 mg/kg twice a day for 30 days. At 7 days after treatment, mice were necropsied and *T. spiralis* larvae were recovered by the press preparation and digestion method. In the control group B1, the total number of *T. spiralis* larvae was 219,378 (an average of 21,937.8 ± 2,224.47 larvae/mouse) while in the treatment, group a total number of 96,272 larvae were found (an average of 6,418.13 ± 1,183.81 larvae/mouse). No adult was identified. The results are shown in Table 2.

During the experiment, no side effects of albendazole such as vomiting were observed.

DISCUSSION

Albendazole is a benzimidazoles derivative. The primary action of drugs in the benzimidazoles group is to inhibit microtubule polymerization by binding to β-tubulin[10]. Although albendazole provides a safe and highly effective therapy against intestinal nematodes[11], it has been shown to be effective against human trichinosis caused by *T. pseudospiralis*[12] but data of albendazole against human *T. spiralis* is limited. In the present study the authors determined the efficacy of albendazole in the early and late stages of mice infected with *T. spiralis*. To determine the efficacy of albendazole against early stage of infection, 10 mg/kg of albendazole was given to infected mice (A2) 7 days after infection twice a day for 15 days. Seven days after treatment, the mice were killed and *T. spiralis* adults and larvae were detected. In this study, the authors were not able to determine the efficacy of albendazole to adults *T. spiralis* because adult *T. spiralis* were not detected in the control group. This is because the life span of an adult *T. spiralis* is approximately 1-2 months. How-

Table 1. Number of *T. spiralis* larvae recovered from the 7 days infected mice, 7 days after treatment with albendazole 20 mg/kg for 15 days.

Mice Number	Control (A1)	Treatment (A2)
1	18,566	0
2	18,876	0
3	18,986	0
4	20,101	0
5	20,446	0
6	21,170	0
7	21,434	0
8	22,096	0
9	22,780	0
10	23,522	0
11	-	0
12	-	0
13	-	0
14	-	0
15	-	0
Total	207,977	0
Mean ± SD	20,797.70 ± 1,704.41	0

Table 2. Number of *T. spiralis* larvae recovered from 30 days infected mice, 7 days after treatment with albendazole 20 mg/kg for 30 days.

Mice Number	Control (A1)	Treatment (A2)
1	18,636	4,244
2	19,238	4,988
3	19,868	5,120
4	20,890	5,441
5	22,128	5,667
6	22,680	5,976
7	23,146	6,233
8	23,441	6,298
9	23,763	6,882
10	25,588	6,894
11	-	7,228
12	-	7,312
13	-	7,882
14	-	7,980
15	-	8,127
Total	219,378	96,272
Mean ± SD	21,937.80 ± 2,224.47	6,418.13 ± 1,183.81

ever, the authors demonstrated that albendazole treatment at the early stage of *T. spiralis* infection gave a 100 per cent cure rate compared to the control group. Albendazole 6.25 mg/kg given to infected mice at 2, 8, 16, 24, 30, 36 and 48 hours after infection demonstrated that adult worms declined from 95 per cent, 91 per cent, 79 per cent, 66 per cent, 27 per cent, 0 per cent and 0 per cent respectively[8] and the same report also demonstrated that albendazole at a dosage of 50 mg/kg/day for 5 consecutive days given to infected mice on day 7 after infection demonstrated 67 per cent reduction of larvae compared to the control[8]. Chung et al demonstrated the efficacy of albendazole against adult *T. spiralis* (infected mice treated with albendazole 20 mg/kg for 5 days at 2 to 6 days after infection) was 46 per cent compared to the control group. They also demonstrated that the efficacy of albendazole against the migratory larvae *T. spiralis* (infected mice treated with albendazole 20 mg/kg for 5 consecutive days at 11 to 15 days after infection) with reduction rate of 80.8 per cent compared to the control[7]. A study in Swiss CD-1 mice infected with *T. spiralis* showed that the efficacy of albendazole at 10 mg/kg against pre-adult stage (day 1 after infection) was 96.5 per cent[6]. The efficacy of albendazole at 100 mg/kg against migrating larvae (day 13, 14 and 15 post infection) was 64.0 per cent reduction[8]. Efficacy of albendazole against the early stage of trichinosis depends on a number of factors including the time of administration, dosage, and duration of treatment. A single dose of 6.25 mg/kg of albendazole given to infected mice 36 hours after infection had no effect on the infection, while the present study with albendazole 20 mg/kg given to mice on day 7 after infection for 15 consecutive days demonstrated 100 per cent efficacy. A study by McCracken demonstrated that albendazole was less effective against mature worms than immature worms in the enteral phase[8]. He also demonstrated that albendazole against adult worms in the enteral phase and larvae in the parenteral phase by using albendazole at a dosage of 50 mg/kg/day for 5 consecutive days to infected mice on day 7 after infection had 67 per cent reduction of larvae[8]. In the present study the authors demonstrated that 20 mg/kg albendazole for 15 days against the early stage of *Trichinella spiralis* infection (7 days after infection) resulted in 100 per cent efficiency.

In experiment B, mice infected with *T. spiralis* were treated with 20 mg/kg (divided into 2 doses) albendazole at 30 days after infection at for 30 days showed that the number of larvae recovered from the treatment group was 71 per cent less than the control group with statistical significance ($p < 0.001$). A study by Chung et al demonstrated the efficacy of albendazole against adult *T. spiralis* (infected mice treated with albendazole 20 mg/kg for 5 days at 21 to 25 days after infection) was 45.4 per cent compared to the control group[7]. A study in Swiss CD-1 mice infected with *T. spiralis* showed that the efficacy of 100 mg/kg albendazole against encysted larvae was 94.75 per cent[6].

In conclusion, for the early stage of *T. spiralis* infection 20 mg/kg albendazole for 15 days is effective in the treatment of infection in mice. The late stage of infection was seen to be tolerant to albendazole although the duration of treatment was longer. The result was similar to a study by McCracken that the Trichinella population becomes less susceptible to treatment when the worms become mature [8]. However, a number of factors may be responsible for the efficacy of albendazole against *T. spiralis* including host status, dosage, time and duration of treatment. Data from the present study indicate that albendazole has high efficacy in both the early and late stage of *Trichinella* infection.

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REFERENCES

1. Capo V, Despommier DD, Siberstein DS. The site of ecdysis of the L1 larvae of *T. spiralis*. J Parasitol 1984; 70: 992-3.
2. Wakelin D, Denham DA. The immune response, *In* Cambell WC. (ed.), *Trichinella* and trichinosis. New York: Plenum Press; 1983: 265-308.
3. Capo V. Despommier DD. Clinical aspects of infection with *Trichinella spp.* Clinical Microbiology Reviews 1996; 9: 47-54.
4. Despommier DD, Sukheo M, Meerovitch E. *Trichinella spiralis*: Site selection of the larva during the enteral phase of the infection in mice. Exp Parasitol 1978; 44: 209-15.
5. Pozio E, Sacchini D, Sacchi L, Tamburrini A. Alberici F. Failure of Mebendazole in the treatment of Humans with *Trichinella spiralis* Infection at the Stage of Encapsulating Larvae. Clin Infect Dis 2001; 32: 638-42.
6. Lopez-Garcia ML, Torrado-Duran S, Torrado-Duran J, Martinez Fernandez AR, Bolas-Fernandez F. Albendazole *versus* ricobendazole (albendazole-sulphoxide) against enteral and parenteral stages of *Trichinella spiralis* in mice. Int J Parasitol 1997; 27: 781-5.
7. Chung MS, Joo KH, Quan FS, Kwon HS, Cho SW. Efficacy of flubendazole and albendazole against *Trichinella spiralis* in mice. Parasite 2001; 8 (Suppl): S195-8.
8. McCracken RO. Efficacy of mebendazole and albendazole against *Trichinella spiralis* in mice. J Parasitol 1978; 64: 214-9.
9. Garcia LS. Practical guide to diagnostic medical parasitology. Washington DC: ASM Press; 1999: 791-2.
10. Lacey E. The mode of action of benzimidazoles. Parasitol Today 1990; 6: 112-5.
11. Hanjeet K. Mathias RG. The efficacy of treatment of albendazole. Acta Tropica 1991. 50; 111-4.
12. Jongwutiwes S, Chantachum N, Kraivichian P, et al. First outbreak of human trichinellosis caused by *Trichinella pseudospiralis*. Clin Infect Dis 1998; 26: 111-5.

ประสิทธิผลของยาอัลเบ็นดาโซล ต่อพยาธิ *ทริคิเน็ลลา สไปรัลลิส* ในหนูถีบจักร

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การศึกษาประสิทธิผลของยาอัลเบ็นดาโซลต่อการติดเชื้อพยาธิ *Trichinella spiralis* ระยะเริ่มแรกและระยะหลังในหนูถีบจักร การทดสอบผลของยาอัลเบ็นดาโซลต่อการติดเชื้อในหนูระยะแรกทำโดยให้ยาอัลเบ็นดาโซล ขนาด 20 มก/กก ของหนู หลังจากทำให้หนูติดเชื้อแล้ว 7 วัน โดยให้ยาติดต่อกันเป็นเวลา 15 วัน หลังจากนั้นอีก 7 วันจึงทำการหาตัวอ่อนพยาธิในหนู ซึ่งพบว่าหนูที่ได้รับยานั้นไม่พบตัวอ่อนแสดงว่ามีการลดลงของตัวอ่อนพยาธิ 100% เมื่อเทียบกับหนูกลุ่มควบคุมที่ไม่ได้รับยา ส่วนการทดสอบประสิทธิผลของยาอัลเบ็นดาโซล ในระยะหลังของการติดเชื้อนั้นทำโดยให้ยาอัลเบ็นดาโซล ขนาด 20 มก/กก ในหนูที่ได้ทำให้ติดเชื้อแล้ว 30 วัน โดยให้ยาเป็นระยะเวลา 30 วันติดต่อกัน หลังจากนั้น 7 วันจึงทำการตรวจหาตัวอ่อนพยาธิในหนูซึ่งพบว่ามีการลดลงของตัวอ่อนพยาธิ 71% เมื่อเทียบกับกลุ่มควบคุม แสดงว่ายาการให้ยาอัลเบ็นดาโซลในระยะแรกของการติดเชื้อมีประสิทธิผลมากกว่าการให้ยาในระยะหลังของการติดเชื้อซึ่งทำให้ตัวอ่อนพยาธิลดลง 100% และ 71% ตามลำดับ

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