EFFICACY OF FLUBENDAZOLE AND ALBENDAZOLE AGAINST *TRICHINELLA SPIRALIS* IN MICE

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Summary :

Efficacy of flubendazole and albendazole against *Trichinella spiralis* in mice were studied. ICR mice were experimentally infected with *Trichinella spiralis* and treated with either flubendazole (FBZ) or albendazole (ABZ) at four different stages of the parasite life-cycle. Oral administration of either FBZ or ABZ at 20 mg/kg and 50 mg/kg on 2 h, 8 h and 24 h (pre-adult stage) after infection eliminated 94.7 ~ 100 % of adults as determined at necropsy on day 7 post infection (p.i.) and 96.9 ~ 100 % of larvae on day 45 p.i. FBZ was more effective than ABZ against adult *T. spiralis* (at 2 to 6 days p.i.), when treated with a dosage of 20 mg/kg for 5 consecutive days (99.4 % and 46.0 % reduction with respect to the control group). Against migrating larval *T. spiralis*, FBZ was more effective than ABZ at 20 mg/kg for five consecutive days (on days 11 ~ 15 p.i.), and the reduction rate of recovered larvae were 99.6 % (FBZ) and 80.8 % (ABZ) respectively. FBZ was more effective against early encapsulated larval *T. spiralis* (at 21 to 25 days p.i.), than ABZ when both were given at 20 mg/kg for five consecutive days (99.8 % and 45.4 % reduction, respectively). In conclusion, flubendazole was more effective than albendazole against adult and parenteral stages of *Trichinella spiralis* in mice.

KEY WORDS : *Trichinella spiralis*, flubendazole, albendazole, efficacy, mice.

Trichinellosis is one of the most widespread helminthic zoonoses. In Korea, three cases of human infection by *Trichinella spiralis* were first confirmed by detecting encysted larvae in the biopsied muscle in December 1997. The patients were treated with flubendazole and albendazole for 15-30 days (Sohn *et al.*, 2000).

Some reports have demonstrated the chemotherapeutic effectiveness of benzimidazole compounds in the treatment of *Trichinella spiralis* infection in mice (Campbell & Cuckler, 1964; Duckett & Denham, 1970; Spaldonova *et al.*, 1974; Fernando & Denham, 1976; Lopez-Garcia *et al.*, 1997). It was demonstrated that mebendazole was highly effective against the immature enteral phase of trichinosis in mice (Fernando & Denham, 1976; McCracken, 1978). McCracken (1978) reported that albendazole was much more effective against pre-adult and had a partial effect on adult

T. spiralis in mice. Lopez-Garcia *et al.* (1997) compared the effect of albendazole and ricobendazole (albendazole sulphoxide) against enteral and parenteral stages of *T. spiralis* in mice. However, there has been few reports that effectiveness of flubendazole against different stages of *Trichinella spiralis* in mice.

The objective of this study was to evaluate the effect of two benzimidazole compounds, flubendazole (FBZ) and albendazole (ABZ) on different stages in the life cycle of *Trichinella spiralis* infection in mice.

MATERIALS AND METHODS

PARASITE

Yamagata strain of *Trichinella spiralis* was used for the experiments. This strain has been maintained in our laboratory by passage through rat since 1993.

EXPERIMENTAL ANIMALS

Female ICR mice weighing 25-30 g were used. Each group was consisted of six animals which was orally infected with 250 larval *T. spiralis*.

DRUGS AND TREATMENTS

Infected mice were treated with either flubendazole (FBZ) or albendazole (ABZ) at four different stages of the parasite life-cycle: pre-adult, adult, migrating larvae and early encapsulated muscle larvae. Oral administration of either FBZ or ABZ at 20 mg/kg and 50 mg/kg on 2 h, 8 h and 24 h (pre-adult stage), 72 h (adult stage), and 20 mg/kg for five consecutive days (adult, migrating and early encapsulated larval stages).

RECOVERY OF WORMS

The number of enteral *Trichinella* in each group of mice was estimated seven days after exposure to infection. At necropsy, each mouse was examined individually for adult worms from gut. All the mice treated with drugs against migrating and early encapsulated muscle larval stage were killed 45 days post infection (p.i.) and the number of diaphragm larvae was counted.

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RESULTS

EFFECT OF THE DRUGS ON PRE-ADULT AND ADULT STAGE *T. SPIRALIS*

Consistent with the earlier report of McCracken (1978), albendazole was highly active against the worms in the immature enteral phase of trichinellosis. A single oral dose of 20 and 50 mg/kg 2 h or 8 h after inoculation totally eradicated the infection as determined by examination of the gut for adult worms on day 7 p.i. (Tables I, II). However, as worms matured, their susceptibility to ABZ diminished; the

same dosages were only partially active at 24, 48 h p.i. (Tables I, II).

FBZ showed similar anthelmintic activity against the worms in intestinal phase. When the infection was 8 h old, a single dose of 20 and 50 mg/kg removed all of the *Trichinella* as determined at necropsy seven day later (Table III, IV). At 48, 72 h after inoculation, activity was not completely curative, at both dosages (Table III, IV). FBZ and ABZ were more effective against pre-adult *Trichinella* than adult worms. When infected mice were treated with the dosage of 20 mg/kg for five consecutive days, there was significant difference in the reduction in worm burden bet-

		Adult*		Larvae**	
		Mean No. worms/mouse	Efficacy of treatment	Mean No. worms/mouse	Efficacy of treatment
control mice		126.5 ± 21.5	—	1,022 ± 17	—
treated mice	2 h	0	100 %	0	100 %
	8 h	0	100 %	0	100 %
	24 h	0	100 %	0	100 %
	48 h	93 ± 9.2	26.5 %	128 ± 5.5	87.5 %
	72 h	59.7 ± 6.1	52.8 %	53 ± 4.5	94.8 %

* Collection at 7 dpi in intestine.

** Collection at 45 dpi in 0.1 g of diaphragm.

Table I. – Number of *T. spiralis* worms recovered from the intestine and diaphragm of mice treated with 20 mg/kg single dose of albendazole at various time after infection.

		Adult*		Larvae**	
		Mean No. worms/mouse	Efficacy of treatment	Mean No. worms/mouse	Efficacy of treatment
control mice		126.5 ± 21.5	—	1,022 ± 17	—
treated mice	2 h	0	100 %	0	100 %
	8 h	0	100 %	0	100 %
	24 h	0.67 ± 0.47	99.5 %	0	100 %
	48 h	20 ± 7.8	84.2 %	29.3 ± 13.9	97.2 %
	72 h	41 ± 21	67.6 %	12.7 ± 11.5	98.7 %

* Collection at 7 dpi in intestine.

** Collection at 45 dpi in 0.1 g of diaphragm.

Table II. – Number of *T. spiralis* worms recovered from the intestine and diaphragm of mice treated with 50 mg/kg single dose of albendazole at various time after infection.

		Adult*		Larvae**	
		Mean No. worms/mouse	Efficacy of treatment	Mean No. worms/mouse	Efficacy of treatment
control mice		126.5 ± 21.5	—	1,022 ± 17	—
treated mice	2 h	2 ± 1.6	98.4 %	0	100 %
	8 h	0	100 %	0	100 %
	24 h	6.67 ± 5.2	94.7 %	32.3 ± 3.4	96.9 %
	48 h	46 ± 12.7	63.6 %	56.5 ± 36.5	94.5 %
	72 h	78.5 ± 8.5	37.9 %	34.3 ± 4.0	96.7 %

* Collection at 7 dpi in intestine.

** Collection at 45 dpi in 0.1 g of diaphragm.

Table III. – Number of *T. spiralis* worms recovered from the intestine and diaphragm of mice treated with 20 mg/kg single dose of flubendazole at various time after infection.

		Adult*		Larvae**	
		Mean No. worms/mouse	Efficacy of treatment	Mean No. worms/mouse	Efficacy of treatment
control mice		126.5 ± 21.5	—	1,022 ± 17	—
	2 h	0	100 %	0	100 %
treated mice	8 h	0	100 %	0	100 %
	24 h	0.33 ± 0.47	99.7 %	0	100 %
	48 h	1 ± 1.41	99.2 %	23 ± 8.4	97.7 %
	72 h	16 ± 4	87.3 %	5.3 ± 1.2	99.5 %

* Collection at 7 dpi in intestine.

** Collection at 45 dpi in 0.1 g of diaphragm.

Table IV. – Number of *T. spiralis* worms recovered from the intestine and diaphragm of mice treated with 50 mg/kg single dose of flubendazole at various time after infection.

	Adult*			Larvae**		
	Control	ABZ	FBZ	Control	ABZ	FBZ
Individual counts	98 126 136 118 106 130	91 43 70 64 72 44	1 0 1 1 1 0	2,131 2,308 1,221 1,267 1,547	98 218 61 131 126	2 0 0 0 1 0
Mean	118.5	64.3	0.67	1,694.8	126.8	0.5
Efficacy of treatment		46.0 %	99.4 %		92.5 %	99.97 %

* Collection at 7 dpi in intestine.

** Collection at 45 dpi in 0.1 g of diaphragm.

Table V. – Number of *T. spiralis* worms recovered from the intestine of mice treated with albendazole and flubendazole at a dosage of 20 mg/kg/day for 5 consecutive days (2 ~ 6 days pi; adult stage).

ween FBZ (99.4 %) and ABZ (46.0 %) (Table V). These results prompted a further examination of the changes in drug susceptibility during the parasite's period of maturation in the gut.

EFFECT OF THE DRUGS ON MIGRATING AND EARLY ENCAPSULATED LARVAL STAGE *T. SPIRALIS*

Despite the decline in drug sensitivity, two drugs were further tested for effectiveness against the invasive phase of trichinellosis because of its special clinical importance. Results are tabulated in Table VI. Mice treated for five days with FBZ yielded a mean of 7.3 larvae, which is 99.6 % less than the control mean of 1,827.5. The average number of larvae in the mice treated with ABZ was 351.4, representing a reduction of 80.8 % as compared to the untreated controls (Table VI).

To determine the efficacy of drugs against early encapsulated muscle larvae, six mice received FBZ and other group of six mice ABZ at 20 mg/kg on each day from the 21st day p.i. during five successive days. All the mice were killed 20 days after the last day of treatment and the number of larvae from diaphragm were

counted. FBZ was more effective than ABZ; 99.8 % and 45.4 % reduction, respectively (Table VII).

Results also show that when given as a series of oral doses, FBZ is more efficacious against the invasive phase than ABZ.

	Larvae		
	Control	ABZ	FBZ
Individual counts	1,622 1,278 1,521 2,458 2,308 1,778	384 405 449 218 301	5 30 3 6 0 0
Mean	1827.5	351.4	7.3
Efficacy of treatment		80.8 %	99.6 %

Table VI. – Number of *T. spiralis* larva recovered at 45 dpi from the diaphragm of mice treated with albendazole and flubendazole at a dosage of 20 mg/kg/day for five consecutive days (11 ~ 15 dpi; migrating larval stage).

	Larvae		
	Control	ABZ	FBZ
Individual counts	1,622	513	0
	1,278	839	18
	1,521	1,662	0
	2,458	998	0
	2,308	979	3
	1,778		0
Mean	1827.5	998.2	3.5
Efficacy of treatment		45.4 %	99.8 %

Table VII. – Number of *T. spiralis* larva recovered at 45 dpi from the diaphragm of mice treated with albendazole and flubendazole at a dosage of 20 mg/kg/day for five consecutive days (21 ~ 25 dpi; early encapsulated muscle larval stage).

DISCUSSION

The *Trichinella*/mouse model has been widely used to assay anthelmintic effectiveness of benzimidazole derivatives (Campbell & Blair, 1974; Fernando & Denham, 1976; McCracken, 1978; McCracken & Taylor, 1980; McCracken *et al.*, 1984; Lopez-Garcia *et al.*, 1997). The present findings, together with the reports cited earlier, indicate that members of this chemical class are much more effective against the pre-adult than adult worm.

In the present study, FBZ was much more active in eliminating adult *T. spiralis*. ABZ, on the other hand, showed a partial effect on adult worms. However, all these differences may be dependent as Campbell & Cuckler (1964) with thiabendazole and Duckett & Denham (1970) with cambendazole found that high level or long term administration of compound caused complete elimination of adult worms. A sex difference was reported for piperazine, which was said to be more potent against male *Trichinella* than against female (Campbell & Blair, 1974). Although adult worms were less removed, larvae were highly reduced. FBZ and ABZ were more effective against female than male (data not shown). Thiabendazole was reported to cause complete cessation of larval production by female *T. spiralis* in mice (Campbell & Cuckler 1964). The related compound cambendazole was also reported to suppress larval production (Duckett & Denham, 1970). Benzimidazoles acted on parenteral forms *Trichinella* (Campbell & Blair, 1974). Despite a low degree of absorption from the gut, mebendazole was highly active against parenteral as well as enteral forms of *Trichinella* in rats (Campbell & Blair, 1974) and mice (McCracken, 1978). McCracken (1978) demonstrated that gavage administration of either mebendazole or albendazole during the invasive phase of *Trichinella* infection significantly reduced by 96 % and 67 %, respectively, the number of

larvae recovered from the host musculature. In this paper, administration of flubendazole at migrating larval *T. spiralis* reduced the number of larvae obtained from diaphragm in mice by 99.6 %. It was reported that mebendazole and albendazole were highly effective against encysted larvae (Fernando & Denham, 1976; Lopez-Garcia *et al.*, 1997). Administration of mebendazole, chemically similar to flubendazole, against encysted larval *Trichinella* reduced the larvae recovered from the host muscle by 99.7 % (Fernando & Denham 1976). In the present study, oral administration of FBZ eliminated 99.8 % of larvae on day 45 p.i. In conclusion, flubendazole was more effective than albendazole against adult and parenteral stages of *T. spiralis* in mice.

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