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Research Note

Study of the reproductive capacity of *Trichinella spiralis* recovered from experimentally infected mice under-dosed with albendazole or mebendazole

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Abstract. The reproductive capacity of *Trichinella spiralis* recovered from experimentally infected mice under-dosed with albendazole (ALB) or mebendazole (MEB) was studied. Different groups of male C57/BL mice were infected with 10 ± 0.5 muscular larvae (ML) per gram of body weight and treated with a single dose by oral (20 mg/kg) of ALB, MEB or praziquantel (PZQ) given at 5th day post infection (DPI), during the intestinal phase of infection. In other group of mice, treatment with the same drugs and dosage was for seven days, starting at day 45 PI through the stage of encapsulating larvae (parenteral phase of infection). A reduction of 72.9 to 89.9% in the parasitic load was observed in ALB or MEB treated groups but not in mice untreated or administered with PZQ. The recovered larvae were used to infect naïve mice and, after 45 DPI, a similar Reproductive Capacity Index (RCI) was observed between the different groups (P=0.323, one-way ANOVA), either from mice infected with larvae recovered from the intestinal treatments (RCI-ALB = 51.6 ± 12.1 and RCI-MEB = 49.2 ± 14.) or from the parenteral ones (RCI-ALB = 52.2 ± 14.0 and RCI-MEB = 51.9 ± 11.8). The RCI of non-treated ML was 59.5 ± 7.7 and 57.9 ± 15.9 for PZQ. This information is significant for practical strategies when under-dosage is dispensed.

To prevent and control parasitic diseases, many strategies have been improved and one of the most popular is the dispensation of antiparasitic drugs, which are highly effective when complete therapeutic dosage is administered. To treat enteric helminth parasites, the most commonly used regimens of benzimidazolic derivatives are 100 – 200 mg twice a day for three days or 400 – 500 mg in a single dose, while to treat tissue helminthes, dosage must be daily administered for two or three weeks (de-Silva *et al.*, 1997). However, it is common when the given treatment does not reach therapeutic levels, and a number of factors contribute to this under-curative dosage, resulting in treatment failure, as seen in the single dose massive treatment campaigns for morbidity control (van Wyk *et al.*, 1997; Geerts & Gryseels, 2000). The factors are: drugs are used in a lower recommended dose, generic products of the drugs are of substandard quality and, drugs are inappropriately repacked or reformulated, expired and of poor-quality. As a result of under-dosing, some parasites can be expected to survive treatment. Studies show that muscular larvae (ML) of *Trichinella spiralis* survive antiparasitic

treatment, such as benzimidazole derivatives, and remain infective to naïve mice (Pozio *et al*., 2000; Marinculic *et al*., 2001; Casulli *et al*., 2006). However, the reproductive capacity of the surviving worms is not sufficiently elucidated by early workers to support the concept of under-dosing. Thus, the aim of this work was to study the reproductive capacity of *T. spiralis* recovered from experimentally infected mice, under-dosed with albendazole (ALB) or mebendazole (MEB).

Male C57/BL mice (6 to 8 weeks old) were maintained in our animal facilities and infected with 10 ± 0.5 ML (strain MSUS/ME/92/CM-92) per gram of body weight. The mice were distributed into 5 experiments; details for the experimental trials are described in Table 1. All used anthelmintics were administered orally. Besides the untreated group as negative control in the experiments, another control group was added: mice administered with PZQ, since it is known that PZQ is highly effective in eradicating cestodes but not nematodes. Newborn larvae (NBL) were obtained from adult worms (AW) recovered from infected mice and maintained in culture media for 18 hours. The ML were recovered by conventional pepsin-HCl artificial digestion of minced mice carcasses.

Results were expressed as reduction rates, calculated as the percent of recovered worms versus those recovered from control mice. For statistical analysis of data, the worm recovery index (WRI) was calculated by dividing the

Table 1. Experimental trials for the treatment with albendazole (ALB), mebendazole (MEB), and praziquantel (PZQ) of mice infected with *Trichinella spiralis*

Experiment number	Source of the Infecting ML	Groups (and dose) (mice per group, n= 9)	Target for treatment (DPI)	Stage of worm recovered (DPI)
1	Non-treated previously infected mice	ALB (20 mg/kg/1day) MEB (20 mg/kg/1day) PZQ (20 mg/kg/1day) WTR	AW (5)	AW (7)
2	Non-treated previously infected mice	ALB (20 mg/kg/1day) MEB (20 mg/kg/1day) PZQ (20 mg/kg/1day) WTR	AW (5)	ML (45)
3	Non-treated previously infected mice	ALB (20 mg/kg/7 days) MEB (20 mg/kg/7 days) PZQ (50 mg/kg/7 days) WTR	ML (45)	ML (60)
4	Experiment 2 ALB	— *	—	ML (45)
	Experiment 2 MEB	—	—	ML (45)
	Experiment 2 PZQ	—	—	ML (45)
	Experiment 2 WTR	—	—	ML (45)
5	Experiment 3 ALB	—	—	ML (45)
	Experiment 3 MEB	—	—	ML (45)
	Experiment 3 PZQ	—	—	ML (45)
	Experiment 3 WTR	—	—	ML (45)

DPI: days post infection, AW: adult worm, ML: muscular larvae, WTR: water.
ALB, MEB and PZQ were commercially acquired from Laboratorios Hormona, Laboratorios A. F. and Merck respectively, all from Mexico City.
— * None

corresponding number of recovered parasites by the number of ML given for infection (Fernandez & Wakelin, 1989). In addition, to establish the relationship between the anthelmintic effect and the treatment starting day, data from experiments 2 and 3 (Table 1) were analyzed with the factorial ANOVA test and the reproductive capacity of surviving larvae (experiments 4 and 5) with the one way ANOVA test (Dawson-Saunders and Trapp, 1994).

Results of the experiments carried out to determine the action of the benzimidazole derivatives on the target developmental stages of parasite are summarized in Table 2. As expected, there was a reduction in the parasite densities at all stages of infection, i. e., at day 5 PI (intestinal, experiments 1 and 2) or at 45 DPI (parenteral, experiment 3, see Table 1). However, analysis of data with the factorial ANOVA test, showed no significant differences (P = 0.733) in relation to the effect of the drug and the day when the treatment was initiated. In addition, no NBL were obtained from AW recovered from infected mice treated during the intestinal phase of infection with ALB or MEB. In contrast, AW from control mice treated during the muscular phase of infection with praziquantel (a drug with very low or without effect on intestinal nematodes) or water, released similar number of NBL (P = 0.237, Student's t test; WRI for PZQ = 0.437 ± 0.122 and WRI for WTR = 0.402 ± 0.127). To determine if the reproductive capacity of *Trichinella* recovered from the experiments 2 and 3 was affected, naïve mice were infected with the surviving larvae and no additional treatment was applied (experiments 4 and 5, Table 1). After 45 days PI, mice of experiments 4 and 5 were found infected and the differences on the reproductive capacity were not statistically significant, according to the one-way ANOVA test (P = 0.122; Figure 1).

Notwithstanding that a single dose of ALB or MEB eliminated the 70 – 80% of AW and an under-curative dose at muscular phase eliminated almost 90% of the ML, a small proportion of surviving parasites was recovered and they were found to be infective to naïve mice. A number of

Table 2. Results of the albendazole (ALB), mebendazole (MEB), and praziquantel (PZQ) treatment on *Trichinella spiralis* developmental stages

Experiment	Group	Starting day of treatment	Reduction Rate (%)		
			Adult Worms	Newborn larvae	Muscle larvae
1	ALB	5	72.9	100	ND
	MEB		80.6	100	ND
	PZQ		17.2	0.0	ND
	WTR[a]		0.0	0.0	ND
2[b]	ALB	5	ND	ND	89.9
	MEB		ND	ND	89.7
	PZQ		ND	ND	19.3
	WTR		ND	ND	0.0
3[b]	ALB	40	ND	ND	75.5
	MEB		ND	ND	82.8
	PZQ		ND	ND	4.4
	WTR		ND	ND	0.0

[a] WTR: water
[b] Factorial ANOVA test, p = 0.733
* ND: Not done

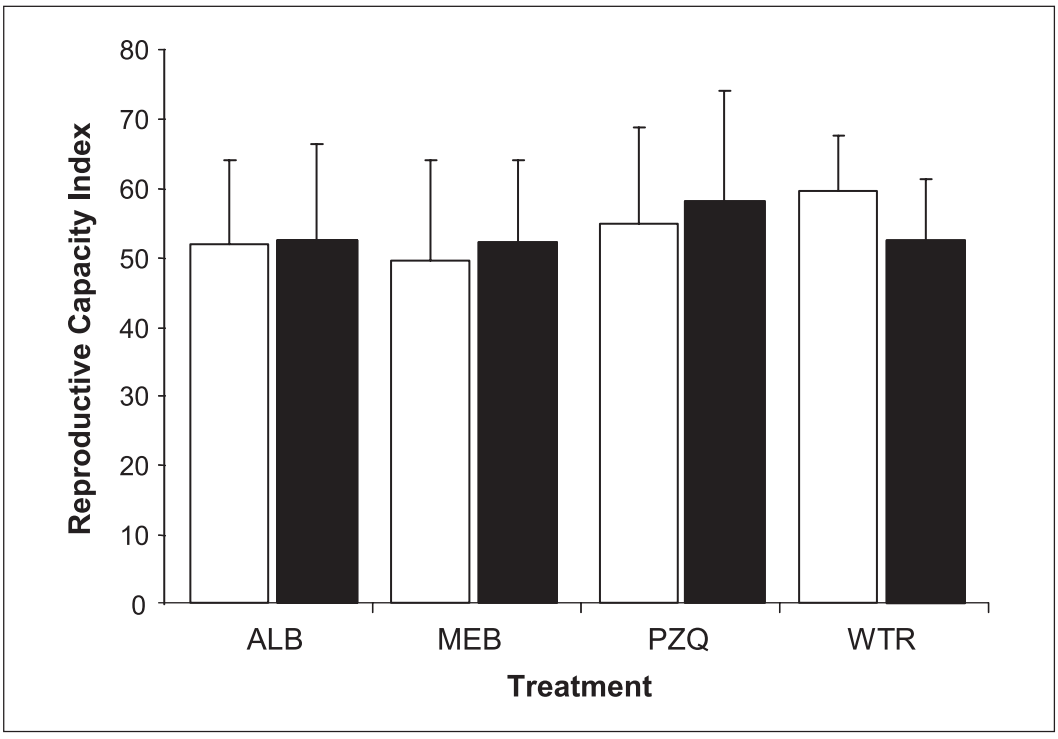

Figure 1. Reproductive capacity of *Trichinella spiralis* muscle larvae recovered after anthelmintic treatment. Larvae recovered from mice experimentally infected and treated with a specific anthelmintic were used to infect naïve mice. Albendazole (ALB), mebendazole (MEB), praziquantel (PZQ) or water (WTR) were given to donors animals at day 5 (open bars) or 45 (close bars) post-infection and larvae were recovered, respectively, at days 45 and 60 post infection. The reproductive capacity index was calculated as the number of ML recovered over the number of ML given as infection.

previous studies have shown that *Trichinella* surviving treatment with benzimidazole derivatives, such as ALB, MEB, or flubendazole, remained infective to naïve mice (Pozio *et al*., 2000; Cheng *et al*., 2001; Marinculic *et al*., 2001; Casulli *et al*., 2006) but the reproductive capacity of the larvae recovered after treatment showed a decrease as reported by Marinculic *et al*. (2001) and Casulli *et al*. (2006). In relation to this, Campbell & Cuckler (1964) reported that larvae surviving to sublethal doses of thiabendazole were not infective to other mice and the larvae harvested from the digested muscles of untreated mice were not killed after immersion in equivalent concentrations of thiabendazole hydrochloride solutions *in vitro*. Our results are in contrast with those previously published because we find that larvae surviving to anthelmintic treatment are able to preserve their reproductive capacity as well as infect new hosts. Consequently, these parasites are able to complete their life cycle with intact fecundity. In this study, AW recovered from mice treated with ALB or MEB at day 5 PI and sacrificed two days later (experiment 1) had inhibited release of NBL, but in experiment 2 with similar treatment, the mice sacrificed at 45 days PI, had ML. Therefore, the results of this study indicate that some worms can evade the effect of the drug when it is treated under-dosage. Although, it is not easily understood how worms escape the effect of the drug, it could be hypothised that the differences in the quantity of drug intake by the worm, both AW and ML due to under-dosing are inadequate to eliminate the parasite. An alternative hypothesis could be that under-

dosage has a temporary effect on the parasite reproductive process which disappears when the drug is cleared from the system. A third hypothesis could be that worms are resistant to ALB and MEB since resistance to antiparasitic drugs has been widely suggested (Geerts & Gryseels, 2000; Torres-Acosta *et al*., 2003). Although several other hypotheses can be suggested, there is a need for additional studies to establish the possible mechanisms supporting parasite evasion and the effect of anthelmintic drugs when given under-dosage.

Though under-dosage is considered to be a useful strategy to control morbidity, caution must be in place as surviving parasites, as shown in this study, are able to preserve their reproductive capacity. This factor in combination with natural wild-types could result in an anthelmintic resistant worm population over a period of time (Geerts & Gryseels, 2000).

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