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Anthelmintic efficacy of tinidazole against the progression of *Toxocara canis* larvae to the brain in mice

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Abstract The anthelmintic effect of tinidazole (100 mg/kg per day for 3 successive days) was tested in male Swiss *CF-1* mice infected with second-stage *Toxocara canis* larvae at challenge doses of 250, 500, 1000, and 1500 embryonated eggs per mouse. The drug was given orally on days 3–5 postinfection (p.i.) to one-half of the animals, and all mice were killed on day 40 p.i. The number of larvae recovered from each mouse's brain and skeletal muscle was then scored in both groups. Tinidazole yielded a highly significant reduction in the total recovery of larvae from the test animals' brains at the second and third inoculum levels but no statistically significant reduction at the highest larval dose as compared with the values obtained in the untreated control animals.

Infection with *Toxocara canis* is common and widespread in dogs and is especially dangerous in puppies (Mackenstedt et al. 1993). Humans become infected by inadvertent ingestion of eggs containing second-stage *T. canis* larvae, which may subsequently spread throughout the body.

A murine experimental model for the study of toxocariasis is especially useful since the course of the infection in mice happens to resemble quite closely the clinical picture in humans. Thus, working with such a system, we and other investigators have found that

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Among the drugs potentially effective for the treatment of human toxocaral disease, only the benzimidazoles and diethylcarbamazine have been investigated in well-controlled studies (Magnaval et al. 1991; Magnaval 1995). Nevertheless, Mackenstedt et al. (1993) have found pyrantel pamoate to be a most effective drug against not only Toxocara spp. but also other helminths in domestic animals. However, since conventional anthelmintics have little activity, if any, against systemically migrating larvae, a single-dose therapy with such drugs however efficacious against intestinal forms of T. canis they might be - is likely to produce only a transient benefit (Jacobs 1987). Accordingly, a number of workers have studied the effects of several anthelmintic drugs in either human or murine systems using different therapeutic procedures (Casarosa and Lugetti 1982; Delgado et al. 1989; Stürchler et al. 1989; Cuellar et al. 1990; Bardon et al. 1995a).

Tinidazole belongs to the nitroimidazole group of antimicrobial agents. The small molecular size and low extent of protein binding of nitroimidazoles favor their distribution throughout the body. Moreover, tinidazole is one of the more lipophilic members of this group (Pyörala et al. 1990). It is bactericidal at low concentrations and has been shown to be effective in trichomonal vaginitis, amebiasis, and giardiasis (Nord and Kager 1983; Martindale 1989; Atias and Werner 1991).

In our experiments, pure tinidazole (1,2-ethylsulfonylethyl-2-methyl-5-nitroimidazole; Elea Laboratories S.A.C.I.F.Y.A., Argentina) was used at a total dose of 300 mg/kg, with 100 mg/kg being given daily on each of days 3–5 p.i. In all, 120 male Swiss *CF-1* mice of 25–30 g average weight, obtained from the Central Laboratory of Public Health of the Province of Buenos Aires, Argentina, were divided into 10 equal groups. All animals were maintained in a temperature- and humidity-controlled environment and were given water and food ad libitum. Mice were infected orally with a suspension of embryonated eggs (Niedfeld et al. 1993).

Tinidazole was also given orally in a 1% (w/v) dextrose solution according to the following dose and treatment schedule. Group 1 consisted of control mice subjected to nothing more than the standard conditions of the vivarium. Group 2 were likewise left uninfected but were thereafter exposed to the same tinidazole dose given to the experimental animals for the screening of possible independent side effects and toxicity of the compound. Groups 3A, 4A, 5A, and 6A comprised mice infected with 250, 500, 1000, and 1500 embryonated eggs, respectively. The corresponding groups 3B-6B were inoculated in the same manner in parallel but were treated with tinidazole on days 3-5 p.i. We gave the agent on those specific days since the larvae would have emerged from the liver by that time but would not have yet begun the neurotropic phase of the infection (Bardon et al. 1995a, b). All mice were killed on day 40 p.i. Brains were removed and quartered by means of an initial longitudinal incision through the corpus callosum followed by a transverse division of the two resulting sections roughly into halves. After preparation of a tissue squash of each quarter between a microscope slide and a coverslip the specimens were scored for larvae by light microscopy at a magnification of 150×. Skeletal muscles were screened for larvae after the peptic digestion (1%, w/v, at pH 1.5) of Oshima (cf. Basualdo Farjat et al. 1995). Larvae counts (log-transformed) in the brain were compared by analysis of variance (one-way ANOVA) through the use of computer software (SAS version 6, SAS Institute, Cary, NC). Values of P < 0.05were considered statistically significant. Results are presented as mean values \pm SEM.

On day 40 p.i. we recovered *T. canis* larvae from the brains of all mice that had been infected with embryonated eggs (groups 3–6, Table 1), whereas we found no larva in the skeletal muscles of the same animals. However, despite the migration of helminth larvae into

Table 1 Number of *Toxocara canis* larvae recovered from the brains of infected mice receiving either tinidazole chemoterapy (*B*) or no treatment at all (*A*). Data were evaluated by one-way AN-OVA, with *P* values of <0.05 being considered significant (*NS* Not significant, \bar{X} geometric mean of *T. canis* larvae recovered, *SEM* standard error of the mean)

$\begin{array}{l} \text{Group} \\ (n = 12) \end{array}$	Inoculum (eggs per mouse)	Number of larvae per mouse brain		Р
		$A \over \bar{X} \pm SEM$	$\frac{\mathbf{B}}{\bar{X}} \pm \mathbf{SEM}$	
1	0	_	_	_
2	0	-	_	_
3	250	$6.99~\pm~2.7$	1.96 ± 3.7	0.01
4	500	$20.46~\pm~4.5$	$3.96~\pm~3.7$	0.01
5	1000	$22.33~\pm~4.41$	$3.29~\pm~2.9$	0.001
6	1500	$38.28~\pm~3.5$	$23.98~\pm~2.2$	NS

the brain, these parasitized mice exhibited neither loss of weight nor obvious neurologic symptoms during the course of the experiment. The animals in groups 1 and 2, which received either no experimental manipulation at all or tinidazole injections in the absence of T. canis infection, manifested no abnormality whatsoever.

Under these experimental conditions, tinidazole displayed anthelmintic action, with the dose used for this study (100 mg/kg per day for 3 days) being especially effective (statistically significantly) in infections with 500 or 1000 embryonated eggs per mouse (groups 4 and 5, respectively; Table 1). Even at the inoculum of 1500 helminth eggs per animal, tinidazole treatment reduced the number of *T. canis* larvae reaching the brain, although this decrement failed to attain statistical significance (Table 1).

The anthelmintic action of tinidazole on *T. canis* observed in this study might occur through alterations in the metabolic activity of this second larval stage after exposure to the drug. Tinidazole penetrates cell membranes and its accumulation is mediated by a conversion of the parent molecule to reactive metabolites that are responsible for its activity (Nord and Kager 1983). The action of tinidazole on toxocariasis, however, has not yet been reported.

We conclude that the administration of tinidazole to experimental mice challenged with up to 1000 embryonated *T. canis* eggs per animal was highly effective in arresting the progression of larvae to the neurotropic phase of infection at the single dose level tested. In this regard, the failure of the drug to effect a statistically significant reduction in the cerebral larval count of mice receiving an inoculum of 1500 embryonated eggs probably resulted from the high parasitic burden of these animals. Under such circumstances, a higher dose of tinidazole within the same therapeutic protocol may well have been more effective.

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