

ACTIVITY OF PRAZIQUANTEL AGAINST HYMENOLEPIS NANA, AT DIFFERENT DEVELOPMENT STAGES, IN EXPERIMENTALLY INFECTED MICE

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SUMMARY

Single doses of praziquantel were administered by oral route, at various time intervals, following the experimental infection of mice with *Hymenolepis nana* eggs (2000 per animal), to investigate the drug action against different development stages of the parasite. It was shown that either 25 or 50 mg/kg given on the 4th day after inoculation had just a partial effect against the cysticercoids. Moreover, 25 mg/kg given on the 7th day was not able to kill all juvenile forms as well. However, this dose administered on the 10th day, when the parasites had reached maturity but oviposition was not yet initiated was 100% efficacious. The same degree of efficacy was achieved with the administration of 25 mg/kg on the 14th day when the fully mature worms already lay eggs. These animal findings indicate that in the treatment of human hymenolepiasis praziquantel, 25 mg/kg, should be taken twice, 10 days apart, so that the second dose kills the larval and juvenile forms which have survived the first one. This should be particularly recommended for treating *H. nana* infection in close communities.

INTRODUCTION

The experimental investigations carried out by THOMAS & GÖNNERT¹⁴ with praziquantel in the treatment of experimental *H. nana* infection in mice have proved the efficacy of this new anthelmintic agent. The authors also have demonstrated that the sensitivity of the parasite varied according to its development stage. The two-day-old parasites are therapeutically less sensitive to a single dose (5 mg/kg) administration than the four-, five- and ten-day-old ones, as shown by the following data.

Moreover, THOMAS¹³ administering 500 mg/kg against cysticercoids 24 and 48 hour old, encysted within the intestinal tissue of rats, achieved an efficacy not higher than 25 and 73%, respectively. At this stage, the larvae do not show any organic differentiation but in

Age of the parasites after infection	Parasite free animals	Number of parasites per mouse	Parasite reduction in %
	Total No. of animals		
2 days	1 / 3	0.6	95.0
5 "	2 / 3	0.3	98.0
10 "	6 / 6	0.0	100.0

proportion that they become developed, their sensitivity to the drug increases. After 72 hours the final stages of development of the scolex and suckers take place. At this time, as well as after 96 hours when the larva leaves the intestinal tissue, it is practically 100% sensitive to praziquantel, 100 and 25 mg/kg single dose, respectively.

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Several clinical trials undertaken in this Country have confirmed the high therapeutic action of this drug in human hymenolepiasis. Nevertheless, the reported cure rates show a relative wide range of variation: BORUCHOWSKI³ 80.0%; PEDRO et al.¹⁰ 82.1%; CARVALHO et al.⁵ 88.2%; CAMILLO-COURA et al.⁴ 88.4%; LOUZADA et al.⁹ 97.0%; FERNANDES et al.⁶ and BARANSKI et al.¹ 100%.

REZENDE¹¹ who reviewed the world-wide clinical experience with praziquantel in hymenolepiasis reports an overall cure rate of 93.7% (663/706) with a single oral dose of 25 mg/kg.

Furthermore, considering the frequency of this cestodiasis in close communities, ROCHA et al.¹² tried to control it through repeated administrations of praziquantel, 25 mg/kg, every second month in a boarding school comprising 145 individuals in which its prevalence was 25%. Although all stool examinations 7 and 14 days after treatment were negative, it was not feasible to eradicate the helminthiasis as its incidence persisted at about 8% in the controls performed at two month intervals during the follow-up period of six months.

On the other hand, FERREIRA et al.⁷ concerned about the occurrence of *H. nana* infection amongst mice kept in laboratory vivarium, administered praziquantel, 15 mg/kg S.C., to 450 animals, all of them lodging this parasitosis. The first examination on the 7th day after the drug administration revealed that 104 mice (23%) had not been cured. They were retreated and at the second examination, one week afterwards, all animals were free from the infection.

Another important aspect refers to the biological cycle of *H. nana*. Once the eggs are ingested by man, the released hexacanth embryo penetrates into the intestinal tissue at the terminal ileum portion. There, the larva grows up into the juvenile stage within four to five days. Then it leaves the tissue to remain fixed at the intestinal wall where it develops into a mature worm in about six to eight days. Approximately on the 12th to the 14th day it starts to lay eggs. Since man is thus, at the same time, the intermediary and definitive host of *H. nana*, the possibility of auto-reinfection exists, which explains the frequent finding of cases with heavy worm burden, as well as the high incidence of this parasitosis in children.

Consequently, taking into consideration, one had, that the sensivity of *H. nana* to praziquantel varies according to its development stage and, on the other hand, that man can lodge, simultaneously, all evolutionary forms of the worm, we have designed this series of experimental investigations to determine the most suitable day after the infection for administering the drug, aiming at a definitive cure of this parasitosis.

MATERIAL AND METHODS

A total of 62 allogenic albino mice were experimentally infected by oral route, inoculating each one with 2000 eggs of *H. nana*. The animals were then distributed into six groups comprising one untreated control and five (A, B, C, D, E) treated with praziquantel at different time intervals after inoculation. Table I shows the group distribution and in Fig. 1 the experimental design is schematically displayed. The 50 mg/kg dose was administered in one group to find out how the cysticeroids, the most resistant forms, would be affected by double the usual dose recommended for treating human hymenolepiasis. The drug, after being emulsioned in water, was administered through an intragastric tube.

We used the Willis' method, the most suitable one, in our own experience, for detecting eggs of *H. nana*. The stools were examined daily from the 8th till the 19th day following the infection. Cysticeroids were investigated using the Hunningen's technique.

At successive periods of time, varying for each group as well as 20 days after the infection, the animals were sacrificed in searching for the presence of parasites at different evolutionary stages. We counted the number of cysticeroids and examined the larvae by compressing the intestinal slice in between two slides. Concommitantly, it was tried to determine their vitality or to find out structural modifications which could indicate a drug effect.

We looked for juvenile and adult worms attached to the intestinal wall or free inside the lumen by cutting along the gut longitudinally and examining it carefully. Morphological changes in the main organs — scolex, suckers, acculeum, collum, proglottis and eggs were also investigated.

T A B L E I
Distribution of groups

Group	Number of mice	Treatment with praziquantel		Scope of the experiment
		Dose	Day after infection	
A	10	50 mg/kg	4th day	To investigate the effect of two different doses upon the cysticercoids of <i>Hymenolepis nana</i>
B	10	25 mg/kg	4th day	
C	10	25 mg/kg	7th day	To investigate the drug action upon the juvenile forms
D	10	25 mg/kg	10th day	To investigate the drug action on the parasites which have reached maturity but have not yet initiated oviposition
E	10	25 mg/kg	14th day	To investigate the drug action on the fully matured worms which are already laying eggs
Control	12	No treatment		To observe the normal development of the parasites for comparison with those affected by the drug

T A B L E II
Results of the daily stool examination

Day after the infection	Groups					
	Control	A	B	C	D	E
	No PZQ Administration	PZQ ON The 4th day 50 mg/kg	PZQ ON The 4th day 25 mg/kg	PZQ ON The 7th day 25 mg/kg	PZQ ON The 10th day 25 mg/kg	PZQ ON The 14th day 25 mg/kg
8 th	0	0	0	0	0	0
9 th	0	0	0	0	0	0
10 th	0	0	0	0	0	0
11 th	0	0	0	0	0	0
12 th	+	0	0	0	0	+
13 th	+	0	0	0	0	+
14 th	+	0	+	0	0	+
15 th	+	0	+	+	0	0
16 th	+	+	+	+	0	0
17 th	+	+	+	+	0	0
18 th	+	+	+	+	0	0
19 th	+	+	+	+	0	0
20 th Autopsy	244 100%	39 16%	67 27%	8 3%	None 0%	None 0%

Living adult parasites found within the intestinal lumen

PZQ = PRAZIQUANTEL

+ = Presence of *H. nana* eggs in the stool

0 = Negative stool examination

RESULTS

Effect upon the larvae (Groups A and B, treated on the 4th day)

In comparison to the control group the counting of cysticercoids in the small intestine of treated animals revealed an average reduction of 35%. Inclusively, many larvae showed

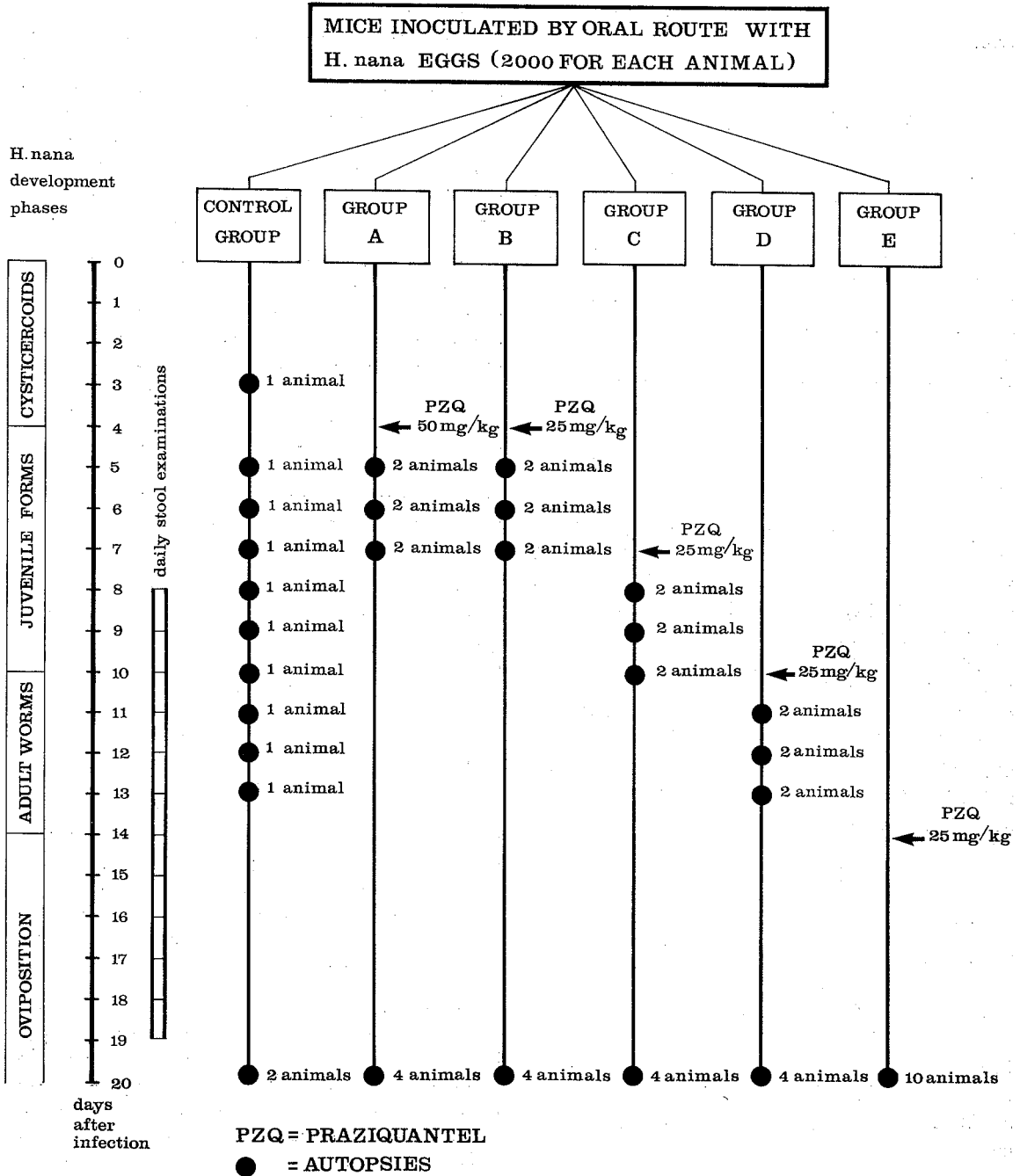


Fig. 1 — Experimental design

structural alterations suggestive of lethal damage. On the 6th day after the infection cysticercoids were no longer found in either treated or untreated groups. Nevertheless, the drug action was not sufficient to prevent that from

the 13th day on in the animals treated with 25 mg/kg and a little later on in those treated with 50 mg/kg, the stool examination started to become positive; and at autopsy, performed on the 20th day, intact adult worms were found

although in smaller number than in the control.

Effect upon the juvenile forms (Group C, treated on the 7th day)

The drug action was quite more pronounced on these forms than on the cyticercoids. It provoked marked alterations and death to most of the parasites as shown by the 97% reduction in the number of intact adult worms found at autopsy on the 20th day. Again, however, it was not yet possible to eliminate the infection completely, since *H. nana* eggs were detected in the stool from the 15th day on.

Effect upon the mature forms (Groups D and E, treated on the 10th and 14th days)

Praziquantel proved to be totally efficacious against both mature parasites, those that had not yet initiated oviposition and those that were already laying eggs. In fact, after treatment it was not feasible to detect the presence of adult worms either by finding out eggs in the stool examination or by observing parasites at autopsy on the 20th day. Solely, in the group treated on the 10th day, in one of the six animals sacrificed within the three days following the drug administration two small and sterile worms were seen.

DISCUSSION

Human hymenolepiasis is regarded by many physicians as an infection of minor relevance because its morbidity and mortality are devoid of significance. In spite of its incidence being relatively high in some regions and of estimations indicating that 43 million individuals are infected (GROLL⁸), in general, hymenolepiasis is considered a parasitosis of low prevalence.

However, some authors attach more importance to this infection stating that when the worm load is heavy a catarrhal enteritis may occur. Others argue that the main aspect to be considered in connection with the pathogenic mechanism refers to the sensitization of the organism induced by the larvae localized inside the intestinal wall.

In Brazil the incidence of *H. nana* infection is low, about 1%, but it may increase sharply amongst individuals living in confined places like boarding school, asylum, rest home, barracks and so forth. KALIL (1965) examining girls at an educational establishment, in Paraná, and CARVALHO et al.⁵ examining children in a close community, in Minas Gerais, found out an incidence of 23 and 25%, respectively.

As a rule, the Brazilian physicians tend to treat *H. nana* infection whether it is diagnosed in isolated cases or in confined population groups, independently of symptoms being present or not. However, until recently there was no efficacious treatment available. The forthcoming of praziquantel has dramatically changed this expectancy. Nevertheless, two questions are still in need of elucidation: the feasibility of attaining 100% cure rates and of accomplishing control of this parasitosis in close communities.

To clear up these questions we have conceived this series of experiments involving mice, an excellent animal model for this purpose. Based on the achieved results the following conclusions can be drawn.

It has been confirmed that praziquantel has only a partial activity against the cysticercoids of *H. nana* and that a two-fold increase of the dose, from 25 to 50 mg/kg, has no significant influence on the outcome of the treatment. The first structural alteration caused by the drug action, observed by us, was a disarray of the acculeum followed by vacuolation of the tegument of the larvae as already described by BECKER et al.².

It was proved that the effect of praziquantel becomes more and more evident as the parasite reaches maturity so that the adult worm is totally destroyed by the drug. Particularly, it was demonstrated that the parasite is fully sensitive from the 10th day of its evolution, before starting oviposition. This is the main contribution of this series of investigations, allowing two inferences of relevant clinical consequences:

- 1st. — it can explain why 100% cure rates have not been achieved in the majority of clinical trials. Taking into account that man is the intermediary and definitive host of *H. nana*, he can lodge,

at the same time, parasites in different evolutionary phases. Since the larval forms are less sensitive to praziquantel they can escape the drug action. Therefore, the infection persists, inclusive by auto-reinfestation. In a close community these remaining cases are source of renewed contamination by elimination of eggs in the stool. They may be the reason for the failure of previous attempts to control hymenolepiasis in such communities;

2nd. — considering the biological cycle of *H. nana* that within 12 days of development initiates oviposition, it should be recommended to administer praziquantel twice, 10 days apart, at the dose of 25 mg/kg, in order to interrupt the epidemiological chain of this parasite. In this way it would be feasible to attain a definitive cure of the infection.

RESUMO

Atividade do praziquantel sobre diferentes estágios evolutivos do *Hymenolepis nana*, em camundongos infectados experimentalmente

O praziquantel foi administrado oralmente, em dose única, a intervalos variáveis de tempo, subseqüentes à inoculação experimental de camundongos com ovos (2000 por animal) do *Hymenolepis nana*, objetivando-se investigar a ação da droga sobre os diferentes estágios evolutivos do parasita. Demonstrou-se que tanto 25 quanto 50 mg/kg, administrados no 4.º dia após a inoculação, apresentavam um efeito apenas parcial sobre as formas cisticercóides. Ademais, a dose de 25 mg/kg empregada no 7.º dia também não era capaz de matar todas as formas jovens. Entretanto, essa mesma dose utilizada no 10.º dia, quando o parasita já atingiu a maturidade mas ainda não iniciou a postura de ovos, mostrou-se 100% eficaz. Igual grau de eficácia foi alcançado com 25 mg/kg administrados no 14.º dia, quando o parasita se encontra em plena fase de oviparidade. Esses achados experimentais indicam que, no tratamento da himenolepiase humana, o praziquantel deve ser empregado em duas doses de 25 mg/kg, administradas com 10 dias de intervalo. Dessa maneira, a segunda dose atuará sobre aquelas formas larvares que tenham es-

capado à primeira. Essa recomendação deve ser seguida, em especial, nos pacientes com infecção pelo *H. nana* que vivem em comunidades fechadas.

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