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EFFECT OF WIDE SPECTRUM ANTI-HELMINTHIC DRUGS UPON *Schistosoma mansoni* EXPERIMENTALLY INFECTED MICE

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SUMMARY

Mebendazole, albendazole, levamisole and thiabendazole are well known as active drugs against several nematode species, and against cestodes as well, when the first two drugs are considered. None of the drugs have proven activity, however, against trematodes. We tested the effect of these drugs on the fecal shedding of schistosome eggs and the recovering of adult schistosomes, after portal perfusion in *Schistosoma mansoni* experimentally infected mice.

Balb/c mice infected with 80 *S. mansoni* cercariae were divided into three groups, each in turn subdivided into four other groups, for each tested drug. The first group was treated with each one of the studied drugs 25 days after *S. mansoni* infection; the second group was submitted to treatment with each one of the drugs 60 days after infection. Finally, the third group, considered as control, received no treatment.

No effect upon fecal shedding of *S. mansoni* eggs and recovering of schistosomes after portal perfusion was observed when mice were treated with either mebendazole or albendazole. Mice treated with either levamisole or thiabendazole, on the other hand, showed a significant reduction in the recovering of adult schistosomes after portal perfusion, mainly when both drugs were given during the schistosomula evolution period, i.e., 25 days after cercariae penetration, probably due to unspecific immunomodulation.

KEY WORDS: Schistosomiasis; *Schistosoma mansoni*; Mebendazole; Albendazole; Levamisole; Thiabendazole.

INTRODUCTION

In several areas of South and Central America, Africa, and Asia mansoni schistosomiasis has been considered a very important parasitic infection in human beings[12], because of its high frequency and the tissue damage caused by the presence of eggs and adult schistosome worms. In Brazil, indigenous mansoni schistosomiasis has already been detected in 17 States, and in 7 of them, prevalence rates may reach levels of 25% or above[2].

There are at least two specific drugs for treating schistosomiasis efficaciously: oxamniquine and praziquantel. Due to their simple administration schedule and to the scarcity of side effects, these drugs have been used in mass treatment programs, and should be considered an important tool for schistosomiasis control[4].

In 1987 AL-WAILI[1] reported the cure of two *Schistosoma haematobium* patients after treatment with mebendazole. On the other hand, KATZ & ARAUJO[9] did not find any effect of this drug upon the quantity and distribution of adult *Schistosoma mansoni* in experimentally infected mice. These authors did not test, however, if mebendazole administration could change, even temporarily, the production and shedding of schistosome eggs by the worms.

Mebendazole, as other drugs such as albendazole and levamisole, has proven activity against several parasitic nematodes. Mebendazole, and albendazole are also effective against cestodes[13]. No proven effect against trematodes has, however, been reported for these drugs. Nevertheless, the practical aspects of their administration and their almost absence of known side effects could be responsible for their very frequent use, even without medical prescription.

This study tested possible changes in either schistosome egg shedding or adult worm recovering after using anti-

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helminthics such as mebendazole, albendazole, levamisole and thiabendazole, in *S. mansoni* experimentally infected mice. The drugs were administered either during the maturation period of schistosomula in mice portal system (25 days after infection) or just after the beginning of schistosome egg shedding by mice (60 days after infection).

MATERIAL AND METHODS

Eighty-one 2 to 3 month-old Balb/c mice, without any enteroparasitic infection, were submitted to percutaneous infection with 80 *S. mansoni* cercariae. After infection, all mice were divided into three groups, each in turn subdivided into four other groups (Table 1).

TABLE 1
Number of Balb/c mice employed in three experiments to test the effect of mebendazole, albendazole, levamisole and thiabendazole upon egg shedding and recovering of adult schistosomes

Groups	1st experiment mebendazole	2nd experiment albendazole	3rd experiment Levam./Thiabend.
1(a)	8	9	8/5
2(b)	7	8	8/8
3(c)	6	7	7*

(a) Group 1: mice treated 60 days after *S. mansoni* infection
(b) Group 2: mice treated 25 days after *S. mansoni* infection
(c) Group 3: mice not submitted to any treatment
(*) Two experimental groups - levamisole and thiabendazole treated mice - but only one control group were used in the third experiment.

Three experiments were then performed. In the first one the effect of mebendazole was tested; albendazole was tested in the second; and, finally, levamisole and thiabendazole, administered to different mice, but compared with the same control group, were studied in the third experiment.

Three groups of mice were used in each experiment. One group was treated with the respective tested drug 60 days after *S. mansoni* infection (G1). The second group was treated 25 days after the cercariae penetration (G2) and the control group was not submitted to any treatment (G3).

The anti-helminthic drugs were orally administered according to the following schedule:

- Mebendazole: 500 mg/kg/day for 4 days.
- Albendazole: 500 mg/kg/day for 2 days.
- Levamisole: 10 mg/kg/day for 2 days.
- Thiabendazole: 100 mg/kg/day for 2 days.

Mice of all groups were submitted to fecal parasitological tests, just after the beginning of *S. mansoni* egg shedding, three times a week, during a 30-day period. Feces were examined after homogenization with a few drops of glycerin, according to the Kato-Katz technique[10]. All mice were sacrificed and submitted to portal perfusion[14] after the 30-day period, to evaluate the number of adult schistosomes in each group.

The geometric mean of *S. mansoni* eggs eliminated from each mouse were recorded every week, as well the number of male and female schistosomes recovered at the end of the experiment. The results were submitted to analysis of variance using Microstat software (Ecosoft Inc.) and level of significance of 95% ($p = 0.05$).

RESULTS

The geometric means of *S. mansoni* eggs eliminated each week by mice submitted to treatment with mebendazole, albendazole, levamisole and thiabendazole are shown in table 2 and figure 1. Levamisole and thiabendazole treated mice showed a significant lower number of *S. mansoni* eggs in all fecal samples examined.

Table 3 shows the median number of adult schistosomes recovered in each mice group at the end of the experiment. A significant lower number of schistosomes were recovered from levamisole and thiabendazole treated mice, mainly when these

TABLE 2
Median number of *Schistosoma mansoni* eggs (geometric mean) eliminated weekly by mice experimentally infected and treated with non-specific anti-helminthics

Drug	Group	1st week		2nd. week		3rd. week		4th. week		Mean	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Mebendazole	G1	237.60	93.44	222.72	64.73	162.00	24.79	139.92	29.68	186.10	47.00
	G2	217.20	20.35	114.48	22.44	147.12	25.10	110.40	34.27	141.76	49.41
	G3	262.08	54.34	202.32	3.04	164.16	51.98	133.68	18.37	184.69	55.33
Albendazole	G1	128.64	13.74	60.00	46.79	73.44	17.66	114.24	29.40	93.36	32.60
	G2	60.72	8.99	43.68	20.85	70.08	23.99	72.24	15.21	60.48	13.00
	G3	59.76	9.45	47.76	14.39	55.44	11.84	59.28	9.37	55.44	5.55
Levamisole	G1	113.28	25.59	73.44	17.80	102.48	36.55	92.16	10.22	94.32	16.96
	G2	65.04	3.17	83.76	49.61	54.48	36.83	66.24	15.67	66.72	12.13
	G3	222.00	32.13	206.88	17.15	307.92	62.06	100.56	11.35	194.16*	85.08
Thiabendazole	G1	44.16	16.59	57.60	26.07	84.48	42.38	42.72	8.70	53.76	19.36
	G2	42.16	7.52	41.28	12.64	41.5	11.93	41.04	9.13	42.00	0.48
	G3	222.00	32.13	206.88	17.15	307.92	62.06	100.56	11.35	194.16*	85.08

* G3 (control group) for Levamisole and Thiabendazole treated mice was the same; s.d. = standard deviation

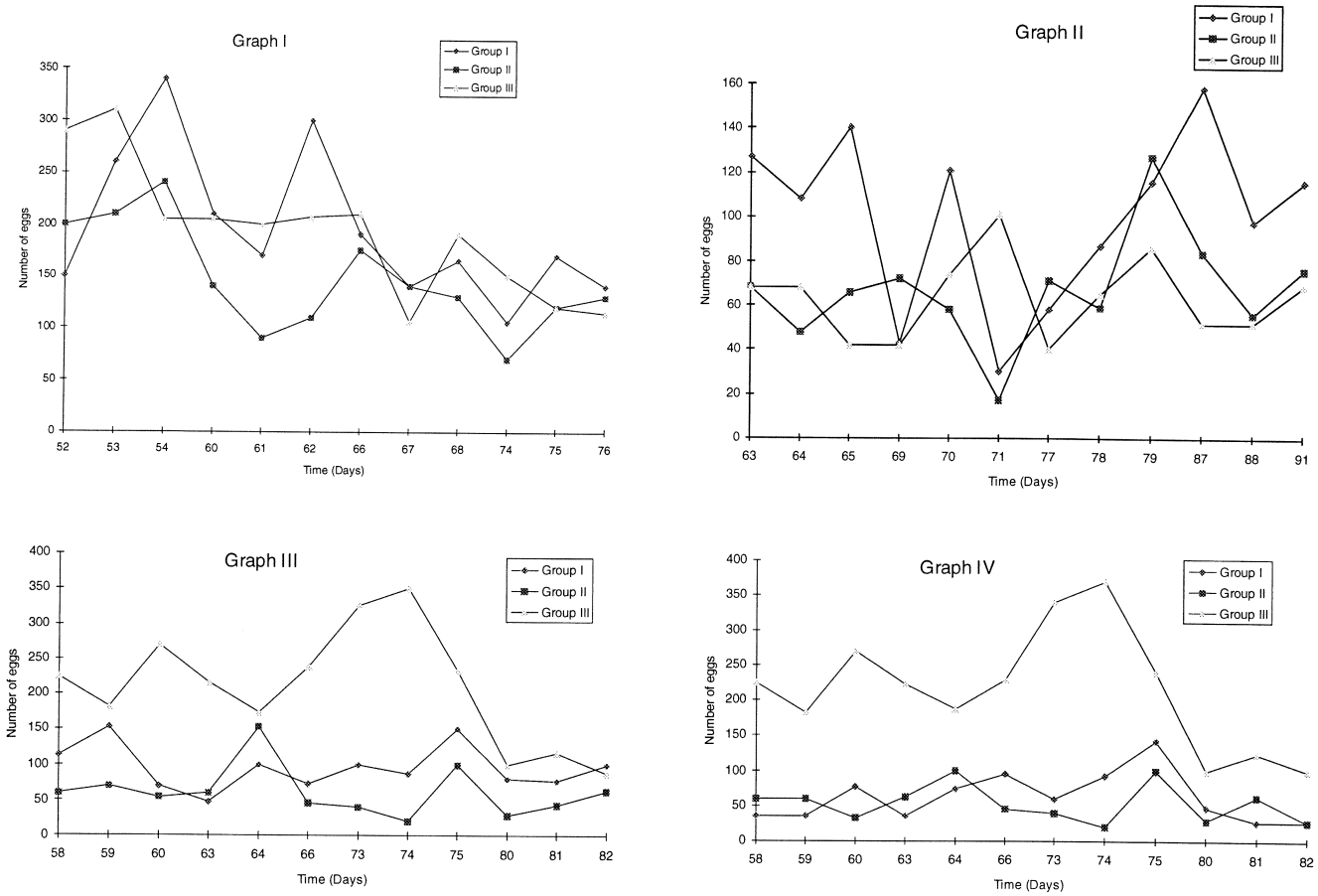

Fig. 1 - Median number of *Schistosoma mansoni* eggs eliminated by mice experimentally infected and treated with Mebendazole (graphic I), Albendazole (graphic II), Levamisole (graphic III) and Thiabendazole (graphic IV).

drugs were administered during the maturation period of schistosomula in the mice portal system, i.e., 25 days after *S. mansoni* infection.

DISCUSSION

Wide spectrum anti-helminthic drugs are usually prescribed by physicians in Brazil to treat enteroparasitic infections. On the other hand, these kind of drugs can frequently be taken without medical prescription to treat supposed worm infections. Thus, we can consider that submitting *S. mansoni* patients to treatment with one of the wide spectrum anti-helminthics available in our country, mebendazole, albendazole or levamisole, is not such a rare event. The administration of thiabendazole, however, is probably more restricted because of its narrower spectrum of action and the common occurrence of side effects.

Besides the AL-WAILI report[1] on the cure of two *S. haematobium* infected patients after administration of mebendazole, and the paper published by KATZ &

TABLE 3

Median number of adult *Schistosoma mansoni* (arithmetic mean) recovered after portal perfusion, in mice experimentally infected and treated with non-specific anti-helminthics

Drug	Group	Male		Female		Total	
		mean	s.d.	mean	s.d.	mean	s.d.
Mebendazole	G1	18.40	9.82	17.13	12.2	35.53	21.94
	G2	14.30	5.20	15.84	7.03	30.14	11.55
	G3	14.94	8.54	14.28	8.54	29.22	16.99
Albendazole	G1	3.54	2.81	3.77	2.98	7.31	5.71
	G2	2.85	1.63	2.48	0.52	5.33	1.86
	G3	2.59	2.06	2.49	2.41	5.08	4.04
Levamisole	G1	8.46	3.80	6.23	2.99	14.69	6.32
	G2	4.78	2.19	4.10	1.38	8.88	4.15
	G3	10.73	5.37	10.65	5.61	21.38*	11.28
Thiabendazole	G1	6.09	2.05	8.71	2.88	14.80	4.51
	G2	3.53	1.28	3.53	1.39	7.08	2.56
	G3	10.73	5.77	10.65	5.61	21.38*	11.28

* G3 (control group) for Levamisole and Thiabendazole treated mice was the same

s.d. = standard deviation

ARAUJO[9] denying any effect of this drug upon *S. mansoni* infected mice, no other studies have been found on the subject. Is is also worth-while to mention that there are no investigations on the effect of wide spectrum anti-helminthics upon the production and shedding of *S. mansoni* eggs by infected vertebrate hosts. One of the aims of the present paper was to evaluate the possibility of occurrence of defective results in fecal quantitative parasitological tests, usually performed for the diagnosis of schistosomiasis. The study also evaluated if these drugs could have either some effect on the evolution of *S. mansoni* or upon the adult worms in their vertebrate host.

Variation in the number of *S. mansoni* eggs eliminated each week, by both treated and control mice was observed initially. Indeed, this kind of variation has already been well established when short time periods are considered[5,6,7], but not for longer periods[3,15]. Thus, the variation of the number of *S. mansoni* eggs eliminated by mice in this experiment should be considered as a normal fluctuation.

The median number of *S.mansoni* eggs weekly eliminated by each mice group (Table 2) was, however, significantly decreased in levamisole and thiabendazole treated mice, mainly in the group which received these drugs during the schistosomula maturation period, i.e., 25 days after *S. mansoni* infection. The same decrease was not observed in mebendazole and albendazole treated mice.

The decrease of the number of adult schistosomes recovered in levamisole and thiabendazole treated mice, mainly in the group which received the drugs 25 days after infection, and the lack of differences in experimental and control groups in mice treated with mebendazole and albendazole (Table 3) strenghten the hypothesis of a partially deleterious effect of levamisole and thiabendazole upon the development of *S. mansoni* in its vertebrate host.

The mechanism responsible for this deleterious effect remains unknown. One can suppose that levamisole and thiabendazole could have an unspecific immunomodulatory action[8]. However, according to MONTENEGRO et al.[11], when levamisole is administered before *S. mansoni* infection, mice are usually more susceptible than resistant to the trematode[11], probably due to the interference in the chemotaxis of blood polymorphonuclear leukocytes[16].

In summary, the results obtained with levamisole and thiabendazole administration to *S. mansoni* infected mice support the hypothesis of a partially deleterious effect upon trematodes, mainly when the drugs are administered during the schistosomula maturation period. Nevertheless, it is important to point out the high anti-helminthic doses employed in this experiment, exceeding significantly the dosage commonly used in human subjects.

RESUMO

Efeito de drogas anti-helmínticas de amplo espectro em camundongos experimentalmente infectados por *Schistosoma mansoni.*

Mebendazol, albendazol, levamisol e tiabendazol são anti-helmínticos ativos contra diversas espécies de nematódeos. As duas primeiras drogas são igualmente eficientes no tratamento de infecções por cestódeos; todavia, não apresentam atividade comprovada no caso de infecções por trematódeos.

No presente trabalho testou-se o efeito desses anti-helmínticos na recuperação de exemplares adultos de *S. mansoni,* bem como sobre a produção e liberação de ovos desse trematódeo, em modelo representado por camundongos experimentalmente infectados.

Camundongos Balb/c, infectados com 80 cercárias de *S. mansoni*, foram divididos em três lotes e cada um subdividido em quatro grupos, correspondentes a cada droga testada. No primeiro lote os camundongos de cada grupo foram tratados, com uma das drogas em questão, 25 dias após a infecção por *S. mansoni*. No segundo lote o tratamento dos camundongos de cada grupo, com a respectiva droga, ocorreu 60 dias após a infecção esquistossomótica. Finalmente, os camundongos do terceiro lote, utilizados como grupo controle, não foram submetidos a nenhum tratamento.

Não se observou qualquer efeito sobre a recuperação de exemplares adultos de *S. mansoni* ou eliminação fecal de ovos do trematódeo nos camundongos tratados com mebendazol ou albendazol. Entretanto, nos animais que receberam tratamento com levamisol ou tiabendazol verificou-se significativa redução na quantidade de exemplares adultos de *S. mansoni* recuperados após perfusão portal, principalmente quando essas drogas foram administradas durante o período de maturação dos esquistossômulos, isto é, 25 dias após a penetração das cercárias, provavelmente em decorrência de processo de imunomodulação inespecífica.

REFERENCES

1. AL-WAILI, N.S. - Mebendazole in the treatment of *Schistosoma haematobium*. **Trans. roy. Soc. trop.Med. Hyg., 81:** 781, 1987.

2. AMARAL, R.S. & PORTO, M.A. - Evolução e situação atual do controle da esquistossomose no Brasil. **Rev. Soc bras. Med. trop., 27** (supl. 3): 73-89, 1994.

3. BARRETO, M.L.; SILVA, J.T.F.; MOTT, K.E. & LEHMAN Jr., J.S. - Stability of fecal egg excretion in *Schistosoma mansoni* infections. **Trans roy. Soc. trop. Med. Hyg., 72:** 181-187, 1978.

4. CHIEFFI, P.P. - Tratamento em massa como medida de controle da esquistossomose no Brasil. **Arq. méd. Hosp. Fac. Ciênc. méd. Santa Casa S. Paulo, 9**: 17-22, 1989.

5. CHIEFFI, P.P.; MARQUES, R.M. & SIQUEIRA, J.G.V. - Avaliação da eficácia do método de Kato-Katz no diagnóstico parasitológico da esquistossomose mansônica. **Rev. Inst. Adolfo Lutz, 41:** 23-30, 1981.

6. DOMINGUES, L.; SILVEIRA, M.; VANDERLEI, M.I. & KELNER, S. - Possíveis fatores que alteram os resultados da coproscopia quantitativa de ovos de *S. mansoni* pelo método de Kato-Katz. **Rev. Inst. Med. trop. S. Paulo, 22:** 114-117, 1980.

7. ENGELS, D.; SINZINKAYO, E & GRYSEELS, B. - Day-to-day egg count fluctuation in *Schistosoma mansoni* infection and its operational implications. **Amer. J. trop. Med. Hyg., 54:** 319-324, 1996.

8. GUERRANT, R.L.; SCHWARTZMAN, S.D. & PEARSON, R.D. - General principles. In: STRICKLAND, G.T. Hunter's Tropical Medicine. 7.ed. Philadelphia, W.B. Saunders, 1991. p. 684-689.

9. KATZ, N. & ARAÚJO, N. - Mebendazole in the treatment of mice experimentally infected with *Schistosoma mansoni.* **Trans. roy. Soc. trop. Med. Hyg., 82**: 873, 1988.

10. KATZ, N.; CHAVES, A. & PELLEGRINO, J. - A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. **Rev. Inst. Med. trop. S. Paulo, 14:** 397-400, 1972.

11. MONTENEGRO, S.M.L.; TEIXEIRA, K.M.; COUTINHO, E.M. et al. - Efeitos imunopotenciadores não específicos na infecção experimental pelo *Schistosoma masoni.* I. Levamisole. **Rev. Inst. Med. trop. S. Paulo, 33**: 69-73, 1991.

12. ORGANIZAÇÃO MUNDIAL DA SAÚDE - O Controle da Esquistossomose. Segundo Relatório do Comitê de Especialistas da O.M.S. Rio de Janeiro, FIOCRUZ, 1994.

13. ORGANIZACIÓN MUNDIAL DA LA SALUD - Medicamentos utilizados en enfermedades parasitárias. Ginebra, 1991.

14. PELLEGRINO, J. & KATZ, N. - Terapêutica experimental. In: CUNHA, A.S., org. Esquistossomose mansoni. São Paulo, EDUSP, 1970. p. 313-326.

15. RABELLO, A.L.T. - Parasitological diagnosis of schistosomiasis mansoni: fecal examination and retal biopsy. **Mem. Inst. Oswaldo Cruz, 87** (suppl. 4): 325-331, 1992.

16. STEIN, A.N.; DIEZ, R.A.; SEN, L. & ESTEVEZ, M.E. - Chemotatic function of polymorphonuclear leukocytes from patients with recurrent infections: partial correction by levamisole "in vitro". **Allergol. Immunopath., 13:** 127-134, 1985.

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