

DEVELOPMENT OF *ASCARIS LUMBRICOIDES* EGGS FROM FEMALES ELIMINATED AFTER CHEMOTHERAPY IN MAN

OMAR S. CARVALHO; H. L. GUERRA* & C. L. MASSARA

Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1745, 30161-970 Belo Horizonte, MG, Brasil

*Fundação Nacional de Saúde-MG, Belo Horizonte, MG, Brasil

The development of Ascaris lumbricoides eggs obtained from females eliminated after treatment of infected individuals with a single oral dose of the antihelminthic drugs thiabendazole (50 mg/kg – 33 patients) or levamisole (250 mg – independent of body weight – 20 patients) was studied. Every female eliminated up to 72 h after treatment were dissected, the uterus isolated and sectioned into small fragments. The eggs were transferred to plastics tubes and incubated at 28 °C in 0.1 N H₂SO₄ for 100 days. Every 20 days, starting from the 20 th up to the 100 th day, the extent of egg embryonation ratio was determined. The culture of A. lumbricoides eggs obtained from females from patients treated with thiabendazole did not contain embryonated eggs until the final period of observation. In contrast, the eggs obtained from females eliminated by patients treated with levamisole (control) presented an embryonation rate of 0.0 – 98.0% in the same period.

Key words: *Ascaris lumbricoides* – therapeutics – eggs embryonation – ovicidal drug

Ascariasis is one of most frequent parasitic infections of man and in some regions, around 90% of children present *Ascaris lumbricoides* eggs in their feces (WHO, 1967). Low social economic level and precarious sanitary conditions are amongst the determining factors contributing to the high prevalence of this parasite. Control programs are aimed mostly at populations resident in areas of poverty, concentrating principally on individual treatment. Although chemotherapy is immediately effective it can also result in a significant increase in the parasite population because the large number of eggs excreted following chemotherapy are normally fully viable and epidemiological conditions remain unaltered (Massara et al., 1991).

Kutsumi & Komiya (1965) and Kutsumi et al., (1966a, b, 1967) tested the action of thiabendazole in water, nightsoil and soil contaminated with *A. lumbricoides*, *Trichuris trichiura* and hookworms eggs. Others (Egerton, 1969; Vanhaelen-Lindhout & Smith, 1971; Wagner

& Peña-Chavarria, 1974a, b; Wagner & Rexinger, 1978; Massara et al., 1991) have studied the action of several antihelminthic drugs, following chemotherapy in man, on the development of various parasite eggs of medical and veterinary interest. In addition, Moreira & Souza (1973), Souza et al. (1985) observed the development of *A. lumbricoides* eggs obtained from the uterus of females eliminated following chemotherapy in man with two antihelminthic drugs. The present paper reports the development of *A. lumbricoides* eggs recovered from the uterus of females eliminated following chemotherapy with thiabendazole and levamisole.

MATERIALS AND METHODS

A total of 53 patients with ascariasis, diagnosed by the Kato-Katz method (Katz et al., 1972), aged between 2-55 years, residents of the metropolitan region of Belo Horizonte (Minas Gerais, Brazil) and Delfim Moreira (south of Minas Gerais) were treated with a single oral dose of thiabendazole (50 mg/kg – 33 patients) or levamisole (250 mg of base salt, independent of body weight – 20 patients) which were used as controls (Massara et al., 1991).

This work was partially supported by CNPq and FAPEMIG.

Received 4 September 1991.

Accepted 5 November 1991.

All worms eliminated up to 72 h after treatment were recovered in 0.75% saline. The females were individually dissected, the uterus isolated in a Petri dish containing distilled water and cut into small fragments. Eggs were then transferred to 50 ml plastic centrifuge tubes and incubated at 28 °C in 0.1 N H₂SO₄ (Fairbairn, 1961) for 100 days on a mechanical shaker (Thermolyne Speci-Mix, Model nr M2612 - SYBRON). The flasks were opened daily for oxygenation.

Every 20 days, starting from the 20th up to the 100th day, the extent of embryonation was determined in eggs kept in 0.025 ml of culture medium. The results shown correspond to the mean of triplicate counts.

The mean rate of embryonation of 20 cultures on each day were compared through the Analysis of Variance (ANOVA), defining $1 - \alpha = 0.05$ (Levin, 1978).

RESULTS

Thirty three cultures of *A. lumbricoides* eggs isolated from females eliminated by patients treated with thiabendazole did not present embryonated eggs until the 100th day of observation. Among the 20 cultures with eggs from patients treated with levamisole, only two did not have embryonated eggs at this final period of observation (cultures no. 9 and 12). In the other 18 cultures, the extent of embryonation varied from 13.0 - 98.0% on the 100th day. The mean value for the percentage of eggs with embryos on the 20th day was 28.0% (varying from 0.0 to 87.0%), on the 40th it was 43.9%; on the 60th, 49.1%; on the 80th, 52.2% and on the 100th, 54.1%, (varying from 0.0 to 98.0%) at each of these time points. From the 40th up to the 100th day the mean value did not vary significantly.

DISCUSSION

The action of thiabendazole in water, nightsoil and soil contaminated with eggs of *A. lumbricoides*, *T. trichiura* and hookworms was studied by Kutsumi & Komiya (1965) and Kutsumi et al. (1966a, b, 1967). They observed a decrease in the number of embryonated eggs in treated areas when compared with the control, as well as a decrease in the rate of infection in the population.

Vanhaelen-Lindhout & Smith (1971), Wagner & Peña Chavarria (1974a, b) studied

the action of thiabendazole and mebendazole on *T. trichiura* and hookworm eggs recovered from the feces of infected individuals. They also observed an ovicidal action with *T. trichiura* and a reduction in the percentage of hookworm eggs with developing embryos. Wagner & Rexinger (1978) demonstrated that mebendazole inhibited the development of *T. trichiura* embryos and decreased the percentage of *A. lumbricoides* eggs with embryos. Maisonneuve et al. (1985) showed that albendazol exerted an ovicidal activity on *A. lumbricoides* and *T. trichiura*. Levamisole did not exhibit a similar action with the eggs of these helminths. Using thiabendazole, levamisole, pyrantel pamoate, cambendazole, mebendazole and praziquantel, Massara et al. (1991) verified the ovicidal activity of thiabendazole on the eggs of *A. lumbricoides* eliminated by children, 48 to 72 h after drug administration. Other drugs demonstrated little or no activity on the development of embryos in the eggs.

TABLE

Percentage of embryonated *Ascaris lumbricoides* eggs obtained from females eliminated by patients treated with levamisole

Cultures no.	Days of counting				
	20	40	60	80	100
01	57.0	84.0	84.0	84.0	85.0
02	39.0	49.0	50.0	53.0	54.0
03	18.0	20.0	25.0	25.0	25.0
04	0.0	49.0	53.0	59.0	63.0
05	0.0	66.0	76.0	81.0	85.0
06	47.0	51.0	53.0	54.0	55.0
07	33.0	39.0	39.0	39.0	40.0
08	50.0	58.0	66.0	69.0	69.0
09	0.0	0.0	0.0	0.0	0.0
10	0.0	3.0	19.0	41.0	48.0
11	3.0	8.0	39.0	54.0	60.0
12	0.0	0.0	0.0	0.0	0.0
13	35.0	60.0	60.0	62.0	62.0
14	43.0	80.0	80.0	80.0	82.0
15	13.0	46.0	52.0	52.0	52.0
16	32.0	46.0	57.0	57.0	57.0
17	87.0	98.0	98.0	98.0	98.0
18	55.0	68.0	73.0	74.0	77.0
19	46.0	52.0	55.0	55.0	57.0
20	2.0	2.0	3.0	7.0	13.0
X	28.0	43.9	49.1	52.2	54.1

Moreira & Souza (1973) and Souza et al. (1985) observed the development of *A. lumbricoides* eggs recovered from uterus of females eliminated following chemotherapy in

man with hexahidrate of piperazina, levamisole, mebendazole and pyrantel pamoate. The use of these drugs did not alter the development of embryos, preserving its ability to develop in vertebrate hosts. The data presented here suggest that although the thiabendazole does not constitute the drug of choice for ascariasis, this antihelminthic demonstrated the capacity to interrupt the *A. lumbricoides* life cycle, acting on the eggs recovered from the feces (Massara et al., 1991) to an equal extent to eggs recovered from the uterus of females after treatment.

ACKNOWLEDGEMENTS

To José Geraldo Amorim da Silva and Antonio Carlos do Prado for technical assistance.

REFERENCES

- EGERTON, J. R., 1969. The ovicidal and larvicidal effect of thiabendazole on various helminthic species. *Tex. Rep. Biol. Med.*, 27 (suppl. 2): 561-580.
- FAIRBAIRN, D., 1961. The *in vitro* hatching of *Ascaris lumbricoides* eggs. *Can. J. Zool.*, 39: 153-162.
- KATZ, N.; CHAVES, A. & PELLEGRINO, J., 1972. A simple device for quantitative stool stick-smear technique schistosomiasis mansoni. *Rev. Inst. Med. Trop. São Paulo*, 14: 397-400.
- KUTSUMI, H.; HARA, T. & YAMAMOTO, K., 1966a. Field trials of thiabendazole as an ovicide in the control of helminth infections. I. Epidemiological analysis of the effectiveness in the control of hookworm infections. *Japan J. Parasitol.*, 15: 399-411.
- KUTSUMI, H.; HARA, T. & YAMAMOTO, K., 1966b. Field trials of thiabendazole as an ovicide in the control of helminth infections. II. Epidemiological analysis of the effectiveness in the control of whipworm infection. *Japan J. Parasitol.*, 15: 516-527.
- KUTSUMI, H.; HARA, T. & YAMAMOTO, K., 1967. Field trials of thiabendazole as an ovicide in the control of helminth infections. III. Epidemiological analysis of the effectiveness in the control of *Ascaris* infection. *Japan J. Parasitol.*, 16: 15-27.
- KUTSUMI, H. & KOMIYA, Y., 1965. Effect of thiabendazole as an ovicide on helminth eggs in nightsoil. *Japan J. Med. Sci. Biol.*, 18: 203-224.
- LEVIN, J., 1978. *Estatística aplicada a Ciências Humanas*, 1^o ed. Harper & Row do Brasil Ltda., São Paulo.
- MAISONNEUVE, H.; ROSSIGNOL, J. F.; ADDO, A. & MOJON, M., 1985. Ovicidal effects of albendazole in human ascariasis, ancylostomiasis and trichuriasis. *Ann. Trop. Med. Parasitol.*, 79: 79-82.
- MASSARA, C. L.; COSTA, H. M. A.; SOUZA, D. W. C.; SOUZA, M. S. L. & CARVALHO, O. S., 1991. Viability of *A. lumbricoides* eggs eliminated after anti-helminthic therapy. *Mem. Inst. Oswaldo Cruz*, 86: 233-237.
- MOREIRA, C. & SOUZA, D. W. C., 1973. Estudo da ação de drogas ascaricidas sobre a embriogênese de ovos retirados de fêmeas expelidas após tratamento humano. *An. Cong. Soc. Bras. Med. Trop.*, 9, Fortaleza.
- SOUZA, M. S. L.; SOUZA, D. W. C.; CARVALHO, O. S.; NEVES, J. & MASSARA, C. L., 1985. Viabilidade de ovos de *Ascaris lumbricoides* após tratamento humano com medicamentos específicos. I. Levamisole e pamoato de pirantel. *Rev. Inst. Med. Trop. São Paulo*, 27: 197-200.
- VANHAELLEN-LINDHOUT, E. & SMIT, A. M., 1971. Abnormally shaped eggs of *Trichuris trichiura* after thiabendazole treatment. *Trop. Geogr. Med.*, 23: 381-384.
- WAGNER, E. D. & PENA CHAVARRIA, A., 1974a. *In vivo* effects of a new antihelminthic, mebendazole (R. 17.635) on the eggs of *Trichuris trichiura* and hookworm. *Am. J. Trop. Med. Hyg.*, 23: 151-153.
- WAGNER, E. D. & PENA CHAVARRIA, A., 1974b. Morphologically altered eggs of *Trichuris trichiura* following treatment with mebendazole. *Am. J. Trop. Med. Hyg.*, 23: 154-157.
- WAGNER, E. D. & REXINGER, D. D., 1978. *In vivo* effects of mebendazole and levamisole in the treatment of trichuriasis and ascariasis. *Am. J. Trop. Med. Hyg.*, 27: 203-205.
- WORLD HEALTH ORGANIZATION., 1967. Lucha contra la ascariasis *Série de Informes Técnicos*, No 379, Geneva.