Rev. Inst. Med. trop. São Paulo
36 (2): 99-103, março-abril, 1994

OOGRAM STUDIES IN MICE INFECTED WITH *SCHISTOSOMA MANSONI* AND TREATED WITH DEXAMETHASONE

Marco Victor HERMETO, Rosilene Siray BICALHO, Roney Elias da SILVA, Alan Lane de MELO & Leógenes Horácio PEREIRA

SUMMARY

Mice infected with about 90 cercariae of *Schistosoma mansoni* (LE strain) were t. .ed during five consecutive days with dexamethasone (50 mg/Kg, subcutaneously), starting on the 42th day of infection. Groups of five mice were then daily sacrificed from the first day after onset of treatment until the first day after. The perfusion of the portal system was performed and a piece of the intestine was processed for qualitative and quantitative oograms. This treatment carries to larger numbers of eggs in the tissues of treated mice, when compared with untreated groups. No changes were observed in the kinetics of oviposition, as all stages of viable eggs were observed in the tissues of treated and control mice. These data reinforce the hypothesis of a partial blockade of the egg excretion in immunossupressed mice.

KEYWORDS: *Schistosoma mansoni*; Schistosomiasis; Dexamethasone; Glucocorticoids; Immunossupression; Oogram.

INTRODUCTION

The anti-inflammatory effect of glucocorticoids (reducing the hypersensitivity state) has been considered as the only explanation for their dramatic effect on the treatment of some cases of acute toxemic schistosomiasis [14]. In addition, it was demonstrated a synergistic effect on the treatment of the acute experimental *Schistosoma mansoni* infection of the laboratory mouse [9]. Significant reduction in the egg counts per gram of faeces, moreover in the intestines and liver of mice treated with schistosomicide plus glucocorticoids were found, when compared with the schistosomicide or the glucocorticoid alone. This effect suggests possible advantages of the use of glucocorticoids associated with schistosomicides for the treatment of human acute toxemic schistosomiasis [9]. However, best therapeutic schemes to acute schistosomiasis need additional investigation [14].

Anyway, some effects of glucocorticoids in the early phases of the experimental *S. mansoni* infection are already established. If these drugs are administered in this period, they reduce the worm burden in infected mice [1, 5, 6, 12, 22], probably by an interference on the adaptation of the early phases of *S. mansoni* to the vertebrate host [6, 7].

Later, when the worms are found in the portal system, the effects of glucocorticoids are matter of controversy. Although they do not reduce the worm burden

This work was supported by CNPq, FINEP (Brazil) and the Parasitic Diseases Programme - W. H. O. (Switzerland).
Correspondence: A.L.M., Grupo Interdepartamental de Estudos sobre Esquistossomose - Departamento de Parasitologia, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. 31161-970, Caixa Postal 486 - Belo Horizonte, MG, Brasil.

when administered in this phase of infection [5], they reduce the numbers of eggs passed in the faeces of the host [8, 9, 11, 15]. This finding could be explained by a possible interference on the fecundity of adult worms [9], although it was also observed that in the tissues of treated mice there is no significant reduction in the egg numbers [9]. Conversely, it was suggested that the glucocorticoids act through a partial blockade of the elimination of *S. mansoni* eggs to the intestinal lumen, with a consequent increase in their numbers in the host tissues [11].

DOENHOFF et al. [2, 3] concluded that there is a partial blockade of egg excretion in mice treated with glucocorticoids. Then the increase of *S. mansoni* eggs in the host tissues could aggravate the pathologic alterations and the course of the disease, even when associated with minor changes in the liver function and partial inhibition of fibrogenesis induced by glucocorticoids [21].

In this paper, the oogram technique was used to observe the effects of dexamethasone (DMS) - a very active glucocorticoid - upon the egg-laying kinetics of *S. mansoni*. This procedure is one of the best methods used to evaluate the activity of drugs on schistosomiasis, providing information about the numbers of eggs, their different evolutive stages and viability, and allows to observe alterations in their morphology in the tissues of vertebrate host [17, 18, 19].

MATERIAL AND METHODS

Cercariae (LE strain of *S. mansoni*) shed by laboratory-reared and infected *Biomphalaria glabrata* were used throughout the study. Albino female mice, weighing about 20 g, were infected by subcutaneous route with about 90 cercariae of *S. mansoni*. Six weeks after the infection, one group of 25 mice was treated daily with dexamethasone (DMS): 50 mg/Kg, subcutaneously, during 5 consecutive days. Another group of 25 mice was kept as control.

Five mice of each group were sacrificed daily by cervical fracture, starting one day after the onset of the treatment until the first day after. The adult worms were recovered by perfusion using the PELLEGRINO & SIQUEIRA [16] technique with minor modifications, and counted to evaluate the worm burden and the trematode distribution within the portal system.

For oogram studies, two 1 cm fragments of the intestine (terminal ileum) were cut off and weighed (results expressed in milligrams). Excess of blood and mucus were removed with absorbent paper, and each fragment was put on glass slide and covered with plastic covers. The preparation was then turned downward and strongly pressed on a rubber surface padded with filter paper (PELLEGRINO & FARIA [18]). In each fragment, all the different stage eggs were. Quantitative evaluation of egg numbers in the intestinal fragments was done according the formula:

$$N^{o} \text{ of eggs/g of tissue} = N^{o} \text{ of eggs per fragment} \times 1{,}000/\text{weigh of fragment (g)}$$

Qualitative oograms of the intestinal fragments were found calculating the percentage of each evolutive stage of eggs. The classification of immature eggs was according to their developmental stages, following criteria previously described by PRATA [19] and PELLEGRINO et al. [17].

Statistical comparisions were done using Student's t test and analysis of variance, and significant $p < 0.05$.

TABLE 1

Percentages of the immature viable *S. mansoni* eggs in the intestinal fragments of mice treated with dexamethasone.

	Days after treatment									
	First		Second		Third		Fourth		Fifth	
Stage of *eggs*	T	C	T	C	T	C	T	C	T	C
First	14	22	13	21	11	23	26	19	17	23
Second	17	18	15	18	8	14	20	15	9	12
Third	39	29	46	40	44	36	50	33	43	29
Fourth	30	31	26	22	36	27	4	33	31	37
Total	100	100	100	100	100	100	100	100	100	100

T= Treated and C = Control

RESULTS

The distribution of the stages of immature viable *S. mansoni* eggs in the intestinal fragments of mice treated with DMS can be seen in Table 1. The kinetics of the egg-laying was not markedly modified by DMS, as the pattern of the oogram was similar, with minor inherent variations, in treated and in non-treated mice. All immature viable stages were present during the five days of observation, in both groups. Alterations in the percentage of different immature viable eggs did not occur, as oviposition and maturation of viable eggs was not interrupted. In addition, Table 2 shows that the mean numbers of immature viable eggs were higher in treated mice, but no statistical significance was found.

The mean numbers of mature viable eggs in the same material can be seen in the Table 2. The number of mature eggs were significantly higher in the tissues of treated animals.

In the same way, the mean numbers of dead eggs (semitransparent, granular, darkened and eggs with retracted embryo) throughout the experiment were significantly higher in the treated animals (Table 2).

During the five days of observation, the total numbers of eggs found in the intestine fragments of mice treated with DMS were significantly higher those from the control ones. These data can be seen in Table 2.

The numbers of worm recovery were not significantly different for both sexes of the worms from control and treated mice (p= 0.76 - Table 3). Significant differences among worm countings from different sites of the portal system were not seem.

DISCUSSION

The observation that, after an active antischistosomal compound is given, oogram changes were present in the liver along with the intestine, and that these alterations were found even when a relatively large proportion of female worms is in the mesenteric veins, indicates that egg production constitutes a sensitive system to be used as indicator for the assessment of drug's activity in schistosomiasis. A dynamic equilibrium between eggs that are being laid, those maturing and those continuously being eliminated with the faeces is attained in a few weeks after the beginning of the egg-laying and is maintained minor changes for a relatively long period. Slight alterations on this equilibrium are easily shown in oograms from intestinal fragments, but are very difficult to conclude by other less sensible methods [17, 18].

As previously demonstrated, in the later phases of infection with *S. mansoni*, DMS does not affect worm burden or sex distribution of the trematode in the portal system of mice. DMS also does not cause "hepatic shift" of these worms. On the other hand, quantitative oograms demonstrated that the mean numbers of mature viable eggs deposited in the intestines of mice treated with DMS were higher than those from the non-treated animals. The numbers of immature viable eggs were also higher in the treated animals, although no differences were observed in the proportional distribution of the different stages of these eggs. This unchanged kinetics of the *S. mansoni* egg-laying in DMS-treated mice agrees with the observations of LENZI et al. [11]. Moreover, the fecundity of *S. mansoni* worms was not reduced as previously suggested [9], even with the high dose of DMS used in the present study. This unchanged fecundity was also observed with *in vitro* studies [13]. On the other hand, these higher numbers of eggs in the tissues of the host may reflect a reduction in the elimination of mature viable eggs to the intestinal lumen, as demonstrated by LENZI et al. [11] and suggested by DOENHOFF et al. [2,3]. LENZI et al. [11] proposed that the glucocorticoids block the elimination of *S. mansoni* eggs by an indirect action upon eosinophils in the intestine wall. These cells

TABLE 2

Mean numbers and standard deviation of immature viable, mature viable, dead (semitransparent, granular, darkned and retracted embryo) and total eggs of *Schistosoma mansoni* in the intestinal fragments of mice treated with dexamethasone

Stage of eggs	Treated	Control	p
Immature eggs	32,494.4 ± 21,006.9	25,442.3 ± 14,824.4	0.22
Mature eggs	23,230.1 ± 13,051.5	14,866.2 ± 8,175.4	0.02
Dead eggs	9,681.1 ± 10,632.6	2,172.3 ± 1,669.4	0.005
Total	65,405.75 ± 37,739.2	42,479.95 ± 19,661.8	0.02

TABLE 3

Mean numbers and standard deviation of the worms recovered from the portal system of mice treated with dexamethasone.

Groups of mice	Mean numbers of recovered worms		
	Male	Female	Total*
Control	7.1 ± 4.3	5.2 ± 5.0	12.3 ± 8.7
Treated	6.2 ± 5.0	5.3 ± 5.1	11.4 ± 9.4

*p=0.76

seem to favor the passage of the parasite eggs to the intestinal lumen and these drugs could interfere with their function [10]. Such experimental observations agree with data obtained from human patients co-infected with HIV and *S. mansoni* [4, 20]. These patients showed an absence or poor peri-ovular inflammatory reaction in fragments of rectal biopsy [4]. Moreover, the parasitological examination of the of stool was much less sensible than the serology for the diagnosis of schistosomiasis in the HIV-infected patients. These findings suggest that the immunossupression carries to an impairment of *S. mansoni* egg's excretion [20].

The higher numbers of immature eggs in the intestinal fragments from treated mice could be explained by a concentration of the oviposition in the terminal ileum of treated animals or by an unexpected stimulus to the oviposition, in sharped contrast to the previous suggested. Moreover, the higher numbers of mature eggs in the same animals do not reflect this possible increase in the oviposition rate, as a period of about 6 days is needed for the embryo to maturate, and the treatment was performed only during 5 days.

Then, a blockade of the excretion of the eggs should not be discharged and even with a possible concentration of the oviposition in the terminal ileum, the numbers of eggs in the tissues of treated host could be higher. Nonetheless, the numbers of dead eggs deposited in the tissues of treated mice oscillates between the period of observation, with a larger difference to non-treated ones in the two first days after the onset of the treatment.

Previous work demonstrated that the numbers of eggs in the tissues of host treated with DMS were reduced, but not significantly [9]. When associated with schistosomicides, this synergistic effect of DMS could be beneficial in the treatment of patients with acute schistosomiasis [9]. However, in the present study it was demonstrated that these numbers can increase with the treatment of mice with DMS. This may reflect the differences in the schemes of administration of the glucocorticoids. LAMBERTUCCI et al. [9] used a very low dose of DMS diluted in the drinking water of mice, starting the administration before the worm maturation. The administration could be variable, as it depends on the volume of the drinking water ingested by each animal throughout the experiment. LENZI et al. [11] also had began the treatment in the early phases of infection, but used the parenteral injection of the drug, a more safe method for the administration of drugs. In the present study, it was used a high dose of DMS by parenteral route, starting 6 weeks after the infection. Moreover, since in mice infected with *S. mansoni* the beginning of egg-laying takes place around the 30th day of infection and since about 6 days are necessary for the eggs reach their full development, it is clear that, only from the 36th day onward, viable eggs in all development stages can be found in the intestinal wall. However, in the first 2 weeks of oviposition, the variability in the percentage of the various stages is very wide, and only after 6 weeks of infection, viable eggs in all stages of development are regularly found [17, 19]. This is the best time for evaluate the effects of drugs upon the oviposition of *S. mansoni* in the vertebrate host [17, 18]. In human acute schistosomiasis, when the glucocorticoids are administered, the egg-laying is also fully achieved [14].

Therefore, this increase in the numbers of eggs, deposited in the tissues of host treated with DMS in this present schedule of treatment, may aggravate the pathology of the liver and gut during the corticotherapy, and thus increasing the repercussions of the parasitism upon the host [14]. Even associated with schistosomicides, glucocorticoids could cause a retention of the *S. mansoni* eggs in the tissues of the host. Then, the use of glucocorticoids in the treatment of patients with acute schistosomiasis mansoni should be best investigated, because this drug may worse the tissue lesions and the pathophysiology of the disease.

RESUMO

Oograma em camundongos infectados com *Schistosoma mansoni* e tratados com dexametasona

Camundongos infectados com cerca de 90 cercárias da cepa LE de *Schistosoma mansoni* foram tratados durante 5 dias consecutivos com dexametasona (50mg/Kg, subcutaneamente) a partir do 42° dia de infecção. Grupos de cinco camundongos foram sacrificados dia-

riamente após o primeiro dia do início do tratamento até o primeiro dia após o término. A perfusão do sistema porta foi feita e fragmentos do intestino foram processados para a realização de oogramas qualitativos e quantitativos. O tratamento leva a um maior número de ovos nos tecidos dos camundongos tratados, se comparado com os grupos não tratados. Nenhuma mudança foi observada na cinética de oviposição, e ovos viáveis em todos os estádios evolutivos foram observados nos tecidos de camundongos tratados e controles. Estes dados reforçam a hipótese de um bloqueio parcial na saída de ovos dos tecidos do intestino para o lúmem intestinal em camundongos imunossuprimidos.

REFERENCES

1. COKER, C. M. - Effects of cortisone on immunity to *Schistosoma mansoni* in mice. **Proc. Soc. exp. Biol.** (N. Y.), **96**: 1-3, 1957.

2. DOENHOFF, M. & LONG, E. - Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse. IV. The inhability of T-cell deprived mice to reinfection, and other *in vivo* studies on the mechanisms of resistance. **Parasitology, 78**: 171-183, 1978.

3. DOENHOFF, M.; MUSALLAM, R.; BAIN, J. & McGREGOR, A. - Studies on the host-parasite relationship in *Schistosoma mansoni*-infected mice: the immunological dependence of parasite egg excretion. **Immunology, 35**: 771-778, 1978.

4. GRECO, D. B.; BAMBIRRA, E. A.; TOLEDO JR., A. C. C. & VITÓRIA, M. A. A. - A esquistossomose mansoni em indivíduos infectados pelo HIV. In: ENCONTRO DE PESQUISA DA FACULDADE DE MEDICINA DA UFMG, 4., Belo Horizonte, 1991. **Resumos**. Res. nº 126.

5. HARRISON, R. A. & DOENHOFF, M. J. - Retarded development of *Schistosoma mansoni* in immunossupressed mice. **Parasitology, 86**: 429-438, 1983.

6. HERMETO, M. V.; BICALHO, R. S.; MELO, A. L. & PEREIRA, L. H. - Kinectics of the pulmonary phase of *Schistosoma mansoni* in mice treated with dexamethasone. **Rev. Inst. Med. trop. S. Paulo, 32**: 168-171, 1990.

7. HERMETO, M. V.; MELO, A. L.; BICALHO, R. S. et al. - Dexamethasone does not reduce the worm burden in mice infected with *in vivo* obtained schistosomules of *Schistosoma mansoni*. **Rev. Inst. Med. trop. S. Paulo, 35**: 389-390, 1993.

8. LAGRANGE, E. - A la recherche de stéroides de synthèse à action antibilharzienne. **C. R. Soc. Biol.** (Paris), **157**: 425-427, 1963.

9. LAMBERTUCCI, J. R.; MODHA, J.; CURTIS, R. & DOENHOFF, M. J. - The association of steroids and schistosomicides in the treatment of experimental schistosomiasis. **Trans. roy. Soc. trop. Med. Hyg., 83**: 345-357, 1989.

10. LENZI, H. L.; ROSMAN, F. C. & LENZI, J. A. - Eosinophil favors the passage of *Schistosoma mansoni* eggs to the intestinal lumen. **Braz. J. Med. biol. Res., 20**: 433-434, 1987.

11. LENZI, H. L.; ROSMAN, F. C. & LENZI, J. A. - Glicocorticóides interferem no mecanismo de eliminação de ovos de *Schistosoma mansoni* em camundongos. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 25., Florianópolis, 1989. **Resumos**. p. 84.

12. LEWERT, R. M. & MANDLOWITZ, S.- Innate immunity to *Schistosoma mansoni* relative to state of connective tissue. **Ann. N. Y. Acad. Sci., 113**: 54-62, 1963.

13. MORRISON, D. D.; VAN DE WAA, E. A. & BENNET, J. L. - Effects of steroids and steroid synthesis inhibitors on the fecundity of *Schistosoma mansoni in vitro*. **J. Chem. Ecol., 12**: 1901-1908, 1986.

14. NEVES, J. - Esquistossomose mansoni. Clínica da forma aguda ou toxêmica. Rio de Janeiro, MEDSI, 1984. p. 143-151.

15. NEWSOME, J. - Observations on corticosteroid treatment of schistosomiasis in hamsters and baboons. **Trans. roy. Soc. trop. Med. Hyg., 57**: 425-432, 1963.

16. PELLEGRINO, J. & SIQUEIRA, A. F. - Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. **Rev. bras. Malar., 8**: 589-597, 1956.

17. PELLEGRINO, J.; OLIVEIRA, C. A.; FARIA, J. & CUNHA, A. S. - New approach to the screening of drugs in experimental schistosomiasis mansoni in mice. **Amer. J. trop. Med. Hyg., 11**: 210-215, 1962.

18. PELLEGRINO, J. & FARIA, J. - The oogram method for the screening of drugs in schistosomiasis mansoni. **Amer. J. trop. Med. Hyg., 14**: 363-369, 1965.

19. PRATA, A. - Biópsia retal na esquistossomose. Bases e aplicações no diagnóstico e tratamento. Rio de Janeiro, 1957. (Tese- Serviço Nacional de Educação Sanitária).

20. TOLEDO, A. A. C.; RABELLO, A. L. T.; NOCE, T. R. et al.- As possíveis interações entre a infecção pelo HIV e a esquistossomose mansoni em Belo Horizonte, MG. IN: ENCONTRO DE PESQUISA DA FACULDADE DE MEDICINA DA UFMG, 4., Belo Horizonte, 1991. **Resumos**. Res. nº 159.

21. WEINER, F. R.; CZAJA, M. J.; GIAMBRONE, M. A. et.al. - Transcriptional and post-transcriptional effects of dexamethasone on albumin and procollagen messengers RNAs in murine schistosomiasis. **Biochemistry, 26**: 1557-1562, 1987.

22. WEINMANN, C. J. & HUNTER, G. W. - Studies on schistosomiasis. XIV. Effects of cortisone upon the *Schistosoma mansoni* burden in mice. **Exp. Parasit., 9**: 239-242, 1960.

Recebido para publicação em 04/01/1993.
Aceito para publicação em 17/01/1994.