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KINECTICS OF THE PULMONARY PHASE OF Schistosoma mansoni IN MICE TREATED WITH DEXAMETHASONE

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SUMMARY

In the experimental schistosomiasis mansoni glucocorticoids cause a reduction in the worm burden when administered in the week of infection or, the longest, at the next week. In order to determinate the probable(s) site(s) of reduction of the worm burden, mice were infected with cercariae of LE strain of **S. mansoni** and dexamethasone was administered daily (50 mg/kg, subcutaneously) starting 1 hour before infection until the eighth day. Mice were sacrificed daily starting on the third day after infection until the ninth day, and schistosomula from lungs were collected. Six weeks after infection, the remaining mice were sacrificed and perfused for adult worm recovery. Analysis of the results showed that the non-treated mice presented larger numbers of lung larvae than the treated ones, and this difference was also found later in the worm burden in the portal system. This difference may reflect the early death of larvae in treated animals, before or after reaching the lungs.

KEY WORDS: Schistosoma mansoni; Schistosomiasis; Dexamethasone; Glucocorticoids.

INTRODUCTION

Many infectious diseases have their course markedly changed by glucocorticoids[6]. Usually, the administration of high doses of these steroids increases the rate of pathogens as a result from depression of the natural host resistance.

However, in schistosomiasis mansoni the treatment with glucocorticoids leads to a reduction in the worm burden[2, 3, 4, 8, 11, 12]. Besides, corticoids have their deleterious effect on **Schistosoma mansoni** worm burden only when they are administered around the time of infection[2, 4], and this decrease is more intense if this therapy is accomplished within the week of infection or, the longest, at the next week[4]. Initially, it was postulated that corticoids exert their effect on worm survival very early in the course of infection, and it was suggested that corticoids could inhibit skin penetration[2, 7] and/or early migration by the larvae[2], the death of a certain number of worms occuring as a result. However, the mechanisms responsible for this paradoxal effect of reduction in the worm burden are not completely known yet. Thus, an enumeration of suppositions rather than a real understanding of the phenomena can be present today: 1 — a direct

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action of the drug upon the larvae of **S. mansoni** in their adaptation and migration processes in the vertebrate host, attributable to a hormonal control of its development or to a chemically, specific and direct schistosomicidal effect upon these larvae[4]; 2 — inhibition of the inflammatory reaction induced by the passage of larvae, that under normal conditions would facilitate their migration by "dissection" of vertebrate host tissues[4]; 3 — the corticotherapy would lead to a thickness of the basal membrane on the subcutaneous connective tissue, as well as an interference on cercarial proteolytic enzymes, with consequent reduction in the infective capacity of **S. mansoni** cercariae[8]; 4 — inhibition of development of **S. mansoni** worms, with their posterior death, could be an indirect consequence of the immunosuppressive activity of the drug in question[4].

Thus, in the present study, the kinectics of the pulmonary phase of **S. mansoni** in mice treated with dexamethasone was investigated aiming to verify the probable(s) site(s) of the effect of the drug, as this phase of **S. mansoni** migration in the vertebrate host is intermediary between the skin and portal phases.

MATERIAL AND METHODS

The LE strain (Belo Horizonte, Brazil) of **S. mansoni**, shed by laboratory-reared and infected **Biomphalaria glabrata**, was used in the present study. Two groups of 30 male albino mice, weighing about 30 g were exposed to **S. mansoni** cercariae by percutaneous route. The abdomen was carefully shaved and washed with dechlorinated water. Exposure to cercariae was carried out according to the technique described by BARBOSA et al.[1]. A cercarial suspension (about 200 organisms/animal) was introduced into the ring and the exposure kept for 50 minutes.

Dexamethasone was administered to the experimental group (50 mg/kg, subcutaneous route) daily, starting 1 hour before infection until the eighth day after.

Three mice of each group were daily sacrificed, starting on the third day after infection until the ninth day. Schistosomula from lungs were collected according to the technique described by BARBOSA et al.[1] and counted under a dissecting microscope. The lungs of mice were perfused with heparinized Hanks-Balanced Salt Solution (hHBSS) (pH 7.2 to 7.6, at a temperature of 37°C) and chopped into small fragments with scissors. These fragments were introduced into glass vessels closed at the lower end by stainless steel screen of mesh 0.09 mm which was supported by three legs inside a beaker with hHBSS at 37°C; the schistosomules migrate from the tissue to warm hHBSS and may be collected and counted after 3 hours.

Six weeks after infection, the remaining mice of each group were sacrificed and perfused for worm recovery, according to the technique of PELLEGRINO & SIQUEIRA[9] with minor modifications, and counted under a dissecting microscope.

The worm burden reduction rate was calculated according to the formula below:

$$\%\ \text{of worm burden reduction} = 100 - \frac{\text{worm burden of the treated group} \times 100}{\text{worm burden of the control group}}$$

The experiment was performed twice.

RESULTS

Data of lung recovery of schistosomules in treated and non-treated mice can be seen in Table 1. There is a significant difference between these two groups throughout the experimental period: 731 larvae in the control group, and 347 in the treated one.

The schistosomules appear in the lungs from the third day of infection until the ninth day in both groups of mice. Besides, the peak of schistosomule recovery from the lungs of mice was reached on the fourth day, for both groups (173

TABLE 1
Recovery of schistosomules from the lungs of mice treated with dexamethasone

Groups of mice	DAYS OF INFECTION*							
	3	4	5	6	7	8	9	
Control	33	173	159	124	64	73	31	731
Treated	16	79	61	57	33	39	15	347

* The numbers represent the total amount of two experiments.

in non-treated and 79 in treated animals). Thus, the parasite dynamics of arriving to the lungs, coming from the skin, was not modified in treated mice. Only the numbers of lung-larvae were changed from the third to the seventh days of infection (about twice more in control than in treated group).

Table 2 summarizes the worm burden from treated and control mice. For both sex of worms, the means of control and treated groups were significantly different ($p < 0.005$). The numbers of worms in treated mice were lower than in control ones.

TABLE 2
Mean numbers and standard deviation of worms recovered from the portal system of mice treated with dexamethasone. Data are the average of two experiments

Group (Nº of mice)	Mean number of worms recovered		
	Male*	Female*	Total
Control (18)	44.1 (15.1)	19.1 (11.8)	34.1 (15.9)
Treated (16)**	25.5 (10.6)	9.1 (6.2)	14.1 (10.4)

* $p < 0.005$; **two mice died during the treatment.

The rates of infection in non-treated and treated groups were 17.6% and 7.15%, respectively. The reduction rate of the worm burden (considering males and females) was 58.38%.

All the data result from two experiments, that were not significantly different.

DISCUSSION

Determination of the site(s) of worm burden reduction in mice infected with **S. mansoni** and treated with glucocorticoids leads to a better understanding of the mechanism(s) responsible for their therapeutic efficacy.

Our results showed that lung larvae of **S. mansoni** were found to be more frequent in non-treated mice, and a marked difference was found between the total numbers recovered from non-treated and treated groups (731 and 347, respectively). The reduction of the worm burden was later confirmed in the portal system (the numbers of worms in treated mice were significantly lower than in those non-treated ones), confirming some previous works[2, 3, 4, 7, 11, 12]. Such difference may reflect the premature death of most larvae at the early phase of infection (skin-lung interval) as on the ninth day of infection only a very reduced number of larvae are found in the portal system[1]. The reduction may occur before the larvae reach the lungs (at the skin or during their migration to the lungs) or after their arrival to this site because this reduction in the number of lung schistosomules was found from the first day until the seventh day of recovery and the technique performed for this finality[1] permits recovery of live larvae only. Moreover, the larvae reached the lungs without delay.

It is well known that pre-treatment with cortisone slow down the penetration of **S. mansoni** cercaria and enhances the natural resistance of mice to this parasite[7]. This result is probably due to an increased polymerization of hyaluronic acid in the connective tissue, an increased density of basal membrane and or an interference on the action of cercarial proteolytic enzimes[8]. However, in our study this effect must be excluded, as the treatment started just one hour before the infection and was prolongated throughout the first week of infection, as this is the more efficacious therapeutic outline[4]. The effect of dexamethasone upon **S. mansoni** in this study is then limited to the skin and or pulmonary phases and, therefore, after cercarial penetration. So, an direct effect of this drug[4] on the worm larvae may be possible as the treatment leads to a real decrease in the rate of parasites. However, more evidences are needed.

The hypothesis of a hormonal control of **S. mansoni** development by glucocorticoids administered to the host are not ruled out. In the peritoneal cavity of mice, dexamethasone causes a delay in the cercaria-schistosomulum transformation process, and this phenomenon may difficult the **S. mansoni** adaptation to the host[5] and therefore may carry to an early death of the larvae by host defenses. Besides, the existence of ecdysteroids in **S. mansoni**, particularly in lung larvae[10], is also interesting because indicates that this parasite stage could be under direct glucocorticoid control[4].

On the other hand, the indirect effect of immunosuppression must be not forgotten, as this depressive effect on immunological system

of the host is the major and complex action of the corticoids. Inhibition of **S. mansoni** development could be an indirect consequence of the immunosuppressive activity of steroids that leads to death some of the parasites[4]. However, it is not known why mice which have been immunosuppressed are less effectively parasitized by **S. mansoni** than the immunological intact controls.

So, we believe that the effect of reduction on the worm burden in mice treated with glucocorticoids may be due to an early action (direct or indirect) upon premature stages of the parasite, during or between skin and pulmonary phases. Further, there is an real drop in the rate of larvae and not a retarded migration of them. Nonetheless, this action is difficult to determinate because the corticosteroids affect general physio-endocrinological balance of the host and parasite, causing major modifications on **S. mansoni**-host relationship.

RESUMO

Cinética da fase pulmonar do Schistosoma mansoni em camundongos tratados com dexametasona

Na esquistossomose mansoni experimental, os glicocorticóides causam uma redução na carga de vermes quando administrados na semana da infecção, ou, no mais tardar, na semana seguinte. A fim de determinar o(s) provável(is) sítio(s) de redução da carga de vermes, camundongos foram infectados com cercárias da cepa LE de **S. mansoni** e dexametasona foi administrada diariamente (50 mg/kg, subcutaneamente), iniciando 1 hora antes da infecção e prosseguindo até o 8º dia. Os camundongos foram sacrificados diariamente, do 3º ao 9º dia pós-infecção, e os esquistossômulos foram coletados. Os camundongos remanescentes foram sacrificados seis semanas após a infecção e perfundidos para recuperação dos vermes adultos. A análise dos resultados mostrou que os camundongos não tratados apresentavam números maiores de larvas pulmonares que os tratados, sendo que esta diferença foi também encontrada mais tarde na carga de vermes do sistema porta. Esta diferença pode representar a morte precoce das larvas nos animais tratados, no período pele-pulmão de seu desenvolvimento.

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