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Schistosoma mansoni: ACQUIRED IMMUNITY IN MICE AFTER THE USE OF OXAMNIQUINE AT THE EVOLUTIVE SKIN AND PULMONARY PHASES

Paulo Marcos Z. COELHO (1), Rômulo T. MELLO (2) & Sílvia E. GERKEN (2)

SUMMARY

Mice infected with 350 cercariae of **Schistosoma mansoni** (LE strain) were treated with oxamniquine, at the dose of 400 mg/kg, 24, 48, 72, and 96 h after infection. Forty days after the treatment, the animals were submitted to a challenge infection with 80 cercariae, through the abdominal and ear skins. The number of immature worms in the animal groups treated 24 and 96 h after the first infection was found to be lower than that in the control group, thus showing that the death of schistosomes by chemotherapy, at the skin and pulmonary phases, causes an acquired resistance state.

KEY WORDS: Schistosoma mansoni; Acquired immunity; Oxamniquine.

INTRODUCTION

Subsequently to the pioneer work by VILLELLA et al.[19] various authors[7, 15, 16] demonstrated the acquisition of immunoprotection by means of a previous infection with irradiated cercariae. BICKLE & JAMES[3] gave an impulse to this research line, when they showed that irradiated and cryopreserved schistosomula keep their ability to induce immunoprotection. This finding, which was corroborated by different authors, made way for and signified a new possibility of obtaining a practicable vaccine, since it was possible to store up live immunizing forms. The comprehensive review elaborated by DEAN[5] showed that higher doses of irradiation, beginning with 20 Krad, are more efficacious in the process of inducing immunoprotection. Based on different works present in the literature, this author suggests that the efficacy of higher doses of irradiation could be related to the parasites death, at the skin phase, thus inducing a more intense immunoresponse. On the basis of these observations, MASTIN et al[10] and BICKLE & ANDREWS[2] were able to get a state of immunoprotection, using chemotherapy with Ro-11.3118, 24 h after infection with 500 cercariae. However, these authors failed to obtain good results when oxamniquine was used.

The scope of the present work is to study the treatment with oxamniquine in detail, using higher doses of the drug aiming at obtaining induction of an immunoprotection state.

(1) Departamento de Parasitologia, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (ICB/UFMG). Belo Horizonte, Minas Gerais, Brasil.
(2) Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia da Universidade Federal de Minas Gerais. Belo Horizonte, Minas Gerais, Brasil.
Address for correspondence: Prof. Paulo Marcos Z. Coelho, Deptº Parasitologia, ICB/UFMG. Caixa Postal 2486. 30161 Belo Horizonte, MG, Brasil.

MATERIAL AND METHODS

Groups of outbred albino mice were infected with about 350 cercariae of **S. mansoni** (LE strain), via the abdominal transcutaneous route. Afterwards, the animals were divided into three groups, and treated with oxamniquine, at a single dose of 400 mg/kg body weight, via oral route, 24, 48, 72 and 96 h after infection. Forty days after the treatment, all the animals of each group were sub-divided into two other groups, and submitted to a challanger infection with 80 cercariae, via transcutaneous route (abdomen and ear), according to the techniques described by BARBOSA et al[1] and GERKEN et al[9]. Twenty days after the challenge infection, all the mice were sacrificed and perfused for recovery and countings of immature worms, as described by PELLEGRINO & SIQUEIRA[14]. Careful observations were carried out in order to detect the absence of adult worms in the mice submitted to chemotherapy, and to search eggs in tissues of their liver and intestines, by means of the oogram method[13].

Statistical analysis was perfomed using the Student's t test[17].

RESULTS

The results obtained in this study are summarized in Tables 1 and 2. As it can be seen, the animals that received chemotherapeutic treatment with oxamniquine, 24 and 96 h after the first infection, presented mean immature worm burdens significantly lower than those found in the control groups. This fact occurred with mice challenged with cercariae both via abdominal (Table 1) and ear (Table 2) skins. However, the same did not occur when treatment was carried out 48 and 72 hours after the first infection. The absence of adult worms and eggs in all the treated animals allows us to infer that the drug was effective, and that the immunity observed was not related with concomitant immunity.

TABLE 1

Recovery of **S. mansoni** worms in mice previously infected* and treated** with oxamniquine, and challenged*** with cercariae through the abdominal skin.

Group	Interval between infection and treatment (hours)	Number of mice per group	Mean and standard deviation of immature worms recovered 20 days after challenge infection	Statistical analysis by the Student's "t" test p<
A	24	10	16.80 ± 6.34	0.01
B	48	9	21.60 ± 9.76	NS
C	72	9	22.00 ± 1.76	NS
D	96	10	17.20 ± 5.87	0.01
Control	—	11	28.91 ± 6.07	—

* Infection with 350 cercariae
** Treatment with oxamniquine (400 mg/kg, single dose)
*** Challenge infection with 80 cercariae
NS = Not significant

TABLE 2

Recovery of **S. mansoni** worms in mice previously infected* and treated** with oxamniquine and challenged*** with cercariae through the ear skin.

Group	Interval between infection and treatment (hours)	Number of mice per group	Mean and standard deviation of immature worms recovered 20 days after challenge infection	Statistical analysis by the Student's "t" test p<
A'	24	9	4.11 ± 4.11	0.05
B'	48	9	6.60 ± 4.83	NS
C'	72	8	4.75 ± 3.59	NS
D'	96	8	3.13 ± 1.89	0.01
Control	—	9	8.33 ± 4.18	—

* Infection with 350 cercariae
** Treatment with oxamniquine (400 mg/kg, single dose)
*** Challenge infection with 80 cercariae
NS = Not significant

DISCUSSION

The results showed that treatment with oxamniquine, at the dose of 400 mg/kg, 24 and 96 h after infection with 350 **S. mansoni** cercariae, causes the development of resistance against reinfection (Tables 1 and 2). BICKLE & ANDREWS[2] could not observe statistically significant resistance in mice treated with a lower dose of oxamniquine (150 mg/kg), 24 h after infection. According to these authors, oxamniquine did not kill **in situ** all the parasites, when it was administered at the dose of 150 mg/kg, at the skin phase, and allowed part of them to migrate to other sites. This assumption was also corroborated by MASTIN et al[10].

Our data showed that oxamniquine was capable of inducing immunoprotection, when administered at a higher dose (400 mg/kg), 24 h

after infection. In a previous experiment, it was verified that treatment with the same drug, and at the same dose, when used 1 h before and 24 h after infection, was effective inducing immunoprotection. When administered within these periods, the drug might have killed the majority of the schistosomes at the skin phase, since they remain at this site up to 24 h after infection[1].

In the same way, resistance to infection could be observed in the animal group treated 96 h after infection, when almost all the schistosomes remain at the lungs[1]. FLISSER & McLAREN[8] have recently demonstrated that treatment with praziquantel, 6 days after infection, produces immediate tegumentary changes, thus allowing a binding between fluorescein marked antibodies and surface antigens. Pulmonary haemorrhages can appear as a result of parasitary enzyme release, as well as of inflammatory reactions around a deposit of immunocomplexes. This inflammatory reaction can contribute to immunoprotection acquisition.

COULSON & MOUNTFORD[4], studying attenuated parasites by irradiation, showed the importance of the administration route in the process of inducing immunoprotection. They failled to obtain immunoprotection, when inoculation of attenuated parasites was performed via intravenous route, in sharp contrast with the results obtained via intradermal route, similar to those achieved when transcutaneous route was used. On the other hand, peritoneal and intratracheal routes were able to promote a partial resistance only. MOUNTFORD et al[12] showed that the death of schistosomes alone (at the skin phase) is not sufficient to induce immunoprotection in mice treated with Ro-11.3128. Further, they showed that the parasites of primoinfection are principally eliminated after reaching the lungs. According to the same research line, MENSON et al[11] showed the importance of vaccination with irradiated cercariae for the pulmonary mobilization of lymphocytes and macrophages towards the infection focus. On the other hand, VIGNALI et al[18] demonstrated the importance of the permanence of the parasites at the lungs for the development of cellular response in rats. Finally, a work very well conducted by ELSAGHIER & McLAREN[6], published in 1989, showed that in a schedule of vaccination with irradiated cercariae, the dose of 20 Krad caused the death of the majority of the irradiated cercariae at pulmonary level (75%), whereas the dose of 50 Krad provoked the death of the great majority of the irradiated cercariae in the skin (only 5% of them reached the lungs). It is important to remark that the use of both doses of irradiation in schedules of vaccination with attenuated parasites induces a significant state of immunoprotection in vaccinated mice.

In the present study, we have failed to obtain acquired resistance in the animal groups treated 48 and 72 h after first infection. In an attempt to explain these results, the most plausible hypothesis is based on the fact that the most part of the parasites had already left the skin on the occasion of the chemotherapeutic treatment, but had not reached the lungs yet[1], consequently dying at other sites where the resultant inflammatory reaction could not produce a suitable immunoprotective response. It is worth noting that the results obtained in this study corroborate both the animal group challenged through the abdominal skin and the other one submitted to challenge infection through the ear skin (Tables 1 and 2). Moreover, it is interesting to remark that differences related to worm recovery in connection with the site of infection could be observed for the first time. Thus, a lower adult worm recovery rate was clearly detected, when reinfection was performed through the ear skin. Finally, the present work allows the reinforcement of the hypothesis that resistance to infection depends on the site where it occurs, both with irradiated cercariae[5, 6] and through the death of schistosomes by chemotherapy. Furthermore, it is well established that chemotherapeutic killing of schistosomes at the lung phase induces immunoprotection against reinfection. These results open the possibility for future successful studies in which the use of chemotherapy at the initial phases of infection could induce an acquired resistance state.

RESUMO

Schistosoma mansoni: **imunidade adquirida em camundongos, após o uso de oxamniquina durante as fases evolutivas da pele e do pulmão.**

Camundongos infectados com 350 cercárias de **Schistosoma mansoni** (cepa LE) foram trata-

dos com oxamniquina, em dose única de 400 mg/kg, 24, 48, 72 e 96 horas após a infecção. Quarenta dias após o tratamento, os animais foram submetidos a uma infecção desafio com 80 cercárias, através da pele abdominal e da orelha. O número de vermes imaturos nos grupos de animais tratados 24 e 96 horas após a primeira infecção foi menor do que o do grupo controle, evidenciando que a morte de esquistossômulos por quimioterapia, durante as fases da pele e do pulmão, causa um estado de resistência adquirida.

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