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BRIEF COMMUNICATION

SYNERGISTIC ACTION OF PRAZIQUANTEL AND HOST SPECIFIC IMMUNE RESPONSE AGAINST *Schistosoma mansoni* AT DIFFERENT PHASES OF INFECTION

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SUMMARY

The interaction between specific immune response to *Schistosoma mansoni* and praziquantel (PZQ) was studied in mice. In mice harboring concomitant immunity, 6-day-old parasites treated with PZQ were more effectively removed than 24h treated parasites despite both had a significant worm burden reduction when compared with respective treated controls. These results show that PZQ can be effective at the skin and lung stages of parasite's development mainly acting with a established specific immune response, and particularly at the lung phase.

KEYWORDS: *Schistosoma mansoni*; Praziquantel; Immune Response; Concomitant Immunity.

The precise mechanisms by which praziquantel (PZQ) acts on *Schistosoma mansoni* are not completely understood. Some of the PZQ effects listed in the literature are: Ca^{2+} influx through the tegument leading to an immediate muscular contraction[6], it reveals hidden antigens in the schistosome surface which are targets to the host immune response[5], it acts as a spacer between lipid molecules[10], it interferes with membrane internalization processes in different stages of the parasite, and also depletes glutathione from the parasite[8,9]. BRINDLEY & SHER (1987)[1] studying the synergistic action of PZQ and host immune response compared the efficacy of PZQ between B cell depleted and immunologically intact mice. It was observed that in B cell-deficient mice the schistosomicide effect of PZQ is strongly reduced or completely abolished. Besides, the drug efficacy was completely restored by passive transfer of immune serum obtained from *S. mansoni* infected donor mice at the acute phase. FARAH *et al.* (2000)[3] examined immunological mechanisms associated with residual protection after chemotherapy and concluded that the pattern of primary cercarial exposure determines whether a secondary infection post PZQ treatment elicits a T helper 1 or a T helper 2-associated immune response. Concomitant immunity is a concept which was first utilized by SMITHERS & TERRY (1969)[11] in order to describe the capacity of adult *S. mansoni* worms surviving in its immunologically intact host. When this host is submitted to a challenge infection, a partial protection against the juvenile parasite is established, thus reducing significantly the worm burden from reinfection.

In the present study the synergistic action of praziquantel and host specific immune response against a *S. mansoni* challenge infection was evaluated in mice bearing a concomitant immunity. *S. mansoni* cercariae (LE strain from Belo Horizonte, Brazil) were used for infection of female Swiss mice. Perfusion of mice for worm burden counts was undertaken as described by PELLEGRINO & SIQUEIRA (1956)[7]. Mice were infected with 40 cercariae, subcutaneous route. One hundred days after primary infection, the animals were reinfected with ± 40 cercariae, transcutaneous route, and distributed into 6 different groups, as follows:

D1- Infected, reinfected and treated with PZQ (500 mg kg^{-1}) 24 h after reinfection.
D2 - Infected, reinfected and treated with PZQ (500 mg kg^{-1}) 6 days after reinfection.
D3 - Infected, reinfected and untreated with PZQ.
D4 - Infected and treated with PZQ (500 mg kg^{-1}) 24 h after infection.
D5 - Infected and treated with PZQ (500 mg kg^{-1}) 6 days after infection.
D6 - Infected and untreated.

Groups D4, D5 and D6 received just one infection, at the same moment when groups D1 and D2 were reinfected. Twenty days after reinfection, all the animals were individually perfused for worm burden evaluation. Adult worms proceeding from primary infection, as well as immature worms from reinfection were counted. Mice were treated with PZQ, single dose by oral route. The worm burden reduction was calculated using the following formula: [(C-T)\C] x 100 where C represents the mean worm recovery from control group, and T represents the mean worm recovery from experimental group.

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As can be seen in Table 1, a more noticeable decrease of worm burden ($p < 0.0002$) was observed in the infected mice group, which was reinfected and treated with PZQ, 6 days after reinfection (lung phase, group D2). This group presented a mean of 3.0 ± 1.7 worms/mouse, whereas its respective control group (D3) showed 12.9 ± 3.6 worms (76% worm burden reduction). Our results also demonstrated that mice bearing concomitant immunity established by infection and reinfection (D1) presented a significant reduction of worm burden ($p < 0.01$), when compared with the group that received a primary infection only (D4). The concomitant immunity was able to potentiate significantly the PZQ activity (D2 x D5 / $p < 0.04$), when treatment of mice was administered at the lung phase, after reinfection. Association between concomitant immunity and PZQ effect against *S. mansoni*, at lung phase after reinfection, demonstrated a clear synergism between drug and host's immune system. Worm burden reduction in mice treated one day (skin phase) after reinfection was not statistically significant when compared with their respective controls. The greater activity of PZQ detected in the group treated at the lung phase is corroborated by the results obtained by WILSON, COULSON & DIXON (1986)[12], who found pronounced evidence that lung would be the main site of the host's immune attack against schistosomules, resulting in a marked elimination of parasites. It seems that the mode of action of PZQ depends on the experimental murine model, on the site of the parasite's location during treatment, and on the type of immunity established against *S. mansoni*. Our results demonstrated an increase in the schistosomicide efficacy of PZQ in association with the immune response, since treatment administered on the mice group bearing concomitant immunity against *S. mansoni*, with a sub-curative dose of 500 mg kg^{-1} PZQ, was able to eliminate significantly more schistosomules at the lung stage than its respective control group. Concomitant immunity was clearly demonstrated by 22% worm burden reduction in infected/reinfected not treated group (D3) compared with infected not treated (D6) control group. In concomitant immunity a part of reduction in worm burden from reinfection would be due to recirculation of juvenile worms - that at the moment of perfusion would be in other sites of the organism (mainly in the lungs) and, therefore, would not be taken into account for statistical estimation - than to a true protective immunity. Juvenile worm recirculation would occur through porto-cava shunts resulting from portal hypertension typical of the disease[2,13]. To achieve 50% worm burden reduction in mice treated at 28 days after reinfection, it was necessary to use 1000 mg kg^{-1} PZQ, a higher PZQ concentration than that required for an effective therapeutic dose (data not shown). ELISA demonstrated that total IgG antibodies level against SWAP (Fig. 1) were higher in groups infected, reinfected and treated with 500 mg kg^{-1} PZQ, either at skin (D1) or lung (D2) phases, and in the group of mice infected, reinfected and not treated (D3). The total IgG antibodies level against SEA were similar to anti-SWAP IgG antibodies level (unpublished data). IgG antibodies anti-schistosomule antigens (Fig. 2) were also detected, however in lower

Table 1

Evaluation of worm burden in Swiss mice previously infected with *S. mansoni,* reinfected with 40 cercariae, and treated with 500 mg kg^{-1} PZQ at skin and lung phases after reinfection

Groups	Mean of immature worms ± SD (n)◆	Worm burden Reduction (%)	Significance level
Inf./untreated (D6)	16.6 ± 2.8 (15)	-	D1 x D2 < 0.007
Inf./Reinf./untreated (D3)	12.9 ± 3.6 (11)	22.6	D6 x D3 < 0.01
Inf./PZQ 24 h (D4)	7.4 ± 2.4 (15)	42.6	D1 x D4 = NS*
Inf./Reinf./PZQ 24 h (D1)	6.7 ± 4.0 (15)	48.1	D1 x D3 < 0.05
Inf./PZQ 6 d (D5)	5.4 ± 3.4 (15)	58.1	D2 x D5 < 0.04
Inf./Reinf./PZQ 6 d (D2)	3.0 ± 1.7 (15)	76.7	D2 x D3 < 0.0002

*NS = Not significant; ◆(n) = Number of mice/group.

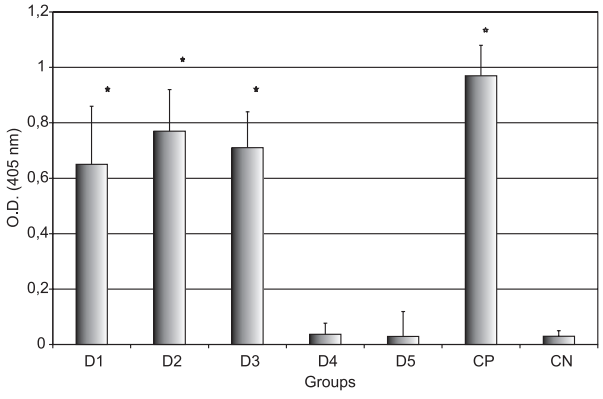

Fig. 1 - Total IgG antibodies against soluble worm antigens preparation (SWAP) of *S. mansoni*. **D1)** infected/reinfected/treated mice with PZQ 500 mg kg^{-1} one day after reinfection. **D2)** infected/reinfected/treated mice with PZQ 500 mg kg^{-1} six days after reinfection. **D3)** infected/reinfected/ not-treated mice. **D4)** infected/treated mice with PZQ 500 mg kg^{-1} one day after infection. **D5)** infected/treated mice with PZQ 500 mg kg^{-1} six days after reinfection. **CP** e **CN** means positive (mice at the chronic phase of infection) and negative control sera, respectively. Bars represent mean + SD.

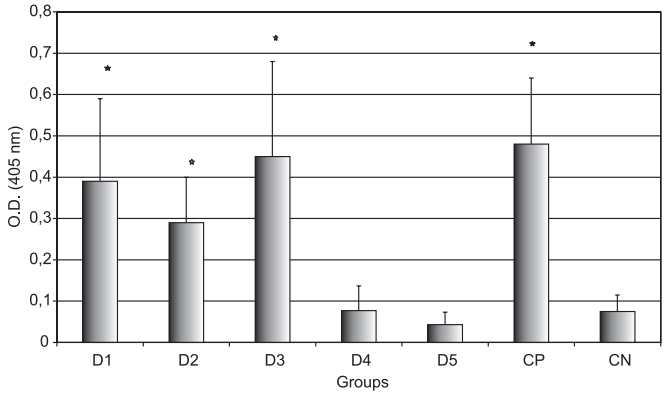

Fig. 2 - Total IgG antibodies against *S. mansoni* schistosomula antigens. **D1)** infected/reinfected/treated mice with PZQ 500 mg kg^{-1} one day after reinfection. **D2)** infected/reinfected/treated mice with PZQ 500 mg kg^{-1} six days after reinfection. **D3)** infected/reinfected/not-treated mice. **D4)** infected/treated mice with PZQ 500 mg kg^{-1} one day after infection. **D5)** infected/treated mice with PZQ 500 mg kg^{-1} six days after reinfection. **CP** e **CN** means positive (mice at the chronic phase of infection) and negative control sera, respectively. Bars represent mean + SD.

level than IgG anti-SWAP antigens. Those groups that received just one infection and were treated at skin and lung phases (D4 and D5) with the same PZQ concentration failed to show a significant antibody response against the tested antigens. The differences in drug activity might be associated with several aspects related to evolutionary phase of the parasite in its host. A better understanding of the *S. mansoni* physiology during its development and the interactions between host specific immune response and parasite will be necessary to explain diminished drug efficacy on 28 day old immature *S. mansoni*.

RESUMO

Ação sinergística do praziquantel e resposta imune específica do hospedeiro ao *Schistosoma mansoni* em diferentes fases da infecção

A interação entre a resposta imune específica ao *Schistosoma mansoni* e praziquantel (PZQ) foi avaliada em modelo murino. Em camundongos portadores de imunidade concomitante, parasitos com 6 dias de idade e tratados com PZQ, foram eliminados mais eficazmente do que parasitos de apenas 24 h, apesar de ambos mostrarem uma redução significativa da carga parasitária quando comparados com os respectivos controles tratados. Estes resultados mostram que o PZQ pode ser eficaz nos estágios de pele e pulmão durante o desenvolvimento do parasita, agindo principalmente com uma resposta imune específica estabelecida e, particularmente, na fase pulmonar.

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