

BRIEF COMMUNICATION

LETHAL EFFECT OF OXAMNIQUINE AND PRAZIQUANTEL ON MICE EXPERIMENTALLY INFECTED WITH *Schistosoma mansoni*

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SUMMARY

Lethality caused by administration of oxamniquine and praziquantel to mice infected with *Schistosoma mansoni*, and their respective controls (uninfected), has been studied. As the results indicate, the infected animals clearly showed higher mortality rates when praziquantel was used. Surprisingly, it may be noted that exactly the contrary occurs in relation to the use of oxamniquine, inasmuch as marked higher mortality rates were seen in the control animals (uninfected). These observations lead to the conclusion that further toxicological studies of antischistosomal drugs using *S. mansoni* infected animals are needed.

KEYWORDS: *Schistosoma mansoni*; LD₅₀; Oxamniquine; Praziquantel.

INTRODUCTION

COELHO et al.⁴ have shown metabolism of sodium pentobarbital to be diminishing in mice bearing experimental schistosomiasis, whereas subsequent papers^{1,2,3,8} have demonstrated various enzymes pertaining to the hepatic microsomal system to be depressed, and that the inflammatory granulomatous reaction around the egg would be the primordial element responsible for the metabolism diminution.

In view of these considerations, it is relevant to study the toxicity of conventional antischistosomal drugs in animals with hepatic lesions caused by the disease, since all the known pharmacological studies (experimental or clinical) have been carried out with individuals and/or animals without schistosome infection.

This paper records lethality caused by oxamniquine and praziquantel, the most used drugs in the treatment for schistosomiasis, using previously infected mice.

MATERIAL AND METHODS

Adult female Balb-C mice were infected with 40 cercariae of *Schistosoma mansoni* (LE strain). This strain has been maintained by serial passage through *Biomphalaria glabrata* and hamster (*Cricetus auratus*) at the laboratories of the Schistosomiasis Research Unit, Federal University of Minas Gerais, Brazil, for more than 30 years.

On day 70 after infection, the infected animals and the uninfected controls were distributed into groups of 15 and 10 animals each, respectively. Two LD₅₀ recorded in literature^{5,6} were employed for drug testing in this work, with some modifications required for preliminary assays, as follows:

- 1) Oxamniquine - 1300mg/kg, oral route⁵ reduced to 800mg/kg, oral route.
- 2) Praziquantel - 2000 to 3000mg/kg⁶, oral route. The dose of 3000mg/kg was employed (oral route).

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The infected animals that died after drug administration during the diurnal period, as well as the surviving ones (24hr after chemotherapy) were perfused for worms, according in general terms to the technique by PELLEGRINO & SIQUEIRA⁷.

Using the same methodology, replications of the experiments were carried out aiming corroborating the first results obtained.

The statistical analysis employing the X² test provided data related to the comparison between the proportions of dead and surviving mice, among the infected and uninfected animals that received treatment. The averages of worms were compared by means of the analysis of variance (since eggs are important in the immunopathology, the mice infected with only male worms were not taken into account).

RESULTS

Data, given in Table 1, concerning the lethal effect of praziquantel in murine schistosomiasis (at 3000mg/kg), show that mice previously infected with *S. mansoni* presented significantly higher mortality (18 out of 44) than the control group (that showed 2 out of 40). The statistical analysis of all the data obtained in three experiments revealed a difference between the two groups ($p < 0.003$).

TABLE 1

Distribution of the frequency of the dead and surviving mice in relation to the infected and uninfected groups which received praziquantel (3000mg/kg and oxamniquine (800mg/kg)

	* Praziquantel (3.000mg/kg)		** Oxamniquine (800mg/kg)	
Mice	Infected	Uninfected	Infected	Uninfected
Dead	18	2	12	37
Surviving	26	38	58	29
total	44	40	70	66

* Total data of three independent experiments ($p < 0.003$)

** Total data of four independent experiments ($p < 0.0001$)

The lethal effect of oxamniquine in murine schistosomiasis (at 800mg/kg), as shown in Table 1, presents a result completely opposite to that obtained with praziquantel (Table 1). So, statistical analysis of the data connected with four experiments shows significantly higher mortality ($p < 0.0001$) in the normal mice (37 out of 66) in relation to the infected group (12 out of 70).

As shown in Table 3, a significantly higher worm burden ($p < 0.026$) in dead animals was conjointly detected in the data of three experiments carried out with praziquantel (dead mice: mean worms = 22.8; living mice: mean worms = 16.3).

As regards the data obtained in four experiments using oxamniquine, they did not present statistically significant differences between the mean number of worms recovered from surviving ($m=20.6$) and dead ($m=18.5$) animals.

DISCUSSION

Results about the LD₅₀ obtained in mice with *S. mansoni* infection and treated with praziquantel and oxamniquine appeared to be surprising regarding oxamniquine.

As mentioned before, praziquantel was employed at the dose of 3000mg/kg, as prescribed by MURMANN⁶, who determined 2000-3000mg/kg as the LD₅₀ for this drug. Serial experiments showed results that corroborate the established hypothesis related to diminution of the metabolic capacity due to hepatic alterations produced by schistosomiasis, mainly owing to the granulomatous reaction around the egg^{3,8}. Increasing of lethality was quite evident and highly significant in all experiments carried out with mice bearing experimental schistosomiasis, when praziquantel was used (Table 1).

Surprisingly, the same event did not occur in relation to oxamniquine. In fact, exactly the contrary happened, that is, significantly higher mortality was observed in the control group in relation to the one with schistosomiasis (Table 1). In this study, the dose of 800mg/kg was our own choice, inasmuch as the dose of 1300mg/kg chosen by FOSTER⁵ resulted in death for all animals in a preliminary test. The explanation for the higher mortality observed in the control (uninfected) animals seems to be supported by the hypothesis of the production of toxic metabolites from oxamniquine, which could be produced more efficiently in the animals with enzymes of the microsomal hepatic system unaltered by schistosomiasis. On the other hand, WEBSTER JR.⁹ records that the main metabolite is formed in the intestines during absorption, and can be encountered in plasma at a 10 times higher dose than the unaltered oxamniquine concentration. It is well known that during schistosomiasis there is a marked diminution of the area of intestinal absorption due to granulomatous lesions, besides irritation of the intestinal mucosa that leads to diarrhoea. Pharmacological studies performed with oxamniquine labelled with radioisotopes would be necessary to elucidate this problem, and they are already devised for further work.

The worm burden evidenced to be statistically significant for determination of mortality of the infected animals treated with praziquantel. So, considering all experiments with praziquantel (Table 2), a significantly higher worm burden could be observed in dead animals in relation to the surviving group. These results corroborate earlier findings⁴ demonstrating a close correlation between worm burden and pentobarbital sleeping-time in mice.

The present results emphasize the need of using animals infected with *S. mansoni* in further pharmaco-

TABLE 2

Worm recovery from dead and surviving mice submitted to treatment with oxamniquine and praziquantel

Groups	Total of all experiments	
	No. of mice	Mean worm \pm SD
*Oxamniquine		
Dead mice	12	18.5 \pm 7.5
Surviving mice	47	20.6 \pm 9.5
** Praziquantel		
Dead mice	16	22.8 \pm 9.6
Surviving mice	26	16.3 \pm 8.6

* 4 experiments

** 3 experiments

n/s = not significant

logical studies aiming at determining toxicity of anti-schistosomal drugs.

RESUMO

Efeito letal de oxamniquina e praziquantel em camundongos experimentalmente infectados com *Schistosoma mansoni*

Pesquisou-se a letalidade causada por administração de drogas (oxamniquina e praziquantel) em camundongos infectados por *Schistosoma mansoni* e seus respectivos controles não infectados. Os resultados indicam que os animais infectados apresentam claramente taxas de mortalidade mais altas, quando foi utilizado o praziquantel. Surpreendentemente, o contrário aconteceu com relação ao uso da oxamniquina, uma vez que taxas de mortalidade marcadamente mais altas puderam ser detectadas nos animais controles (não infectados). Estas observações levam à conclusão de que são necessários mais estudos toxicológicos sobre drogas esquistossomicidas, usando-se animais infectados com *S. mansoni*.

ACKNOWLEDGEMENTS

This study was supported in part by CNPq, FINEP, FAPEMIG, PRPq-UFMG, Brazil, WHO, Basle, Swit-

zerland. We are grateful to Mrs. Vera de Paula Ribeiro for translating the manuscript. We also acknowledge Mr. Alberto G. Santos, Miss Zenir de Souza, Miss Florence M. Rosa, Mr. José Carlos R. Santos and Mr. Atenágoras N. Silva for technical assistance.

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Recebido para publicação 13/02/1995

Aceito para publicação 18/04/1995