Rev. Inst. Med. trop. São Paulo
39 (1): 11-14, janeiro/fevereiro, 1997

IMMUNOSTIMULATION AS ADJUVANT FOR THE CHEMOTHERAPY OF EXPERIMENTAL SCHISTOSOMIASIS

Luciana M. SILVA & Zilton A. ANDRADE

SUMMARY

Immunosuppressed animals respond poorly to schistosomal chemotherapy and a proper response can be restored by the administration of immune serum. Present study attempts to search whether immunological stimulation would increase drug effectiveness.

Swiss mice infected with 50 *S. mansoni* cercariae were later treated with complete Freund's adjuvant. Treatment with oxamniquine was made with 100 mg/kg.b.w., 25 mg/kg.b.w. and 50 mg/kg.b.w., the last two doses representing a fourth and a half of the recommended curative dose. Appropriate controls for the drug, the adjuvant and the infection were also studied. The serum-level of anti-*S. mansoni* antibodies (ELISA) and recovery of worms by perfusion of the portal vein system were the evaluated parameters. Statistical analysis of the results failed to reveal significant differences in worm recovery between adjuvant-stimulated animals treated with oxamniquine and any of the treated controls receiving the same amount of the drug. Although total lack of immunity interferes with curative treament the usual immune response seems to be sufficient to allow for curative drug action in schistosomiasis and thus apparently does not need to be artificially stimulated.

KEYWORDS: Schistosomiasis; Immunostimulation; Chemotherapy; Oxamniquine.

INTRODUCTION

It has become apparent that there exists a synergism between chemotherapy and immunity during the treatment of parasitic diseases[1]. Studies on experimental schistosomiasis have demonstrated that curative drugs cause relatively mild damage to the worm tegument, especially at the tubercles and sensory organs[8,9]. However, these exfoliative lesions expose important sub-tegumental epitopes that are preferential targets for antibody mediated immune damage, which ultimately causes the worm death[2]. Immunosuppressed mice respond poorly to curative chemotherapy, but their full capacity can be restored by the administration of immune serum[2,3].

During treatment of patients with schistosomiasis, especially during large scale operations, there are usually a group of individuals exhibiting poor responses. Although some of these failures can be ascribed to drug resistance[6,7], others may be linked to poor immune responses. One wonders whether pre-treatment immunostimulation would not have benefited the latter. As a matter of fact, suggestions have been advanced that a vaccine could also be used as an adjuvant for the drug treatment of schistosomiasis[4].

Present investigation attempts to answer whether immunostimulation would improve chemotherapy of schistosomiasis.

MATERIALS AND METHODS

Outbred Swiss mice of both sexes, 18 to 22 g, were submitted to infection with 50 *Schistosoma mansoni* cercariae each, by the transcutaneous route. After 2 months, the animals were seen to excrete viable schistosome eggs in the stools. Half of the animals received then weekly foot-pad injections of 0.1 ml Freund's adjuvant diluted in half with saline, for 3 weeks. Complete adjuvant was used for the first injection, and incomplete for the other two. One week after the last injection, the animals were treated with either 25 or 50 and 100 mg of oxamniquine per kilogram of body weight, administered by gavage in a single dose.

Laboratory of Experimental Pathology, Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Salvador, Bahia, Brasil.
Correspondence to: Z.A. Andrade, Rua Valdemar Falcão 121, 40295-001 Salvador, BA, Brasil.

Two control groups were studied: all the other details being the same as above, the first group consisted of infected-only animals. The second group of animals was infected and immunostimulated, but not drug-treated.

Serum *S. mansoni* antibody levels were tested by an ELISA method employing whole worm antigen, in mouse sera taken at three occasions: before the administration of adjuvant, before the beginning of treatment and at the time of sacrifice. Animals were tested separately.

One month after treament the animals were killed by exanguination under ether anesthesia. Perfusion was made by Duval & DeWitt's method[5] for worm recovering and counting. After perfusion a thorough search for worms was made on the peritoneal surface, mesenteric veins and liver, this latter being crushed between two slides and examined under the low power of the microscope.

An analysis of variance (ANOVA) was the method utilized for statistical evaluation.

TABLE 1
Recovery of *S. mansoni* worms according to groups of treatment.

Oxamniquine		No Adjuvant	With Adjuvant
0 mg/kg	n	34	29
	X	11.82	7.84*
	SEM	1.28	0.86
25 mg/kg	n	32	44
	X	4.90*	4.05**
	SEM	0.85	0.48
50 mg/kg	n	21	37
	X	3.29*	3.00**
	SEM	1.11	0.68
100 mg/kg	n	15	15
	X	0.47**	0.00**
	SEM	0.19	0.00

SEM = $SD/\sqrt{n}$; *$p < 0.05$; **$p < 0.01$; n = number or animals and X = average of worms.

RESULTS

Treatment efficacy was evaluated by counting the number of worms recovered. Table 1 shows the number of mice per group, as well as the average of worms recovered. The number of worms recovered from the animals treated with the subcurative dose of 50 mg and 25 mg per kg/body weight of oxamniquine was similar for both groups, regardless the use of Freund's adjuvant.

Obviously, the major recovery was attained in animals which were not treated with oxamniquine and the lowest average was obtained from the group receiving the recommended therapeutic dose of oxamniquine for mice (100 mg/k.b.w).

No statistically significant difference was seen between the groups treated with lower doses of oxamniquine, regardless the presence of adjuvant. The data also show that animals treated only with the adjuvant exhibited a significant reduction of worm burden (Table 1), if compared with the control group of infected-only animals. However if the groups "adjuvant-only" and those treated with subcurative doses of oxamniquine are compared, the reduction in worm burden becomes non-significant.

Table 2 presents the isolated results of each factor, by comparing the data from the several groups. Although a statistically significant result was noted for the group which was only drug-treated and that which received adjuvant alone as well, no synergistic drug-immunostimulation result was obtained. Antibody levels were seen to vary considerably, with titles going from 1:200 up to 1:25,600 regardless of immunostimulation. No correlation was detected between serum antibody levels and anti-schistosomal chemotherapeutic efficacy under the present experimental conditions.

Serum antibody levels in infected mice showing low titles of antibodies increased only slightly after Freund's adjuvant administration, but exhibited a rebound at the time of sacrifice. On the contrary, those animals presenting the highest antibody titles and that did not receive adjuvant, showed a considerable fall in antibody level after treatment. In spite of that decrease, the average antibody level measured at the time of sacrifice was still more elevated than in those animals showing low titres and receiving adjuvant (Table 3).

TABLE 2
Summary of statistical analysis for the several factors involved in the treament of schistosomiasis.

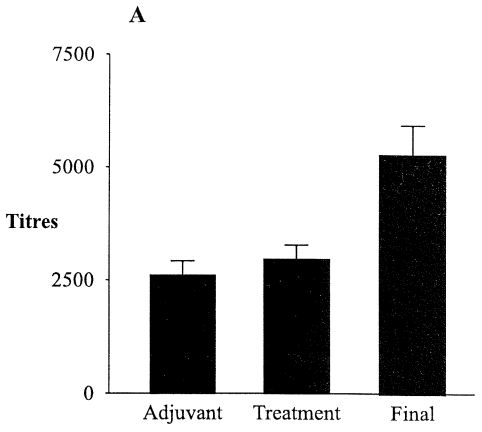
Source	Sum sqres	DF	Mean sqres	F-ratio	Prob.
Drug	2641.18	3	880.39	35.20	< 0.0001
ADJ	125.74	1	125.73	5.03	0.0259
Drug X ADJ	156.35	3	52.12	2.08	0.1033
within cell.	5476.92	219	25.01		
TOTAL	8400.18	226			

DISCUSSION

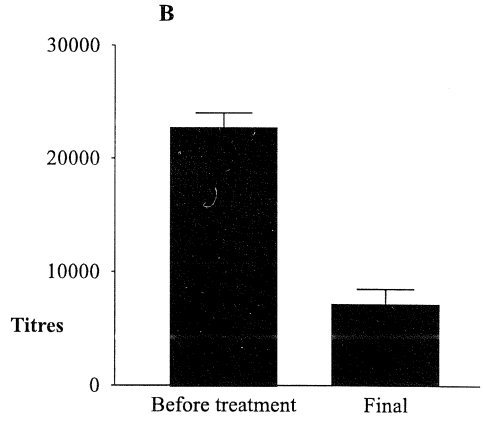
Data obtained with the present investigation indicate that non-specific immunostimulation does not enhance oxamniquine efficacy against schistosomiasis. Although experimental data have revealed that lack of immune capacity clearly interferes with cure-rates, either with oxamniquine[10] or praziquantel[2,4], an intact immunological capacity seems to be all that is required for the proper drug action. Present results are at variance with data observed by others in which the simultaneous administration of foreign immune sera resulted in enhancement of chemotherapy, even with suboptimal doses of either oxamniquine[10] or praziquantel[4]. An increase in cure-rate of about 25% or higher has been observed in studies that used rabbit polyclonal anti-schistosomal antibodies[4]. When monoclonal antibodies were utilized in similar investigations, it was seen that they could bind to different location on the worm tegument and also induce different degrees of damage[1,2,4]. Our data do not discard the possibility that the administration of a targeted anti-schistosomal antibody or of a foreign serum with high concentration of such antibody may result in chemotherapeutic enhancement. However, the cooperation of the immune cofactor for the curative action of anti-schistosomal drugs can be taken for granted in the usual circumstances, inasmuch as the beginning of worm death may further stimulate antibody production, as we have observed in animals with pre-treatment low serum antibody levels (Table 3).

TABLE 3

Antibody levels observed in animals treated with 50 mg/K body weight of oxamniquine: A - with adjuvant; B - without adjuvant.



Means	SD	n
2811.764	2524.352	17
3188.235	2629.135	17
5741.176	4420.274	17



Means	SD	n
23771.420	4837.945	7
8228.571	5726.463	7

RESUMO

Imunoestimulação como adjuvante na quimioterapia da esquistossomose

Animais imunodeprimidos respondem mal ao tratamento anti-esquistossomótico. A capacidade para responder adequadamente pode ser restaurada pela administração de soro imune. Não se sabe se a imunoestimulação de animais normais pode aumentar a eficácia da droga. Camundongos infectados com 50 cercárias do *Schistosoma mansoni* e tratados 2 meses depois com adjuvante de Freund responderam da mesma forma que os controles não imunestimulados ao tratamento com 25, 50 e 100 mg/kg pc de oxamniquine. Não houve correlação, entre níveis de anticorpos séricos e a eficácia da droga. Conclui-se que embora a imunodepressão afete a eficácia da quimioterapia anti-esquistossomótica, uma resposta normal do hospedeiro parece ser o suficiente para servir como co-fator da quimioterapia, não havendo portanto indicação para a imunoestimulação artificial nas condições habituais.

ACKNOWLEDGEMENTS

We thank Dr. Irismar Reis de Oliveira for statistical analysis, and Marcia M. Souza, Tereza Almeida and Antonio C.S. Santos for technical help.

REFERENCES

1. BRINDLEY, P.J. – Relationship between chemotherapy and immunity in schistosomiasis. **Advanc. Parasit., 34**:133-161, 1994.

2. BRINDLEY, P.J. & SHER, A. – The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. **J. Immunol., 139**:215-220, 1987.

3. DOENHOFF, M.J. – The immune-dependence of chemotherapy in experimental schistosomiasis. **Mem. Inst. Oswaldo Cruz, 84**(suppl. 1):31-37, 1989.

4. DOENHOFF, M.J.; MODHA, J. & LAMBERTUCCI, J.R. – Anti-schistosome chemotherapy enhanced by antibodies specific for a parasite esterase. **Immunology, 65**:507-510, 1988.

5. DUVALL, R.H. & DeWITT, W.B. – An improved perfusion technique for recovering adult schistosomes from laboratory animals. **Amer. J. trop. Med. Hyg., 16**:483-486, 1967.

6. KATZ, N. – Experiência com quimioterapia em grande escala no controle da esquistossomose no Brasil. **Rev. Inst. Med. trop. S. Paulo, 22**:40-51, 1980.

7. KATZ, N.; DIAS, E.P.; ARAÚJO, N. & SOUZA, C.P. – Estudo de uma cepa humana de *Schistosoma mansoni* resistente a agentes esquistossomicidas. **Rev. Soc. bras. Med. trop., 7**:381-387, 1973.

8. KOHN, A.; LOPEZ-ALVAREZ, M.L. & KATZ, N. – Transmission electron microscopical studies in the tegument of male *Schistosoma mansoni* after oxamniquine treatment. **Ann. Parasit. hum. comp., 57**:285-291, 1982.

9. KHON, A.; SERAPIÃO, J.C.; KATZ, N. & DIAS, E.P. – Ação da oxamniquine sobre o *Schistosoma mansoni* em camundongos experimentalmente infectados. **Rev. Inst. Med. trop. S. Paulo, 21**:217-227, 1979.

10. LAMBERTUCCI, J.R.; MOHDA, J. & DOENHOFF, M.J. – *Schistosoma mansoni*: the therapeutic efficacy of oxamniquine is enhanced by imune serum. **Trans. roy. Soc. trop. Med. Hyg., 83**:362-363, 1989.

Recebido para publicação em 06/08/1996
Aceito para publicação em 28/01/1997