Rev. Inst. Med. trop. São Paulo
16(3):132-134, maio-junho, 1974

UDC 616.995.122

INFECTION OF MICE WITH CERCARIAE AND SCHISTOSOMULA OF *SCHISTOSOMA MANSONI* BY INTRAVENOUS AND SUBCUTANEOUS ROUTES

J. C. HOLANDA [1], J. PELLEGRINO [2] and G. GAZZINELLI [3]

SUMMARY

Two groups of 10 mice were injected with 100 cercariae of *Schistosoma mansoni* per animal, by the intravenous route. A group of 10 mice, injected subcutaneously, with the same number of cercariae, served as control. All animals were sacrificed 7 weeks infection. The mean number of schistosomes recovered from mice infected intravenously was 9.4 ± 5.5 and 9.3 ± 4.0, respectively. Mice infected subcutaneously showed a mean burden of 23.6 ± 10.9 schistosomes. These data show that *S. mansoni* cercariae are infective to mice when injected intravenously, although to a lesser extent than by the subcutaneous route. The infectivity of schistosomula (*S. mansoni*) for mice was checked by injecting (intravenous and subcutaneous routes) 100 larvae, obtained *in vitro*, in two groups of 10 mice. The results obtained (44.0 ± 10.1 and 40.1 ± 9.8 schistosomes, respectively) showed that schistosomula are able do infect mice, with a great percentage of recovery, either by the intravenous or by the subcutaneous route.

INTRODUCTION

It is well known the infective capacity of *Schistosoma mansoni* cercariae for mice when the animals are exposed via the skin or the cercariae are injected either by the subcutaneous or the intraperitoneal routes (Cf. PELLEGRINO & KATZ [2]). No reference was found in the literature on the infectivity of *S. mansoni* cercariae when injected intravenously.

With regard to the infectivity of schistosomula (*S. mansoni*), obtained *in vitro*, STIREWALT & UY [8] mentioned that these larvae produce infections in mice following intravenous, but not intracutaneous or intraperitoneal inoculation.

The present paper reports the inoculation of mice with cercariae and schistosomula by the intravenous and subcutaneous routes, in order to check the infective capacity of cercariae when injected intravenously and to verify whether schistosomula obtained *in vitro*, by a physical method, are able to infect mice.

MATERIAL AND METHODS

Cercariae of S. mansoni — Cercariae were obtained by exposing *Biomphalaria glabrata* to light at the temperature of 28°C. The snails were reared and infected (L.E. strain) in the laboratory.

(1) Fellow from CNPq, Brazil
(2) Schistosomiasis Research Unit, Institute of Biological Sciences, Federal University of Minas Gerais
(3) Department of Biochemistry and Immunology, Institute of Biological Sciences
Contribution no. 48 from the Schistosomiasis Research Unit
This work was supported, in part, by the "Conselho Nacional de Pesquisas" (MINIPLAN)
Address for reprints: Caixa Postal 1404 — 30000 Belo Horizonte, Brazil

Schistosomula — Schistosomula were prepared according to the technique of GAZZINELLI et al. [1] as modified by RAMALHO-PINTO et al. [7]. Briefly, the method consists of three steps. First of all, a cooled suspension of cercariae in 10 ml volume of Hank-BSS pH 7.2-7.4 was whorled in a Vortex mixer for 1 min (step one-tail loss). The tail-loss bodies separated by gravity sedimentation were packed by 1 min low-speed centrifugation and incubated at 30°C for 40 min (step two — secretion induction). Finally, the larvae from step two were suspended in inactivated guinea-pig serum and incubated under agitation at 37°C for 1 hour (step three — coat removal). The larvae so obtained display the characteristics of schistosomula as established by STIREWALT et al. [8].

Infection of animals — Cercariae and schistosomula were concentrated following the technique of PELLEGRINO & MACEDO [3] in order to obtain about 1,000 organisms per ml. A volume of 0.1 ml of the suspension was then injected into the dorso-lateral veins of the tail. Seven weeks after injection the animals were sacrificed and the liver and mesenteric veins perfused (PELLEGRINO & SIQUEIRA [5]) for schistosome collection. Fragments from the small intestine were taken for oogram studies (PELLEGRINO et al. [4]).

RESULTS AND COMMENTS

Table I shows the results obtained after injection of a cercarial suspension (100 organisms per animal) in mice by intravenous and subcutaneous routes. Comparable results were obtained in 2 experiments when the intravenous route was employed. Actually, the mean worm burden was 9.4 ± 5.5 and 9.3 ± 4.0 schistosomes, respectively. In the animals infected by the subcutaneous route a mean of 23.6 ± 10.9 was observed. These results show that cercariae of *S. mansoni* are infective for mice when injected intravenously but the percentage of worm recovery is very small.

The results obtained after infection of mice with schistosomula using the intravenous or the subcutaneous routes were very similar (Table II), the mean worm recovery being about 40%.

TABLE I

Recovery of adult *Schistosoma mansoni* from mice injected intravenously and subcutaneously with 100 cercariae per animal

Number of mice	Routes of inoculation and number of schistosomes recovered		
	Intravenous	Intravenous	Subcutaneous (control)
1	18	4	34
2	13	7	21
3	12	9	15
4	3	14	7
5	4	10	31
6	5	10	44
7	19	13	15
8	9	16	28
9	5	3	12
10	6	7	29
Total	94	93	236
Mean	9.4 ± 5.5	9.3 ± 4.0	23.6 ± 10.9

TABLE II

Recovery of adult *Schistosoma mansoni* from mice injected intravenously and subcutaneously with 100 schistosomula, obtained *in vitro*, per animal

Number of mice	Routes of inoculation and number of schistosomes recovered	
	Intravenous	Subcutaneous
1	66	42
2	45	31
3	56	34
4	43	26
5	35	47
6	32	39
7	36	41
8	35	58
9	48	30
10	44	53
Total	440	401
Mean	44.0 ± 10.1	40.1 ± 9.8

It is interesting to mention that the adult schistosomes recovered from mice infected either with cercariae or with schistosomula were of normal size and the female worms

presented eggs of normal morphology. The oograms from rectal fragments showed viable eggs in all stages of maturation.

According to STIREWALT & UY [9], *S. mansoni* schistosomula produce infections following intravenous, but not intracutaneous or intraperitoneal inoculation. Our findings agree with those of PEREIRA [6] which studied the migratory capacity of schistosomula recovered *in vivo*, but are in contrast with those reported by STIREWALT & UY [9] in what concerns the infection of mice after subcutaneous inoculation of larvae obtained *in vitro*.

RESUMO

Infecção do camundongo com cercárias e esquistossômulos do Schistosoma mansoni *pelas vias endovenosa e subcutânea*

Dois grupos de 10 camundongos foram injetados com 100 cercárias de *Schistosoma mansoni* pela via endovenosa. Um outro grupo de 10 camundongos, injetados com o mesmo número de cercárias, por via subcutânea, serviu como controle. Todos os animais foram sacrificados 7 semanas depois da infecção. O número médio de esquistossomos recuperados dos camundongos infectados por via endovenosa foi de 9,4 ± 5,5 e 9,3 ± 4,0, respectivamente. Os camundongos infectados por via subcutânea apresentaram, em média, 23,6 ± 10,9 esquistossomos. Esses dados mostram que a cercária do *S. mansoni* é infectante para o camundongo quando inoculada por via endovenosa, embora em menor grau do que pela via subcutânea.

A infectividade do esquistossômulo (*S. mansoni*) para o camundongo foi testada pela injeção endovenosa e subcutânea de 100 larvas obtidas *in vitro*. Os resultados obtidos (44,0 ± 10,1 e 40,1 ± 9,8 esquistossomos) mostram que o esquistossômulo é capaz de infectar o camundongo, com grande percentagem de recuperação, tanto pela via endovenosa como pela via subcutânea.

REFERENCES

1. GAZZINELLI, G.; OLIVEIRA, C. C.; FIGUEIREDO, E. A. & PELLEGRINO, J. — *Schistosoma mansoni*: Biochemical studies of the steps involved in the process of transformation of cercariae into schistosomula. *Exp. Parasitol.* (In press), 1973.

2. PELLEGRINO, J. & KATZ, N. — Experimental chemotherapy of schistosomiasis mansoni. *Advances Parasitology* **6**:233-290, 1968.

3. PELLEGRINO, J. & MACEDO, D. G. — A simplified method for the concentration of cercariae. *J. Parasitol.* **41**:329-330, 1955.

4. PELLEGRINO, J.; OLIVEIRA, C. A.; FARIA, J. & CUNHA, A. S. — New approach to the screening of drugs in experimental schistosomiasis mansoni in mice. *Amer. J. Trop. Med. & Hyg.* **11**:201-215, 1962.

5. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Brasil. Malariol.* **8**:589-597, 1956.

6. PEREIRA, L. H. — *Capacidade de migração das larvas de* Schistosoma mansoni *Sambon, 1907, no camundongo albino*. Thesis. Institute of Biological Sciences, Federal University of Minas Gerais, 1971, 38 pp.

7. RAMALHO-PINTO, F. J.; GAZZINELLI, G.; HOWELLS, R. E.; MOTA-SANTOS, T. A.; FIGUEIREDO, E. A. & PELLEGRINO, J. — *Schistosoma mansoni*: a defined system for the step-wise transformation of the cercariae to schistosomules *in vitro*. (Submitted for publication), 1973.

8. STIREWALT, M. A.; MINNICK, D. R. & FREGEAU, W. A. — Definition and collection in quantity of schistosomules of *Schistosoma mansoni*. *Trans. Royal Soc. Trop. Med. & Hyg.* **60**:352-360, 1966.

9. STIREWALT, M. A. & UY, A. — *Schistosoma mansoni*: Cercarial penetration and schistosomule collection in an *in vitro* system. *Exp. Parasitol.* **26**:17-28, 1969.

Recebido para publicação em 23/10/1973.