Rev. Inst. Med. trop. São Paulo
6(4):167-170, julho-agôsto, 1964

UDC 616.995.122.22-085

CHEMOTHERAPY OF EXPERIMENTAL SCHISTOSOMIASIS

V. Studies of some techniques used for the assessment of drug activity

Z. BRENER

SUMMARY

The rapid hepatic shift of *Schistosoma mansoni* worms which occurs two hours after the administration of active compounds was investigated as a possible screening method in experimentally infected mice. Good results were obtained with soluble antimonial compounds administered by intraperitoneal route but poor results were achieved with drugs given by oral route.

A comparative study of the methods of intestinal oogram and distribution of worms was performed on mice treated on the same conditions. The mice were examined three days after a 7-day treatment and results seem to prove that both methods have the same sensitivity concerning drug activity.

INTRODUCTION

Many different criteria are being used for the evaluation of drug activity in experimental schistosomiasis and this variety of techniques makes it very difficult to compare all the recommended methods. In this paper an attempt is made to study some new aspects of this important problem, chiefly the contributions of BUTTLE & KAHYYAL [2] and PELLEGRINO et al. [5].

MATERIAL AND METHODS

Infection of animals: albino mice, weighing 18-20 g, were infected, through cutaneous route, with 100 cercariae according to the technique previously described (BRENER [1]).

Drug toxicity: mice weighing 18-20 g were used and the mortality observed for 3 days. The LD_{50} was determined graphically by the probit method.

Distribution of worms: the distribution of worms in the hepatic portal system of treated mice was studied through a perfusion method already described (BRENER [1]).

Comparison between intestinal oogram and distribution of worms: infected mice were treated, from the 50th day of infection, with 7 consecutive doses of different active compounds. Three days after treatment groups of 5 mice were sacrificed and the distribution of worms in the liver, portal vein and mesenteric veins was determined. Fragments of the small intestine of the same animals were examined microscopically between slide and cover slide. A minimum of 300 viable eggs were counted and classified according to their evolutive stages. An oogram showing more than 50% of mature eggs was considered as an evidence of drug activity (PELLEGRINO et al. [5]).

Observation on the rapid shift of the worms to the liver: the infected animals were treated with a single dose corresponding to about 50 per cent of the LD_{50} and sacrificed after two hours. The distribution of worms was determined through perfusion.

Centro de Pesquisas de Belo Horizonte, Instituto Nacional de Endemias Rurais.

Drugs used: sodium antimony III bis-pyrocatechol-3-5-disulphonate ("Fuadin"); sodium antimonyl III gluconate ("Triostib"); antimony III EDTA; lithium antimony III thiomalate ("Anthiomaline"); antimony III dimercapto succinate, sodium salt (TWSb); potassium antimony III tartrate (tartar emetic); di-hidro-emetine; 1-(diethylamino ethylamino)-4,6,8-trimethyl-5-azothioxanthone ("Ciba 17'581"); pararosaniline, pamoate salt (TAC); 1-methyl-4-diethylamino-ethylaminothioxanthone ("Lucanthone"); 1: 7-bis (p-amino-phenoxy) heptane.

RESULTS

Table 1 shows the results of distribution of worms in mice treated with a single dose corresponding to about half of the LD_{50} and examined, by perfusion, two hours after drug administration. Table 2 shows the results obtained with the intestinal oogram and worm distribution in groups of animals treated with 7 consecutive doses of different drugs and examined three days after drug administration.

DISCUSSION

Most of the criteria used for the evaluation of drug activity in experimental schistosomiasis are based on two important phenomena which follow the administration of active drugs: the shift of the worms to the liver (SCHUBERT [6], STANDEN [7]) and the decrease or interruption of egg elimination (KIKUTH & GÖNNERT [4], DE CARNIERI [3]). Changes of the worm distribution occurs after administration of every active drug and shows a clear dose-effect relationship. An extremely rapid hepatic shift of worms which takes place about two hours after medication was described by BUTTLE & KAHYYAL [2] and represents an interesting contribution to the dynamics of the migration of *S. mansoni* worms. The possibility of applying this early migration of the parasites as a screening test was investigated. Very good results are obtained with soluble antimonial compounds given by intraperitoneal route but poor results were achieved with insoluble drugs given by oral route and this makes this technique unreliable for screening purposes.

According to PELLEGRINO et al. [5], in his new method of screening drugs, a compound

TABLE 1

Percentage of worms in the liver of mice experimentally infected with *S. mansoni* and examined two hours after the administration of a single dose corresponding to about half of the LD_{50} of some active compounds.

Drugs	Dosis (mg/kg)	% worms in the liver			Nº of worms (mean)
		♂	♀	Paired	
Sodium antimony bis pyrocatechol 3:5 disulphonate ("Fiadin")	500	100	100	95	18,0
Sodium antimonyl III gluconate ("Triostib")	50	98	100	97	24,6
Sodium antimonyl III ethylen diamino tetracetate	40	100	100	100	22,5
Lithium antimony III thiomalate ("Anthiomaline")	50	64	87	88	21,2
Antimony III dimercaptosuccinate, sodium salt ("TWSb")	1000	80	73	34	20,8
Potassium antimony III tartrate	25	100	98	94	21,4
Di-hidro-emetine	10	57	75	10	27,5
1-(diethylaminoethylamino) 4,6,8-trimethyl-5-azothioxanthone-hydrochloride ("Ciba 17'581")	500	80	73	36	20,8
Pararosaniline, pamoate salt	800	30	—	5	21,7
1:7-bis (p-amino-phenoxy) heptane	400	39	25	36	20,4
Controls	—	20	38	3	22,3

TABLE 2

Distribution of worms in the liver, portal and mesenteric veins and percentage of mature intestinal eggs in mice experimentally infected with *S. mansoni*, treated during 7 days and examined three days after treatment.

Drugs	Dosis (mg/kg)	Distribution of paired worms (%)			Percentage of mature intestinal eggs in the 5 mice of each group				
		L	P	M	1	2	3	4	5
1-(diethylaminoethylamino) 4,6,8-trimethyl-5-azothloxanthone-hydrochloride ("Ciba 17'581")	50	100	0	0	100	100	100	100	100
	25	93	0	7	90	75	100	100	100
	12,5	81	0	19	50	87	98	84	88
	6	12	0	88	36	15	25	31	42
	—	6	18	76	32	17	28	30	27
1-methyl-4-diethylaminoethylaminothioxanthone-hydrochloride ("Lucanthone")	50	80	0	20	100	100	68	65	72
	25	80	6	14	100	79	27	60	51
	—	8	16	76	38	33	24	12	27
Di-hydro-emetine	10	65	30	5	72	75	70	78	72
	5	41	27	30	38	80	79	66	70
	—	8	31	60	27	15	30	41	13
Pararosaniline, pamoate salt	0.5 % (diet)	20	8	70	88	96	68	85	76
	0.25% (diet)	26	21	52	100	69	24	68	74
	—	4	20	75	39	28	43	17	21
Potassium antimony III tartarate	20	100	0	0	100	100	100	100	100
	10	73	10	16	100	100	100	100	100
	5	50	22	28	100	100	100	100	100
	2,5	13	27	60	60	100	21	16	41
	—	1	28	71	32	20	10	28	26
Antimony III dimercaptosuccinate, sodium salt ("TWSb")	100	80	8	12	100	100	100	100	100
	50	54	21	27	25	100	100	100	63
	25	35	21	44	100	31	26	79	45
	—	8	16	76	38	33	24	12	27
Sodium antimonyl III gluconate ("Triostib")	25	73	0	26	100	100	100	100	100
	12,5	21	32	47	86	100	100	28	86
	—	6	33	60	35	14	25	14	39

L — liver. P — portal vein. M — mesenteric veins.

is considered active when the intestinal oogram of treated mice shows 50 per cent or more of mature eggs when examined three days after a 7-day treatment. The comparative study performed on mice treated on the same conditions seems to prove that the method depending on changes of the worm distribution is as sensitive as the oogram method. This experience shows also that there is no need to delay the study of the worm distribution until the 5th or 7th day after drug administration, as commonly done, because a marked shift is observed as soon as three days after treatment. The choice of the best method would be determined by their practicability in the local conditions of the laboratories engaged on screening programs.

BRENER, Z. — Chemotherapy of experimental schistosomiasis. V. Studies of some techniques used for the assessment of drug activity. *Rev. Inst. Med. trop. São Paulo* **6**:167-170, 1964.

SUMÁRIO

Quimioterapia da esquistossomose experimental; V. Estudo de algumas técnicas usadas para a triagem de drogas.

A possibilidade do emprêgo, para a triagem de drogas, do fenômeno da migração rápida dos vermes para o fígado, que ocorre cêrca de duas horas após a administração de medicamentos ativos, foi investigada em camundongos experimentalmente inoculados com *Schistosoma mansoni*. Resultados satisfatórios, foram obtidos com antimoniais solúveis aplicados por via intraperitoneal mas resultados menos evidentes foram obtidos com medicamentos insolúveis administrados por via oral.

Estudando, comparativamente, com vários compostos ativos, os métodos do oograma intestinal e da distribuição de vermes em camundongos experimentalmente inoculados e sacrificados três dias após a administração de 7 doses consecutivas, foi observado que ambos os métodos apresentam sensibilidade semelhante.

REFERENCES

1. BRENER, Z. — *Contribuição ao estudo da terapêutica experimental da esquistossomose mansoni*. Belo Horizonte, Tese da Fac. Odontologia e Farmácia da Univ. de Minas Gerais, 1962.

2. BUTTLE, G. A. H. & KAHYYAL, M. T. — Rapid hepatic shift of worms in mice infected with *Schistosoma mansoni* after a single injection of tartar emetic. *Nature* **194**:780-781, 1962.

3. CARNERI, I. de — Osservazioni sugli indici dell'azione chemioterapica di sostanze attive su *Schistosoma mansoni* nel topo. *Arch. Ital. Sc. Med. Trop. & Parasitol.* **39**:400-424, 1958.

4. KIKUTH, W. & GÖNNERT, R. — Experimental studies on the therapy of Schistosomiasis. *Ann. Top. Med. Parasitol.* **42**:256-267, 1948.

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

6. SCHUBERT, M. — Conditions for drug testing in experimental schistosomiasis mansoni in mice. *Am. J. Trop. Med. & Hyg.* **28**: 121-136, 1948.

7. STANDEN, O. D. — Experimental schistosomiasis. III. Chemotherapy and mode of drug action. *Ann. Trop. Med. Parasitol.* **47**: 26-43, 1953.

Recebido para publicação em 20 janeiro 1964.