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EXPERIMENTAL CHEMOTHERAPY OF SCHISTOSOMIASIS VIII — LABORATORY AND CLINICAL TRIALS WITH SODIUM ANTIMONY DIMETHYLCYSTEINE TARTRATE (NaP)

Naftale KATZ (1) and J. PELLEGRINO (1, 2)

SUMMARY

The antischistosomal effect in mice experimentally infected with *Schistosoma mansoni* and dosed with 5 i.p. or i.m. injections of sodium antimony dimethylcysteine tartrate (NaP) was very evident. At a dose level of 25 mg/kg/day x 5 practically all animals presented oogram changes accompanied by a pronounced hepatic shift of schistosomes. When treatment consisted of a single injection (i.p. or i.m.) the anti-schistosomal activity was very poor.

In hamsters the antischistosomal activity was very evident at the level of 16 mg/kg/day x 5, i.p. All animals presented oogram changes and practically all schistosomes were shifted toward the liver.

In *Cebus* monkeys, NaP was inactive when administered as a single i.m. injection of 15 mg/kg. A persistent interruption of egg laying (parasitological cure) was achieved in monkeys treated with 75 and 7.5 mg/kg x 5, i.m.

The results of a limited clinical trial were very disappointing. Three patients dosed with 4 mg/kg/day x 5, i.m., continued passing *S. mansoni* eggs 4 months after treatment. Side effects were: abdominal pain, nausea, vomiting, sialorrhea and electrocardiographic changes. Only one patient treated with 8 mg/kg/day x 4, i.m. could be considered as parasitologically cured. However, this patient presented subendocardial ischemia.

Owing to the side effects — including cases of death after NaP administration reported in Brazil — it was concluded that clinical trials with this antischistosomal agent should not be pursued.

INTRODUCTION

In 1968 ERCOLI [2] developed a preparation containing 1 mole potassium antimonyl tartrate to 3.4 or 4.5 moles of dimethylcysteine. The Author claims that this preparation is less toxic to animals than the same amount of antimony given as tartar emetic. In mice the LD_{50} (given as Sb mg/kg) for tartar emetic and for the cysteine compound were 19 and 73, respectively. The ED_{50}, as judged by the shift of *S. mansoni* worms to the liver were 8 and 14 mg Sb/kg, respectively. According to ERCOLI [2] the chelation of tartar emetic reduces the toxicity, while only slightly reducing the therapeutic activity on schistosomes. Similar results had been reported by FRIEDHEIM [3] and KAYYAL et al. [6], when peni-

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(1) "Instituto de Endemias Rurais", "Centro de Pesquisas René Rachou", Belo Horizonte, Brazil

(2) Schistosomiasis Research Unit, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil
Address for reprints: C. Postal 1743 — 30000 Belo Horizonte, Brazil

cillamine (dimethylcysteine) and antimony potassium tartrate (APT) were administered simultaneously.

TARRANT et al.[14] demonstrated that penicillamine increases the LD_{50} in uninfected mice and that it was more effective when given immediately after APT. In mice infected with *S. mansoni* a daily dose of 20 mg/kg of APT for 5 days produced a 56% reduction in the number of worms. Most of schistosomes were found in the liver 5 days after dosing and all animals presented oogram changes. Penicillamine given 3 times daily at a level of 25 mg/kg did not modify the activity of 20 mg/kg of APT. However, at 100 mg/kg it nearly abolished the effect of APT. According to TARRANT et al.[14], penicillamine, at doses sufficient to reduce the acute toxicity of APT, also reduces its effectiveness.

In this paper the results obtained with NaP (Fig. 1) in the treatment of laboratory animals as well as in a limited clinical trial will be presented.

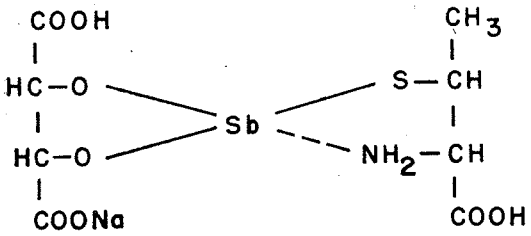

Fig. 1 — Chemical structure of NaP.

MATERIAL AND METHODS

Infection of animals — Cercariae shed by laboratory-reared and infected *Biomphalaria glabrata* (L.E. strain of *S. mansoni*, Belo Horizonte) were used throughout this study. Albino mice, weighing 18 to 20 g, were exposed to 100 ± 10 cercariae by the tail immersion method (PELLEGRINO & KATZ[10]). Adult hamsters (*Cricetus auratus*) were infected with 60 ± 10 cercariae via the cheek pouch (PELLEGRINO, DE MARIA & FARIA[8]). The percutaneous route was used for exposing adult *Cebus apella macrocephalus* (200 cercariae per animal).

Treatment of animals — Four groups of mice, each one consisting of 12 animals per dose, were used (Table I). Treatment with NaP was performed by the intraperitoneal route (100, 50 and 25 mg/kg/day, for 5 consecutive days; 200, 100, 50 and 25 mg/kg as a single dose) and by the intramuscular route (100, 50, 25 and 12.5 mg/kg/day, for 5 consecutive days; 200, 100, 50 and 25 mg/kg as a single dose). Two groups of 10 mice were left as untreated controls.

Groups of 6 hamsters were treated for 5 consecutive days, by the intraperitoneal route, at the dose-levels of 16, 8, 4 and 2 mg/kg/day.

Three adult *Cebus* monkeys, 5 to 6 months after exposure, were treated with NaP at the levels of 75, 15 and 7.5 mg/kg/day, for 5 consecutive days (intramuscular route). One monkey received a single i.m. injection of 15 mg/kg.

Oogram and distribution of schistosomes — Assessment of therapeutic activity — Mice and hamsters were killed by a blow on the neck 3 days after the end of treatment. The schistosomes in the portal and mesenteric veins and in the liver were recovered by perfusion, using PELLEGRINO & SIQUEIRA's[13] technique adapted for mice and hamsters. For oogram studies, press preparations from intestinal fragments (PELLEGRINO & FARIA[9]) were microscopically examined and a minimum of 200 viable eggs per mouse or hamster counted and classified according to the criteria described elsewhere (PELLEGRINO et al.[12]).

Rectal snips (usually four) from *Cebus* monkeys were taken by mucosal curettage, as described elsewhere (PELLEGRINO et al.[11]). The whole press preparation was examined and all schistosome elements counted and classified. It was then possible to calculate the number of viable eggs per gram of rectal tissue.

Changes in the oogram from intestinal fragments of mice and hamsters were considered as indicative of antischistosomal activity when one or more developmental stages of immature eggs were absent. The number of worms retained in the liver after perfusion was routinely determined by squashing the

whole organ between two glass plates and examining the preparation with a dissecting microscope.

In *Cebus* monkeys the assessment of drug activity was based on the gradual disappearance of immature and mature eggs in rectal snips as well as by the decrease of the number of viable eggs per gram of rectal tissue (KATZ, PELLEGRINO & MEMÓRIA [5]). A monkey was considered as parasitologically cured when no viable eggs were found in rectal snips within a period of at least 120 days after treatment.

Relapse — Relapse was studied in 2 groups of mice harboring mature *S. mansoni* infections. One group was treated at the dose-level of 50 mg/kg/day i.p. x 5 and the other group with the schedule of 100 mg/kg/day x 6. One day before treatment and at different periods within treatment and thereafter, groups of 4 mice were sacrificed. Schistosomes were recovered by perfusion of the hepatic-portal system and intestine fragments taken for oogram studies.

Clinical trials — Five male adult patients with active schistosomiasis mansoni (hepato-intestinal form) were treated with NaP at the following schedules: 4 mg/kg/day x 5 (4 patients) and 8 mg/kg/day x 4 (1 patient) by the intramuscular route. In the former schedule treatment was interrupted in one patient after the first injection due to toxic effects. Electrocardiographic tracings were performed before and after completion of treatment. Therapeutic activity was assessed by 4 to 6 quantitative stool examinations (KATZ, CHAVES & PELLEGRINO [4]).

RESULTS

Mice — The results obtained in mice experimentally infected with *S. mansoni* and treated with NaP are summarized in Table I.

The antischistosomal effect in the group of mice dosed with 5 i.p. injections of NaP was very evident. The hepatic shift of worms was accompained by oogram changes in all treated animals. The activity was much less evident when one single dose was administered by the intraperitoneal route. Only 25% of treated animals (200 mg/kg) presented oogram changes and there was no evidence of hepatic shift of schistosomes. When the intramuscular route was employed, the results have shown a high percentage of oogram changes at the levels of 100, 50, and 25 mg/kg/day x 5, the same occurring with the hepatic shift of worms. On the other hand, the activity after a single i.m. dose was very poor (Table I).

Hamsters — In hamsters the antischistosomal activity was very evident at the level of 16 mg/kg/day x 5, i.p. In fact, all animals presented oogram changes and practically all worms were found in the liver (Table II).

Monkeys — As can be seen in Table III, NaP was inactive when administered as a single i.m. injection of 15 mg/kg. A persistent interruption of egg laying was achieved in monkeys treated with 75 and 7.5 mg/kg/day x 5 (parasitological cure). Relapse was observed in the animal treated with NaP at the dose level of 15 mg/kg/day x 5.

Relapse — In Fig. 2 were plotted the data concerning the percentage of immature eggs (oogram) as well as the percentage of worms located in the liver (hepatic shift). It is clearly shown that interruption of egg laying was of very short duration in the group of mice dosed with 50 mg/kg/day x 5 in comparison with the group dosed with 100 mg/kg/day x 6. Relapse occurred in both groups, oogram and distribution of schistosomes returning to normal figures after about 3 weeks.

Clinical trials — In the group of patients treated with 4 mg/kg/day x 5, treatment was interrupted in one patient due to an increase of bradycardia (52 to 38 bpm). Two patients presented abdominal pain, nausea, vomiting, dizziness and sialorrhea. Accentuated and discreet flattening of T wave were observed in 1 and in 2 patients, respectively. Parasitological control indicated that all patients of this group continued to excrete *S. mansoni* eggs after treatment (Table IV).

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TABLE I

Antischistosomal activity of NaP in mice experimentally infected with *Schistosoma mansoni*: oogram changes, distribution of schistosomes, and percentage of worms retained in the liver after perfusion

Number of mice	Dose (mg/kg/day x days)	Route	Animals dead	Mean worm burden	Distribution of schistosomes (%)			Percentage of worms retained in the liver	Percentage of animals with oogram changes
					Liver	Portal vein	Mesenteric vessels		
12	100 x 5	i.p.	4	9.5	83.0	7.9	9.1	37.0	100.0
12	50 x 5	i.p.	4	8.6	66.7	14.5	18.8	20.3	100.0
12	25 x 5	i.p.	4	10.7	59.3	4.7	36.0	11.0	100.0
Control (10)		—	0	15.8	11.4	12.6	76.0	0.0	0.0
12	200 x 1	i.p.	8	7.5	13.3	3.3	83.4	0.0	25.0
12	100 x 1	i.p.	5	5.7	27.5	35.3	37.2	0.0	14.3
12	50 x 1	i.p.	3	5.3	25.0	48.0	27.0	0.0	0.0
12	25 x 1	i.p.	1	6.9	25.4	47.6	27.0	0.0	0.0
Control (10)		—	0	10.4	20.2	21.0	58.8	0.0	0.0
12	100 x 5	i.m.	5	10.3	79.2	11.1	9.7	43.0	100.0
12	50 x 5	i.m.	5	8.6	78.3	8.3	13.4	55.0	100.0
12	25 x 5	i.m.	2	10.2	46.1	14.7	39.2	3.9	80.0
12	12.5 x 5	i.m.	2	18.5	11.9	28.1	60.0	0.5	0.0
Control (10)		—	0	15.8	11.4	12.6	76.0	0.0	0.0
12	200 x 1	i.m.	8	7.5	13.3	3.3	83.4	0.0	25.0
12	100 x 1	i.m.	5	5.7	27.5	35.3	37.2	0.0	0.0
12	50 x 1	i.m.	3	5.3	25.0	48.0	27.0	0.0	0.0
12	25 x 1	i.m.	1	6.9	25.4	47.6	27.0	0.0	0.0
Control (10)		—	1	10.4	20.2	21.0	58.8	0.0	0.0

TABLE II

Antischistosomal activity of NaP in hamsters experimentally infected with *Schistosoma mansoni*: oogram changes, distribution of schistosomes and percentage of worms retained in the liver after perfusion

Number of hamsters	Dose (mg/kg/day x days)	Route	Animals dead	Mean worm burden	Distribution of schistosomes (%)			Percentage of worms retained in the liver	Percentage of animals with oogram changes
					Liver	Portal vein	Mesenteric vessels		
6	16 x 5	i.p.	0	21.8	96.9	3.1	0.0	53.4	100.0
6	8 x 5	i.p.	0	23.0	40.6	29.7	29.7	0.0	83.3
6	4 x 5	i.p.	0	38.2	14.4	33.6	52.0	0.0	0.0
6	2 x 5	i.p.	0	33.8	11.4	38.4	50.2	0.0	0.0
Control (6)	—	—	0	29.7	21.3	32.6	46.1	0.0	0.0

TABLE III

Antischistosomal activity of NaP in *Cebus* monkeys experimentally infected with *S. mansoni*. Serial mucosal curettages.

Monkey	Schedule of treatment	Duration of infection before treatment	Days before (−) or after (+) the beginning of treatment	Stages of viable eggs					Dead eggs and shells	Number of viable eggs per gram of rectal tissue	Remarks
				1st	2nd	3rd	4th	Mature			
1	75 mg/kg/ day x 5, i.m.	5 months	− 89	18	23	21	4	175	85	8827	Parasitological cure
			− 68	24	16	32	12	257	113	13861	
			− 13	36	30	25	43	308	99	24419	
			+ 3	0	0	1	4	186	35	8268	
			+ 9	0	0	0	0	7	26	333	
			+ 20	0	0	0	0	0	0	0	
			+ 29	0	0	0	0	0	1	0	
			+ 56	0	0	0	0	0	1	0	
			+ 90	0	0	0	0	0	0	0	
			+ 115	0	0	0	0	0	0	0	
			+ 140	0	0	0	0	0	0	0	
2	15 mg/kg/ day x 5, i.m.	6 months	− 78	26	29	17	5	271	139	13231	Transitory interruption of egg laying. Relapse.
			− 28	30	12	23	15	130	82	9251	
			− 1	6	2	21	16	57	31	3953	
			+ 3	0	0	0	3	62	12	3457	
			+ 9	0	0	0	0	9	11	463	
			+ 36	0	0	0	0	0	2	0	
			+ 66	0	0	0	0	1	3	52	
			+ 108	0	2	2	41	13	18	3022	
			+ 120	4	1	12	23	41	16	3443	
3	7.5 mg/kg/ day x 5, i.m.	6 months	− 70	0	1	17	36	63	77	4398	Parasitological cure
			− 6	3	23	37	9	91	61	5276	
			− 1	37	2	12	12	122	44	8486	
			+ 8	0	0	0	0	25	9	980	
			+ 18	0	0	0	0	0	0	0	
			+ 83	0	0	0	0	0	0	0	
			+ 108	0	0	0	0	0	3	0	
			+ 140	0	0	0	0	0	17	0	
4	15 mg/kg/ day x 1, i.m.	6 months	− 70	8	13	32	3	130	192	7438	No antischistosomal activity
			− 6	9	15	56	43	93	132	16119	
			− 1	14	11	35	48	89	43	10591	
			+ 8	15	18	6	5	71	118	3993	
			+ 18	16	45	97	30	97	163	12231	
			+ 78	60	45	66	32	221	139	10341	
			+ 108	3	3	17	17	48	47	9888	

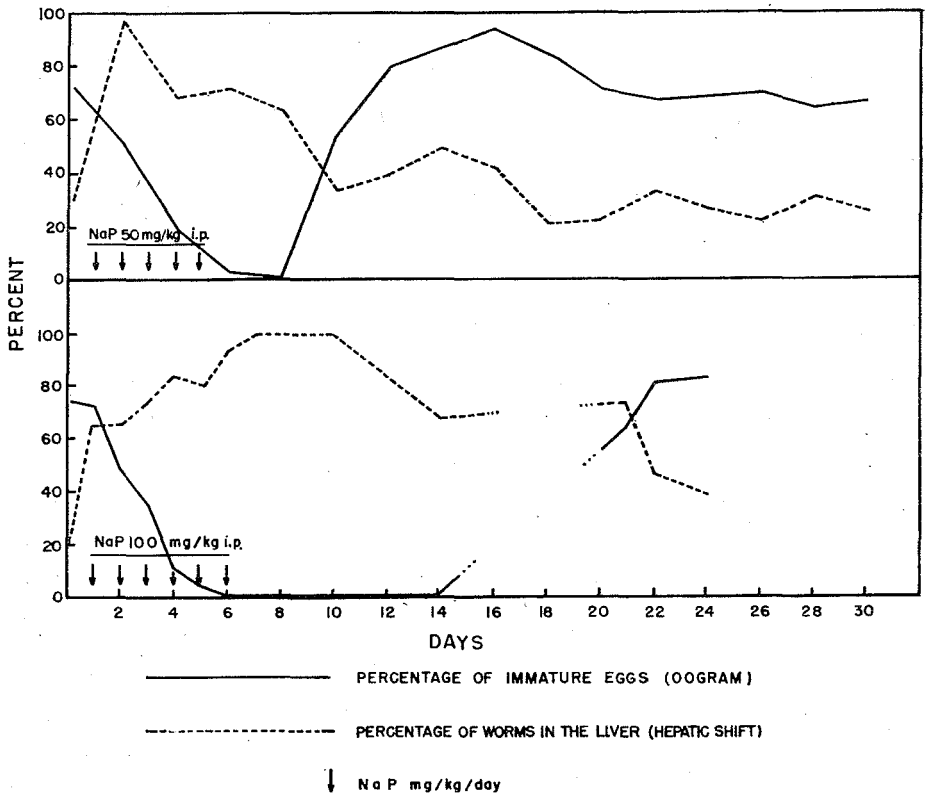

Fig. 2 — Relapse in two groups of mice treated with NaP. It is clearly shown that interruption of egg laying was of very short duration in the group dosed with 50 mg/kg/day x 5 in comparison with the group treated with 100 mg/kg/day x 6.

TABLE IV

Assessment of therapeutic activity of NaP in 5 schistosome patients

Patients	Schedule of treatment (mg/kg/day x days)	Stool examination (eggs/g)	
		Before treatment	4 to 6 months after treatment (*)
1	4 x 5	140	100
2	4 x 5	240	160
3	4 x 5	480	240
4	4 x 1	60	80
5	8 x 4	200	0 (**)

(*) Mean of 4 quantitative stool examinations

(**) Six stool examinations were performed within the 4th month after treatment

The patient treated with 8 mg/kg/day x 4 presented nausea, vomiting, headache, muscle pain and, after the 4th injection, a severe precordial pain which lasted for 4 days. The electrocardiogram showed subendocardial ischemia but returned to normal 10 days after the last injection. Parasitological cure was achieved in this patient (Table IV).

DISCUSSION

In an attempt to reduce the high toxicity displayed by antimonials used in the treatment of schistosomiasis, ERCOLI [2] developed a preparation containing 1 mole potassium antimonyl tartrate to 3.4 to 4.5 moles of dimethylcysteine (penicillamine). Although the chelation of antimony by penicillamine drastically reduces the toxicity of potassium antimonyl tartrate, it was later shown by TARRANT et al. [14] that at doses sufficient to reduce the acute toxicity of APT, dimethylcysteine also reduces the effectiveness of APT on schistosomes.

PEDRIQUE et al. [7], in a rural area of Venezuela, treated 108 patients with a schedule of daily intramuscular injections (400 mg of NaP) for 5 days. In all patients but 4, treatment was completed. Nausea and vomiting occurred after 94 (17.7%) of the 530 injections. Fever, myalgia, skin vesicles or rashes of mild nature were also observed in 11% of the individuals. Among 101 patients, followed-up by a stool concentration method, 1, 2, and 3 months after treatment, 95 (94%) were seen to be free from *S. mansoni* ova.

Since the antischistosomal activity of NaP (sodium antimony dimethylcysteine tartrate) was very evident in mice, hamsters and *Cebus* monkeys experimentally infected with *S. mansoni* and pharmacological and clinical studies in volunteers resulted favourable (trials performed in the United States) it was decided to conduct a limited study in patients suffering from chronic schistosomiasis mansoni. The results were disappointing from the beginning. Actually, one patient was forced to interrupt treatment after the first injection due to an increase in bradycardia (52 to 38 bpm) (group of 4 patients treated with 4 mg/kg/day x 5). One patient dosed with 8 mg/kg/day x 4 presented nausea, vomiting, myalgia and, after the last injection, a severe precordial pain which lasted for 4 days. The electrocardiogram showed subendocardial ischemia. As far as parasitological cure is concerned, it was achieved only in the patient dosed with 8 mg/kg/day. Owing to the side effects observed and the poor antischistosomal activity, clinical trials with NaP were discontinued. On this regard it is important to note that 2 fatal cases were observed by COUTINHO [1] in patients treated with NaP (26 patients, daily dose of 400 mg of NaP for 5 consecutive days).

RESUMO

Quimioterapia experimental da esquistossomose. VIII — Ensaios de laboratório e clínicos com o tartarato de sódio-antimônio dimetilcisteína (NaP)

A atividade terapêutica do NaP, quando administrado em 5 injeções por via i.p. ou i.m., em 5 dias consecutivos, a camundongos experimentalmente infectados pelo *Schistosoma mansoni*, foi muito evidente. Na dose de 25 mg/kg/dia x 5, praticamente todos os animais apresentaram alterações do oograma, acompanhadas por pronunciado deslocamento dos esquistossomos para o fígado. Quando o tratamento se limitou a uma única injeção (i.p. ou i.m.) a atividade terapêutica foi muito fraca.

Em "hamsters" a atividade anti-esquistossomótica, na dose de 16 mg/kg/dia x 5, i.p., foi muito evidente. Todos os animais apresentaram alterações do oograma e praticamente todos os esquistossomos foram deslocados para o fígado.

Em macacos *Cebus* o NaP mostrou-se inativo quando administrado, por via intramuscular, em dose única de 15 mg/kg. Cura parasitológica da infecção foi observada em macacos tratados com 75 e 7.5 mg/kg/dia x 5, i.m.

Os resultados de um ensaio clínico limitado foram altamente desapontadores. Três pacientes tratados com 4 mg/kg/dia x 5, i.m., continuaram eliminando ovos de *S. mansoni* 4 meses após o tratamento. Os efeitos colaterais observados foram: dor abdominal, náu-

sea, vômito, sialorréia e alterações do eletrocardiograma. Somente um paciente tratado com 8 mg/kg/dia x 4, i.m., pôde ser considerado como parasitologicamente curado. Todavia, esse paciente apresentou isquemia sub-endocárdica.

Devido aos efeitos colaterais — incluindo casos fatais relatados no Brasil — após o tratamento com o NaP, foi concluido que ensaios clínicos com este agente esquistossomicida devem ser interrompidos.

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