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EXPERIMENTAL CHEMOTHERAPY OF SCHISTOSOMIASIS IV — OOGRAM STUDIES WITH NICARBAZIN, AN EGG-SUPPRESSIVE AGENT

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SUMMARY

Interruption of egg laying occurred in mice experimentally infected with *Schistosoma mansoni* and treated for 5 consecutive days with nicarbazin (complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine) incorporated in the diet at the concentrations of 1.5 and 1.0%. When the medicated diet contained nicarbazin in the proportion of 0.5%, oogram changes were found in 50% of the treated mice. Oviposition was resumed as soon as the drug was withdrawn. Nicarbazin was ineffective when administered by gavage even at the dose level of 1,000 mg/kg/day x 5.

No antischistosomal activity could be detected in hamsters after treatment with medicated diet (1.0%) for 36 days or after two courses of 5 consecutive days, separated by a 2-day interval (2.0% nicarbazin in the diet).

In *Cebus* monkeys experimentally infected with *S. mansoni* and treated with nicarbazin by oral route a temporary interruption of egg laying could be detected by serial mucosal curettages.

It was stressed the importance of the development of drugs which can be applied as suppressives for the control of Schistosomiasis.

INTRODUCTION

It was recently reported by CAMPBELL & CUCKLER [1] that in mice experimentally infected with *Schistosoma mansoni* egg production is suppressed by treatment with nicarbazin (equimolecular complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine, Fig. 1). When fed to mice at 0.2% in the diet, nicarbazin completely inhibited the deposition of schistosome eggs, but did not kill the worms. However, the inhibitory activity was reversible, egg production being resumed when the drug was withdrawn.

Since the mode of action of nicarbazin is rather unusual in that it seems to be chiefly directed against the reproductive system of schistosomes, it was found worth while to investigate the oogram changes produced by this drug in mice, hamsters and *Cebus* monkeys experimentally infected with *S. mansoni*. The results obtained are herein presented.

MATERIALS AND METHODS

Infection of animals

Cercariae shed by laboratory-reared and infected *Biomphalaria glabrata* (L. E. strain

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of *S. mansoni*) were used in the present study. Albino mice, weighing 20 g, were exposed to 100 ± 10 cercariae by the tail immersion method [4]. Adult hamsters (*Cricetus auratus*) were infected with 50 ± 10 cercariae via the cheek pouch [9]. The percutaneous route was used for exposing adult *Cebus apella macrocephalus* Spix, 1823 to 150 ± 20 *S. mansoni* cercariae.

Treatment of animals

Seven weeks after exposure, groups of 8 to 12 mice were fed, *ad libitum*, with nicarbazin incorporated in the diet at the concentrations of 1.5, 1.0, 0.5, 0.2, and 0.1%, for 5 consecutive days. All animals were killed and examined 3 days after the end of treatment. A group of 12 untreated mice was left as control.

Nicarbazin was also administered, by stomach tube, to groups of 15 mice, harboring mature *S. mansoni* infection, at the daily dose levels of 250 mg/kg (divided in 5 daily doses of 50 mg/kg) and 1,000 mg/kg (single daily dose), for 5 consecutive days.

In order to investigate the speed of action of nicarbazin and the resumption of egg laying (relapse), a group of 80 mice received, for 5 consecutive days, medicated diet containing the drug in the proportion of 1.5%. Groups of 4 mice were sacrificed before treatment and on successive days after the beginning of dosing for oogram studies.

A group of 6 hamsters was treated with nicarbazin (1.0% in the diet) for 36 consecutive days, starting 3 weeks after exposure. Another group of 10 hamsters received, 7 weeks after exposure, two courses of treatment (2.0% medicated diet) of 5 consecutive days, separated by a 2-day interval. In both groups the animals were killed and examined one day after the end of treatment. Five untreated hamsters were left as control.

Three adult *Cebus* monkeys were treated, by gavage, as follows. Monkey no. 1: one course of treatment, 5 months after exposure, consisting of 400 mg/kg/day (divided in 5 doses) x 2 days; 200 mg/kg/day (divided in 5 doses) x 10 days. Monkey no. 2: two courses of treatment (17 months after exposure): lst course — 400 mg/kg/day (divided in 2 doses) x 2 days; 200 mg/kg/day (divided in 2 doses) x 10 days; 2nd course (3 months after the lst course) — 200 mg/kg/day (single dose) x 30 days. Monkey no. 3: one course of treatment (19 months after exposure) of 400 mg/kg/day (single dose), for 10 consecutive days. Monkey no. 4 was left as untreated control.

Assessment of antischistosomal activity

Mice and hamsters were killed by a blow on the neck. The schistosomes in the portal and mesenteric veins and in the liver were recovered by perfusion using PELLEGRINO & SIQUEIRA'S technique [6] adapted to mice and hamsters. For oogram studies, press preparations of intestinal fragments were microscopically examined and 200 viable eggs, per mouse or hamster, counted and classified according to their developmental stages [7, 8]. Rectal snips, usually 4, from *Cebus* monkeys were taken by mucosal curettage as described elsewhere [3, 10].

Changes in the oogram from intestinal fragments of mice and hamsters were considered significant when one or more developmental stages of immature eggs were absent. In *Cebus* monkeys the assessment of drug activity was based on the gradual disappearance of immature and mature viable eggs in rectal snips.

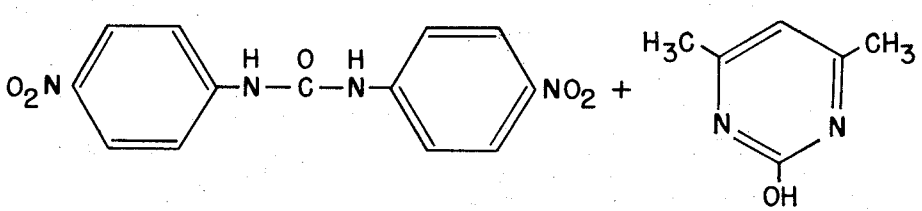

Fig. 1 — Chemical structure of nicarbazin

RESULTS

The results obtained in mice experimentally infected with *S. mansoni* and treated with nicarbazin are summarized in Table I. As can be seen, when the drug was incorporated in the diet in the proportions of 1.5 and 1.0%, oogram changes were found in all treated animals. A slight hepatic shift of schistosomes was observed at these dose levels. Half of the mice treated with 0.5% medicated diet presented oogram changes. Treatment by stomach tube, even at the daily dose level of 1,000 mg/kg, for 5 consecutive days, was ineffective.

Figure 2 shows that the percentage of immature eggs in intestinal fragments from mice treated with nicarbazin (1.5% in the diet) is progressively reduced dropping to zero on the 6th day. However, resumption

TABLE I

Therapeutic activity of nicarbazin in mice experimentally infected with *S. mansoni*
The animals were killed and examined 3 days after the end of treatment

Schedule of treatment (% nicarbazin in the diet, for 5 consecutive days)	Number of mice	Animals dead	Mean number of worms	Distribution of schistosomes (%)			Percentage of mice with oogram changes
				Liver	Portal vein	Mesenteric vessels	
1.5	12	4	16.6	36.0	29.4	34.6	100.0
1.0	12	6	17.7	30.2	8.5	61.3	100.0
0.5	12	4	18.8	27.3	23.5	49.2	50.0
0.2	8	3	19.2	26.0	13.5	60.5	40.0
0.1	8	3	16.6	17.3	22.9	59.8	0.0
Control	12	2	20.1	20.2	24.9	54.9	0.0

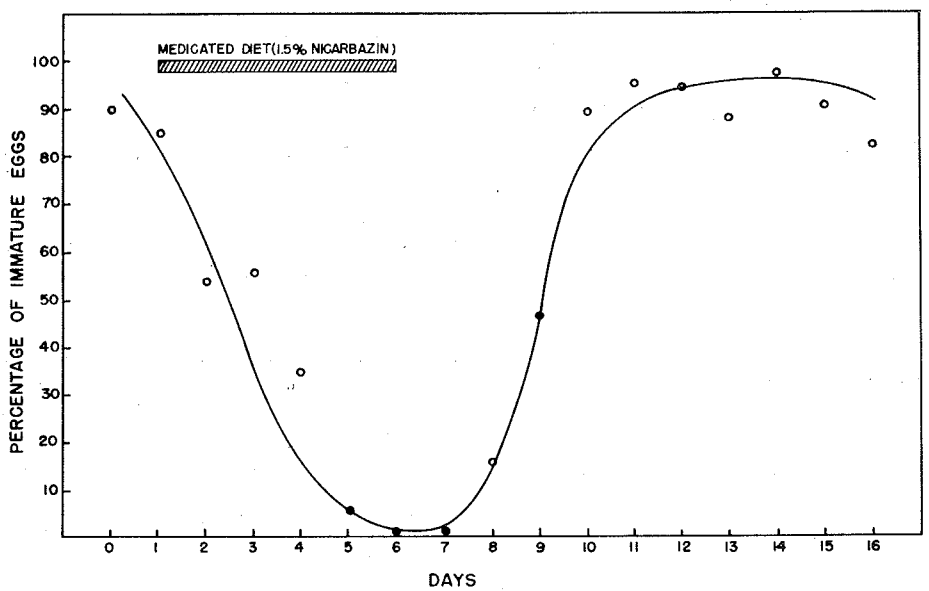

Fig. 2 — Curve obtained by plotting the percentage of immature viable eggs from intestinal fragments of mice treated with nicarbazin (1.5% in the diet for 5 consecutive days). Rasumption of egg laying (relapse) took place soon after treatment had been stopped. Each point represents the average from four animals

of egg laying (relapse) occurred soon after the drug was withdrawn.

In hamsters experimentally infected with *S. mansoni* and treated with nicarbazin (1.0 and 2.0% in the diet) no antischistosomal activity could be detected (Table II).

A transient interruption of egg laying was observed in *Cebus* monkeys treated with nicarbazin (administered by gavage) at different schedules (Table III). The suppressive activity occurred within the period of treatment or soon after the completion of dosing and was clearly demonstrated by the disappearance of immature viable eggs from rectal snips and by the reduction in the number of viable eggs per gram of rectal tissue (quantitative oogram). However, in all treated animals relapse took place after medication was discontinued. Loss of appetite and apathy were observed in monkeys 1 and 2 within the period of treatment.

DISCUSSION

It has been shown that the rather complex problem of chemotherapy of schistosomiasis probably demands three types of drugs [5, 12]: a) a *prophylactic* drug which will prevent infection; b) a *suppressant* which may not necessarily impose irreversible harm upon the schistosomes but will prevent egg laying and thus interrupt transmission; c) a *curative* drug which will destroy most, or all, of the adult worms. According to STANDEN [12], the development of a good suppressive drug would offer a major contribution to control of the disease.

Although some of the available schistosomicidal agents may inhibit egg laying when administered in spaced small doses, nicarbazin represents the first truly egg-suppressant drug reported in the literature (CAMPBELL & CUCKLER [1]). In fact, this compound inhibits egg production at a dosage which can be increased at least tenfold without killing the worms. Besides, egg laying could be completely suppressed for a period of months without producing appreciable harm to the schistosomes [1].

Even though the data so far available seem to indicate that nicarbazin acts chiefly, and perhaps exclusively, on the reproductive system of *S. mansoni*, some puzzling aspects have arisen from our studies. Firstly, in mice harboring mature *S. mansoni* infection, nicarbazin was only effective when incorporated in the diet. Administered by gavage in single daily doses up to 1,000 mg/kg or in multiple daily doses of 50 mg/kg, no

TABLE II

Therapeutic activity of nicarbazin in hamsters experimentally infected with *S. mansoni*. The animals were killed and examined one day after the end of treatment.

Schedule of treatment	Number of hamsters	Animals dead	Mean number of worms	Distribution of schistosomes (%)			Percentage of hamsters with oogram changes
				Liver	Portal vein	Mesenteric vessels	
1% in the diet, for 36 consecutive days, starting 3 weeks after exposure	6	2	23.7	11.3	18.6	70.1	0.0
2% in the diet; two courses of 5 consecutive days, separated by 2-day interval*	10	1	28.6	24.0	15.3	60.7	0.0
Control (7 weeks of infection)	5	0	29.7	16.0	31.9	52.1	0.0

* Treatment was started 7 weeks after exposure

TABLE III

Therapeutic activity of nicarbazin in hamsters experimentally infected with *S. mansoni*

Monkey	Duration of infection when treatment was started	Schedule of treatment (by gavage)	Days before (—) dosing or after (+) the beginning of treatment	Stages of viable eggs					Dead eggs and shells	Number of viable eggs per gram of rectal tissue
				1st	2nd	3rd	4th	Mature		
1	5 months	*One course of treatment*: 400 mg/kg/day (divided in 5 doses) x 2 days; 200 mg/kg/day (divided in 5 doses) x 10 days.	— 45	2	6	17	6	27	22	2533
			— 3	10	39	37	8	59	24	5709
			+ 4	0	0	2	3	143	48	4836
			+ 8	0	0	0	0	46	33	1393
			+ 12	0	0	0	0	9	31	264
			+ 15	0	0	0	0	2	16	660
			+ 17	0	0	0	0	0	8	0
			+ 23	0	0	0	0	0	3	0
			+ 32	25	20	1	2	0	2	1481
			+ 36	0	2	1	0	7	4	358
			+ 106	2	10	0	0	10	22	1222
			+ 117	2	8	11	5	16	41	1653
2	17 months	*Two courses of treatment*: *1st course*- 400 mg/kg/day (divided in 2 doses) x 2 days; 200 mg/kg/day (divided in 2 doses) x 10 days; *2nd course*- (started on day + 93): 200 mg/kg/day (single dose) x 30 days.	— 81	14	6	13	3	38	9	2761
			— 66	1	4	8	0	49	11	2831
			— 5	3	4	18	8	162	50	8904
			+ 8	0	0	3	1	27	41	1359
			+ 14	0	0	0	0	6	7	297
			+ 21	1	15	4	0	8	19	1052
			+ 37	22	1	1	1	45	25	3448
			+ 66	3	1	28	4	39	11	3456
			+ 84	0	1	3	0	52	16	2043
			+ 93	1	4	16	13	130	45	7922
			+ 105	0	0	0	0	3	5	155
			+ 119	0	0	0	0	2	0	100
			+ 126	4	0	0	0	10	39	744
			+ 130	31	16	9	18	62	43	6903
3	19 months	*One course of treatment*: 400 mg/kg/day (single dose) x 2 days; 200 mg/kg/day (single dose) x 10 days.	— 112	5	5	9	10	86	18	4978
			— 30	11	18	8	0	9	7	2690
			+ 12	0	0	0	0	0	0	0
			+ 18	0	0	0	0	0	1	0
			+ 33	0	3	24	6	0	0	1487
			+ 46	12	20	52	14	72	55	6273
4	Control	—	9*	1	18	53	23	33	10	4460
			10	7	1	5	10	38	46	2696
			16	20	9	31	23	219	36	8229
			18	4	4	9	5	87	27	4209
			22	16	8	12	21	296	215	11517

* Months after exposure to *S. mansoni* cercariae

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oogram changes could be detected. Secondly, no suppressive activity was evidenced in hamsters experimentally infected after feeding the animals for a long period with medicated diet (1.0% nicarbazin). Finally, nicarbazin was effective in suppressing egg laying in *Cebus* monkeys when administered by gavage. These results strongly suggest that the equimolecular complex of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP) is metabolized in different ways when administered to mice, hamsters and *Cebus* monkeys. It is known that in chickens nicarbazin is more readily absorbed from the intestine than DNC but less readily absorbed than the HDP portion of the complex. Besides, the HDP portion of nicarbazin is excreted or metabolized more rapidly than DNC[11]. There is evidence that in calves, after absorption, nicarbazin splits into its components which are then metabolized separately. The dinitrocarbanilide is first reduced to soluble diaminocarbanilide and the acetylated to insoluble 4,4'-bisacetamidocarbanilide which forms crystalline deposits in the renal tubules [2]. A similar pattern of degradation was observed in rats but not in dogs.

Since in schistosome infections the pathological effects are chiefly due to deposition of eggs with consequent tissue damage, the development of drugs able of interrupting egg production might conceivably play an important role in the control of the disease in hyperendemic areas. Researches along this promising line are in progress in our laboratories.

RESUMO

Terapêutica experimental da esquistossomose. IV — Ensaios com nicarbazin, agente supressor da oviposição

Ensaios terapêuticos com o nicarbazin (complexo equimolecular de 4,4'-dinitrocarbanilide e 2-hidroxi-4,6-dimetilpirimidina) foram feitos em camundongos, hamsters e macacos *Cebus* experimentalmente infetados com *Schistosoma mansoni.*

Quando incorporado à dieta, na proporção de 1,5 e 1,0%, o nicarbazin provocou interrupção da postura em camundongos infetados. Entretanto, esta interrupção foi apenas temporária, recomeçando tão logo o tratamento de cinco dias foi suspenso.

O nicarbazin não exerceu nenhuma atividade terapêutica em hamsters, mesmo quando administrado na dieta (1,0%) durante 36 dias.

Em macacos *Cebus* tratados com o nicarbazin por via oral, oogramas seriados evidenciaram interrupção temporária da postura.

Foi salientada a importância de estudos visando o encontro de agentes supressores para o contrôle da esquistossomose.

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