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The Prophylactic and Curative Activity of a nitrothiazole Derivative, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone, in Rhesus Monkeys Experimentally Infected with *Schistosoma mansoni* and in Mice Infected with *S. japonicum*

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Introduction

The treatment of schistosomiasis has been carried out primarily by the use of trivalent antimonial compounds. However, since these antimonial compounds are highly toxic the search for new types of drugs of lower toxicity has been actively pursued. Recently a non-antimonial compound was synthesized in the research laboratories of CIBA Limited, Basle, Switzerland. This substance, designated as CIBA 32644-Ba, was reported to have an effective schistosomicidal activity against adult *Schistosoma mansoni in vitro* in a concentration of 10 μg/ml and to inhibit egg-laying by female schistosomes in a concentration of 1 μg/ml (LAMBERT, 1964).

In the course of the preliminary studies conducted in mice infected with *S. mansoni,* no living worms were found when the drug was administered orally in a dose of 100 mg/kg daily for 10 consecutive days. The full therapeutic course of this compound appeared to have no significant hepatotoxic action in normal or infected mice. The only toxic action observed after therapeutic doses was a temporary and reversible inhibition of spermatogenesis. The serum of rabbits which were given the drug orally was found to exhibit a maximal schistosomicidal action *in vitro* when collected 6 hours after administration. The drug was found to be concentrated in the vitellaria of the schistosomes as well as in the eggs deposited in the liver and intestinal wall of the host. Morphological

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changes in the ootype and vitellaria of the females and the testes of the male worms have been observed.

In view of these considerations, the present studies were begun to determine the prophylactic, suppressive, and therapeutic activities of CIBA 32644-Ba in *Schistosoma mansoni* infections in monkeys and in *S. japonicum* infections in mice.

Materials and Methods

A. Studies with Schistosoma mansoni

A total of 25 monkeys *(Macaca mulatta)* weighing between 2.5 and 3.5 kg each at the beginning of the experiment were used for these studies and divided into 5 groups. The animals of Groups I, II, III and IV (Table 1) were each exposed percutaneously to 400 cercariae of a Puerto Rican strain of *S. mansoni* maintained in the laboratory in albino mice and *Australorbis glabratus* snails. The cercariae were collected from pools of 100 or more snails. Exposures were carried out by placing the cercariae on the previously shaven abdominal area of the monkeys. The monkeys of Groups I, II and III were treated with various regimens of the drug at different times during the course of infection. Animals of Group IV were exposed but not treated and served as untreated controls; those of Group V were treated but not exposed to infection and served as drug controls. The drug was given by means of a stomach tube. In the animals of Group I, the drug was administered in daily doses of 100 mg/kg for 4 days, beginning with the day before exposure. In the animals of Group II, the drug was given in the amount of 50 mg/kg twice daily for 6 consecutive days, beginning on the 56th day following exposure. The first of the daily treatments was given between 09.00 and 10.00 hours and the second one was given between 16.00 and 17.00 hours. The animals of Group III were given 6 doses of 100 mg/kg, beginning with the 56th day following exposure. Likewise, the animals of Group V were given 6 consecutive daily doses of 100 mg/kg and were used as uninfected drug controls.

Throughout the experiment, observations were made for changes in body weight and reactions such as weakness, ataxia, paralysis, marked changes in appetite, or other obvious signs of intoxication. Stools from all animals were examined for the presence and number of schistosome eggs twice weekly from the 32nd day after exposure to the end of the experiment. The direct smear and the AMS III Concentration Technique (HUNTER et al., 1948) were employed.

The animals used in the prophylactic studies were necropsied

TABLE 1

The prophylactic and curative effect of CIBA 32644-Ba in 25 monkeys exposed to 400 Schistosoma mansoni cercariae each

Group No.	Monkey No.	Dose (mg/kg)	Rx started (from day of exposure)	Results of necropsy				No. eggs/gm feces*	
				Number live worms recovered					
				Female	Male	Total	Percent	Maximum	Mean
I	1	100 for	—1	1	20	21	5	0	0
	2	4 days		1	25	26	6	0	0
	3			0	4	4	1	0	0
II	4	50 b.i.d.	56	3	19	22	6	15	2
	5	for 6 days		0	2	2	0.5	0	0
	6			0	0	0	0	2	0.2
	7			0	0	0	0	0	0
	8			0	0	0	0	0	0
	9			0	0	0	0	0	0
III	10	100 for	56	6	31	37	9	1	0.1
	11	6 days		1	6	7	2	0	0
	12			1	6	7	2	0	0
	13			0	4	4	3	11	1
	14			0	2	2	0.5	0	0
	15			0	0	0	0	0	0
	16			0	0	0	0	0	0
IV	17	—	—	49	56	105	26	31	18
	18	—	—	40	46	86	21	32	18
	19	—	—	17	62	79	20	39	21
	20	—	—	23	48	71	18	18	13
	21	—	—	21	47	68	17	30	20
	22	—	—	24	34	58	14	21	12
	23	—	—	29	44	73	18	35 **	16 **
V	24	100 for	—	—	—	—	—	—	—
	25	6 days	—	—	—	—	—	—	—

* From two weeks after completion of treatment to end of experiment.
** From earliest egg appearance to necropsy.

49 days after exposure and those used in the therapeutic studies were necropsied 126 days after exposure to infection. The Perf-o-Suction method (RADKE et al., 1961) was used for recovery of schistosomes. In addition, the intestine and liver were removed and examined for the presence of living or dead schistosomes which might not have been recovered by perfusion.

Determination of the therapeutic effectiveness of the drug was

based on the presence, number, and viability of schistosome eggs in the feces, intestine, and liver of the animals, and also the number, sex ratio, and appearance of the living schistosomes recovered, and the gross pathology of liver, spleen, and intestine. Immediately after perfusion, the recovered worms were placed in warm alcohol-formalin-acetic acid (AFA) fixative, cleared, stained with Delafield and Ehrlich hematoxylin, mounted, and studied microscopically for possible morphological changes. The testes and the ovaries of the monkeys were removed at necropsy, fixed in buffered 10 % formalin, sectioned at 7 μ thickness, and stained with hematoxylin and eosin for histopathological studies.

B. Studies with Schistosoma japonicum

Female albino mice (SM strain) weighing 25 to 30 g each were divided into 5 groups of 20 each (Table 2). Each group was further subdivided into treated and untreated controls. All of the mice were exposed percutaneously to 25 male and 25 female cercariae each of a Japanese strain of *S. japonicum* obtained from infected *Oncomelania nosophora*. The drug was suspended in corn oil and administered orally in the amount of 100 mg/kg daily for 10 consecutive days. Treatment of the animals began at different times with respect to exposure to infection and varied from the 1st (Group I) to the 42nd day (Group V). Microscopic examinations of the pooled stools from each group were performed on the

TABLE 2

The prophylactic and curative effect of CIBA 32644-Ba in 100 albino mice exposed to 50 Schistosoma japonicum cercariae each

Group No.	Rx started * (from day of exposure)	Surviving 10 weeks	Results of necropsy (all mice)				Viable eggs in stools (67th day)	Viable eggs in liver (hatching test)
			Number live worms recovered					
		Total	Female	Male	Total	Percent		
I	0	10/10	0	0	0	0	Neg.	Neg.
Ic	—	4/10	92	89	181	36.2	Pos.	Pos.
II	5	10/10	7	5	12	2.4	Pos.	Pos.
IIc	—	5/10	87	105	192	38.4	Pos.	Pos.
III	10	8/10	67	71	138	27.6	Pos.	Pos.
IIIc	—	7/10	98	82	180	36.0	Pos.	Pos.
IV	15	9/10	67	72	139	27.8	Pos.	Pos.
IVc	—	8/10	75	81	156	31.2	Pos.	Pos.
V	42	10/10	0	0	0	0	Neg.	Neg.
Vc	—	7/10	63	133	196	39.2	Pos.	Pos.

* 100 mg/kg for 10 days.

67th day following exposure. These examinations were conducted only to determine the presence or absence of eggs. No attempts were made to estimate the number of eggs present. All animals which died before the end of the experiment were necropsied, and the worm burden was recorded. All of the surviving animals were necropsied 70 days after exposure and the recovered worms were counted and separated by sex. Eggs recovered from pooled mouse livers from each group were tested for viability by the hatching technique.

Results

No obvious signs of toxicity were observed in any of the treated animals. There were no significant changes in the weights of any of the monkeys during the course of this experiment. Pathological studies of the testes and ovaries of treated and untreated monkeys showed no obvious gross or microscopical differences. The testes and ovaries of all animals, treated and untreated, showed a histological pattern consistent with normal prepubertal or early pubertal stages and gave no evidence of degenerative changes.

1. *Schistosoma mansoni*

The results of the effect of treatment on the number of living schistosomes found at necropsy have been summarized in Table 1. Forty-nine days after exposure, a significant reduction in the worm burden was observed in the animals which received the drug prophylactically (Group I). No eggs appeared in the stools of these animals and no gross pathological changes attributable to the infection were seen in any of the organs.

The control monkeys showed the typical egg-production pattern of *S. mansoni* in *M. mulatta:* a peak at approximately 8 weeks; a marked reduction for 4 weeks; and then a moderate number persisting for the duration of the experiment (Fig. 1). The animals treated with 2 daily doses of 50 mg/kg each for a total of 6 days (Group II) showed a parallel course for the first two periods, but the eggs disappeared during the third. Essentially the same results were obtained in the animals which received 100 mg/kg each.

In both of these groups the passage of eggs was completely suppressed in all but 4 monkeys, and the worm burden was significantly reduced. Only a few worms, almost all of them males, were found in 2 of 6 monkeys of Group II and 5 of 7 monkeys of Group III. Both male and female worms appeared to be stunted, and considerable morphological changes, particularly in the re-

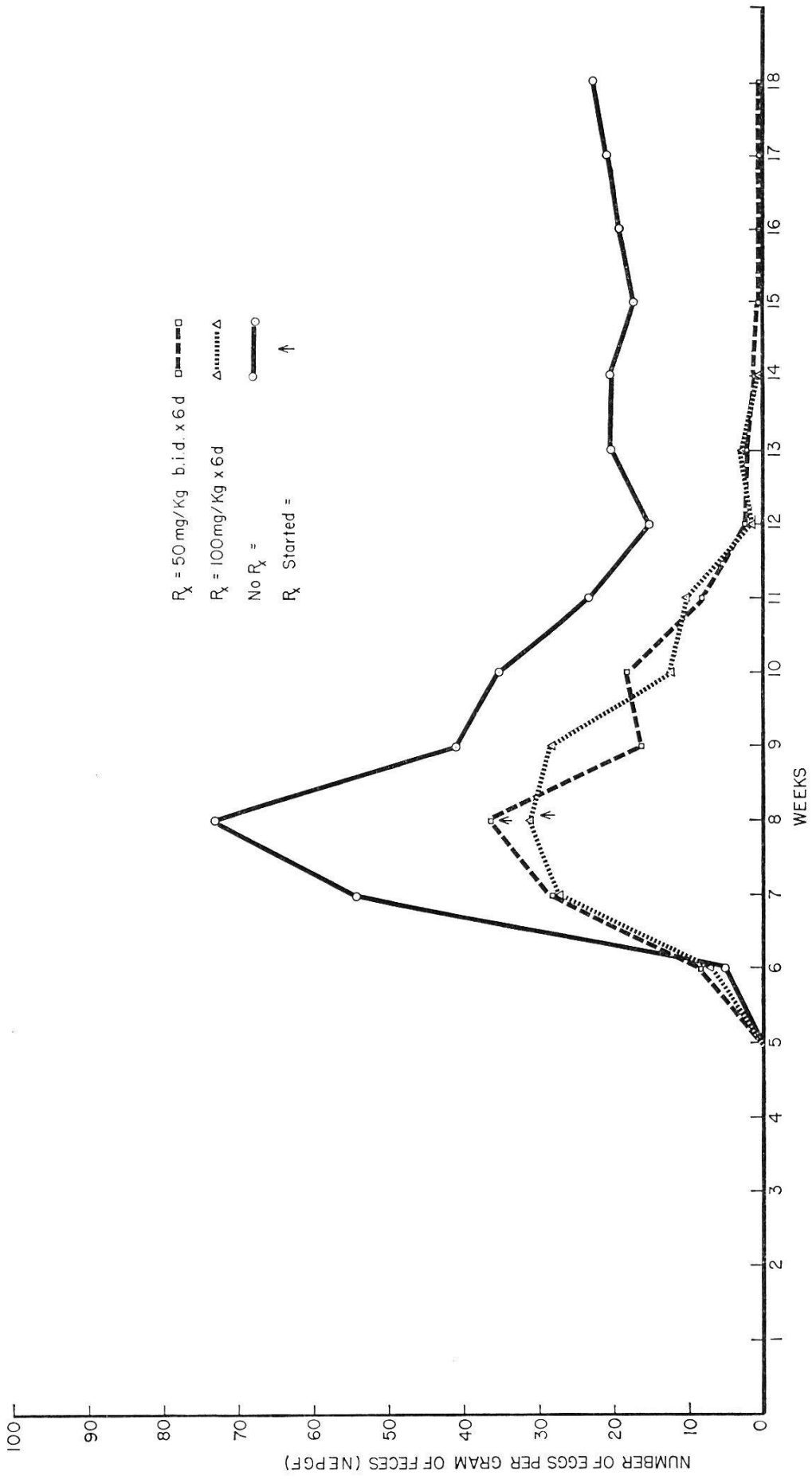

Fig. 1. Passage of *Schistosoma mansoni* eggs in *Macaca mulatta* before and after treatment with CIBA 32644-Ba.

productive organs, were observed. Hatchability studies conducted each week throughout the experiment showed that miracidia could be recovered from those specimens containing eggs.

Gross pathological observations in the untreated controls showed enlargement of livers and spleens. Brownish discoloration and moderate spotting due to egg accumulation were also apparent in these organs. The gross appearance of lungs and both small and large intestines was essentially normal, except for the presence of petechial hemorrhages in the intestines of one animal (No. 23). By contrast, no gross abnormalities were observed in the livers, lungs, and intestines of the treated and infected monkeys, although a moderate splenomegaly was noticeable in all of these animals.

2. *Schistosoma japonicum*

The results obtained in mice exposed to *Schistosoma japonicum* (Table 2) were essentially the same as those observed in monkeys infected with *S. mansoni*. No viable eggs and no living worms were observed in any of the animals which were treated prophylactically beginning with the day of exposure (Group I), or in those which were treated 42 days after exposure (Group II). There was a significant reduction in the number of worms in the animals in which treatment began 5 days after exposure (Group II), but this was not true of the groups in which drug administration was initiated on the 10th and 15th days (Groups III, IV).

Discussion

The results in general indicate that the compound, CIBA 32644-Ba, has a marked prophylactic and therapeutic activity against infections with *S. mansoni* in monkeys and *S. japonicum* in mice. In doses which appear to be well tolerated, this activity was manifested by a marked reduction in the number of worms developing and by a stunting of those few worms which survived the effect of the drug. Although the specific mode of action of this drug could not be determined in these experiments, there was evidence that in the monkeys infected with *S. mansoni,* the female worms were more sensitive to the drug than males, and that egg-laying was arrested even in those few females which survived the effect of the drug. The heavier dosages used on *S. japonicum* in mice eliminated all male and female worms when administered during susceptible stages of the infection. Preliminary microscopic observations of the surviving worms indicate that the compound

produces morphological changes in the ootype and the vitellogenous cells of the female. These results are in agreement with and support those reported previously by LAMBERT (1964) with *S. mansoni,* except that the prophylactic effect appears to be much greater than he indicated.

Preliminary studies conducted by LAMBERT (1964) on drug absorption revealed that the blood level in man reached a peak 6 hours after administration, and that absorption of the drug was better when it was given in two divided daily doses than when a single daily dose was used. In view of these findings, a comparison was made of the activity of this drug in monkeys treated for 6 days with 100 mg/kg given in two daily doses (Group II, 50 mg/kg b.i.d.) and with 100 mg/kg given in a single daily dose. As indicated in Table 1, no significant difference in results was observed in the two groups.

In both *S. mansoni* and *S. japonicum* infections, the drug was very effective when treatment started at the time of infection or when it was administered after oviposition had begun. Conversely, only little effect was observed when the treatment was given after the early schistosomular development and before the worms had reached sexual maturity in the hepatic circulation. This apparent resistance to the effects of therapy in the immature stages is in agreement with observations conducted with other antimonial and non-antimonial drugs (LÄMMLER, 1964).

Of special interest was the observation that the marked prophylactic and curative activity of CIBA 32644-Ba occurred both against infections with *S. mansoni* in monkeys and *S. japonicum* in mice. This contrasts with results obtained with antimony compounds such as TWSb which was very active against experimental schistosomiasis in monkeys but relatively inactive in mice (BRUCE et al., 1962), or with non-antimonial compounds, such as Hoechst S-688, which was very effective against *S. mansoni* in mice but relatively ineffective in monkeys (LUTTERMOSER et al., 1960).

Summary

Oral regimens of the compound 1-(5-nitro-2-thiazolyl)-2-imidazolidinone were found to have a strong prophylactic and chemotherapeutic effect on experimental infections with *Schistosoma mansoni* in Rhesus monkeys and with *Schistosoma japonicum* in mice. The dosage levels necessary to bring about satisfactory results in these animals were well below toxic levels. Egg suppression and a significant reduction in worm burdens was observed

in the animals in which therapeutic treatment began at the time of exposure or a few days thereafter. No noticeable effect was demonstrated in the animals in which drug administration was initiated 10 or 15 days after exposure. When the drug was given after the worms had reached sexual maturity, a marked therapeutic activity was manifested by the reduction in number, or complete elimination, of worms, by the complete suppression of egg deposition, and by a stunting effect in those few worms which survived the treatment. In *S. mansoni* infections in monkeys, female worms appeared to be more sensitive to the drug than males.

Résumé

Le traitement oral par le 1-(5-nitro-2-thiazolyl)-2-imidazolidone a une importante activité prophylactique et chimio-thérapeutique dans l'infestation expérimentale du Rhésus avec *S. mansoni* et de la souris avec *S. japonicum*. La dose nécessaire à l'obtention de résultats satisfaisants est nettement inférieure à la dose toxique. La suppression de la ponte ovulaire et une réduction significative du nombre de parasites furent observées chez les animaux où le traitement fut appliqué au moment ou quelques jours après l'infestation. Quand le produit est administré après la maturation sexuelle des parasites, une activité thérapeutique marquée fut observée par la réduction du nombre ou l'élimination complète des vers, par la suppression totale de la ponte ovulaire et par un effet inhibant la croissance des quelques parasites qui avaient survécu au traitement. Dans l'infestation des Rhésus par *S. mansoni,* les vers femelles se sont montrés plus sensibles au traitement que les mâles.

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