EXPERIMENTAL CHEMOTHERAPY OF SCHISTOSOMIASIS. III — LABORATORY AND CLINICAL TRIALS WITH TRICHLORPHONE, AN ORGANOPHOSPHORUS COMPOUND *

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Oogram studies have been carried out on mice, hamsters, and Cebus *monkeys experimentally infected with* Schistosoma mansoni *and treated with trichlorphone (0,0-dimethyl 1-hydroxy-2, 2, 2-trichloroethylphosphonate). In mice, despite a slight hepatic shift of schistosomes, all animals presented oogram changes when dosed,* per os, *at the schedules of 200, and 100 mg/kg/ /day × 7. In hamsters, antischistosomal activity could be detected only at toxic levels. In monkeys, trichlorphone showed insignificant action even after oral administration of 30 mg/kg/day for 10 consecutive days.*

In 5 volunteers, a sharp drop in cholinesterase plasma level was observed 24 hours after a single oral dose of 7.5 mg/kg. However, cholinesterase levels returned to the initial values within a period of 11 to 27 days. Trichlorphone was then administered to 12 schistosome patients (7.5 mg/kg/day, every fortnight, × 5). One month after therapy, interruption of egg laying was observed in 6 patients. Late parasitological control showed that all treated patients continued to pass viable S. mansoni *eggs with their stools.*

In 1962, Cerf and collaborators (2) investigated the possibility of using organophosphorus compounds less injurious to mammals for the treatment of human parasitic helminths. It was found that trichlorphone gave encouraging results in the treatment of ancylostomiasis, ascariasis, trichuriasis, creeping eruption, and intestinal schitosomiasis. These findings were subsequently confirmed by Talaat *et al.* (11). More recently, Forsyth & Rashid (4, 5) reported that spaced administration of trichlorphone to patients infected with *Schistosoma haematobium* is highly successful on reducing the levels of urinary egg outputs. However, no antischistosomal activity could be detected by Abdalla *et al.* (1) on mice, gerbils and *Cercopithecus aethiops* experimentally infected with *Schistosoma mansoni*. In view of these conflicting results it was felt worth while to conduct a thorough study on laboratory animals as well as a limited clinical trial with trichlorphone (0,0--dimethyl 1-hydroxy-2, 2, 2-trichloroethylphosphonate, fig. 1) in schistosomiasis mansoni. The results are herein reported.

MATERIALS AND METHODS

Infection of animals

Mice, hamsters and *Cebus* monkeys were infected with *S. mansoni* cercariae (LE strain, maintained in the laboratory) as described elsewhere (9).

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Treatment of infected animals

Seven weeks after exposure to *S. mansoni* cercariae (10 organisms per animal), groups of 12 mice were treated with trichlorphone, *per os*, for 7 consecutive days, at the dose levels of 200, 100, 50 and 25 mg/kg/day. A group of 8 untreated mice was left as control.

Groups of 7 hamsters (*Cricetus auratus*) were treated for 7 consecutive days, *per os*, at the dose levels of 200, 100, and 50 mg/kg/day, 8 weeks after exposure (80 cercariae per animal via the cheek pouch). A group of untreated hamsters was left as control.

Four *Cebus apella macrocephalus* were dosed *per os*, 8 to 11 months after exposure (150 to 200 cercariae per animal), with 15, and 30 mg/kg/day x 10 (2 animals), 30 mg/kg/day x 5 (1 animal), and 30 mg/kg/day every fortnight x 5 (1 animal). One untreated monkey was left as control.

Oogram and distribution of schistosomes

The methods and criteria used for oogram studies and worm distribution within the hepatic portal system have been described elsewhere (9).

Alterations 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 disappearance of immature viable eggs in rectal snips obtained by mucosal curettage (8).

Clinical studies

Cholinesterase plasma levels were determined in 5 male adult volunteers before and at different periods (1, 4, 7, 11, 14, 18, and 27 days) after a single oral dose of trichlorphone (7.5 mg/kg). The method used was that developed by Rappaport *et al.* (Gradwohl (6) and is based on the change in colour of m-nitrophenol.

Twelve adult male patients with active schistosomiasis mansoni (hepato-intestinal form) were treated with trichlorphone (tablets of 50 mg) at the dose level of 7.5 mg/kg/day, every fortnight x 5. Therapeutical activity was assessed by one rectal biopsy performed 1-2 months after completion of treatment and stool examinations 150 days after therapy.

RESULTS

Mice

The data concerning the therapeutical activity of trichlorphone on mice experimentally infected with *S. mansoni* are summarized in Table I. All mice dosed with 200, and 100 mg/kg presented oogram changes. At the dose levels of 50,, and 25 mg/kg, the percentages of animals with altered oogram were 37.5 and 11.1, respectively. Only a slight hepatic shift was observed at the highest dose level employed.

Hamsters

Table II shows the results obtained in hamsters. Oogram changes were observed only in animals dosed at toxic levels (200, and 100 mg/kg/day x 7). No significant hepatic shift occurred with the schedules used. Dead worms were found in the liver of the only surviving animal of the group treated with 200 mg/kg.

Cebus monkeys

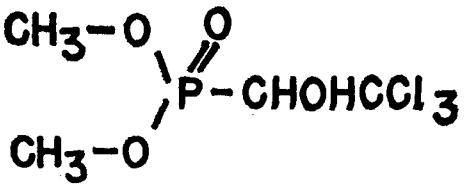
As can be seen from Table III, a slight but transient antischistosomal activity, as judged by oograms provided by mucosal curettages, could be detected in *Cebus* n.º 1, within the period of treatment, and in *Cebus* n.º 3, just after the last dose.

Cholinesterase plasma level

A sharp drop in plasma cholinesterase level occurred in all volunteers one day after trichlorphone administration. The return to pretreatment levels was observed after 11 to 27 days (Fig. 2).

Clinical trials

Only minor and transient side effects such as nausea, vomiting (2 cases), dizziness, and abdominal pain were observed. Rectal biopsies performed 1-2 months after the end of treatment revealed in-



Chemical structure of trichlorphone

terruption of egg laying (absence of viable eggs) in 6 out of 12 patients (Table IV). Late parasitological control showed viable *S. mansoni* eggs in all treated patients (Table IV).

DISCUSSION

It is known that trichlorphone is active against a variety of gastro-intestinal nematodes in sheep and cattle (3, 10) and cholinesterase inhibition is probably involved in its mechanism of action (12).

Our results obtained in mice partly agree with those reported by Abdalla *et al.* (1). In fact, these authors did not disclose any antischistosomal activity in mice infected with *S. mansoni* and treated with trichlorphone at the daily dose level of 100 mg/kg for 3, 4, and 6 consecutive days. The criteria used by Abdalla and coworkers to assess therapeutic activity were a) comparison of the average number of excreted eggs and their viability, b) worm burden, c) separation of sexes, and d) hepatic shift of schistosomes. By using the oogram method, which is very sensitive to detect even slight disturbances on egg-laying (7), we were able to demonstrate an unequivocal action of trichlorphone against *S. mansoni* infections in mice and hamsters. Our poor results in monkeys are in accordance with those reported by Abdalla *et al.* (1) and Thompson (12).

The administration of trichlorphone at the schedule of 5 mg/kg/day x 12 to schistosomose patients was followed by a very marked decrease of plasma and red-cell cholinesterase levels with a slow recovery

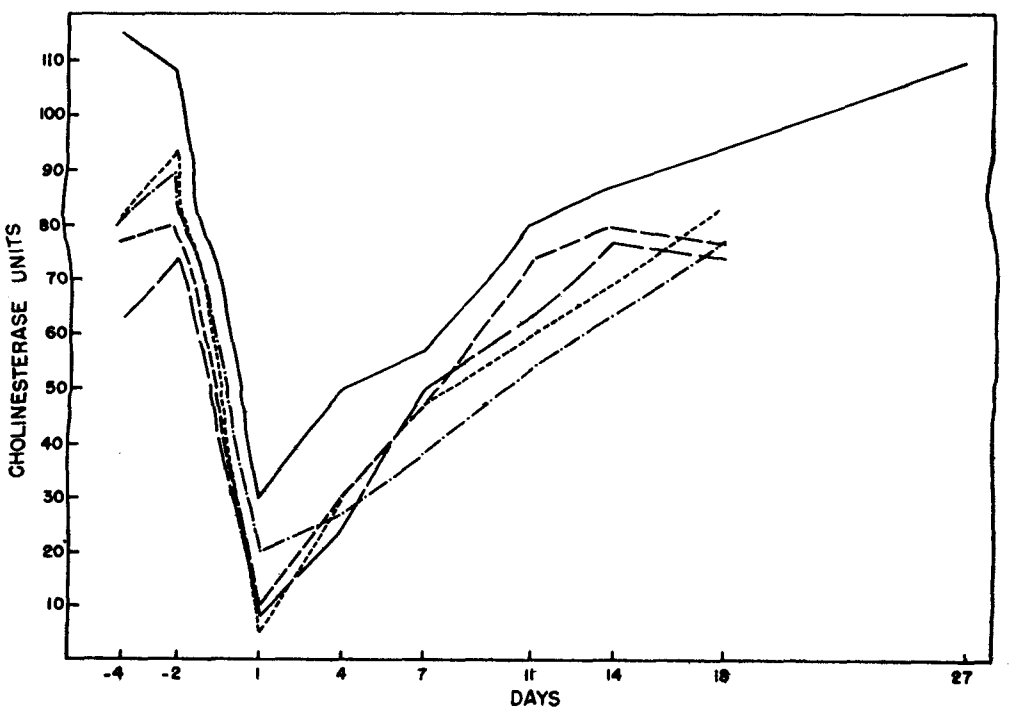


Fig. 2 — *Cholinesterase plasma level in 5 volunteers before and after a dose of thichlorphone (7,5 mg/kg).*

Table I — Antischistosomal activity of Trichlorphone on mice experimentally infected with *S. mansoni*. The animals were sacrificed 3 days after the end of treatment.

Schedule of treatment (mg/kg/day x 7) *per os*	Number of mice	Animals dead	Mean worm burden	Distribution of schistosomes (%)			Percentage of animals with oogram changes
				Liver	Portal vein	Mesenteric vessels	
200	12	3	16.2	35.6	16.4	48.0	100.0
100	12	2	18.4	22.8	15.8	61.4	100.0
50	12	4	28.1	26.2	15.1	58.7	37.5
25	12	3	24.5	19.0	19.4	61.6	11.1
Control	8	0	15.0	13.3	34.2	52.5	0.0

Table II — Antischistosomal activity of Trichlorphone on hamsters experimentally infected with *S. mansoni*. The animals were sacrificed 3 days after the end of treatment.

Schedule of treatment (mg/kg/day x 7) *per os*	Number of mice	Animals dead	Mean worm burden	Distribution of schistosomes (%)			Percentage of animals with oogram changes
				Liver	Portal vein	Mesenteric vessels	
200	7	6	10.0*	10.0	20.0	70.0	100.0
100	7	6	41.0	26.8	12.2	61.0	100.0
50	7	1	25.1	13.2	33.8	53.0	0.0
Control	7	0	37.5	18.2	29.3	52.5	0.0

*Presence of dead worms in the liver

Table III — Antischistosomal activity of Trichlorphone on *Cebus* monkeys experimentally infected with *S. mansoni*.

Oograms from rectal snips obtained by curettage.

Monkey and schedule of treatment	Days before (—) or after (+) the beginning of treatment	Viable eggs. Stages					Dead eggs and shells
		1st	2nd	3rd	4th	Mature	
1	— 5	16	2	3	1	141	10
(Treated 8 months after exposure)	— 3	22	17	16	3	11	24
	+ 2	45	52	63	49	86	48
	+ 8	0	0	0	0	26	8
15 mg/kg/day x 10	+ 11	5	5	5	0	11	11
	+ 16	12	54	18	60	76	31
	+ 36	14	4	52	34	119	48
	+ 160	4	10	19	15	72	18
2	— 19	8	31	95	163	94	32
(Treated 9 months after exposure)	— 6	10	15	33	48	109	11
	0	8	3	17	17	19	11
	+ 9	0	44	47	0	98	84
30 mg/kg/day x 5	+ 17	7	0	3	12	101	42
	+ 25	35	30	3	14	142	52
	+ 145	32	10	34	27	51	7
3	— 30	17	16	14	18	47	20
(Treated 8 months after exposure)	— 18	7	2	0	5	38	15
	+ 3	51	36	3	5	20	29
	+ 9	5	0	103	0	21	4
30 mg/kg/day, every fortnight, x x 5	+ 25	2	4	52	0	2	6
	+ 56	1	55	7	4	72	21
	+ 64	0	0	68	30	45	13
	+ 77	0	0	0	0	23	4
	+ 104	10	14	54	50	77	24
	+ 158	2	0	11	22	8	13
4	— 63	36	39	58	43	83	44
(Treated 11 months after exposure)	— 1	40	4	38	6	105	26
	+ 13	20	29	26	0	57	22
	+ 70	36	15	68	9	260	50
30 mg/kg/day x 10	+ 82	35	45	73	27	209	34
5	+ 82*	4	10	36	4	80	17
Control	+ 88	29	9	7	5	17	10
	+ 108	14	17	46	20	100	44
	+ 258	2	4	41	5	70	33

* Days after exposure to *S. mansoni* cercariae

Table IV — Results of the treatment of 12 adult patients suffering from schistosomiasis mansoni with Trichlorphorme

Schedule: 7.5 mg/kg, every fortnight x 5 (Total dose = 37.5 mg/kg).

Patients	Days before (—) or after (+) the completion of treatment	Rectal biopsy Viable eggs/ Dead eggs	Stool examination for viable *S. mansoni* eggs
1	— 80 + 32 + 150	300/93 0/56	Positive
2	— 84 + 48 + 150	58/34 0/0	Positive
3	— 90 + 43 + 90	55/26 82/12	Positive
4	— 81 + 34 + 150	169/106 0/26	Positive
5	— 83 + 50 + 150	264/0 106/0	Positive
6	— 82 + 55 + 150	148/61 127/0	Positive
7	— 80 + 60	131/45 150/0	Not done
8	— 80 + 57	137/50 139/0	Not done
9	— 77 + 41 + 150	107/171 107/60	Positive
10	— 77 + 38 + 150	170/41 0/0	Positive
11	— 77 + 41 + 150	158/126 0/239	Positive
12	— 71 + 35 + 150	113/112 0/0	Positive

which did not reach its normal values even at 45 days after the end of treatment (1). Since in the present study, confirming early reports by Forsyth and Rashid (4, 5), the recovery of cholinesterase plasma level was observed to occur about a fortnight after a single oral dose of 7.5 mg/kg of trichlorphone, it was decided to treat schistosome patients with a 2-week interval between consecutive doses. Although side effects were of minor clinical significance, only a temporary interruption of egg laying could be detected. All treated patients continued to pass viable eggs after the completion of treatment.

According to Forsyth and Rashid (5), the conflicting reports from workers who have used trichlorphone for treating schistosomiasis are in part expained by the inclusion of both *S. mansoni* and *S. haematobium* patients in their series. When trichlorphone was given by mouth in single spaced doses of 7.5 mg/kg to 76 patients infected with *S. haematobium*, all ceased voiding eggs of the parasite in the urine (5). However, as far as *S. mansoni* infections are concerned, the poor therapeutical activity of trichlorphone and the well known toxic properties of higher dosages of this organophosphorus compound for man are very discouraging factors to support the continuation of further clinical trials.

R E S U M O

Ensaios pré-clinicos foram realizados em camundongos, hamsters e macacos Cebus *experimentalmente infectados com* Schistosoma mansoni *e tratados com trichlorphone (0-0-dimetil 1-hidroxi-2, 2, 2-tricloroetilfosfonato). Em camundongos, apesar de ter sido discreto o deslocamento dos vermes para o fígado, em todos os animais houve alterações do oograma, quando foram administrados 200 e 100 mg/kg/dia x 7,* per os. *Em hamsters, a atividade sôbre os esquistossomos, só pôde ser detectada com doses tóxicas. Em macacos, o trichlorphone mostrou ação insignificante, mesmo após administração oral de 30 mg/kg/dia durante 10 dias consecutivos.*

Em 5 voluntários, uma acentudada baixa da colinesterase plasmática, foi observada 24 horas após uma única dose oral de 7,5 mg/kg. Todavia, o nível da colinesterase voltou aos valôres iniciais no período de 11 a 27 dias. O trichlorphone foi administrado a 12 pacientes com esquistossomose mansoni (7,5 mg/kg/dia, quinzenalmente, x 5). Um mês após o fim do tratamento, a interrupção da postura dos vermes foi observada em 6 pacientes. O contrôle parasitológico tardio revelou a ineficácia do tratamento em todos os pacientes.

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