

BODY OF REPORT

SEATO Medic Study No. 29 Studies on Opisthorchis viverrini in Thailand -
Chemotherapy of Liver Fluke

Project No. 3A 025601 A 811 Military Medical Research Program
S. E. Asia

Task 01: Military Medical Research Program
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Subtask 01: Military Medical Research Program
SEASIA (Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory
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 Division of Medical Research Laboratories

 Department of Medical Zoology

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Objectives: To test chemotherapeutic agents for efficacy against O. viverrini infections in man.

Description: Two drugs have been evaluated sufficiently to present a crude analysis of the data. These are Dithiazanine iodide and Propoquine.

Progress: PROPOQUINE. (CI 135, Parke Davis Co.). During the period of 20

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Table 1
HISTORICAL DATA FOR 42 PATIENTS

Medication Received	Number of patients	CI-356, Protocol 356-3					
		Age		Height in Cm.		Weight	
		Median	Range	Median	Range	Median	Range
450 mg/day	6	34	24-50	173.0	159-180	135.0	132-154
300 mg/day	28	29	20-49	160.0	140-175	112.5	95-136
Placebo	8	28	20-43	161.5	157-174	125.0	110-150
Total	42	30	20-50	161.0	140-180	120.0	95-154

October 1964 to 13 January 1965, 42 male inmates of the Udorn Prison took part in the testing of CI-135 against *O. viverrini*. Those weighing greater than 60 kg were given 450 mg/day, while those weighing from 40 - 59 kg were given 300 mg/day. In addition there was a placebo control group with patients in the same weight range described for the treatment group. Treatment was continued for 30 days.

Subjects had one pretreatment observation, and they were also observed on days 7, 14, 21, 28, 35, 42 and some subjects had a final observation ranging from day 57 to 84. At each period observations were made on the weight, blood pressure, hepatomegaly, splenomegaly, heart, lungs, mean ova count (eggs per gram stool), hemoglobin, hematocrit, total WBC, differential, bilirubin, cholesterol, esters, total protein, A/G ratio, thymol turbidity, SGOT, SGPT, CCF, zinc turbidity, and alkaline phosphatase. In all, 6 patients received 450 mg/day. The age of this group was 24-50 (mean 34). The height was 159-180 cm (mean 173), and the weight was 132-154 lbs (mean 135). A total of 28 persons received 300 mg/day. Of these, the mean age was 29 (range 20 - 49), the mean height was 160 cm (range 140 - 175) and the mean weight was 112 lbs (range 95 - 136). Eight persons were given the placebo. The average age, height and weight was 28 years, 161 cm and 125 lbs (Table I).

Concerning arterial blood pressure, among the 6 persons receiving 450 mg/day the median pressures before treatment and at 7, 14, 21, 28 and 42 days after

treatment were, respectively, 91, 94, 94, 97, 84 and 84. In the group of 28 persons receiving 300 mg/day, the pressures at these same intervals were 94, 92, 94, 94, 94 and 89. Of the 7 persons receiving a placebo the respective pressures were 90, 86, 94, 94, 97 and 84. It is evident that no untoward arterial blood pressure changes were manifested by use of this drug (Table 2).

The percent of ova reduction is presented in Table 3. Among the 6 persons receiving 450 mg/day, 1 experienced no reduction in egg count as measured by the Stoll technique. One each experienced reductions in the 41-60, and the 61-80% groups, while two of the 6 had egg count reductions of between 81 and 92%. Of the 28 persons receiving 300 mg/day, 6 experienced no egg count reduction, 3 had from 1 to 20% reduction, 6 had from 21 to 40%, 3 had from 41 to 60%, 8 had from 61 to 80 and 2 had from 81 to 95% egg reduction. For the placebo group, one showed no reduction, 1 was between 1 and 20%, 4 were 41 to 60% and 1 each were in the 61-80 and 81-100% reduction groups.

The biochemistry test results are tabulated in Table 4. In some cases the pre-treatment level was used as the "normal" because these data have not been determined for the population under test. Table 5 presents the greatest changes for certain tests, both increases and decreases. In Table 6 are data on subjects who had various tests outside the expected range. In the 450 mg/day group was one high WBC, 1 high basophile, one high bilirubin both direct and 30 minutes. In the 300 mg/day group was a single low hemoglobin, two high hematocrits, a low WBC, four high WBC, 2 low polys, one high poly, 9 high basophiles, three high bilirubins (all 30 minutes) and 2 high cholesterols. In the placebo group was a single high poly, a high lymphocyte, a high monocyte and 2 high basophiles. It should be noted that the high bilirubins all occurred on day 7 of the study, probably indicating an error in procedure or calculation. Likewise, 4 of high basophiles occurred on day 21 and 5 more were on day 42. A large number of high alkaline phosphatase were recorded (Table 6 continued), but most of these occurred on day 28.

Conclusion: Further statistical analyses are in progress to more fully determine the effectiveness of Propoquine against *O. viverrini* under the experimental conditions of this study. It appears on the basis of the data presented here that the agent is effective to a limited degree and that it is nontoxic within the time/dosage limits of the study.

Table 2

MEAN ARTERIAL BLOOD PRESSURE FOR EACH OBSERVATION BY TREATMENT GROUP

Medication Received	Number of patients	CI-356, Protocol 356-3											
		Pre-treatment		Day 7		Day 14a		Day 21		Day 28		Day 42	
		Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
450 mg/day	6	91.0	84-100	94	80-94	94	87-116	97	84-113	84	84-94	84.0	70-89
300 mg/day	28	93.5	70-116	92	67-116	94	81-113	94	84-110	94	80-103	88.5	70-97b
Placebo	7c	90.0	84-103	86	73-90	94	90-103	94	87-110	97	84-103	84.0	63-97
Total	42	90.0	70-116	90	67-116	94	81-116	94	84-113	94	80-103	84.0	63-97

- a. Patient 33 in the 300 mg/day group was discharged from prison before Day 14.
 b. Six patients did not have blood pressure specified.
 c. Patient 41 - systolic blood pressure not specified.

Table 3

PERCENT OVA REDUCTION FOR 42 PATIENTS

Medication Received	Number of patients	CI-356, Protocol 356-3							Range
		Percent Reduction							
		0	1-20	21-40	41-60	61-80	81-100		
450 mg/day	6 a	1	0	0	1	1	2	0-92	
300 mg/day	28	6	3	6	3	8	2	0-95	
Placebo	8	1	1	0	4	1	1	0-84	
Total	42	8	4	6	8	10	5	0-95	

- a. One patient discharged from prison.

Table 4

RANGES OF LABORATORY DETERMINATIONS FOR 42 PATIENTS

CI-356, Protocol 356-3				
Determinations	Pre-treatment Range	Normal Range	Expected Range	Post-treatment Range
Hemoglobin	11.9-18.7	9-16	9.0-18.7	8.2-17.4
Hematocrit	35-56	25-50	25-56	31-58
WBC	3,850-14,600	4,500-10,000	3,850-14,600	3,700-21,050
Differential (%)	Polys	30-74	50-70	24-76
	Lymphs	13-57	20-40	17-62
	Monos	0-2	0-4	0-4
	Eos	0-27	1-40	0-40
	Bas	0-1	0-1	0-1
Bilirubin 1'	0.08-2.44	0-2	0-2.44	.08-3.70
Bilirubin 30'	0.16-2.80	0-2	0-2.80	.16-5.20
Cholesterol	37-303	120-250	37-303	80-365
Esters	12.6-100.0	77.5*	12.6-100.0	26.1-98.6
Total protein	5.9-9.5	7.6*	5.9-9.5	5.9-9.2
A/G Ratio	1.0:1-5.3:1	1.8:1*	1.0:1-5.3:1	1.0:1-4.5:1
Thymol Turbidity	1.2-11.8	0-5	0-11.8	1.0-10.4
Albumin	0-1	0	0-1	0-1
Sugar	0	0	0	0-2
Ketones	0	0	0	0-Trace
Bile	0-2	0	0-2	0-1
WBC/hpf	0-4	0-10	0-10	0-15 a
RBC/hpf	0-2	0-2	0-2	0-15
Casts/ 100	Granular	0	0	0
	WBC	0	0	0
	RBC	0	0	0
Ceph. Flocc.	0-4	.5*	0-4	0-4
Zn. T.	6-33	22*	6-33	5-36
Iodine 2	0-4	.20*	0-4	0-3
Blood Albumin	2.45-7.75	4.52*	2.45-7.75	2.98-6.00
Blood Globulin	0-5.63	3.12*	0-5.63	1.18-4.55
Alkaline Phosphotase	0.4-2.3	.93*	0.4-2.3	0.6-6.2
SGOT	13-215	0-50	0-215	3-152
SGPT	10-125	0-50	0-125	0-125

* These values represent the pre-treatment mean for these patients since the normal value was not known.

a. Patients 32 and 6 reported many.

b. Patients 18 and 21 reported few.

Table 5

LISTING OF PATIENTS WITH LARGEST CHANGES FOR CERTAIN LABORATORY DETERMINATIONS

CI-356, Protocol 356-3										
Determination	Largest Increase					Largest Decrease				
	Subject Number	Treatment Received	Pre-trial Value	During Trial Value	Study Day	Subject Number	Treatment Received	Pre-trial Value	During Trial Value	Study Day
Hemoglobin	25	300mg/day	12.6	16.8	80	11	300mg/day	13.0	8.2	7
Hematocrit	8	300mg/day	45	58	14	32	450mg/day	56	33	21
WBC/hpf	6	300mg/day	11,250	21,050	7	4	300mg/day	14,600	4,850	14
SGOT	7	300mg/day	13	152	28	15	300mg/day	215	30	7
SGPT	15	300mg/day	48	125	14	37	Placebo	120	11	14

Table 6

LISTING OF SUBJECTS WHO HAD VARIOUS LABORATORY DETERMINATIONS OUTSIDE THE EXPECTED RANGE ^a

Treatment Received	Subject Number	Hemo-globin		Hema-tocrit		WBC		Polys		Lymphs	Monos	Bas	Bilirubin 1'	Bilirubin 30'	Cholesterol
		Low	High	Low	High	Low	High	Low	High	High	High	High	High	High	High
450mg/day	30														
	33					20,450(28)						2 (84)			
	34					20,450(28)							3.70(7)	4.80(7)	
	1														
	3														
	4							28(42)							3.00(7)
	5											2 (7,21)			
	6											3 (42)			
	7														
	8														
	10														
300mg/day	11	8.2(7)												5.20(7)	365(83)
	12													3.00(7)	
	16								76(28)						
	18											2(21)			
	19														
	21														
	23														
	26														
	28														
	35														
	36														
Placebo	39														
	41									27(7)	62(7)				
	42														

a. Study day is in parentheses. If patient had more than one value outside the expected range, the most discrepant value is given.

Table 6 (Contd)

LISTING OF SUBJECTS WHO HAD VARIOUS LABORATORY DETERMINATIONS OUTSIDE THE EXPECTED RANGE ^a

Treatment Received	Subject Number	Sugar	Ketones	WBC/hpf	RBC/hpf	Granular	Zinc turbidity	Alkaline Phos.
		High	High	High	High	High	Low High	High
450mg/day	30							
	31							2.8(14)
	32							4.0(28)
	34	Trace(28)		Many(35)				2.7(84)
	1	1(21)						2.7(28)
	2	1(28)					Trace(28)	
	3	1(42)						
	4	1(35)						
	5	1(28)						
	6			Many(35)	3(28)			3.3(28)
	7							3.3(28)
300mg/day	8							5.6(83)
	9						Trace(28)	2.5(28)
	10	1(83)						3.5(21)
	11	1(21,28,35)		15(7)				3.7(28)
	12							3.1(42)
	13	1(21)	Trace(21)					2.8(21,28)
	14							2.8(28)
	15	1(7)						3.1(28)
	16							
	17	1(35)						3.7(28)
	18						1(28)	3.0(21)
Placebo	19							2.6(14)
	20	1(21)						
	21	1(80)						
	22							
	23							
	25							
	26	1(80)						3.5(80)
	27	2(84)						3.2(14)
	28	1(21)		15(7)	15(7)			2.7(84)
	29	1(84)						5.3(80)
	35	1(28,35)						3.3(42)
37	2(42)						6.2(28)	
38	1(21,35)						2.6(14)	
39	1(21,35,84)							
40								
41								

a. Study day is in parentheses. If patient had more than one value outside the expected range, the most discrepant value is given.

Table 1

ANALYSIS OF DITHIAZANINE IODIDE AGAINST *OPISTHORCHIS VIVERRINI*
IN HUMANS
GROUP A (30 mg/Kg Bw/da x 5 da)

Pre Rx Mean' EPGF	Post Rx 1-28 da*	% Reduct- ion	Post Rx 29-56 da**	% Reduct- ion	Post Rx 57-84 da**	% Reduct- ion	Post Rx 85-112 da**	% Reduct- ion
336	40 (5)	88	100	70	Neg.	100	-	-
6650	240 (5)	96	-	-	1200	82	5200	22
175	60 (5)	66	-	-	Neg.	100	100	43
2125	3733 (3)	0	-	-	-	-	-	-
1166	500 (3)	57	-	-	-	-	-	-
3300	4500 (4)	0	1700	48	400	9	-	-
10250	3204 (5)	69	-	-	-	-	-	-
4866	4000 (4)	18	3300	32	-	-	1100	77
1866	1766 (4)	5	-	-	-	-	-	-
3166	2400 (3)	24	3700	0	-	-	-	-
42600	2625 (4)	94	21200	50	-	-	-	-
6600	4925 (4)	25	11400	0	4200	36	5600	15
1866	24575 (4)	0	2500	0	400	79	400	79
25	20 (4)	20	Neg	100	100	0	300	0
650	650 (4)	0	700	0	-	-	3900	0
366	3900 (4)	0	800	0	600	0	300	18
6400	3400 (4)	47	-	-	-	-	-	-
1150	1025 (4)	11	400	65	600	48	300	74
1400	4666 (3)	0	Neg	100	-	-	Neg.	100
8825	4025 (4)	54	5500	37	9900	0	1400	84
800	Neg (2)	100	-	-	-	-	-	-
3900	9066 (3)	0	7800	0	13300	0	6600	0
3333	2675 (4)	20	900	73	5100	0	200	9
500	300 (4)	40	1800	0	200	60	400	20

* No. in () is total number specimens examined, 2 counts per specimen.

** One specimen (2 counts) on each.

Progress: Dithiazanine Iodide. Treatment was divided into 3 groups. A total of 24 persons received 30 mg/Kg body weight over a period of five days (Group A). The same dosage, over a period of 10 days was given to 19 additional persons (Group B), while the same dosage for 15 days was given to 28 patients (Group C). Pre-treatment egg counts (based on at least two Stoll egg counts made from 4 to 1 day pre-treatment), ranged from 25 to 42600 eggs per gram feces (EPGF) for Group A; from 233 to 43300 for Group B, and from 1025 to 30300 EPGF for Group C. Counts during the first 28 days after treatment were made on from 2 to 10 separate stool specimens with two separate Stoll counts made on each specimen. During the remaining post treatment examination periods a single specimen was examined by making 2 Stoll counts on it. The total number of specimens examined during the 1-28 day post treatment period is shown on Tables 1, 2 and 3. For Group A, 18 of the 24 persons had 4 or 5 stool specimens (8 or 10 counts). The count ranged from 20 to 24575 EPGF. The percent egg reduction over the pre-treatment period ranged from 0 (7 cases) to 100% (1 case). From 29-56 days after treatment, of 16 examinees, two were negative and the maximum count was 21200. The percent reduction over the pre-treatment period ranged from 0 (7 cases) to 100% (2 cases). During the 57-84 post treatment period, of 13 examinees, the counts ranged from 0 to 13300 EPGF with egg reduction percentages of from 0 (5 cases) to 100% (2 cases). During the maximum period of post treatment observation (85-112 days) the counts of 14 examinees ranged from 0 to 6600 with egg reduction percentages over the pre-treatment group of from 0 (3 cases) to 100 (1 case). For Group B (30 mg/Kg BW/day x 10 days), the pre-

Table II
ANALYSIS OF DITHIAZANINE IODIDE AGAINST OPISTHORCHIS VIVERRINI
IN HUMANS

GROUP B (30 mg/Kg Bw/da x 10 da)

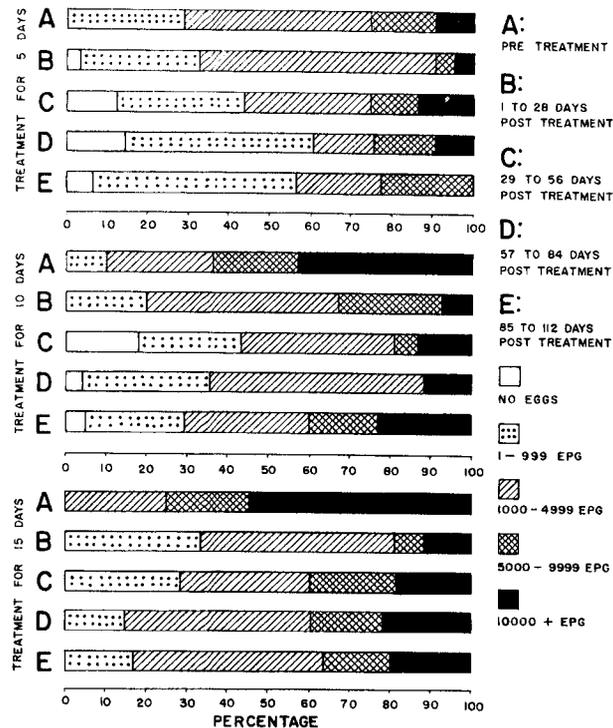
Pre Rx Mean EPGF	Post Rx 1-28 da *	% Reduct-ion	Post Rx 29-56 da**	% Reduct-ion	Post Rx 57-84 da**	% Reduct-ion	Post Rx 85-112 da**	% Reduct-ion
21950	1066 (5)	95	3000	86	1700	92	4700	78
4600	940 (5)	80	12100	0	1400	70	6900	0
10375	5583 (6)	46	1400	86	12000	0	13700	0
4400	1380 (5)	69	600	86	800	82	500	89
1925	140 (5)	93	Neg.	100	300	84	300	84
8400	2440 (10)	71	3200	62	1700	80	3000	64
35730	8844 (9)	75	3700	89	3400	90	8300	76
22250	8720 (5)	61	5000	78	-	-	-	-
43300	1980 (5)	95	700	98	2300	95	-	-
2250	12 (8)	99	Neg.	100	Neg.	100	Neg.	100
1466	2160 (5)	0	-	-	1800	0	1300	11
9100	1840 (5)	80	-	-	3700	60	9100	0
10766	17033 (3)	0	-	-	-	-	13800	0
7700	2800 (5)	64	3600	53	1200	84	2200	71
16866	7860 (5)	53	3200	81	3100	82	2500	85
533	540 (5)	0	100	81	100	81	200	62
233	1016 (5)	0	Neg.	100	400	0	100	57
7766	2300 (4)	70	12100	0	100	98	12800	0
29070	5240 (5)	82	300	99	58800	0	14400	50

Table III
ANALYSIS OF DITHIAZANINE IODIDE AGAINST OPISTHORCHIS VIVERRINI
IN HUMANS

GROUP C (30 mg/Kg Bw/da x 15 da)

Pre Rx Mean EPGF	Post Rx 1-28 da *	% Reduct-ion	Post Rx 29-56 da **	% Reduct-ion	Post Rx 57-84 da **	% Reduct-ion	Post Rx 85-112 da **	% Reduct-ion
19375	340 (5)	98	400	98	4300	78	3300	83
5100	60 (5)	98	300	94	400	92	2000	61
3300	3675 (5)	0	8000	0	14900	0	1900	42
14050	220 (6)	98	700	95	1600	89	500	96
9250	2060 (5)	78	300	97	700	92	200	98
13875	1660 (5)	88	3100	78	1500	89	7800	44
14000	2120 (5)	84	2400	83	4900	65	4000	71
14525	3740 (5)	74	2600	82	4100	72	2100	86
5250	540 (5)	9	7800	0	600	89	-	-
2530	8140 (5)	0	13000	0	-	-	-	-
2675	2860 (5)	0	3300	0	-	-	-	-
15466	160 (5)	99	32000	0	2100	86	1400	91
11176	80 (5)	99	3100	72	1400	87	2800	75
24133	6260 (5)	74	9800	59	4100	83	12700	47
12450	3520 (5)	71	1500	88	9700	22	62400	0
13500	3860 (5)	71	45500	0	10100	25	6200	54
1025	200 (5)	80	100	90	1200	0	1800	0
4525	2180 (5)	48	6900	0	2100	54	2100	54
2500	780 (5)	68	800	68	700	72	800	68
8000	1460 (5)	82	1000	87	5500	31	4000	50
10750	622 (9)	94	1300	88	1000	91	800	93
2536	2464 (9)	3	1800	29	3300	0	17100	0
8325	3288 (9)	60	200	98	5100	39	9400	0
16366	11512 (9)	30	800	95	11600	29	-	-
30300	12840 (9)	58	18100	40	7200	76	7500	75
5470	1855 (9)	66	9600	0	12000	0	21100	0
62150	1683 (9)	97	22300	71	14300	76	21900	65
10400	10975 (9)	0	7100	32	6100	31	2400	77

FIGURE 1
EFFECT OF TREATMENT WITH DITHIAZANINE IODIDE ON
INTENSITY OF INFECTION WITH
OPISTHORCHIS VIVERRINI



treatment counts ranged from 233 to 43300 EPGF. The first period of post treatment study (1-28 days) revealed that of 19 examinees the lowest count average was 12 EPGF while the highest was 17033. The number of counts made on each patient ranged from 6 to 20. The percentage egg reduction over the pre-treatment period ranged from 0 (4 cases) to 99%. During the 29-56 day post treatment examination period the counts ranged from 0 (3 cases) to 12100 EPGF, representing percentage decreases over the pre-treatment period of from 0 (2 cases) to 100% (3 cases). During the 85 to 112 day post treatment period, the counts ranged from 100 to 14400 representing percentage of egg production reduction of from 0 (5 cases) to 100% (1 case). For Group C, the pre-treatment counts ranged from 1025 to 62150 EPGF. During the 1-28 day post treatment period the counts ranged from 60 to 12840 EPGF representing egg reductions of from 0 (4 cases) to 99% (2 cases). At the 29-56 day post treatment period the counts were from 100 to 32000 EPGF (0 to 98% reduction over pre-treatment levels). From 57 to 84 days after treatment, counts were from 400 to 14900 EPGF (reduction percentages 0 (3 cases) to 92%.) During the final period of observation (84 to 112 days post treatment) counts were from 200 to 62,400 EPGF (percentage reduction of from 0 (5 cases) to 98%). The data are summarized in graphic form in Figure 1.

Conclusions: Dithiazanine iodide in the quantity given over the period of observation is effective in reducing the number of eggs produced by *O. viverrini*. While it must be borne in mind that a reduction in egg count does not necessarily

indicate a comparable reduction in worm burden, the counts made during the 85-112 post treatment days period indicates that in all cases the egg count was considerably lower than at the pre-treatment levels.

Table IV

ANALYSIS OF DITHIAZANINE IODIDE AGAINST OPISTHORCHIS VIVERRINI
IN HUMANS

PERCENT OVA REDUCTION AT GRADED PERIODS FOLLOWING TREATMENT

Medication received	No. days after termination of treatment	No. persons	Percent reduction					
			0	1-20	21-40	41-60	61-80	81-100
30 mg/Kg/da for 5 da	1-28*	24	7	5	3	3	2	4
	29-56**	16	7	0	2	2	3	2
	57-84	13	5	1	1	2	1	3
	85-112	14	3	4	1	1	3	2
30 mg/Kg/da for 10 da	1-28*	19	4	0	0	2	8	5
	29-56**	16	2	0	0	1	2	11
	57-84	17	4	0	0	1	2	10
	85-112	17	5	1	0	2	5	4
30 mg/Kg/da for 15 da	1-28*	28	4	2	1	3	8	10
	29-56**	28	8	0	3	1	4	12
	57-84	26	4	0	6	1	6	9
	85-112	24	5	0	0	6	7	6

* From 3 to 10 separate specimens, 2 counts on each specimen.

** This and subsequent groups, one specimen, two counts.