

Chemotherapy of Gnathostomiasis

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OBJECTIVE : To continue to search for chemicals with chemotherapeutic activity against advanced third-stage larvae of *Gnathostoma spinigerum*.

BACKGROUND : These studies are a continuation of the work reported in previous years. Many antihelminthic drugs have been evaluated for possible chemotherapeutic activity against experimental *G. spinigerum* infection of white mice with advanced third-stage larvae or migrating stages of the worm. All drugs tested have been ineffective so far.

METHODS : Mice of the ICR strain were infected by oral administration each with five advanced third-stage larvae of *G. spinigerum*. After the infection was allowed to progress for some days, the test drug was administered parenterally in a predetermined regimen. Infected control mice were given the infection but no drug. After completion of the treatment regimen, the mice were sacrificed and necropsied. Parasites were counted in the liver, in other visceral organs, and in body muscles, and the results recorded.

Similarly, adult domestic cats, after being proved negative with natural *Gnathostoma* infection by monthly examination of stool for the *Gnathostoma* eggs by Formalin Ether Sedimentation technique (Ritchie) were infected each with 50-60 *G. spinigerum* advanced third-stage larvae (obtained from the experimentally infected mice) through skin penetration and oral infection. After the infection was allowed to proceed for 45-50 days, the test drug was administered parenterally according to the predetermined regimen. Infected control cats were given the infection but no drug.

The drug tested during this reporting period was Ancylool disophenol (2, 6-diiodo-4-nitrophenol) parenteral 4.5% (American Cyanamid Company, Princeton N.J.)

RESULTS : The drug screening test on white mice infected with *G. spinigerum* advanced third-stage larvae gave the following results :

1. The drug was administered subcutaneously five doses to each mouse, one dose per week, with the first dose of 0.1 ml/lb. body weight followed by 0.01 ml/lb. for 4 successive doses to Group A of 25 treated mice, Group B of 20 mice also received five doses each with the first and second doses had 0.1 ml/lb. body weight each, but other three successive doses had 0.01 ml/lb. body weight. These regimens were found to be ineffective in significantly reducing the numbers of the larvae in treated mice comparing with the control (Table 1).

2. The drug was administered subcutaneously eight doses to each mouse, one dose/week, with each of the first three doses of 0.1 ml/lb. body weight and

followed by each of five successive doses of 0.02 ml/lb. body weight to 19 white mice (Group A). A similar regimen was administered to another group of 20 white mice (Group B) after a shorter duration of the infection with the larvae than those of Group A mice. The numbers of larvae in treated mice of Group A were reduced compared with the control, but treated mice of Group B showed no significant reduction of the larvae compared with the control (Table 2).

Multiple subcutaneous administration of Ancylosol disphenol at the dosage of 0.1 ml/lb. body weight for 5-6 doses at weekly interval has been giving good therapeutic effects on some cats and dogs experimentally infected with migrating stages of *G. spinigerum*, but some treated animals have shown signs of drug toxicity (1, 2). The study initiated during this reporting period was to determine the multiple minimum effective doses of the drug for treating the migrating stages of the worm in cats without causing any toxicity.

With another treatment regimen, each of the eight negative cats was infected by skin penetration and oral feeding with 50-60 *G. spinigerum* advanced third-stage larvae, two of these cats were designated as control. The six infected cats were treated in group of two by multiple subcutaneous injections of Ancylosol at 10-day intervals for 12 doses with the dosages of 0.05 ml, 0.03 ml, and 0.02 ml per lb. body weight.

The results showed no worm in the organs of two cats treated with 0.05 ml parenteral Ancylosol on necropsy 20 and 23 days after the last dose. Of two cats treated with the 0.02 ml dose, one had 10 living worms and 1 dead larva, and the other had 6 living larvae in the organs on necropsy 19 and 24 days after the last dose. The two control cats each had infection with 8 worms in the tissue. Thus 12 doses of 0.05 ml/lb. body weight of parenteral Ancylosol given to cats at 10-day intervals is very effective chemotherapy in eliminating all migrating stages of *Gnathostoma spinigerum* in cats.

However, of the two cats treated with 12 doses of 0.03 ml/lb. body weight, one showed no worm and the other had only one living larva in muscles of the leg (Table 3). It now remains to determine further that 0.04 ml/lb. body weight of the chemical given in total of 12 doses to each cat would be the minimum effective chemotherapeutic treatment for *G. spinigerum* migrating stages infection in cats without causing toxicity.

All six cats treated with parenteral Ancylosol in this experiment showed no signs of toxicity due to the drug on necropsy.

REFERENCES

1. Daengsvang, S., Sermswatsri, B., Youngyi, P., Guname, D., Sirichakwal, P., Yingyourd, P., Machimasatha, R. : The SEATO Annual Progress Report, April 1969 to March 1970, p. 157.
2. Daengsvang, S., Chularerk, U., Sirichakwal, P., Yingyourd, P., Machimasatha, R. : The SEATO Annual Progress Report, April 1970 to March 1971, p. 150.

Table 1. Treatment of *Gnathostoma spinigerum* infected mice with Ancylo1 disophenol (2, 6-diiodo-4-nitrophenol) subcutaneous injection.

<u>Drug dose per week</u>		No. of mice treated	Mice positive with larvae	Third-stage larvae found no. (%)	Duration of infection of white mice before treatment (days)	Time of necropsy after treatment (days)	Remarks
0.1 ml/lb. body weight	0.01 ml/lb. body weight						
<u>Group A</u>							
1	4	25	25	48 (38.4)	16	11-13	
	Control	10	10	20 (40.0)	18	18	
<u>Group B</u>							
2	3	20	20	42 (42.0)	61	1-13	
	Control	10	10	41 (42.0)	61	0-15	

Table 2. Treatment of *Gnathostoma spinigerum* infected mice with Ancylool disophenol (2, 6-diiodo-4-nitrophenol) subcutaneous injection.

<u>Drug dose per week</u>		No. of mice treated	Mice positive with larvae	Third-stage larvae found no. (%)	Duration of infection of white mice before treatment (days)	Time of necropsy after treatment (days)	Remarks
0.1 ml/lb. body weight	0.02 ml/lb. body weight						
<u>Group A</u>							
3	5	19	18	28(29.4)	48-150	11-35	
	Control	15	15	31(41.3)	164-170	0-36	
<u>Group B</u>							
3	5	20	20	42(42.0)	15-58	12-27	
	Control	15	15	35(46.6)	7-15	0-27	

Table 3 Chemotherapy of *Gnathostoma spinigerum* larval and immature stages infection in 6 adult cats each treated with 12 subcutaneous doses of Ancylosol disophanol (2, 6-diido-4-nitrophenol) on the basis of 0.02 to 0.05 ml. per pound body weight at 10-day intervals.

Cat no.	Nos third-stage larvae given to cats (infection method)	Age of the worm in days in cats before treatment	Dose of Ancylosol in ml. per lb body weight	Autopsy findings after completion of treatment		Remarks		
				Nos/stage of living worms	Organ found infected	Days of Sacrifice after last dose	Days of sacrifice after infection	Others
149	50(46 skin, 4 feeding)	45	0.05	0	0	20	176	
150	50(47 skin, 3 feeding)	45	0.05	0	0	23	179	
151	60(50 skin, 10 feeding)	46	0.03	0	0	24	181	
152	52(35 skin, 17 feeding)	48	0.03	1 larva	Muscles of hind leg	22	181	
153	55(35 skin, 20 feeding)	48	0.02	9 larvae	Muscles of abdominal wall, front and hind legs, chest	19	177	1 dead larva in muscles of chest
154	56(45 skin, 11 feeding)	50	0.02	1 immature 6 larvae	Diaphragm Muscles of chest, back and hind legs	24	184	
155 (Control)	50(40 skin, 10 feeding)			4 larvae	Muscles of front & hind legs, chest, liver		191	
156 (Control)	50(40 skin, 10 feeding)			4 immature (3♂, 1♀)	Muscles of chest, back & hind legs		190	
				4 larvae	Muscles of front & hind legs, abdominal wall			
				4 immature (3♂, 1♀)	Muscles of chest, back & diaphragm, stomach wall			
				7				