# Chemotherapy of Gnathostomiasis

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OBJECTIVE : To continue to search for chemicals with effective chemotherapeutic activity against advanced third-stage larvae of *Gnathostoma spinigerum* in experimentally infected mice and cats.

BACKGROUND : These studies are a continuation of the work reported in previous years. Many anthelminthic 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 except Ancylol disophenol given in 8 subcutaneous injections at one week intervals to infected white mice which showed significantly reduced number of *G. spinigerum* larvae compared with control mice. However, a second group of infected white mice showed no significant reduction of the larvae after being injected, also with 8 doses of the same drug (1). The screening test with this drug on the mice infected with the *G. spinigerum* larvae was then suspended.

It was also shown previously (1) that 12 doses of 0.05 ml/lb body weight of parenteral Ancylol given to 2 cats at 10-day intervals were very effective in reducing numbers of *G. spinigerum* larvae; the dosage of this drug at 0.04 ml/lb body weight remained to be tested.

Etrenol (Hycanthone) was previously tested on mice infected with *G. spinigerum* advanced third-stage larvae by oral administration but showed no therapeutic effect on the infection (2).

METHODS :

Ancylol : Two adult domestic cats, after being kept at the Veterinary Medicine facility for about one year and proved negative for natural Gnathostoma infection [monthly stool examination for Gnathostoma eggs by Formalin Ether Sedimentation technique (Ritchie)] were infected each with 71 and 99 *G. spinigerum* advanced third-stage larvae obtained from experimentally infected mice. After the infection was permitted to continue for 37 and 42 days, each cat was administered, parenterally, 12 doses of 0.04 ml per lb body weight at 10-day intervals.

Mice of ICR strain were infected by oral administration, each with five *G. spinigerum* advanced third-stage larvae. After the infection became established, the following drugs and regimens were tested :

1. Etrenol (Hycanthone, Winthrop Products Inc) : This drug is used for schistosomicidal activity. The drug was given by intramuscular injection and the dosage guide for treating bilharziasis was followed.

2. Trodax : (Nitroxynil aqueous solution of the eglumine salt for subcutaneous administration) proved to give therapeutic results against fascioliasis of sheep and cattle and against Ancylostoma caninum in dogs. This is produced by May and Baker Ltd, England.

3. Jonit (Oral administration) or phenylene-diisothiocyanate-(1, 4), proved to give therapeutic results on nematodes and cestodes infesting a variety of animals. The drug is produced by Farbwerke Hoechst AG, Germany.

Infected control mice for every drug tested were injected only with sterile distilled water. After completion of the treatment schedule, all mice were sacrificed and necropsied. Parasites found in various tissue were counted and the results recorded.

RESULTS : The two cats treated with 12 doses of Ancylol 0.04 ml/lb body weight at 10-day intervals showed an effective therapeutic result, as one was negative and the other had only 12 living larvae in the diaphragm, compared with 16 larvae and immatures found in the two control cats (Table 1). The treated animals showed no gross pathological changes or toxic effects of the organs caused by the drug. It is suggested that Ancylol be tested parenterally in non-human primates infected with migrating larval stage of *G. spinigerum*, using the same regimen of multiple dosages of the drug and longer intervals between the doses. The results obtained from the experiment on primates should lead to consideration of further trials, on man if possible.

Etrenol (Hycanthone) : This drug was given intramuscularly to the infected mice in one and two dose regimens (one dialy dose) of 5 mg, 10 mg, 15 mg, 20 mg per kg body weight. The results with these small dosages are shown in Table 2. There was no significant reduction in the number of gnathostome larvae in the treated mice compared with the control mice for any regimen. Therefore, Etrenol (Hycanthone) given intramuscularly at these lower dosage is considered to have no therapeutic effect. Further trials on the infected mice at larger dosages of the drug, especially with 50 mg, 100 mg, 150 mg, per kg body weight given intramuscularly, are now in progress.

Jonit : This drug was administered orally one dose for one day and one daily dose for two days using dosages of 50, 100, 150 mg/kg body weight on mice infected with *G. spinigerum* advanced third-stage larvae. The results are shown in Table 3. The drug is considered to have no therapeutic value in the treatment of *G. spinigerum* infection.

Trodax (Nitroxynil) : One dose of the drug was administered subcutaneously using doses of 5, 10, 15 and 20 mg/kg body weight on mice infected with *G. spinigerum* advanced third-stage larvae. The results are shown in Table 4. The drug is considered no therapeutic value in the treatment of *G. spinigerum* infection.

SUMMARY : Two cats infected with *G. spinigerum* migrating stage larvae were successfully treated with 12 doses of Ancylol 0.04 ml/lb body weight at 10-day intervals. Neither cat showed any evidence of gross pathological changes caused by the drug. It is suggested that Ancylol be tried on primates with the same

regimen before trials be begun in man. Intramuscular administration of Etrenol (Hycanthone) to infected mice with small dosages was ineffective and further trials at larger dosages are in progress.

Trosax (Nitroxynil) subcutaneous administration and oral administration of Jonit (Phenylene-diisothiocynate-(1, 4) were ineffective in the chemotherapy of *Gnathostoma spinigerum* in experimentally infected mice.

This is a final report from this Laboratory. Work in progress will be continued at the Faculty of Tropical Medicine, Mahidol University.

REFERENCES :

1. Daengsvang, S. and Yingyourd, P. AFRIMS (SEATO) Medical Research Laboratory, Annual Report, April 1976 - September 1977.

2. Daengsvang, S. and Yingyourd, P. SEATO Medical Research Laboratory, Annual Report, April 1974 - March 1975.

Table 1. Treatment of *Gnathostoma spinigerum* migrating stage larvae infected cats with multiple subcutaneous doses of Ancylol Disophenol, 0.04 ml per 1b body weight per dose for 12 doses at 10-day intervals

| Drug Dose (mg/kg) | Total Doses of Drug to Each Cat | Number of Cat Treated | Number of Third-stage Larvae Given to Each Cat | Age of the Worm in Cat Before Treatment (in day) | Autopsy Finding | | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | | Worm Survival Rate % | Number and Stage of Living Worm & Infected Organs | |
| 0.04 | 12 | 2 | 77,99 | 37,42 | 0,2.02 | 0, 2 Living larvae in diaphragm | 1 Dead larva in diaphragm. |
| - | - | 2 | 50 | - | 16 each | 8 Living larvae in muscles and liver 8 immatures in muscles, stomach wall and diaphragm | *2 Control cats |

* Results obtained from the Annual Progress Report, April 1976 - September 1977.

Table 2. Treatment of *Gnathostoma spinigerum* advanced third-stage larvae infected mice each with one dose and two doses (one daily dose) of Etreno. (Hycanthone) intra-muscular injection

| Hycanthone Drug Dose (mg/kg) | No. of Drug Dose | No. Mice Infected with the Larvae | Total Larvae Infected the Mice | Necropsy Findings | | Time of Necropsy (Days)* |
|---|---|---|---|---|---|---|
| | | | | Total Larvae (%) | Organ Found Infected | |
| 5 | 1 | 20 | 100 | 41(41) | Livers, muscles | 22-36 |
| 5 | 2 | 5 | 25 | 11(44) | Livers, muscles | 22-36 |
| Control | - | 10 | 50 | 21(42) | Livers, muscles | 12-36 |
| 10 | 1 | 20 | 100 | 43(43) | Livers, muscles | 40-41 |
| 10 | 2 | 5 | 25 | 12(48) | Muscles | 40-41 |
| Control | - | 10 | 50 | 23(46) | Livers, muscles | 35-42 |
| 15 | 1 | 20 | 100 | 49(49) | Livers, muscles | 6-49 |
| 15 | 2 | 5 | 25 | 12(48) | Muscles | 6-49 |
| Control | - | 10 | 50 | 26(52) | Livers, muscles | 6-51 |
| 20 | 1 | 20 | 100 | 48(48) | Livers, muscles | 20-50 |
| 20 | 2 | 5 | 25 | 12(48) | Muscles | 20-50 |
| Control | - | 10 | 50 | 25(50) | Livers, muscles | 27-51 |

* Days after administration of last drug dose.

Table 3. treatment of *Gnathostoma spinigerum* infected mice with Jonit oral administration for one and two day courses

| Jonit Drug Dose (mg/kg) | No. of Infected Mice Treated | | Third-stage Larvae Found After One Dose Number (%) | Third-stage Larvae Found After Two Doses Number (%) | Time of Necropsy (Days)* |
|---|---|---|---|---|---|
| | One Dose | Two Doses | | | |
| 50 | 10 | 10 | 24(48) | 24(48) | 23 |
| 100 | 10 | 10 | 25(50) | 25(50) | 21 |
| 150 | 10 | 10 | 26(52) | 24(48) | 8-35 |
| Control | 20 | 20 | 47(47) | 47(47) | 21-37 |

* Days after administration of the drug.

Table 4. Treatment of *Gnathostoma spinigerum* infected mice with one subcutaneous dose of Trodax (Nitroxynil)

| Trodax (Nitroxynil basis) Drug Dose (mg/kg) | No. of Infected Mice Treated | Third-stage Larvae Found Number (%) | Time of Necropsy (Days)* |
|---|---|---|---|
| 5 | 20 | 46(46) | 22 |
| Control | 10 | 25(50) | 22 |
| 10 | 20 | 51(51) | 20 |
| Control | 10 | 23(46) | 20 |
| 15 | 20 | 48(48) | 21 |
| Control | 10 | 24(48) | 21 |
| 20 | 20 | 42(42) | 23 |
| Control | 10 | 25(50) | 23 |

* Days after treatment