

Chemotherapy of Gnathostomiasis

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OBJECTIVE: To determine (1) the effect of multiple subcutaneous doses of Ancyolol on larval and immature stages of Gnathostoma spinigerum in infected dogs and cats, (2) the effect of oral administration of Bithionol, Thiabendazole and Niridazole on white mice previously infected with G. spinigerum larvae. These drugs have been used effectively in the treatment of certain helminthic diseases but have yet to be used in treating gnathostome infections.

DESCRIPTION: (1) Ancyolol, Disophenol (2, 6-ditodo-4-nitrophenol) Parenteral 4.5%.

Previous results suggest that 3 to 6 subcutaneous doses of Ancyolol kill many larvae and immature G. spinigerum located in the tissue (1970 Annual Report). The effect of 4-7 subcutaneous doses, of the same chemotherapeutic agent, on the migrating stage of the worm in various organs of dogs and cats is again undertaken. The drug was administered to 7 cats and 4 dogs, previously infected with G. spinigerum. Five infected cats and four dogs were used as controls. Each dose amounted to 0.1 ml of drug per pound of body weight; directions given by the manufacturer for the treatment of canine hookworm. Treated animals were given 4 to 7 weekly doses of the drug, 1 and 3 months after being transcutaneously infected with the larvae. Experimental animals were sacrificed and examined for the presence of worms in the various organs 12-22 days after the last dose was given.

(2) Bithionol or bitin, 2, 2'-thiobis (4, 6-dichlorophenol) oral administration.

This phenolic compound has been used effectively in treating human paragonimiasis in Japan (Yokogawa et al., 1963) and Thailand (Charoenarb et al., 1964). Bithionol is now being studied to determine its effectiveness by oral administration in distilled water and/or in 10% ethanol administered through a polyethylene tube to adult white mice infected with G. spinigerum. Dosage, 40 mg/kg body weight every other day. Adult laboratory bred white mice weighing 25-38 grams were used for the experiment. Autopsies were performed on mice after completing the experiment. An electrically illuminated examination box and microscope were used to determine the presence of worms in the muscles and visceral organs.

(3) Thiabendazole (MK-360), 2-(4-Thiazolyl benzimidazole) oral administration.

This drug has been shown to be effective in treating experimental trichinosis in swine (Campbell and Cuckler, 1962) at a dosage of 86 mg/kg body weight for 7 days. Papasarathorn et al. (1964) found that Thiabendazole was effective for subclinical ascariasis and trichuriasis in man when given for one day and two days and without any serious untoward reactions.

A preliminary study on the effect of Thiabendazole on induced gnathostomiasis in laboratory bred white mice was undertaken. Fifteen white mice were infected orally with advanced third-stage larvae and 15 with fully developed larvae of G. spinigerum, 10 from each group were treated, the remaining 5 used as controls. Autopsies were performed on all treated and control mice to examine for the presence of worms in the tissues.

Table 1. Chemotherapy of *G. spinigerum* larval and immature stages infecting definitive hosts (cats and dogs) by multiple subcutaneous doses of Ancyol disphenol (2, 6-dilodo-4-nitrophenol) on the basis of 0.1 ml per pound body weight per dose at 7-day intervals.

| No. Animal | No. third-stage larvae penetrated thru skin/percent | Age of worm in host before treatment | Total weekly doses of Ancyol | Autopsy findings 7-22 days after treatment | | | Remarks |
|------------------------|---|--------------------------------------|-------------------------------|--|---|--|--|
| | | | | Survival rate of worm | No/stages of living worm | Organs found infected | |
| 2 cats (#106,111) | 42(70%), 44(85%) | 1 month | 5 | 0%,16% | 7 larvae | abdominal flesh, legs, chest, diaphragm | 1 dead larva in right hind leg flesh. Sacrificed 14 days after last dose Ancyol |
| 2 cats (#117,118) | 48(92%), 44(88%) | 1 month | 6 | 0%, 9% | 4 larvae | diaphragm | Sacrificed 21 days after last dose Ancyol |
| 2 cats (#125,112) | 52(76%), 49(83%) | - | Control, (1 mo.) no treatment | 69%,78% | 1 mature female 7 immature males & females and 28 larvae in one cat, 38 larvae in liver of other | abdominal flesh, flesh of legs, chest, diaphragm, liver, stomach, lung | 1 cat sacrificed 76 days the other died 43 days after first skin infection. 1 dead larva in abdominal muscle |
| 3 cats (#126,113, 114) | 73(70%), 57(90%), 47(84%) | 3 months | 4, 7, 7 | 1%, 9%, 21% | 16 larvae | abdominal flesh, chest, diaphragm | 1 cat died 4 days after 4th dose, other 2 sacrificed 22 days after 7th dose Ancyol |
| 1 cat (#115) | 44(88%) | - | Control, (3 mo.) no treatment | - | - | - | First egg positive stool 97 days after first skin infection, second egg positive 209 days after first infection. Kept for supplying eggs |
| * 2 cats (#38,74) | 53(62%), 46(90%) | 5, 6 $\frac{1}{2}$ months | Control no treatment | 51%,56% | 53 immature adult males & females and larvae | stomach, diaphragm, chest wall, abdominal and back muscles, abdominal fat, omentum | Sacrificed 154, 195 days of prepatent period |
| 2 dogs (#23,24) | 67(89%), 64(80%) | 1 month | 5 | 6%, 2% | 5 larvae | diaphragm, liver | Sacrificed 12 and 14 days respectively after last dose Ancyol |
| 1 dog (# 21) | 98(93%) | - | Control, (1 mo.) no treatment | 25% | 4 mature and immature males, 5 immature females, 16 larvae | abdominal wall, chest, and back muscles, stomach, liver, diaphragm | Sacrificed 83 days after infection |
| 2 dogs (#19,22) | 71(92%), 66(88%) | 3 months | 7 | 6%,0% | 4 larvae (1 doubtful living) | diaphragm, abdominal fat | Sacrificed 14 days after 7th dose Ancyol, 1 dead larva in diaphragm. Dog #19 showed moderate jaundice with loss of weight disappearing after two interruptions of treatment, one week each |
| 1 dog (# 20) | 54(95%) | - | Control, (3 mo.) no treatment | - | - | - | First ova positive stool 110 days after infection followed by 81 days of patent period. Negative since 18/12/70. |
| ** 2 dogs (#1,18) | 65(79%), 64(100%) | 8 $\frac{3}{10}$ months | Control, no treatment | 63%,70% | 86 mature males and females | stomach, lung, omentum | Sacrificed 262 and died 297 days after the infection |

* From skin penetration by *G. spinigerum* advanced third-stage larvae (transcutaneous infection) project reported in Annual Progress Report 1969 and 1970.

** From skin penetration by *G. spinigerum* advanced third-stage larvae (transcutaneous infection) projected in Annual Progress Report 1970 and in the present Report 1971.

(4) Niridazole (Ambilhar, Ciba), 1-(5-nitro-2-thiazoly)-2-imidazolidinone or Ciba 32,644-Ba oral administration.

Niridazole was originally introduced for the treatment of schistosomiasis; it is also known to be active against Entamoeba histolytica (Lambert 1964, Powell et al., 1966). The drug was prepared fresh at 1 mg in 1 ml distilled water for administration to each experimental mouse, at a daily dosage of 25 mg per kilogram body weight. Fifteen laboratory bred white mice were orally infected; 10 were treated with the drug, the other 5 were used as controls.

PROGRESS: Table 1 summarizes the results of Ancylosol in 5 treated cats and 4 dogs. The results show that 5-6 weekly doses effectively cured 3 (one cat reported last year) of the 5 infected cats beginning the treatment 1 month after the infection with the larvae.

Three of the four dogs beginning treatment 1 month and 3 months after infection showed markedly lower worm survival rates than corresponding controls. One animal was completely cured. It is apparent that Ancylosol may be used with good results if administered under close and careful supervision, but no further studies are planned because of the drug's potential toxicity.

(2) Bithionol or Bitin, oral administration. The preliminary finding of Bithionol chemotherapy on induced gnathostomiasis in white mice is shown in Tables 2 and 3. The drug appears to have little or no effect on the parasite, but because so few mice were studied, repeated studies using more infected mice are now being developed.

(3) Thiabendazole, oral administration. The findings indicate that no significant difference exists between the treated mice and controls. The 10 treated mice showed a total of 42 (84%) living larvae encysted in the muscles and liver. The 5 control mice killed and examined showed 23 (92%) living encysted larvae in muscles and liver.

(4) Niridazole (Ambilhar, Ciba) oral administration. This experiment on mice infected with G. spinigerum fully developed larvae in cyclops is still in progress.

SUMMARY: Three of 5 adult cats treated with 5-6 subcutaneous doses of Ancylosol disophenol were cured of infection with G. spinigerum; the remaining cats showed significant reductions of the worm survival rates. Similarly, all four infected dogs showed either a significant reduction in the survival rates of worms or were completely cured. Toxic effects were noted in only one animal. Oral administration of Bithionol was only slightly effective in worm load reduction. Thiabendazole seems to have no therapeutic value in experimentally induced gnathostomiasis. The study on the therapeutic effect of Niridazole (Ambilhar, Ciba) on white mice is still in progress.

Table 2. Preliminary finding of chemotherapy on experimental white mice infected with fully developed *Gnathostoma spinigerum* larvae in cyclops by oral administration of Bithionol (bitin), 2, 2'-thiobis (4, 6-dichlorophenol).

| Dose of bithionol per mouse | No. of mouse | No. of larvae in cyclops | No. advanced third-stage larvae found on autopsy | Organs Infected | Remarks |
|--|--------------|--------------------------|--|--|---|
| — | 4 | 120 | 32 (27%) (unencysted) | liver, chest, abdomen, hind-leg | Died before treatment began. |
| <u>Drug in distilled water</u> | | | | | |
| 1-6 | 6 | 180 | 93 (52%) (unencysted) | liver, lung, abdomen, chest, back fore-hind-legs | Died after 1-6 doses. |
| 12-20 | 9 | 270 | 75 (28%) (encysted) | liver, abdomen, chest, back, fore-hind-legs. | Killed 2-6 days after the last dose. |
| 12 & 20 | 2 | 58 | 11 (19%) (encysted) | liver, chest, back abdomen | Died and killed 2 days after the last dose. |
| <u>Drug in distilled water and 10% ethanol</u> | | | | | |
| 13-20 | 4 | 120 | 26 (22%) (encysted) | liver, abdomen, chest, back, fore-leg | Died and sacrificed 2 days after the last dose. 1 mouse negative. |
| 20 (control-no drug) | 2 | 60 | 16 (26%) (encysted) | liver, abdomen, back, fore-hind-legs | Killed 2 days after the last dose. 1 mouse negative. |
| <u>Drug in 10% ethanol</u> | | | | | |
| 20 | 6 | 90 | 28 (31%) (encysted) | skin, abdomen, chest, back | Killed 1-2 days after the last dose. |
| 20 (control-no drug) | 2 | 30 | 12 (40%) (encysted) | liver, back | Killed 1-2 days after the last dose. |

Table 3. Preliminary finding of chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third-stage larvae obtained from other infected white mice by oral administration of Bithionol (bitin), 2, 2'-thiobis (4, 6-dichlorophenol).

| Dose of Bithionol per mouse | No. of mouse | No. of all advanced third-stage larvae fed | No. of all advanced third-stage larvae found on autopsy | Organs infected | Remarks |
|-----------------------------|--------------|--|---|--------------------------------|--|
| <u>Drug in 10% ethanol</u> | | | | | |
| 20 | 7 | 45 | 19 (42%) (encysted) | liver, chest, back, hindleg | Killed 1 day after the last dose. |
| 13-20 (control-no drug) | 3 | 17 | 13 (76%) (encysted) | liver, back, hindleg. | Died or killed 1-2 days after the last dose. |

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