## Chemotherapy of Gnathostomiasis

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OBJECTIVE: To determine the effect of oral administration of drugs on white mice infected with Gnathosma spinigerum larvae. The drugs selected have been used effectively in the treatment of certain helminthic diseases but have yet to be used in treating gnathostome infections.

DESCRIPTION: (1) Bithionol or Bitin, 2, 2'—thiobis (4,6—dichlorophenol) oral administration.

Previously, (Annual Progress Report 1971) oral administration of Bithionol to a small number of adult white mice, each weighing 23—38 grams, infected with Gnathostoma spinigerum larvae (40 mg/kg body weight) showed little or no effect on the parasite. During this reporting year, 1972, repeated studies were undertaken by oral administration of the drug in distilled water, in 10% ethanol and in 0.5% methocel (methyl cellulose) to adult white mice infected with G. spinigerum larvae. Dosage, 40 mg/kg body weight every other day. Autopsies were performed on mice after completing the experiment. An illuminated examination box and microscope were used to determine the presence of worms in the muscles and visceral organs.

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

A preliminary study on the effect of Thiabendazole chemotherapy in distilled water with or without 0.05% hydrochloric acid on induced gnathostomiasis in white mice by oral administration was reported in the Annual Progress Report 1971. No effect on the parasite was observed. During 1972 repeated studies were undertaken by oral administration of the drug in distilled water and in 0.05% hydrochloric acid to more adult white mice infected with G. spinigerum larvae.

Eleven white mice were infected orally with G. spinigerum fully developed larvae in cyclops, of which 7 were treated by 14 doses of 50 mg/kg body weight of the drug in distilled water and 4 used as controls. Seventy-one white mice were infected each with 5 G. spinigerum advanced third stage larvae obtained from other infected mice, of which 10 were treated by 14 doses of 50 mg/kg body weight of the drug in 0.05% hydrochloric acid and 5 used as controls; 37 were treated by 14 doses of 100 mg/kg body weight of the drug in distilled water and 19 used as controls. Autopsies were performed after completing the experiment.

(3) Niridazole (Ambilhar, Ciba), 1—(5—nitro—2—thiazolyl)—2—imidazolidinone or Ciba 32, 644—Ba oral administration.

Previously, (Annual Progress Report 1971), Niridazole was prepared fresh at 1 mg/ml distilled water for oral administration at a daily dosage of 25 mg/kg body weight. During this reporting period, continuation of this experiment and a new study on additional mice with increased concentration of the drug in distilled water at a daily dosage of 50—100 mg/kg body weight for 10 days were undertaken. In total, 132 adult white mice were orally infected with G. spinigerum larvae: 39 were treated with 25, 50 and 100 mg of the drug per kilo body weight daily for 10 doses, and 39 were used as controls. Autopsies were performed.

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(4) Banocide (Hetrazan) or Diethylcarbamazine citrate (1—diethylcarbamyl—4—methylpiperazine dihydrogen citrate) oral administration.

Diethylcarbamazine administered per os is rapidly absorbed and excreted in various forms in the urine within 1—2 days. This drug rapidly eliminates circulating microfilariae and tends to act more slowly on the adult worms of Wuchereria bancrofti. Large doses of Hetrazan killed the adults and developing stages of Dracunculus medinensis (Faust, Russell and Jung 1970). The drug usually destroys the microfilariae of Onchocerca volvulus in the skin within a few days but has little effect upon the adult worms (Hawking 1958). Banocide is now being studies to determine its effectiveness by oral administration at a daily dosage of 6 mg/kg body weight for 15 doses in distilled water and administered to 39 adult white mice infected orally with 5 G. spinigerum advanced third stage larvae. Twenty infected white mice were used as controls. Autopsies were performed.

(5) Niridazole (1—(5—nitro—2—thiazolyl)—2—imidazolidinone or Ciba 32, 644 Ba) combined with Hetol (1,4—bis—trichloromethylbenzol) oral administration.

Hetol has been used for treating cattle infected with liver flukes with satisfactory results (Enigk and Duwel 1960). Yokogawa et al. (1965) successfully treated Clonorchis sinensis infections in animals with Hetol (Hoechst). The combination of Niridazole 100 mg and Hetol 50 mg/kg body weight in distilled water was orally administered, daily for 10 doses, to 40 experimental white mice after each being infected with 5 G. spinigerum advanced third stage larvae. Ten infected white mice were used as controls. Autopsies were performed.

(6) Banocide (Hetrazan) or Diethylcarbamazine citrate combined with Bithionol (Bitin) oral administration.

The combination of these 2 drugs was prepared in distilled water for oral administration to white mice after being infected with 5 G. spinigerum advanced third stage larvae at a daily dose of Banocide 10 mg and Bithionol 50 mg/kg body weight for 15 doses.

PROGRESS: (1) Bithionol or Bitin. Table 1 summarizes the results of chemotherapy on induced gnathostomiasis in adult white mice by oral administration every day for 20 doses of Bithionol in distilled water, in 10% ethanol and in 0.5% methocel (methyl cellulose). The results of autopsies on the treated and control white mice after completing the treatment showed (a) drug in distilled water: found in 54 treated and 20 control mice 14% and 13% G. spinigerum living advanced third stage larvae respectively, (b) drug in 10% ethanol: found in 59 treated and 23 control mice 22% and 12% G. spinigerum living advanced third stage larvae respectively, (c) drug in 0.5% methocel: found in each group of 42 treated and 22 control mice 21% G. spinigerum living advanced third stage larvae. The drug appears to have no therapeutic effect on the infected mice.

(2) Thiabendazole. The findings of the repeated studies of chemotherapy on white mice infected with G. spinigerum larvae treated by oral administration of this drug in distilled water and in 0.05% hypochloric acid at dosage of 50 mg/kg body weight and in distilled water at dosage of 100 mg/kg weight showed no significant difference between the treated mice and controls (Tables 2 and 3). The drug appears to have no therapeutic effect on the infected mice.

(3) Niridazole Ambilhar, Ciba). Daily oral administration of Niridazole in distilled water to white mice infected with G. spinigerum advanced third stage larvae at dosages 25 mg, 50 mg and 100 mg/kg body weight for 10 doses showed no effect on the parasite. In a total of 10 treated mice administered 25 mg/kg body weight and 53 treated mice given 50 mg/kg body weight found on autopsies after completing the treatment 17% and 23% living advanced third stage larvae respectively, compared with 17% living larvae found in the 25 controls. Another 30 infected white mice treated with 100 mg/kg body weight showed on autopsies 51% advanced third stage larvae compared with 54% of the larvae found in 14 control mice (Tables 4 and 5). This drug appears to have no therapeutic effect on the infected mice.

Table 1.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae after each being infected with 15 fully developed larvae in cyclops by oral administration of Bithionol (bitin), 2, 2'—thiobis (4, 6—dichlorophenol). The dosage is 40 mg/kg body weight every day for 20 doses.

| Dose of Bithionol per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found (%) | Organs infected | |
| Drug in distilled water | | | | | |
| 20 | 54 | 32 (59) | 114 (14) | livers and/or body muscles | Autopsies 19—25 days after the last dose. |
| 20 doses distilled water (control—no drug) | 20 | 10 (50) | 38 (13) | " " | Autopsies 19—25 days after the last dose. |
| Drug in 10% ethanol | | | | | |
| 20 | 59 | 39 (66) | 195 (22) | livers and/or muscles | Autopsies 1—25 day after the last dose. |
| 20 doses 10% ethanol (control—no drug) | 23 | 15 (65) | 43 (12) | " " | " " |
| Drug in 0.5% methocel | | | | | |
| (methyl cellulose) 20 | 42 | 33 (79) | 130 (21) | livers and/or body muscles | Autopsies 5—26 days after the last dose. |
| 20 doses 0.5% methocel (control—no drug) | 22 | 17 (77) | 69 (21) | " " | Autopsies 7—26 days after the last dose. |

Table 2.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third–stage larvae after each being infected with fully developed larvae in cyclops by oral administration of Thiabendazole (MK–360), 2–(4–Thiazolyl benzimidazole). The dosage is 50 mg/kg body weight every day for 12 doses.

| Dose of Thiabendazole per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found % | Organs infected | |
| Drug in distilled water | | | | | |
| 14 | 7 | 7 (100) | 48 (46) | livers and/or body muscles | Autopsies 23–39 days after the last dose. |
| 14 doses distilled water (control–no drug) | 4 | 4 (100) | 31 (52) | " " | Autopsies 40 days after the last dose. |

Table 3.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae after each being infected with 5 advanced third stage larvae obtained from other infected white mice by oral administration of Thiabendazole (MK–360), 2–(4–Thiazolyl benzimidazole).

| Dose of Thiabendazole per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found (%) | Organs infected | |
| Drug in 0.05% HCL | | | | | |
| (50 mg/kg body weight) | | | | | |
| 14 | 10 | 10 (100) | 42 (84) | livers and/or body muscles | Autopsies 5 days after the last dose. |
| 14 doses 0.05% HCL (control–no drug) | 5 | 5 (100) | 23 (92) | " " | Autopsies 1–5 days after the last dose. |
| Drug in distilled water | | | | | |
| (100 mg/kg body weight) | | | | | |
| 14 | 37 | 37 (100) | 117 (63) | " " | Autopsies 12–29 days after the last dose. |
| 14 doses distilled water (control–no drug) | 19 | 19 (100) | 61 (64) | " " | Autopsies 7–30 days after the last dose. |

Table 4.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae obtained from other infected white mice by oral administration of Miridazole (Ambilhar, 1—(5—nitro—2—thiazolyl) 2—imidazolidinone or Ciba 32, 644 Ba). The dosage is 190 mg/kg body weight every day for 10 doses.

| Dose of Niridazole per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found (%) | Organs infected | |
| Drug in distilled water | | | | | |
| 10 | 30 | 28 (93) | 76 (51) | livers and/or body muscles | Autopsies 3—29 days after the last dose. |
| 10 doses distilled water (control—no drug) | 14 | 13 (93) | 38 (54) | ,, ,, | Autopsies 29 days after the last dose. |

Table 5.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae after each being infected of Niridazole (Ambilhar, 1—(5—nitro—2thiazolyl) 2—imidazolidinone or Ciba 32, 644 Ba). The dosages are 25—50 mg/kg body weight every day for 10 doses.

| Dose of Niridazole per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found (%) | Organs infected | |
| Drug in distilled water (25 mg/kg body weight) 10 | 10 | 10 (100) | 25 (17) | livers and/or body muscles | Autopsies 33—34 days after the last dose. |
| Drug in distilled water (50 mg/kg body weight) 10 | 53 | 39 (74) | 185 (23) | " " | Autopsies 7—25 days after the last dose. |
| 10 doses distilled water (control—no drug) | 25 | 17 (68) | 62 (17) | " " | Autopsies 1—34 days after the last dose. |

Table 6.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae after each being infected with 5 advanced third stage larvae obtained from other infected mice by oral administration of Banocide (Hetrazan) or diethylcarbamazine citrate (1—diethylcarbamy 1—4—methylpiperazine dihydrogen citrate). The dosage is 6 mg/kg body weight every day for 15 doses.

| Dose of Banocide per mouse | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advance third stage larvae found (%) | Organs infected | |
| Drug in distilled water | | | | | |
| 15 | 39 | 39 (100) | 133 (68) | livers and/or body muscles | Autopsies 1—18 days after the last dose |
| 15 doses distilled water (control—no drug) | 20 | 19 (95) | 67 (67) | " " | Autopsies 15—19 days after the last dose. |

Table 7.

Chemotherapy on experimental white mice infected with Gnathostoma spinigerum advanced third stage larvae after each being infected with 5 advanced third stage larvae obtained from other infected white mice by oral administration of Niridazole (Ambilhar) combined with Hetol (1, 4—bis—trichloromethyl—benzol). The dosage is 100 mg Niridazole combined with 50 mg Hetol/kg body weight in distilled water every day for 10 doses.

| Dose of Niridazole combined with Hetol | No. mice treated | Autopsy findings | | | Remarks |
|---|---|---|---|---|---|
| | | No. mice positive with advanced third stage larvae (%) | No. advanced third stage larvae found (%) | Organs infected | |
| Drug in distilled water | | | | | |
| 10 | 40 | 38 (95) | 130 (65) | livers and/or body muscles | Autopsies 9—19 days after the last dose. |
| 10 doses distilled water (control—no drug) | 10 | 9 (90) | 26 (52) | livers and/or body muscles | Autopsies 5—19 days after the last dose. |

(4) Banocide (Hetrazan) or Diethylcarbamazine citrate. The findings showed no significant difference between the treated mice and controls. The 39 treated mice yielded a total of 133 (68%) living advanced third—stage larvae in the livers and/or body muscles. The 20 control mice examined showed 67 (67%) living advanced third—stage larvae also in the livers and/or body muscles (Table 6). It therefore appears to have no therapeutic effect in infected mice.

(5) Niridazole (1—(5—nitro—2—thiazolyl)—2—imidazolidinone or Ciba 32, 644 Ba) combined with Hetol (1, 4—bis—trichloromethylbenzol). The findings on autopsies of the experimental mice showed no significant difference between the treated mice and controls (Table 7). The 40 treated mice found in a total of 130 (65%) living advanced third stage larvae in the livers and/or body muscles. The daily oral administration of combined Hetol and Niridazole for 10 doses showed no therapeutic value in the infected mice.

(6) Banocide (Hetrazan) or Diethylcarbamazine citrate combined with Bithionol (Bitin). This experiment on mice infected with G. spinigerum advanced third stage larvae is still in progress.

SUMMARY: Oral administration of Bithionol, Thiabendazole, Niridazole, Banocide, and Niradazole combined with Hetol seems to have no therapeutic value in experimentally induced gnathostomiasis in white mice. The oral administration to mice infected with G. spinigerum advanced third stage larvae with combined Banocide and Bithionol is now in progress.