Chemotherapeutic effects of 4-isothiocyanato-4′-nitrodiphenylamine (C9333-Go/CGP 4540) on infections with *Nematospiroides dubius, Hymenolepis diminuta, Hymenolepis nana* and *Spirometra mansonoides*[1]

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Summary. 4-Isothiocyanato-4′-nitrodiphenylamine possessed anthelmintic activity against *Nippostrongylus dubius* and *Hymenolepis nana* in mice and *H. diminuta* in rats as determined by both egg counts and recovery of parasites at autopsy. No activity was detected against the cestode, *Spirometra mansonoides,* in cats.

4-Isothiocyanato-4′-nitrodiphenylamine (C9333-Go/CGP 4540) possesses antischistosomal and antifilarial activities[4-6]. The drug also is active chemotherapeutically in infections with *Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides* and *Trichuris trichiura*[7]. A dual mode of action is suggested by the findings that, at the dose levels employed, the schistosomes and adult filariids require approximately 2 months before death of the parasite occurs, whereas the drug effects upon the other helminths are manifested within several days. To study the short-term effect of the isothiocyanate, a laboratory model would be desirable. Therefore, possible chemotherapeutic effects of CGP 4540 were examined on laboratory infections with the nematode *Nematospiroides dubius* and the cestodes *Hymenolepis diminuta, H. nana* and *Spirometra mansonoides.*

The drug, formulated as previously described[5], was administered orally to anesthetized animals by gastric intubation. *N. dubius* infections were in Lobund mice which had been infected with 250 third stage larvae. Male Holtzman rats and ICR mice were infected with 10 cysticercoids of *H. diminuta* and *H. nana,* respectively. In the cases of the Hymenolepids, examination of feces and drug treatment were initiated at least 14 days post infection. Cats were infected with 5 or 6 *S. mansonoides* plerocercoids each. Egg estimations were accomplished employing a saturated sodium chloride flotation technique except for *S. mansonoides* infections where estimations were made by scanning wet fecal smears.

Extending earlier findings[8], *N. dubius* and *H. nana* were both sensitive to the isocyanate, as was *H. diminuta* (table). 4 days post treatment no *N. dubius* eggs were present in the feces even at a dose of 25 mg/kg. At autopsy, worm burdens were greatly reduced, but present, up to a level of 100 mg/kg. Interestingly, the female parasites appeared to be more sensitive to the drug than the males, since almost all of the recovered worms from treated animals were males. This would account for the apparent higher sensitivity of egg counts.

At 50 mg/kg, *H. diminuta* destrobilization was apparent during the first 40 h post therapy. By 5 days, no eggs could be detected in the feces. That scolices were not completely removed was indicated by the reappearance of eggs in the feces after 21 days. In accord with this possibility, 1 adult parasite was recovered in 1 animal of each of the treated groups of 3 animals.

Effect of nitrodiphenylaminoisothiocyanate on the recovery of eggs and adults of *Nematospiroides dubius, Hymenolepis diminuta* and *Hymenolepis nana*

Parasite and stage	Day post administration	Animal number*	Recovery at indicated total drug dose (mg/kg)** 0	25	50	100	150	200
N. dubius	1	1, 2, 3, 4, 5	++++	++++	++++	++++	++++	++++
Eggs in feces	2	1, 2, 3, 4, 5	++++	+	+	+++	++	++
	3	1, 2, 3, 4, 5	++++	0	0	+	0	++
	4	1, 2, 3, 4, 5	++++	0	0	0	0	0
N. dubius	8	1	85	8	7	2	0	0
Adults at autopsy	8	2	105	4	3	0	0	0
	8	3	>80	6	0	0	0	0
	8	4	>80	1	0	0	0	0
	8	5	>80	2	5	0	0	0
	1	1, 2, 3	+++		++++	++++		
H. diminuta	3	1, 2, 3	+++		++	++++		
Eggs in feces	4	1, 2, 3	+++		+	+		
	5	1, 2, 3	+++		0	0		
	6	1, 2, 3	+++		0	0		
	8	1, 2, 3	+++		0	0		
	21	1, 2, 3	+++		+	+		
H. diminuta	21	1	8		0	0		
Adults at autopsy	21	2	3		0	0		
	21	3	6		1	1		
H. nana	1	1, 2, 3, 4	++++		0	0		0
Eggs in feces	2	1, 2, 3, 4	++++		0	0		0
H. nana	3	1	17 (2)***		35 (3)	0		0
Adults at autopsy	3	2	40		14 (2)	0		0
	3	3	1 (1)		0	0		0
	3	4	60 (4)		2	4		0

*Animal numbers represent animals housed in each cage. Eggs were collected from the pooled feces from each cage. For example, at each dosage level there were 5 mice infected with *N. dubius.* **Dosage schedules: 0: vehicle only in a volume equivalent to that administered to highest dosage group; 25 and 50: single morning dose; 100: 50 morning and 50 evening; 150: 100 morning and 50 evening; 200: 100 morning, 50 evening and 50 morning of 2nd day. ***In the *H. nana* experiments, numbers in parentheses represent mature worms recovered.

Immediately upon receipt of ICR mice (Mogul Corp.), they were infected with 10 *H. nana* cysticercoids and drug therapy was inaugurated after 14 days with the first appearance of eggs in the feces. The high recovery of worms in the control and 50 mg/kg groups at autopsy is indicative of animal room reinfections, particularly since most of the parasites recovered were immature. At 100 mg/kg, worms were recovered from only 1 animal.

CGP 4540 had no demonstrable effect upon either *S. mansonoides* egg counts or worm recovery when CGP 4540 was administered to the host cats at dose levels up to 150 mg/kg.

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Effect of aspirin and vitamins C and E on synovial rheumatoid arthritic and other cells[1]

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Summary. Normal and rheumatoid arthritic human synovial cells, normal rat muscle and bone cells, were cultured with combinations of aspirin (acetylsalicytic acid), vitamins C and E. Aspirin reduced percent growth of all cells by about 1/5 relative to controls. High vitamin C eradicated arthritic cells. In combinations, vitamin C was most important in eradicating arthritic cells. A low-aspirin, low-vitamin C combination was most effective in reducing arthritic cell populations, while having little effect on normal cells. Vitamin E retarded but did not prevent the action of vitamin C.

Amongst many effects aspirin (acetyl-salicytic acid) is believed to inhibit prostaglandin synthesis[2,3]. Prostaglandins are believed to be modulators of the activity of the body[4], perhaps mainly involved in local communication between cells, especially in defensive reactions induced by damage or stress. Aspirin is an anti-defensive drug inhibiting many of the reactions of the body to noxious agents. The potency of aspirin relative to sodium salicylate differs in different sites and for other reasons[5,6]. Aspirin probably exerts its anti-defensive effect locally rather than on 1 central mechanism, its local action seeming to be a blockage of humoral mediators of defensive reactions, an interference with some step in a sequence in which the mediator, which may sometimes be a kinin or a slow-reacting substance, is involved. Piper and Vance[6] have shown that such a sequence includes the release of an unidentified smooth muscle contracting substance and that this release is blocked by aspirin[6].

It has been demonstrated by investigations with acetylsalicytic acid (aspirin), and other analgesics, that the analgesic effect of these drugs involves a peripheral component of action due to interference with the function of pain producing substances[8]. Distinct actions on peripheral nerves of analgesics with and without anti-inflammatory properties have been reported by Schorderet[9]. He found out that salicylic acid of 2 mM (0.36 mg/ml) blocked the conduction of excitation, increased the content of inorganic phosphate and decreased potassium concentration. Hartman supposes that nonnarcotic analgesics, amongst which he numbers acetanilide, phenacetin and phenzone, behave in a distinct manner as analgesics with anti-inflammatory properties.

On the other hand vitamin C (ascorbic acid) has been reported to be a cofactor in certain specific enzymatic reactions and is needed for all normal function. Some species are unable to synthesize vitamin E and must obtain it from exogenous sources. The function of vitamin E compounds (tocopherols) is not known with certainty but there is some good evidence that they are antioxidants, preventing the destructive nonenzymatic attack of molecular oxygen on the double bonds of the polyunsaturated fatty acid components of tissue lipids.

Material and methods. The rheumatoid and nonrheumatoid synovial cell line was obtained with the courtesy of Dr D. Ford, Director of Canadian Arthritis and Rheumatism Society, B.C. The normal rat muscle cells and the bone cells were obtained from 1-day-old Western Albino rats. After using ether for anesthesia, under sterile conditions pieces of the muscle were removed from 4 rats, placed in a petri dish containing Hank balanced salt solution (BSS) plus antibiotics. Pieces of the femur after removal of the tissue surrounding it were also transferred to a petri dish with BSS, mincing the muscle pieces as well as the bone with scissors to small fragments, washing them several times in BSS, then culturing the tissues with standard cell culture procedure for muscle[10]. After trypsinizing the tissue, the cells were counted using a hemocytometer and trypan blue for viability, with 10^4–10^5 cells/flask inoculated in Falcon flasks using Eagle modified media supplemented with 10% calf serum and antibiotics (penicillin, streptomycin and fungazone; Grand Island Biological Company). Some explant cultures were used, but no experiment used a primary culture, but only after reinoculation. A method for bone culture[11] previously reported was utilized, using the same media. The cells were maintained in a 37 °C incubator, replacing the flask atmosphere with 5% CO_2 + air. When the cells reached confluency standard trypsinizing procedures were used to remove them from the flask surface where they grew as monolayers. The cells were counted and reinoculated for different experiments.

Aspirin solution. 2 types of solution were used, with low and high aspirin concentration. The high concentration was 0.18 g/100 ml (10^{-2} M solution), the low 0.018 g/100 ml. More diluted concentrations down to 10^{-7} M have been utilized.

Vitamin C. Ascorbic acid was used with concentrations of 1 g/ml and 0.1 g/ml.

Vitamin E. Vitamin E was used in the succinate form with 1000 IU/100 ml. Since it is lipophilic, it does not dissolve in