Effect of albendazole therapy in cats infected with Paragonimus kellicotti

Cat No.	Albendazole (mg/kg b.wt)	Eggs per g of feces on post-treatment days 0	3	6	9	12	14	Autopsy examination DAIP	No. of patent cysts	No. of live flukes*
1	100	860	200	80	0	0	0	107	None	None
2	100	2120	340	40	0	0	0	107	None	None
3	20	720	700	200	20	60	20	108	5	9
4	20	2660	960	780	220	80	20	108	3	7
5	None	1300	2360	960	920	1460	1240	106	9	17**
6	None	1420	480	980	1440	1060	1240		Not done	

* Each cat given 25 metacercariae. DAIP = Days after inoculation with Paragonimus metacercariae. ** Percent recovery of adult flukes from 7 cats not included in this paper was: 87, 84, 80, 68, 60, 58 and 50.

inoculation with Paragonimus (DAIP). Ova of Paragonimus were seen beginning 56–59 DAIP. Starting 80 DAIP, 4 Paragonimus-infected and 2 uninfected control cats were administered an oral aqueous suspension of albendazole in 2 divided doses of 20 or 100 mg/kg b.wt daily for 14 days. Faeces were examined daily for trematode ova[6] and radiographs and hemograms were taken weekly. 4 weeks after the start of chemotherapy 5 cats were killed and necropsied. The results are shown in the table.

It is apparent that the administration of 100 mg/kg b.wt of albendazole for 14 days (total dose, 1400 mg) killed the adult flukes and stopped shedding of ova. A dramatic resolution of the patent cysts in the lungs was apparent both radiographically and at necropsy. The administration of 20 mg/kg b.wt of albendazole (total dose, 280 mg) killed half of the flukes and partially suppressed the shedding of ova.

The control cat number 6 that was not necropsied (table) was then treated with albendazole 50 mg/kg b.wt, daily for 21 days beginning 101 DAIP. Paragonimus ova were not detected in the feces of this cat 9 days after administering the drug and there was a dramatic resolution of lesions in the lungs as detected by radiographic examination and necropsy 2 weeks after the cessation of chemotherapy.

No clinical signs related to treatment were recognized. Hematological examination of all 8 cats were within normal limits except at one sampling interval (92 DAIP) when cat numbers 1 and 2 were leukopenic and neutropenic. The cause of the transient neutropenia was not determined. Histopathological examination of sections from all major organs of all treated cats failed to reveal any changes related to albendazole treatment.

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Dichloropyrimidines: Specific inhibitors of virus growth[1]

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Summary. Dichloropyrimidines can be considered as a new group of antiviral substances having a common spectrum of inhibitory action.

The antiviral activity of 2-amino-4,6-dichloropyrimidine has been reported previously[2–4]. Certain features of this action deserve consideration: 2-amino-4,6-dichloropyrimidine acts on virus growth at concentrations which have little or no effect on macromolecular metabolism of unifected cells; rather unrelated viruses, such as Polio, Vaccinia and Herpes simplex viruses, are inhibited; inhibitory action is not due to fraudulent replacement of nucleic acid precursors. Research now in progress indicates that the antiviral action of 2-amino-4,6-dichloropyrimidine is shared by other bichlorinated pyrimidines. Preliminary data from this research are referred to below.

Material and methods. Compounds studied are listed in tables, for brevity. Virus strains (NIH, Bethesda) were: Polio 1 Brunenders, Coxsackie B_1, Encephalomyocarditis (EMC), Newcastle Disease (NDV), Vesicular stomatitis (VSV), Vaccinia and Herpes simplex 1 (HSV). Experiments were carried out on human aneuploid HEp 2 cells (American type culture collection, Rockville) and on primary mouse embryo cells, both grown in Eagle's MEM (Hank's base, pH 7.3) supplemented with 7% calf serum. Eagle's MEM (Earle's base) and aminoacid free Eagle's MEM (AFE) both brought up to pH 7.3 and supplemented with 2% calf serum were used in the tests. Maximum non-cytotoxic doses (MNCTD) of the drugs were determined by incubating 16-h-old HEp 2 cell monolayers (10^7 cells/sample) at 37 °C in Eagle's MEM 2% serum in the presence of scalar drug dilutions. After 48 h, gross cell damages were checked under light microscope and cell vitality was determined by measuring intracellular incorporation of neutral red[4]. Drug ability to interfere with cell growth was established by adding colchicine (Simes, 0.1 µg/ml) to the cultures 3 h after

1 This work has been supported by a grant of Consiglio Nazionale delle Ricerche, Rome (Italy).

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3 M. A. Marcialis, M. L. Schivo, A. Atzeni, A. Garzia and B. Loddo, Experientia *29*, 1559 (1973).

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Table 1. Effect of chlorinated derivatives of cyclic and heterocyclic compound on the growth of Polio 1 and Vaccinia viruses

Compounds in Eagle's MEM		µg/ml*	Inhibitory effect on Polio 1	Vaccinia
2-Chloro-4,6-dimethylpyrimidine	(A)	1000	–**	–
2-Amino-4-hydroxy-6-chloropyrimidine	(A)	1000	–	–
2-Amino-4-ethanol-6-chloropyrimidine	(A)	66	–	–
2,4-Dimethoxy-6-chloropyrimidine	(A)	132	–	–
2,4-Dichloropyrimidine	(A)	3	+**	+
2,4-Dichloro-6-methylpyrimidine	(A)	12	+	+
4,6-Dichloropyrimidine	(A)	12	+	+
2-Amino-4,6-dichloropyrimidine	(A)	100	+	+
2,4,6-Trichloropyrimidine	(B)	0.66	–	–
2,4,5,6-Tetrachloropyrimidine	(B)	3.3	–	–
2,6-Dichloropurine	(C)	2.2	–	–
2,6-Dichloro-7-methylpurine	(C)	0.12	–	–
2,6,8-Trichloropurine	(C)	20	–	–
2,6-Dichloropyridine	(D)	25	–	–
3,6-Dichloropyridazine	(D)	30	–	–
0-Chlorobenzoic acid	(E)	300	–	–
1-Chloro-2,4-dinitrobenzene	(B)	4	–	–
1-Chloro-3,4-dinitrobenzene	(D)	4	–	–
2,4-Dichlorobenzene	(B)	500	–	–
2,5-Dichlorohydroquinone	(B)	3	–	–
2,4,5-Trichlorobenzensulphonic acid	(B)	100	–	–
3,5-Dichlorosalicylic acid	(B)	50	–	–
3,5-Dichlorosalicylaldehyde	(B)	25	–	–
2,4-Dichloroaniline	(D)	20	–	–

*Corresponding to 2/3 of one MNCTD. ** –, less than 50% inhibition; +, more than 90% inhibition. A, given by Istituto Chemioterapico Italiano, Lodi; B, furnished by Eastman; C, by Sigma; D, by Fluka; E, by Fisher.

drug treatment and by counting, 36 h later, C-metaphases thus accumulated. One MNCTD was considered the maximum drug concentration unable to produce gross cell damages and to inhibit neutral red uptake and mitotic cycle by more than 20%. $^2/_3$ of the MNCTD thus established were used in tests for antiviral action. Cell monolayers (10^6 cells/sample) were infected at 20 °C for 1 h with 10 infectious units (IU) per cell, washed 3 times in Hank's base and incubated at 37 °C in Eagle's MEM or in AFE in the presence of the drugs; 24 h later, the entire cultures were frozen and thawed (–70 °C and 20 °C) 3 times and freed of cell debris at 3000 rpm for 3 min. Infectious units produced by Polio 1, Coxsackie B_1 and VSV were titrated in HEp 2 cells by the agar method[5], while the end point titration (6 stationary tube cultures of HEp 2 cells per decimal dilution) was used for EMC, NDV, HSV and Vaccinia virus. The first method was found to have an error of less than 10%, the second of 33%. More details of technique have been given previously[2–4].

Results. Preliminarily drugs were screened for antiviral action on Polio 1 and Vaccinia viruses. Data from these experiments, referred to in table 1, show that, as already observed for 2-amino-4,6-dichloropyrimidine[2–4], all dichloropyrimidines tested inhibit the growth of both viruses by more than 90%. On the other hand, no other drugs reduce the yield of either viruses by more than 50%. The latter, practically inactive drugs, include monochlorinated, trichlorinated, tetrachlorinated pyrimidines as well as bichlorinated derivatives of purines, pyridine, pyridazine, benzene and salicylic acid.

Dichloropyrimidines share a common antiviral spectrum. Besides Polio 1 and Vaccinia, these drugs also inhibit Coxsackie B_1, and HSV, while they are ineffective on EMC, NDV, VSV, irrespective of the type of cell substrate adopted in the tests (table 2).

Table 2. Antiviral spectrum of dichloropyrimidines

Pyrimidine in Eagle's MEM	µg/ml*	Virus yield (in IU) in % of untreated controls									
		Polio 1	Coxs. B1	Vaccinia		HSV		EMC		VSV	NDV
		H**	H	H	M**	H	M	H	M	H	H
2,4-Dichloro-	3	7.9	5.0	6.3	5.1	7.8	5.0	> 50	> 50	> 50	> 50
4,6-Dichloro-	12	1.2	0.5	2.5	1.6	6.2	5.0	> 50	> 50	> 50	> 50
6-Methyl-2,4-dichloro-	12	7.9	6.3	6.3	5.2	7.9	6.2	> 50	> 50	> 50	> 50
2-Amino-4,6-dichloro-	100	0.5	0.3	1.5	1.2	5.1	3.9	> 50	> 50	> 50	> 50

* Corresponding to 2/3 of 1 MNCTD; ** H, in HEp 2 cells; M, in mouse embryo cells.

Table 3. Antagonism and potentiation produced by glutamine and cysteine and by 2-mercaptoethanol on antiviral effect of dichloropyrimidines

Pyrimidine in the medium	µg/ml	Poliovirus yield (in IU)			Poliovirus yield (in IU)		Vaccinia virus yield (in IU)	
		in AFE*	in AFE+ glutamine+ cysteine (20 µg/ml)	in AFE+ thymidine+ uridine+ cytidine (20 µg/ml)	in MEM**	in MEM+ mercapto-ethanol (3 µg/ml)	in MEM	in MEM+ mercaptoethanol (3 µg/ml)
–		6.6×10^7	8×10^7	5.4×10^7	8.5×10^7	7.6×10^7	2.1×10^7	2.3×10^7
2,4-Dichloro-	3	1.4×10^5	9.5×10^6	1.8×10^5	7.1×10^6	6.0×10^5	8.2×10^5	3.3×10^5
4,6-Dichloro-	12	3.9×10^4	6.8×10^6	4.4×10^4	7.8×10^5	6.1×10^4	6.6×10^5	1.3×10^5
6-Methyl-2,4-dichloro-	12	2.5×10^5	9.2×10^6	1.9×10^6	3.5×10^6	4.9×10^5	9.2×10^5	2.5×10^5
2-Amino-4,6-dichloro-	100	2.8×10^4	1.1×10^7	4.7×10^4	3.9×10^5	4.1×10^4	4.8×10^5	8.6×10^4

* Amino acid free Eagle's MEM. ** Complete Eagle's MEM.

Table 4. Comparison of the antipolio effects of dichloropyrimidines

Pyrimidine in AFE* medium	MNCTD** (μg/ml)	ID 95*** (μg/ml)	ID 95/MNCTD
2,4-Dichloropyrimidines	5	2.1	0.42
4,6-Dichloropyrimidine	20	4.6	0.23
6-Methyl-2,4-dichloro-pyrimidine	20	9.2	0.46
2-Amino-4,6-dichloro-pyrimidine	150	31.5	0.21

*Amino acid free Eagle's MEM. **Maximum non-cytotoxic dose. ***Minimum dose producing 95% inhibition on virus growth.

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Moreover, as already observed for 2-amino-4,6-dichloropyrimidine, the antiviral effect of the dichloropyrimidines is antagonized, in amino-acid-free-medium, by glutamine and cysteine, but not by pyrimidine precursors of nucleic acids. In complete media, containing glutamine and cysteine (or cystine) in the amino acid supplement, the antiviral effect is potentiated by 2-mercaptoethanol (table 3).

The limited number of compounds tested so far does not permit any conclusion on structure activity relationship inside the dichloropyrimidine group. As shown in table 4, it can only be said, at present, that the 4,6 positions of Chlorine atoms in the pyrimidine ring are to be preferred to the 2,4 (2,6) positions in that the former enhance the therapeutic index of the molecule.

Bacteriophage T4 mutants which propagate on E. coli K12 but not on E. coli B

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Summary. We have isolated and characterized 2 mutants of coliphage T4 which are able to propagate on E. coli K12 but not on E. coli B. We have assigned the mutations to genes 8 and 53, both structural genes. The products of genes 8 and 53 are found in the baseplate.

In an effort to understand the bacterial functions which are necessary for proper phage development, we have isolated and characterized many bacterial mutants unable to propagate bacteriophage λ. We have found 2 classes of such mutants which block λ DNA replication[2,3], another 2 classes which block λ RNA transcription[4,5] and a fifth class which affects the morphogenesis of several phages including λ, T4 and T5[6]. In the present report, we have extended our studies of host-phage interactions and show that one can isolate T4 mutants which discriminate between 2 naturally occurring hosts, E. coli K12 and E. coli B.

Materials and methods. The E. coli B^E sup$^-$ (called B) and E. coli K12 W3101 sup$^-$ (called K12) were the bacterial hosts. In order to isolate T4 mutants which propagate on K12 but not on B, a nitrosoguanidine mutagenized stock of bacteriophage T4rI was absorbed to K12 cells and the infected cells plated on a mixed bacterial lawn of K12 and B. We anticipated that derivatives of T4rI which grow on K12 but not on B would form small, turbid plaques on this mixed bacterial lawn as opposed to the large, clear plaques made by the parent strain. Plating efficiency of transfer and phage yield were as previously described[2]. Complementation tests were done in liquid by infecting the non-permissive Bsup$^-$ bacteria with 5 phage of each type per bacterium, and allowing the culture to lyse at 37 °C.

Results and discussion. 2 T4rI derivatives, called No. 4 and No. 20, were isolated as being able to propagate on K12 bacteria but not on B bacteria. The frequency of occurrence after nitrosoguanidine mutagenesis was approximately 5×10^{-4}. Table 1 shows that the growth of the 2 mutants is slightly depressed on the K12 host, but is severely inhibited on B (regardless of whether they are sup$^-$ or sup$^+$).

Preliminary experiments showed that both T4 No. 4 or T4 No. 20 infected bacteria lysed after 20 min of growth at 37 °C, indicating that the early events of infection as well as cell lysis functions occur normally during the abortive infection, and that the failure to yield phage results from a block at the level of phage morphogenesis. Subsequently we tested by spot complementation on Bsup$^-$ T4 No. 4 and T4 No. 20 against amber mutations in all T4 late genes. We found that T4 No. 4 complemented phage mutants in all genes except gene 8 and that T4 No. 20 did not complement phage mutants in gene 53 (table 2). From recombination data obtained on the K12 sup$^+$ host, it appears that the T4 No. 4 mutation is very

Table 1

Phage	e.o.p.* on B	e.o.p.* on K12	e.o.t.** on B	e.o.t.** on K12	Phage yield*** on B	Phage yield*** on K12
T4rI	1.0	1.0	1.0	1.0	65	95
T4rI No. 4	2.0×10^{-6}	1.0	0.4	1.0	1.1	29
T4rI No. 20	3.0×10^{-6}	1.0	0.3	1.0	1.2	27

*e.o.p., the efficiency of plating, denotes the number of plaques produced by a phage strain on a given bacterial host relative to the number on K12; **e.o.t., the efficiency of transmission, denotes the probability that an infected bacterial host will produce at least one viable phage progeny; ***phage yield denotes the average number of viable phage progeny released per infectious center.

1 Supported by grant No 3.519.75 from the Fonds National Suisse de la Recherche Scientifique.

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