

CARYOLOGICAL EFFECT OF VERMICULINE ON HELA CELLS

Sir:

The antibiotic vermiculine belongs to the group of aglycoside macrolide dilactones^{1,2}. The substance was isolated from the filtrate of a culture of *Penicillium vermiculatum* DANGEARD ATCC 26005³. In concentrations of 50 µg/ml the substance slightly inhibits the growth of Gram-positive bacteria and of some protozoa (*Trypanosoma cruzi*, *Leishmania braziliensis*), the growth of yeasts and *Mycobacteria* was suppressed by concentrations from 100 to 500 µg/ml. In *in vitro* experiments the substance showed a strong cytotoxic effect against the following cells: EHRlich ascites carcinoma, lymphoma NK/Ly, sarcoma 37, leukemia L 5178 and L-1210⁴. In HeLa cells proliferating *in vitro* the antibiotic inhibited the DNA synthesis in the first place⁵. In the present paper we studied the chromosomal abnormalities caused by vermiculine in HeLa cells.

HeLa cells cultivated *in vitro* at 37°C in the basal EAGLE'S medium (BEM) were used. Portions of the cell suspension were removed after 24, 48, and 72-hours' action of vermiculine (2.5 µg/ml) and caryological changes in treated cells were evaluated. In the given time intervals 50 microphotographs of the c-metaphase of the control as well as of the treated cells were evaluated. In every photographed cell the numbers of chromosomes and their distribution according to the position of the centromere and number and type of chromosomal abnormalities were established. The results are presented in Tables 1 and 2.

Caryological abnormalities caused by vermiculine in HeLa cells were found already after 24 hours and then during the whole experiment. Due to multiple chromatid exchanges and centromeric spreading many of the chromosomes were strongly deformed so that it was not possible to classify them according to the centromere position (Table 1). In most of the treated cells aggregations of chromatin material without chromosomal structure were found. In Table 2 these abnormalities are described as chromatine clumps. In the control cells these changes were not observed. In the treated cells also fragments of the chromosomes and centromeric spreading were found in a relatively high frequency.

Table 1. Chromosome distribution in control and vermiculine-treated HeLa cells according to the centromere position. In experiment and control per 50 c-metaphases were checked.

Type of chromosome*	Duration of treatment (hours)	Concentration of vermiculine	
		2.5 µg/ml	Control
M	24	2	7
	48	6	10
	72	4	9
m	24	8	15
	48	5	14
	72	9	12
sm	24	21	19
	48	17	20
	72	18	17
st	24	6	8
	48	7	9
	72	5	9
t+T	24	7	9
	48	5	10
	72	7	8

* Chromosomes are classified into 5 groups according to morphology: mediocentric (M), metacentric (m), submetacentric (sm), subtelocentric (st), acrocentric (t) and telocentric (T).

Vermiculine decreased the number of chromosomes which could be arranged according to the centromere position. The total number of deformed and non-deformed chromosomes in the treated cells was also slightly decreased.

The pronounced mutagenic effect of vermiculine in animal cells is in good correlation with the effect of other antibiotics having a lactone ring in their molecules^{6,7}. Theoretical structural considerations suggest that, in view of the ability of lactones to combine with nucleic acids, vermiculine could react in a similar way as known alkylating compounds⁸.

VLADIMÍR FRANK

JÁN FUSKA

Department of Technical Microbiology and Biochemistry, Slovak Technical University, Jánska 1, 880 37 Bratislava, Czechoslovakia

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Table 2. Types and frequency of chromosome aberrations in vermiculine treated HeLa cells. In experimental and control cells per 50 metaphases were checked.

Type of aberration	Duration of treatment (hours)	2.5 μ g of vermiculine per ml		Control	
		Number of cells with aberration	Total number of aberrations	Number of cells with aberration	Total number of aberrations
Deformed chromosomes	24	48	330	0	0
	48	46	292	0	0
	72	48	277	0	0
Centromeric spreading	24	42	174	5	17
	48	34	136	8	27
	72	30	117	9	31
Chromatin clumps	24	48	392	0	0
	48	45	248	0	0
	72	49	303	0	0
Fragments	24	27	56	2	2
	48	36	59	0	0
	72	33	64	0	0
Chromatid breaks	24	24	48	1	1
	48	33	51	1	1
	72	36	42	0	0
Chromatid exchanges	24	32	54	0	0
	48	22	49	0	0
	72	26	61	0	0
Dicentrics	24	13	13	0	0
	48	10	12	0	0
	72	13	16	0	0

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