MOLECULAR-BIOLOGICAL EFFECTS OF THALLIUM CARBONATE

G. D. Zasukhina, G. N. Krasovskii, I. M. Vasil'eva, N. I. Sdirkova, V. V. Sokolovskii, U. I. Kenesariev, and L. Ya. Vasyukovich

UDC 612.6.052:612.398.145.1]-014.46:546.683

KEY WORDS: thallium carbonate; breaks in DNA; mutagenesis of vaccinia virus; chromosomal aberrations; dominant lethals.

Environmental pollution by heavy metals insistently demands a comprehensive study of their biological properties, with special reference to the establishment of hygienic norms. Since many of them have been shown to give genetic effects [1], evaluation of the genetic activity of various compounds of heavy metals which may contaminate the human environment is extremely important.

This paper describes the results of an investigation of the genetic activity of thallium carbonate (Tl_2CO_3), using as the criterion the action of this substance on DNA, by comparison with other methods: induction of chromosomal aberrations and mutability in viruses, and also the appearance of dominant lethals. Thallium was chosen as the model because of the widespread use of this element in industry and, consequently, the possibility that it may enter the external environment, by discharge into watercourses of industrial effluents, for example [2]. In regions where thallium ores are found its concentration in reservoir water may reach 2.7 mg/liter [4]. The concentration of this element in water and mud deposits of rivers and lakes in the USA and Western Europe may reach 88.2 μg/liter [6].

EXPERIMENTAL METHOD

Experiments were carried out on cultures of rat embryonic fibroblasts prepared in the usual way. To determine the number of single-stranded breaks the cell DNA was labeled with thymidine-^3H (1 mCi/ml medium) for 24 h and covered with fresh medium containing Tl_2CO_3. After incubation with the mutagen the cells were suspended in 0.1 M NaCl in a concentration of $1 \cdot 10^5$ to $1 \cdot 10^6$ cells/ml. Lysis of the cells and chromatography of the cell lysates on columns with hydroxyapatite were carried out by Rydberg's method [5]. The DNA content in the fractions was determined by the amount of label precipitated by 5% TCA on Synpore No. 4 millipore filters.

To determine the survival rate and mutability of vaccinia virus in the test culture it was infected with this virus (Lister strain); the duration of contact was 40 min. Intracellular virus was treated with Tl_2CO_3 solution and the cells were washed and titrated by the plaque method on a culture of chick fibroblasts.

TABLE 1. Action of Tl_2CO_3 on DNA of Rat Fibroblasts (M ± m)

Tl_2CO_3, M	Double-stranded DNA, % of control	
	mutagen 24 h	postincubation 24 h
$1 \cdot 10^{-5}$	82±2	88±2
$1 \cdot 10^{-6}$	73±2	78±2
$1 \cdot 10^{-4}$	47±2	64±4

Laboratory of Genetics of Viruses, Institute of General Genetics, Academy of Sciences of the USSR. Laboratory of Toxicology of Water Pollution, A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 723-725, December, 1980. Original article submitted February 11, 1980.

0007-4888/80/9012-1731$07.50

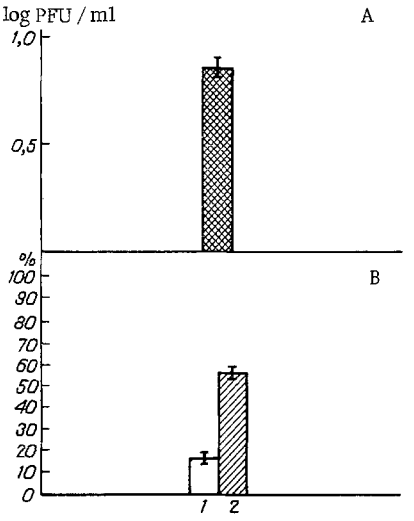

Fig. 1. Effect of thallium salt (Tl_2CO_3) on vaccinia virus in rat fibroblasts. A) Difference between survival rate of vaccinia virus in rat embryonic fibroblasts in control and after treatment with $1 \cdot 10^{-4}$ M Tl_2CO_3) for 24 h. Ordinate, survival rate (in log PFU/ml). B) Spontaneous mutagenesis (1) and mutagenesis induced (2) by $1 \cdot 10^{-4}$ M Tl_2CO_3 for 24 h in vaccinia virus in culture of rat embryonic cells. Ordinate, small plaque variants (in %).

The optimal time of addition of the mutagen to the culture to obtain the largest number of metaphases among the cells when studying chromosomal aberrations was after 48 h. Exposure to the mutagen lasted 24 h. Colchicine (0.5 μg to 1 ml of culture) was added 4 h before fixation. Cells were taken by active pipeting with EDTA solution and medium 199 consecutively. Hypotonia was produced with 0.54% KCl solution for 15 min at 37°C. Fixation with a mixture of methyl alcohol and glacial acetic acid (3 : 1) was carried out twice for 15 min each time, at 22°C. The cells were stained with azure–eosin. Each experiment was repeated three times. Induction of dominant lethals was studied by the method described in [3]; the poison was administered to the animals perorally.

EXPERIMENTAL RESULTS

The ability of Tl_2CO_3 to induce single-stranded breaks in DNA was studied by a chromatographic method. Cultures of rat fibroblasts were treated for 24 h with different concentrations of Tl_2CO_3. The number of breaks arising in the DNA molecule was found to depend on the concentration of the salt used (Table 1). Post-incubation of the culture in growth medium for 24 h partially restored the double-stranded structure of DNA.

As Table 1 shows, immediately after treatment of the cells with Tl_2CO_3 in a concentration of $1 \cdot 10^{-4}$ M nearly half of the DNA was single-stranded, whereas in a concentration of $1 \cdot 10^{-6}$ M only 18-20% of the DNA was single-stranded. With the concentrations of the compound used, complete restoration of the original double-stranded structure of DNA was not observed after postincubation for 24 h. However, repair was more marked in the case of low concentrations of thallium.

The second method used to determine the mutagenicity of Tl_2CO_3 was to study the survival rate and mutability of vaccinia virus in rat fibroblasts treated with this salt. Viruses can be used as a test system for the evaluation of environmental mutagens. Determination of the mutagenic potential of compounds in the cells of higher organisms correlates as a rule with the mutagenic response in viruses.

Rat embryonic fibroblasts, infected with vaccinia virus, were treated with Tl_2CO_3 ($1 \cdot 10^{-4}$ M) for 24 h. The number of plaque-forming units in 1 ml (PFU/ml) was counted by the titration method and the size of the plaques was measured. The survival rate of the virus reflected the difference in titers of the virus (log PFU/ml) between the control and Tl_2CO_3 treated material. The yield of small plaque variants characterized the number of induced mutations (Fig. 1). Analysis of the results showed that the survival rate of vaccinia virus in rat fibroblasts treated with Tl_2CO_3 was significantly lower than in the control. The percentage of

TABLE 2. Induction of Chromosomal Aberrations in Rat Fibroblasts under the Influence of Tl_2CO_3

Tl_2CO_3, M	Number of repetitions	Number of metaphases analyzed	Number of aberrant cells	Number of aberrations	Number of aberrant cells per 100	Number of aberrations per 100 cells
Control	3	300	5	5	1,66	1,66
$1 \cdot 10^{-6}$	3	275	17	20	6,18	7,27
$1 \cdot 10^{-5}$	3	350	43	55	12,29	15,71

TABLE 3. Frequency of Dominant Mutations in Intact Female Albino Rats after Mating with Males Poisoned with Tl_2CO_3 for 8 Months ($M \pm m$)

Dose, mg/kg	Number of pregnant rats	Number of postimplantation sites	Number of corpora lutea of pregnancy (C)	Number of living embryos (A)	Number of resorptions (B)	Mortality before implantation, $C-(A+B)/C$	Mortality after implantation, $\frac{B}{A+B}$	Over-all embryonic mortality, $\frac{C-A}{C} \cdot 100\%$
Control	16	10,93±0,39	11,75±0,33	10,87±0,37	0,87±0,13	0,06±0,2	0,013±0,009	6,16±1,64
$5 \cdot 10^{-6}$	18	11,05±0,49	11,72±0,35	10,83±0,47	0,22±0,12	0,06±0,02	0,018±0,010	7,42±1,60
$5 \cdot 10^{-5}$	18	10,38±0,48	11,11±0,35	10,05±0,49	0,33±0,13	0,07±0,02	0,031±0,012	10,03±2,10
$5 \cdot 10^{-4}$	18	10,11±0,48	11,55±0,40	9,77±0,48	0,27±0,11	0,08±0,22	0,027±0,010	10,97±1,80

small plaque variants in the experimental material was three times greater than the spontaneous virus mutagenesis in the control cells.

The same concentrations of Tl_2CO_3 and the same exposure were used to study the yield of chromosomal aberrations in test cell cultures (Table 2). The yield of chromosomal aberrations after exposure to Tl_2CO_3 in a concentration of $1 \cdot 10^{-5}$ M was about six times, and in a concentration of $1 \cdot 10^{-6}$ M three times, higher than the spontaneous level. The aberrations were chromosome and chromatid fragments, except in only one case when a dicentric chromosome was identified (in $1 \cdot 10^{-5}$ M Tl_2CO_3).

Chronic administration of the thallium salt perorally to male rats followed by mating with intact females showed a tendency for the overall embryonic mortality to rise after doses of $5 \cdot 10^{-5}$ and $5 \cdot 10^{-4}$ mg/kg body weight (Table 3). After administration of the thallium salt in a dose of $5 \cdot 10^{-5}$ mg/kg the changes observed affected only certain parameters specific for this element: this dose can be regarded as on the threshold of action of thallium under conditions of chronic poisoning.

As a result of the action of Tl_2CO_3 on cultures of rat fibroblasts definite correlation was thus observed between induction of DNA breaks, the formation of chromosomal aberrations, and inactivation and mutability of vaccinia virus. At the same time, the compound was shown to possess mutagenic activity in the dominant lethals test in rats.

LITERATURE CITED

1. G. N. Krasovskii and V. V. Sokolovskii, Gig. San., No. 9, 56 (1979).
2. V. V. Korsunskaya, in: Proceedings of a Republican Conference on Hygienic Water Supplies and Safeguarding of Reservoir Hygiene [in Russian], Perm (1969), p. 110.
3. World Health Organization Technical Reports Series, No. 333. The Chemistry and Physiology of Gametes, Geneva (1968).
4. S. L. Shvartsev and L. N. Gomonova, Dokl. Akad. Nauk SSSR, 220, 1183 (1975).
5. B. Rydberg, Radiat. Res., 61, 274 (1975).
6. V. Zitko and W. V. Garson, Bull. Environ. Contam. Toxicol., 14, 530 (1975).