# INTERCHANGEABILITY OF MUTAGENS DURING FRACTIONAL EXPOSURE TO UNEQUAL CONCENTRATIONS OF ALKYLATING AGENTS

A. N. Tulina and A. N. Chebotarev

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Recent investigations [1, 2] have shown the ability of certain mutagens to replace one another in the formation of the effect of "crossed clastogenic adaptation," as a result of which preliminary treatment with a low concentration of one mutagen "protects" cells against the powerful mutagenic action of the basic concentration of another mutagen. The selectivity of the interchangeability effect relative to different combinations of mutagens in the "crossed clastogenic adaptation" phenomenon is evidence that certain criteria exist on the basis of which a combination of substances will be able or unable to potentiate the "clastogenic adaptation" effect. It was accordingly decided to study from this aspbct thiotepa and dipin,* for despite the evident similarity of their chemical structure, they have different numbers of mutagenic centers and, consequently, different mechanisms of formation of chromosomal aberrations. This paper gives data on the interchangeability of dipin and thiotepa in the formation of the effect of "clastogenic adaptation" of cells.

## EXPERIMENTAL METHOD

Experiments were carried out on a culture of human lymphocytes at the $G_0$ stage before stimulation by phytohemagglutinin (PHA). The basic concentration of the mutagens (monocentric thiotepa and dicentric dipin) was 20 μg/ml. The preliminary concentrations were 10, $10^2$, $10^3$, $10^4$, and $10^5$ times below the basic concentration. The schedule of the experiments was the same for both variants: 10 ml of cultural mixture, consisting of 1 part of whole blood, 3 parts of bovine serum, and 12 parts of Eagle's medium, was treated with the five preliminary concentrations of thiotepa (in the variant without preliminary treatment 1 ml of Hanks' solution was added to the cultural mixture) and incubated at 37°C for 1 h. The cells were then washed twice with Eagle's medium, the cultural mixture was changed, and incubation was repeated without the mutagen at 37°C for 2 h. Treatment with the basic concentration of dipin was then carried out, with an exposure of 1 h at 37°C (in the second version of the experiment, conversely, the preliminary treatment of the cells was with dipin and the basic treatment with thiotepa). The cells were then washed 3 times with Eagle's medium to remove the mutagen, the cultural mixture was changed, and PHA ("Difco P") was added at the rate of 0.015 ml to 10 ml of the cultural mixture. Colchicine was added in a concentration of 0.5 μg/ml 2 h before fixation. The culture was fixed at the 56th hour of incubation with a mixture of methanol and glacial acetic acid in the ratio of 3:1. The preparations obtained were stained with azure-eosin. In each version from 100 to 200 metaphases were analyzed. The 95% confidence intervals for the percentage of aberrant metaphases and the number of aberrations were calculated by Eqs. (1) and (2) respectively. The significance of differences was estimated by the chi-square test:

$$\left.\begin{matrix}P_2\\P_1\end{matrix}\right\} = \frac{1}{n+3{,}84}\left[nP + 1{,}92 \pm 0{,}5 \pm 1{,}96 \times \right.$$
$$\left. \times \sqrt{\frac{(nP \pm 0{,}5)(n - nP \pm 0{,}5)}{n}} + 0{,}86\right], \quad (1)$$

$$\left.\begin{matrix}X_2\\X_1\end{matrix}\right\} = 4\sum_{i=1}^{n} Xi/(\sqrt{4n-1} \pm 1{,}96)^2 \quad (2)$$

*Tetraethylenimide of piperazine-N,N'-diphosphoric acid.

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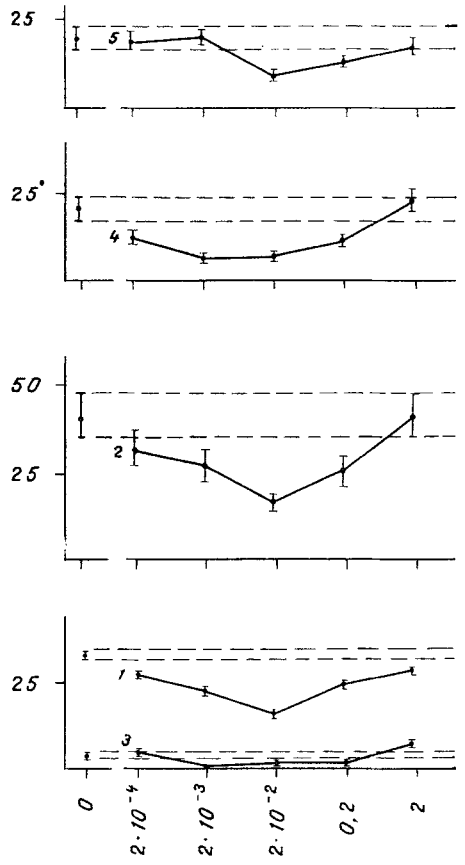


Fig. 1

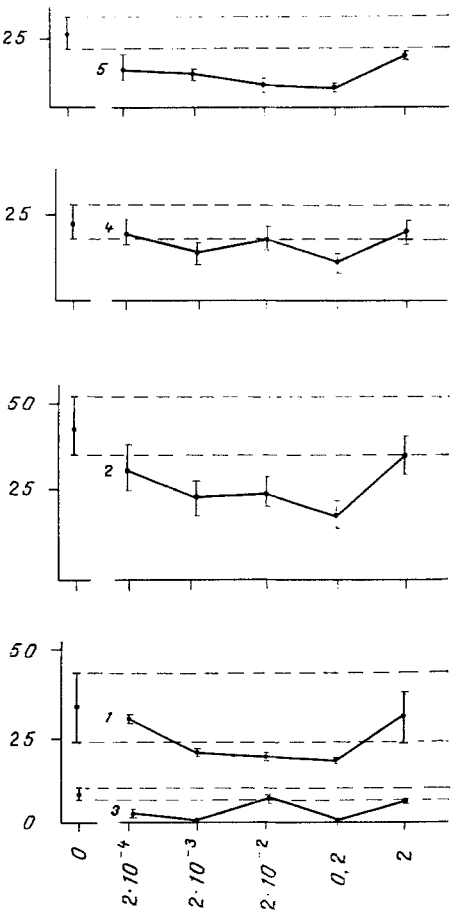


Fig. 2

Fig. 1. Frequency of chromosomal aberrations due to the action of 20 μg/ml of dipin preceded by treatment with various concentrations of thiotepa. Abscissa, concentration of thiotepa for preliminary treatment; ordinate, number of aberrations per 100 cells (for fraction of aberrant metaphases — %). 1) Fraction of aberrant metaphases, 2) total number of breaks, 3) number of breaks in exchanges, 4) number of chromatid breaks, 5) number of chromosomal and isochromatid breaks.

Fig. 2. Frequency of chromosomal aberrations during the action of 20 μg/ml of thiotepa preceded by treatment with various concentrations of dipin. Abscissa, dipin concentration for preliminary treatment; ordinate, number of aberrations per 100 cells (for fraction of aberrant metaphases — %). 1) Fraction of aberrant metaphases, 2) total number of breaks, 3) number of breaks in exchanges, 4) number of chromatid breaks, 5) number of chromosomal and isochromatid breaks.

where n is the number of cells analyzed, P the fraction of aberrant metaphases, and $\Sigma X_i$ the number of chromosomal aberrations.

## EXPERIMENTAL RESULTS

The experimental results are illustrated in Fig. 1 for the thiotepa/dipin version and in Fig. 2 for the dipin/thiotepa version. The vertical lines correspond to 95% confidence intervals. The zone bounded by the broken lines corresponds to the 95% confidence intervals for versions treated with the mutagen, but without preliminary treatment. It will be clear from Fig. 1 that the effect of the basic concentration of dipin was reduced after preliminary treatment with thiotepa in concentrations equal to 0.2 μg/ml for the number of chromosome breaks ($p < 0.01$), the number of single breaks ($p < 0.05$), and $2 \times 10^{-2}$ μg/ml for the fraction of aberrant metaphase ($p < 0.001$), the number of chromosomal breaks ($p < 0.001$), the number of single breaks ($p < 0.001$), the number of paired breaks ($p < 0.001$), and $2 \times 10^{-3}$ μg/ml for the number of chromosomal breaks ($p < 0.05$) and the number of single breaks ($p < 0.001$). The same effect was observed for the version in which dipin was used for preliminary treatment and the

basic exposure was to thiotepa, except in the case when preliminary treatment was given with thiotepa and the number of chromosomal breaks was significantly reduced mainly on account of a fall in the level of chromatid (single) breaks, and in the version in which preliminary treatment with dipin was given and the decrease in the number of chromosomal breaks took place as a result of a decrease in the number of paired breaks. The results of the experiments indicate clearly that a combination of dipin and thiotepa leads to the appearance of an effect of crossed "clastogenic adaptation."

The following hypothesis can be put forward on the basis of analysis of the results. Despite differences in the mechanisms of formation of chromosomal aberrations, both dipin and thiotepa can weaken each other's mutagenic effect due to the phenomenon of "crossed clastogenic adaptation," but at the same time the character of the mutagen which was used for preliminary treatment affects the predominant repair of single or paired breaks. This fact suggests that the preliminary concentration not only activates a set of repair enzymes, but also "tunes" the repair system for eradication of injuries typical of that particular mutagen. For the "crossed clastogenic adaptation" effect to arise between two mutagens, it is evidently essential that similar types of lesions be found in the spectrum of chromosomal aberrations induced by these mutagens.

LITERATURE CITED

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# CHANGES IN GENE EXPRESSION IN THE RAT BRAIN INDUCED BY ZAJDELA'S ASCITES HEPATOMA

V. P. Shelepov, V. L. Moiseev,
M. M. Zaboikin, V. A. Chekulaev,
A. V. Likhtenshtein, and V. S. Shapot*

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The development of a malignant neoplasm is accompanied by progressive and varied disturbances of homeostasis, which even affect such a "protected" organ of the body as the brain. The writers showed previously that changes in carbohydrate and lipid metabolism in rat brain tissue are among the principal manifestations of the "distant" action of transplantable hepatomas H-27 and ZAH [3]. The possibility cannot be ruled out that weakening of the functional capacity of the brain regulating centers (especially those controlling the internal medium of the body), arising as a result of this action may take a contribution to the formation of metabolic "vicious circles" leading ultimately to death.

The aim of this investigation was to study expression of certain genes in the brain of animals with a rapidly growing transplantable Zajdela's ascites hepatoma (ZAH; the animals survived for 5-6 days).

The test objects were the genes of actin (a constitutively expressed "household" gene, the c-Ha-ras protooncogene conjecturally coding for G-proteins involved in signal transmission from a surface receptor to the internal medium of the cell [5]; the "stressor" hsp70 gene, induced by various unfavorable factors and, in particular, by heat shock (and, consequently, by a febrile state), by toxic compounds, and disturbances of the energy supply [6, 9, 10], i.e., by factors whose existence can be regarded as perfectly feasible in a tumor-bearing organism.

*Corresponding Member, Academy of Medical Sciences of the USSR.

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