DYNAMICS OF CHANGES IN FREQUENCIES OF CYTOGENETIC EFFECTS AFTER EXPOSURE *IN VIVO* TO MUTAGENS

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UDC 615.28.015.4:575.224.5

KEY WORDS: thiophosphamide; chromosomal aberrations; sister chromatid exchanges.

Accuracy and adequacy of evaluation of the effects of chemotherapy and exposure to mutagens *in vivo*, with the use of analysis of chromosomal aberrations (CA) and sister chromatid exchanges (SCE), depend on the correct choice of time for carrying out analysis after mutagenic (or chemotherapeutic) treatment. In turn the time interval during which the frequencies of SCE and CA must be analyzed to allow conclusions to be drawn on the cytogenetic activity of the substance depends on the time of appearance and the duration of existence of a raised level of frequencies of SCE and CA among circulating lymphocytes.

Data in the literature on the study of frequencies of SCE and CA after a single exposure to a mutagen *in vivo* are contradictory. Accordingly, the object of the present investigation was to make a detailed study of the time course of changes in frequencies of SCE and CA in the immediate period after exposure to chemicals.

EXPERIMENTAL METHOD

Experiments were carried out on three grey chinchilla rabbits into which thiophosphamide (T) was injected intravenously in a dose of 3 mg (rabbit 1), 5 mg (rabbit 2), and 8 mg (rabbit 3) per kilogram body weight. At different time intervals (from 5 to 360 min) blood was taken from the rabbits for lymphocyte culture in the presence of 5-bromodeoxyuridine dR) and subsequent analysis of the frequencies of SCE in the second mitosis and of CA in the first mitosis after treatment.

EXPERIMENTAL RESULTS

It was shown in previous communications [1] that the fraction of aberrant metaphases (ρ) and the number of chromosomal breaks per cell (X) increase exponentially with an increase in the dose of mutagenic action, defined as the integral with respect to time of the dependence of concentration T on time after administration of the substance.

The relationship of X and ρ with dose of mutagenic action *in vivo* is in the form:

$$X = e^{(a+kD)^2} - 1 \tag{1}$$

$$\rho = 1 - e^{-(a+kD)^2}, \tag{2}$$

where a and k are parameters of the equation. If instead of the value of D (the dose of mutagenic action) we substitute an expression for its determination

$$D = \frac{C_0}{h}(1 - e^{-ht}), \tag{3}$$

where C_0 is the initial concentration of the substance; h, the elimination constant of T, which includes a variable t, we obtain the dependence of X and ρ on time after administration of the mutagen:

$$X = e^{\left[k\frac{C_0}{h}(1-e^{-ht})+a\right]^2} - 1 \tag{4}$$

$$\rho = 1 - e^{-\left[k\frac{C_0}{h}(1-e^{-ht})+a\right]^2}. \tag{5}$$

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 11, pp. 91-93, November, 1983. Original article submitted January 20, 1983.

 0007-4888/83/9611-1602$07.50

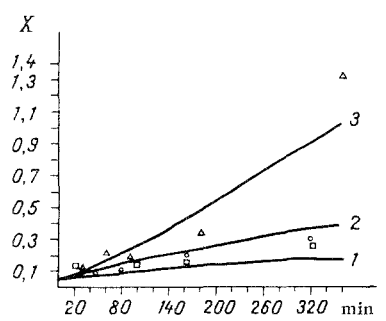

Fig. 1

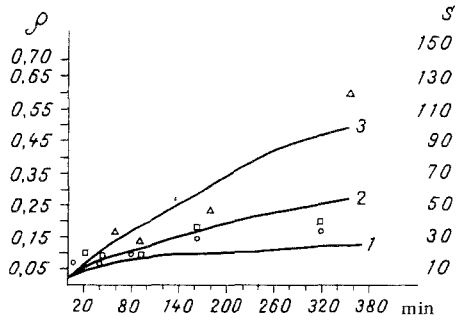

Fig. 2

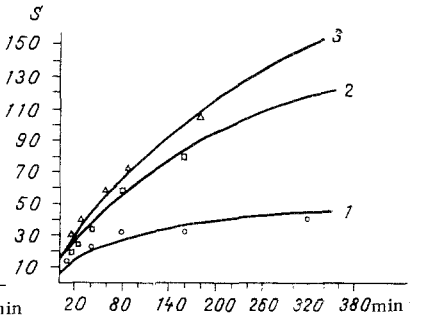

Fig. 3

Fig. 1. Dynamics of change in number of chromosomal breaks per cell (X) after administration of T *in vivo*. Here and in Figs. 2 and 3: 1 (circles) — 3 mg/kg, 2 (squares) — 5 mg/kg, 3 (triangles) — 8 mg/kg.

Fig. 2. Dynamics of change in fraction of aberrant metaphases (ρ) after administration of T *in vivo*.

Fig. 3. Dynamics of change in number of SCE (S) per cell after administration of T *in vivo*.

The experimental points and regression lines calculated by equation (4), showing dependence of X on the time of taking blood after injection of thiophosphamide are illustrated in Fig. 1. The same for dependence of ρ is shown in Fig. 2, in which the regression lines were calculated by equation (5). As will be clear from Figs. 1 and 2, the theoretical lines describe the experimental points well.

The number of SCE is a linear function of the dose given *in vivo* [2]; this relationship is in the form:

$$S = b_0 + b_1 D. \tag{6}$$

If the expression (3) for determining dose is substituted in this equation, we obtain a relationship between S and the time after injection of the substance:

$$S = b_0 - b_1 \frac{C_0}{h}(1 - e^{-ht}). \tag{7}$$

Experimental points and regression lines calculated by equation (7) are given in Fig. 3.

As will be clear from Figs. 1, 2, and 3, the theoretical lines suggested describe the experimental points well.

The frequency of CA and SCE tends toward a certain maximal value corresponding to maximal value of dose. These values can be calculated if, in equations (4, 5, and 6) the value of t is assigned an infinitely large value (i.e., $t \to \infty$), in which case these equations assume the form:

$$X = e^{\left(k\frac{C_0}{h} + a\right)^2} - 1, \tag{8}$$

$$\rho = 1 - e^{-\left(k\frac{C_0}{h} + a\right)^2} \tag{9}$$

$$S = b_0 + b_1 \frac{C_0}{h}. \tag{10}$$

In the experiment described maximal values of X, ρ, and S close to the calculated values were observed only after injection in a dose of 3 mg/kg. After injection of doses of 5 and 8 mg/kg the frequencies of SCE and CA continued to rise for the next 6 h; the number of SCE after 180 min reached very high values (100 exchanges per cell or more), which could not be recorded sufficiently accurately.

On reaching a certain value the frequencies of SCE and CA begin to decline [5, 6]. It is not known when the decline in the frequency of SCE and CA begins, whether this moment depends on the quantity of thiophosphamide injected, and whether maximal frequencies of SCE and CA reach the values which may be expected by equations (8, 9, and 10).

In the course of 6 h after injection of thiophosphamide in this investigation a logarithmic increase in the frequencies of SCE and CA with time after injection of the mutagen was thus observed. The dynamics of the change in frequencies of the cytogenetic effect depends on the initial quantity of thiophosphamide injected, but depends only a little on the elimination constant of the compound, for with a change in the dose injected, the values of the constant change only negligibly.

Equations for determining maximal cytogenetic effects of treatment with mutagens (8, 9, and 10) can be used to determine the maximal cytogenetic effects of small doses of mutagens, such as are used in chemotherapy and which do not induce an appreciable cytogenetic effect when about 100-200 cells are counted in the usual way [3]. In fact, if it is necessary to estimate the degree of cytogenetic damage which can be induced by administration of chemotherapeutic agents, this can be done before treatment. To do this it is necessary to know the spontaneous levels of the number of SCE and frequency of CA for a given individual or to use these parameters determined for the population. The efficacy constant of the given preparation, determined *in vitro*, and the illumination constant of the substance, determined by a study of its pharmacodynamics, and also the quantity of the substance injected must also be known.

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COMPARISON OF LEVEL OF SISTER CHROMATID EXCHANGES AND CHROMOSOMAL ABERRATIONS INDUCED BY CHEMICAL MUTAGENS *IN VITRO*

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UDC 575.224.46.044:576.312.36:578.083

KEY WORDS: mutagen; aberrations; chromatid exchange.

The use of analysis of sister chromatid exchanges (SCE) as a quantitative test for mutagenicity of chemicals has several advantages, the most important of which is the high sensitivity of the method [4, 5]. However, a great handicap to the wide use of this method is still uncertainty regarding the mechanisms of their appearance [12] and the connection with the formation of chromosomal aberrations, despite the many investigations devoted to this question [6, 12].

One approach to the solution of this problem may be a simultaneous quantitative study of dose dependences for the appearance of SCE and chromosomal aberrations induced by different chemical compounds, and the investigation described below was undertaken for that purpose.

EXPERIMENTAL METHOD

Dose dependences of five compounds with different numbers and types of alkylating groups were studied: mitomycin C — a bifunctional alkylating antibiotic, E39 — a quinone derivative with two ethylenimine groups, thiophosphamide and dipin — derivatives of aminophosphoric acid

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 11, pp. 93-95, November, 1983. Original article submitted April 29, 1983.

0007-4888/83/9611-1604$07.50