GENETICS

Effect of Mitomycin C on Distribution of Chromatid Exchanges and Their Dose Dependency in Resting Human Lymphocytes

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Cells distribution by the number of chromatid exchanges induced by mitomycin C corresponds to geometric distribution. The dependences of the number of chromatid exchanges and the number of fragments on the dose of the mutagen are described by exponential and linear curves, respectively. Quantitative regularities of mitomycin C-induced chromosome aberrations correspond to those of "monocentric" mutagens.

Key Words: *chromatid exchanges; mitomycin C; human lymphocytes*

It is currently accepted that environmental mutagens increase the frequency of mutations. Study of quantitative regularities of mutagen action is necessary for the prognosis of mutagen effect of the environment. The most well studied factor is ionizing radiation [7, 9,10]. However, chemical mutagens are more abundant. The effects of chemical mutagens, their dose dependency, and distribution of the cells by the number of aberrations are also intensively studied [1]. Chemical mutagens cause different aberrations, including fragments and exchanges, in particular, chromatid exchanges. Study of quantitative regularities of exchange aberrations is important, because this type of aberrations frequently occurs in various oncological diseases [11,12].

However, quantitative regularities of exchange aberrations caused by chemical factors are poorly studied. The purpose of our study was to examine quantitative regularities of chromatid exchange aberrations induced by chemical mutagens *in vitro* in cultures of human peripheral lymphocytes.

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MATERIALS AND METHODS

The culture of peripheral blood lymphocytes from a healthy donor was used in the study. Routine methods of culturing, preparation, staining, and cytogenetic analysis were used [8].

G_0 lymphocytes were incubated with mitomycin C (Sigma) for 1 h at 37°C, washed 3 times to remove mutagen, and incubated in a medium containing phytohemagglutinin (PanEko) for 51 h. Colchicine (0.5 μg/ml; Calbiochem) was added 2 h before fixation.

The correspondence between the observed and expected distributions was evaluated using χ^2 test. Regression equations were found by the method of least squares. The correspondence between regression curves and experimental data was evaluated by *F* test, the significance of regression parameters was evaluated using Student *t* test.

RESULTS

Chromosome aberrations were presented by chromatid and isochromatid fragments and chromatid exchanges (Table 1). The ratio of chromatid to chromosome and isochromosome aberrations was 8:1, which correspon-

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ded to the effect of "monocentric" mutagens [1]. No dicentric chromosomes and centric rings were found. This suggests that in all experiments only metaphases of the first cell division were analyzed.

Geometric, Poisson, and Pascal distributions were chosen as the expected distributions. Poisson distribution ($P(x)=m^x\times\exp(-m)/x!$) well describes cell distribution with radiation-induced aberrations, in particular, chromosome exchanges (dicentrics and rings) [3]. The cells exposed to chemical mutagens by the number of breaks are distributed according to geometric distribution ($P(x)=(1-\theta)\times\theta^x$) typical for monocentric mutagens and Pascal distribution for dicentric mutagens ($P(x)=C^x_{n+x-1}\times\theta^m\times(1-\theta)^x$) [1,5,6]. In these formulae m is the mean number of aberrations per cell, x in the number of aberrations in a cell, $P(x)$ is the number of cells with x aberrations, n=2 in Pascal distribution, θ is distribution parameter calculated as $m/(m+1)$ for geometric and $2/(m+2)$ for Pascal distribution, x=0, 1, 2, … .

In our experiments, distribution of cells by the number of chromatid exchanges induced by all mitomycin C concentrations fitted a geometric distribution (Table 2).

It was previously shown that cell distribution by the number of mitomycin C-induced breaks could be also described by geometric distribution [2] typical of monocentric mutagens [1,4,5].

Dose dependency of the total number of breaks induced by monocentric mutagens can be described by the formula:

$$M=\exp(a+bC)^2-1 \qquad \textbf{(1)},$$

where M is the number of breaks per cell, C is the concentration of mutagen, a and b are parameters.

Figure 1 presents experimental values of the number of chromatid exchanges induced by mitomycin C in various concentrations and regression dependency calculated by formula **(1)**. This formula adequately describes experimental data (F=1581.1, $P=3.0\times10^{-5}$); while a and b values are highly significant (t=5.365; P=0.0127 and t=21.58; P=0.00022, respectively). This figure also presents experimental values of the number of fragments and regression equation $M=a\times C+b$ with the same designations as in formula **(1)**. Regression analysis revealed linear increase in the number of fragments with increasing the dose of mitomycin C (F=83.35; P=0.00278). At the same time, only a parameter significantly differs from 0 (t=9.13; P=0.00278), while b value is insignificant (t=-0.78; P=0.49). Thus,

TABLE 1. Cell Distribution by the Number of Mitomycin C-Induced Chromatid Exchanges

Number of exchanges per cell	Mitomycin C concentration, μg/ml			
	0.5	1.0	2.0	4.0
0	686	1016	374	97
1	9	36	40	32
2	0	1	1	7
3	0	0	0	4
4 and more	0	0	0	2

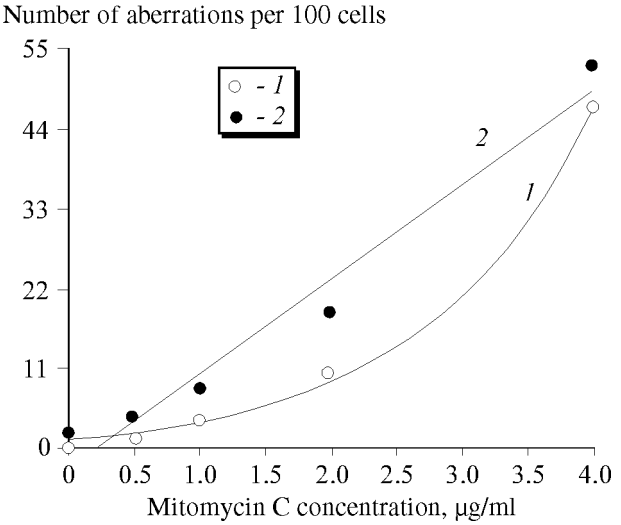

Fig. 1. Experimental data and calculated dose-response curves for the number of chromatid exchanges (*1*) and fragments (*2*) induced by mitomycin C.

TABLE 2. Correspondence between the Observed and Theoretical Cell Distributions by the Number of Chromatid Exchanges

Distribution		Mitomycin C concentration, μg/ml			
		0.5	1.0	2.0	4.0
Geometric	χ^2; *df*	2.541×10^{-3}; 0	0.1838; 1	2.644; 1	1.217; 3
	P	—	0.054	0.8961	0.2654
Poisson	χ^2; *df*	9.625×10^{-4}; 0	—	0.603; 1	14.314; 2
	P	—	—	0.5626	0.9992
Pascal	χ^2; *df*	1.67×10^{-3}; 0	—	1.599; 1	3.748; 2
	P	—	—	0.7940	0.8465

the number of fragments induced by mitomycin C directly correlates with mutagen concentration.

These results suggest that the dependency of the number of exchanges per cell on the dose of mutagen is adequately described by equation **(1)**, proposed for dose dependency of the number of breaks per cell induced by monocentric mutagens. The dependency of the number of fragments on the dose of mitomycin C shows linear character similar to that of the effect of ionizing radiation. In contrast to radiation causing Poisson distribution of exchange aberrations and linear-square dose dependency, mitomycin C induces geometric distribution and exponential dose response.

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