ANALYSIS OF TYPES OF CHROMOSOMAL ABERRATIONS FOLLOWING THE COMBINED ACTION OF CHEMICAL MUTAGENS

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Types of chromosomal aberrations in cultures of human lymphocytes exposed to the combined action of various concentrations of thiophosphamide and dipin, with different proportions of each, were studied. The mutagens acted on the G_0 stage. The range of concentrations used was from $3.17 \cdot 10^{-5}$ to $22.19 \cdot 10^{-5}$M. Equimolar concentrations of thiophosphamide inhibited more chromatid exchanges and fewer sister-strand (isolocus) unions than dipin, and it also induced a greater proportion of single breaks and a greater proportion of breaks in chromatid exchanges relative to the total number of chromosome breaks. Both the absolute and the relative frequencies of chromosomal aberrations depended on the concentration of the mutagens. A change in the ratio between thiophosphamide and dipin, if the total number of molecules of the two mutagens at the different concentration levels remained constant, gave rise to an effect whose level was between the effects of action of equimolar concentrations of the pure mutagens. This effect depended on the proportion of each mutagen in the combined treatment. It is concluded that the action of thiophosphamide and dipin is additive.

KEY WORDS: combined action of mutagens; thiophosphamide; dipin; chromosomal aberrations; lymphocytes.

There is no general agreement in the literature on the mechanisms of the combined action of mutagens. Synergism [2, 8], and the additive [9] or protective action [3, 4] of different combinations of mutagens have been described.

In this investigation the types of chromosomal aberrations following combined action of thiophosphamide* and dipin,† which possess cytogenetic specificity [1, 5, 6], were analyzed in order to elucidate the mechanisms of their combined action.

EXPERIMENTAL METHOD

Experiments were carried out on cultures of peripheral blood lymphocytes from the same donor. Two typical alkylating agents were used as mutagens, namely thiophosphamide and dipin, solutions of which were made up in Hanks' solution. Altogether 49 combinations of different concentrations of thiophosphamide and dipin (from $3.17 \cdot 10^{-5}$ to $22.19 \cdot 10^{-5}$ M) were tested. The experiments were planned so that with each of the above concentrations the total number of molecules of the two mutagens remained constant although the ratio between them varied. Blood was treated with the mutagens for 1 h, after which the cells were twice washed with 15 volumes of Hanks' solution. The conditions of culture of the lymphocytes with the addition of phytohemagglutinin (PHA) were standard. Each combination of mutagens was investigated in three experiments, in each of which 300 metaphases were analyzed. Before analysis, the preparations were coded. The experimental results were subjected to dispersion analysis. To stabilize the dispersions of the absolute and relative values the data were transformed by taking the square root and using the equation: $\varphi = 2 \arcsin \sqrt{p}$, where p is the fraction of a particular type of chromosomal aberration.

*2,4,6-tris(aziridine)-s-triazine.

†N,N′-bis(diaziridinyl-phosphinylidine)piperazine.

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TABLE 1. Number of Breaks in Chromatid Exchanges (I) and Sister-Strand Unions (II) in 100 Cells

Combined concentration of mutagens ($\cdot 10^{-5}$ M)	Ratio of thiophosphamide: dipin							
	6:0		4:2		2:4		0:6	
	I	II	I	II	I	II	I	II
3,17	0,7	0,7	0,7	0,7	0,7	2,0	0,7	1,0
6,34	6,7	2,0	2,7	3,7	1,3	1,0	5,3	4,0
9,51	4,7	2,0	4,0	3,7	2,0	5,0	0,7	4,7
12,68	21,0	3,0	8,3	4,3	10,0	6,7	12,7	6,3
15,85	26,0	5,3	20,7	7,7	16,7	11,3	19,3	10,0
19,02	38,7	9,3	36,3	9,0	35,0	14,3	28,0	18,7
22,19	45,0	11,3	39,3	11,7	36,0	18,3	19,7	14,3

TABLE 2. Types of Chromosomal Aberrations with Different Proportions of Thiophosphamide and Dipin (in fractions)

Ratio of thiophosphamide: dipin	Fraction of chromosomal breaks	
	single	in chromatid exchanges
6 : 0	0,60	0,14
5 : 1	0,60	0,12
4 : 2	0,54	0,10
3 : 3	0,50	0,10
2 : 4	0,47	0,08
1 : 5	0,47	0,08
0 : 6	0,45	0,07

EXPERIMENTAL RESULTS

Data for the two main types of exchanges induced by thiophosphamide and dipin, namely chromatid exchanges and sister-strand (isolocus) unions, are given in Table 1. Clearly thiophosphamide induced more breaks in chromatid exchanges and fewer in sister-strand unions (by a factor of about 2) compared with equimolar concentrations of dipin.

Dispersion analysis showed that during the combined action of thiophosphamide and dipin the cytogenetic effect depended essentially on the concentration of the mutagens ($P < 0.001$) and also on the ratio between them ($P < 0.05$). With an increase in the concentration of mutagens the frequency of breaks in both types of exchanges increased in a nonlinear fashion. Meanwhile, with each total concentration, if the proportion of dipin was increased (and that of thiophosphamide reduced) in the combined treatment, the number of breaks in the chromatid exchanges was reduced and the number in sister-strand unions was increased relative to the level observed following the action of thiophosphamide alone, until the effect of dipin alone was reached. The existence of some fluctuations in frequencies can evidently be explained by the influence of random factors. Since the total number of molecules of the two mutagens remained constant with each combined concentration, but their relative proportions were changed, the result points unambiguously to summation of the contribution of each mutagen in the effect of the combined action of these agents.

Analysis of the relative frequencies of the chromosomal aberrations was carried out by the "fraction of single breaks" and "fraction of breaks in chromatid exchanges" tests, relative to the total number of chromosome breaks. Dispersion analysis showed that these indices were dependent on concentration ($P < 0.01$) and the ratio between thiophosphamide and dipin ($P < 0.05$). The dependence of the mean values of the results of the cytogenetic tests on the ratio between the mutagens is shown in Table 2. Thiophosphamide can be seen to induce a greater fraction of single breaks and breaks in chromatid exchanges than dipin (Table 2). A change in the ratio between thiophosphamide and dipin gave an effect the level of which was intermediate between the effects of equimolar concentrations of the pure mutagens and which changed proportionally to the fraction of each agent. These results rule out interaction between thiophosphamide and dipin, i.e., any strengthening or weakening of the effects following the combined action of these alkylating compounds on chromosomes of human lymphocytes.

The experimental scheme used in this study thus demonstrated the additive nature of the action of thiophosphamide and dipin against the background of the nonlinear relationship between the cytogenetic effect and the concentration of mutagens. The need for taking into account the precise form of the concentration dependence when the combined action of mutagens is studied has been emphasized previously [7].

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