EFFECT OF DEOXYCYTIDINE ON FREQUENCY OF SISTER CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES DETECTED WITH 5-BROMODEOXYURIDINE

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The frequency of sister chromatid exchanges (SCE) during abolition of the inhibitory action of 5-bromodeoxyuridine (BdU, 0.05 mM) on DNA synthesis by means of deoxycytidine (dC, 0.1 and 0.01 mM) was investigated in cultures of lymphocytes from the blood of three normal individuals. The frequency of SCE was significantly increased in all three experiments in the presence of dC in a concentration of 0.1 mM. Parallel with the increase in the frequency of SCE, dC also increased the yield of second-division metaphases. Elevation of the SCE level under the influence of dC was connected with its normalizing action on DNA replication.

KEY WORDS: sister chromatid exchanges; 5-bromodeoxyuridine; deoxycytidine.

In modern investigations to study differential staining of sister chromatids and sister chromatid exchanges (SCE) 5-bromodeoxyuridine (BdU) is widely used. Analysis of their results shows that with an increase in the dose of BdU added to the cell cultures, the frequency of SCE increases [3, 5, 10]. On the other hand, as the dose of thymidine or its analog BdU increases, the synthesis of endogenous deoxycytidine (dC) is blocked, with consequent delay or interruption of DNA synthesis and of cell multiplication [2]. This disturbance is abolished by the addition of exogenous dC [6, 10]. On the basis of these facts it can be postulated that the increase in the yield of SCE compared with their spontaneous level observed when BdU is used is connected with the disturbance of DNA synthesis under the influence of BdU. To test this hypothesis the frequency of SCE was studied when the inhibitory action of BdU was abolished by simultaneous admistration of dC.

EXPERIMENTAL METHOD

Experiments were carried out on peripheral blood lymphocytes from three normal individuals. The cells were cultured by a semimicromethod with the addition of phytohemagglutinin (PHA) for 72 h. The BdU and dC were dissolved in Eagle's medium immediately before use and were added to the growing culture 28 h before fixation. The final concentration of BdU was 0.05 mM and of dC 0.1 or 0.01 mM. The blood culture was kept in darkness. Colcimid (0.06 μg/ml) was added 2 h before fixation. Standard air-dried chromosomal preparations were used. Differential staining of the sister chromatids to detect SCE was carried out with the fluorochrome Hoechst-33258 and with Giemsa's stain. In each variant of the experiment 35 metaphases were analyzed to count the SCE and 100 metaphases to determine the fraction of second division metaphases, counting from the time of addition of the DNA precursors.

EXPERIMENTAL RESULTS

Three analogous experiments were carried out at different times, and the blood of one of three normal individuals was used in each of them. On the basis of the results the mean frequencies of SCE and the percentage of second division metaphases in the different variants of the same experiment, and also for all three experiments together, were compared. The results are given in Tables 1 and 2.

Comparison of the tests in Table 1 in which BdU was added alone or together with the last dose of dC (0.1 mM) revealed statistically significant differences (by the Fisher–Student criterion) in the frequency of SCE in all three experiments (experiment No. 1: $0.05 > P > 0.01$; experiments Nos. 2 and 3: $P < 0.001$). Comparison of the percentage relationships revealed a significant acceleration of movement of the cells through

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TABLE 1. Mean Frequency of SCE in Lymphocytes During Culture in Presence of BdU and of BdU+dC (M ± m)

Experiment No.	Precursors added		
	BdV	BdU+dC 0.01 mM	BdU+dC 0.1 mM
1	7,00±0,395	8,97±0,599	9,77±0,519
2	7,03±0,532	8,34±0,664	9,57±0,532
3	8,69±0,602	8,90±0,660	10,90±0,742

TABLE 2. Frequency of Second Division Metaphases (in %) in Lymphocytes Cultured in Presence of BdU and of BdU+dC

Experiment No.	Precursors added		
	BdU	BdU+dC 0.01 mM	BdU+dC 0.1 mM
1	68	80	79
2	69	77	82
3	71	75	88

the cycle (Table 2) in experiments No. 2 ($0.05 > P > 0.02$) and No. 3 ($0.01 > P > 0.002$). Statistical analysis (by comparison of pairs) of the combined data for all three experiments showed a significant increase in the frequency of SCE ($0.01 > P > 0.002$) and an increase in the fraction of second division metaphases ($0.02 > P > 0.01$). A highly significant difference ($0.01 > P > 0.001$) in the frequency of SCE was found in experiment No. 1 between the variants BdU+dC 0.01 mM and BdU. No difference was found between the analogous tests ($P > 0.05$) in experiments Nos. 2 and 3 whether considered separately or together ($P > 0.1$). No significant difference likewise was found in the number of second division metaphases, whether in the individual experiments ($P > 0.05$) or when considered together ($P > 0.05$).

Comparison of variants of the experiments in which dC was added in concentrations of 0.1 and 0.01 mM revealed a statistically significant difference ($0.05 > P > 0.01$) in the SCE level in experiment No. 3. In the same experiment an increase in the yield of second division metaphases was observed ($0.02 > P > 0.01$). However, in two other experiments no such differences were found either in the change in level of SCE ($P > 0.05$) or in the yield of second division metaphases ($P > 0.1$). No differences in the above-mentioned indices likewise were found when all three experiments were considered together ($P > 0.1$).

The folowing explanation, which may at first sight appear to be paradoxical, can be given for the action of deoxycytidine in increasing the SCE level. The potential molecular events which lie at the basis of SCE are known to be realized as such if the cell passes through a period of DNA synthesis [4, 9]. It can tentatively be suggested that in the presence of a deficiency of cytosine precursors, which arises when BdU is added to the cell cultures, cells with a larger number of potential exchanges will experience greater difficulty in passing through the mitotic cycle, and their relative proportion among the metaphases will decrease. The addition of exogenous dC to the culture medium, normalizing DNA synthesis and movement of the cells through the cycle, abolishes the obstacle to the entry into mitosis of that fraction of the cell population in which the frequency of SCE is greater. The final result will be an increase in the mean values of exchanges calculated per metaphase. This explanation is in good agreement with Shafer's hypothesis [8] of the mechanisms of SCE, according to which SCE are the cytogenetic expression of the overcoming of the obstacle to DNA replication at the molecular level. This obstacle is created by cross-linking of the complementary strands in the DNA molecule and is overcome by recombination of the DNA strands in the two sister chromatids at the site of cross-linking. If this hypothesis is true, a disturbance of the metabolism of DNA precursors and their normalization must be reflected in the level of SCE recorded in metaphase of mitosis. However, it is difficult to assert that the effect of dC can be attributed to this factor only, for thymidine or its analog have a manifold action on metabolism in the cell [1].

It followed from the results of this experiment that in investigations that are widely carried out for the determination of the frequencies of SCE and, in particular, during the action of mutagens and the study of hereditary diseases with chromosomal instability, BdU should be used in conjunction with dC. In that case the frequency of SCE discovered corresponds more closely to the true frequency than that found by the use of BdU alone.

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