Cytogenetic Anomalies in Cultured Human Lymphocytes Exposed to Halogen Analogs of Thymidine

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 9, pp. 326-328, September, 1995
Original article submitted November 30, 1994

Allocyclic chromosomes and monochromatid breaks are observed during testing of a number of halogen analogs of thymidine. The first effect depends directly and the second inversely on the molecular weight of the analog.

Key Words: *human lymphocytes; halogen analogs of thymidine; allocyclic chromosomes*

Long-term exposure of heteroploid Chinese hamster cells to colcemid, an agent that causes the development of cells with micronuclei, and to halogen analogs of thymidine: 5-iododeoxyuridine (5-IUdR), 5-bromodeoxyuridine (5-BUdR), and 5 chlorodeoxyuridine (5-CUdR), induces the formation of dicentric chromosomes joined end-to-end [2-4]. These dicentrics result from delayed disruption of telomeric links in interphase chromosomes [7].

We studied cytogenic abnormalities in cultured human lymphocytes exposed to low doses of 5-IUdR, 5-BUdR, and 5-CUdR.

MATERIALS AND METHODS

Lymphocytes from a healthy 44-year-old man and a healthy 35-year-old woman were used. In order to initiate cell culture, 0.2 ml phytohemagglutinin (Bulgaria), 6.0 ml Eagle's medium, and 1.5 ml bovine serum were added to 0.5 ml heparinized blood. Cells were grown at 37°C. 5-IUdR, 5-BUdR (both from Serva), and 5-CUdR (Sigma) were added to a final concentration of 20 μg/ml in the 48th hour of culturing. The cells were fixed at 68 h. Colcemid (0.1 μg/ml, Serva) was added 1.5 h before fixation. The cells were then treated with 0.65% KCl for 11 min and fixed with a methanol:acetic acid (3:1) mixture. Chromosomal preparations were obtained by the standard air-dried method and stained with azure-eosin. One hundred metaphases were analyzed in each experiment.

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RESULTS

Delay of spiralization in the region of structural heterochromatin of chromosome 9 is the most obvious cell response to halogen analogs of thymidine. Previously, this was demonstrated for high doses of 5-BUdR added at the end of the S-phase [1]. The numbers of metaphases with such a response are shown in Table 1. The reaction is seen to be most pronounced after the addition of 5-IUdR and minimal after the addition of 5-CUdR. With the use of the χ^2 test it was found that the frequency of such metaphases is statistically different for 5-IUdR and 5-BUdR on the one hand and 5-BUdR and 5-CUdR on the other, independently of the sex of the lymphocyte donor. Thus, an intermediate value was found for 5-BUdR. The heterochromatin region of chromosome 9 often looked like a prophase region or like "pulverization". Quite often it resembled a break, i.e., it was achromatic (Fig. 1, *a, b*).

In contrast to 5-BUdR and 5-CUdR, 5-IUdR sometimes caused the heterochromatin regions in chromosomes 1 and 16 to have similar breaks.

The formation of monochromatid breaks or deletions in regions other than the structural heterochromatin of chromosomes 1, 9, and 16 (Fig. 1, *c*) is another interesting phenomenon. It can be seen from Table 1 that the number of such breaks

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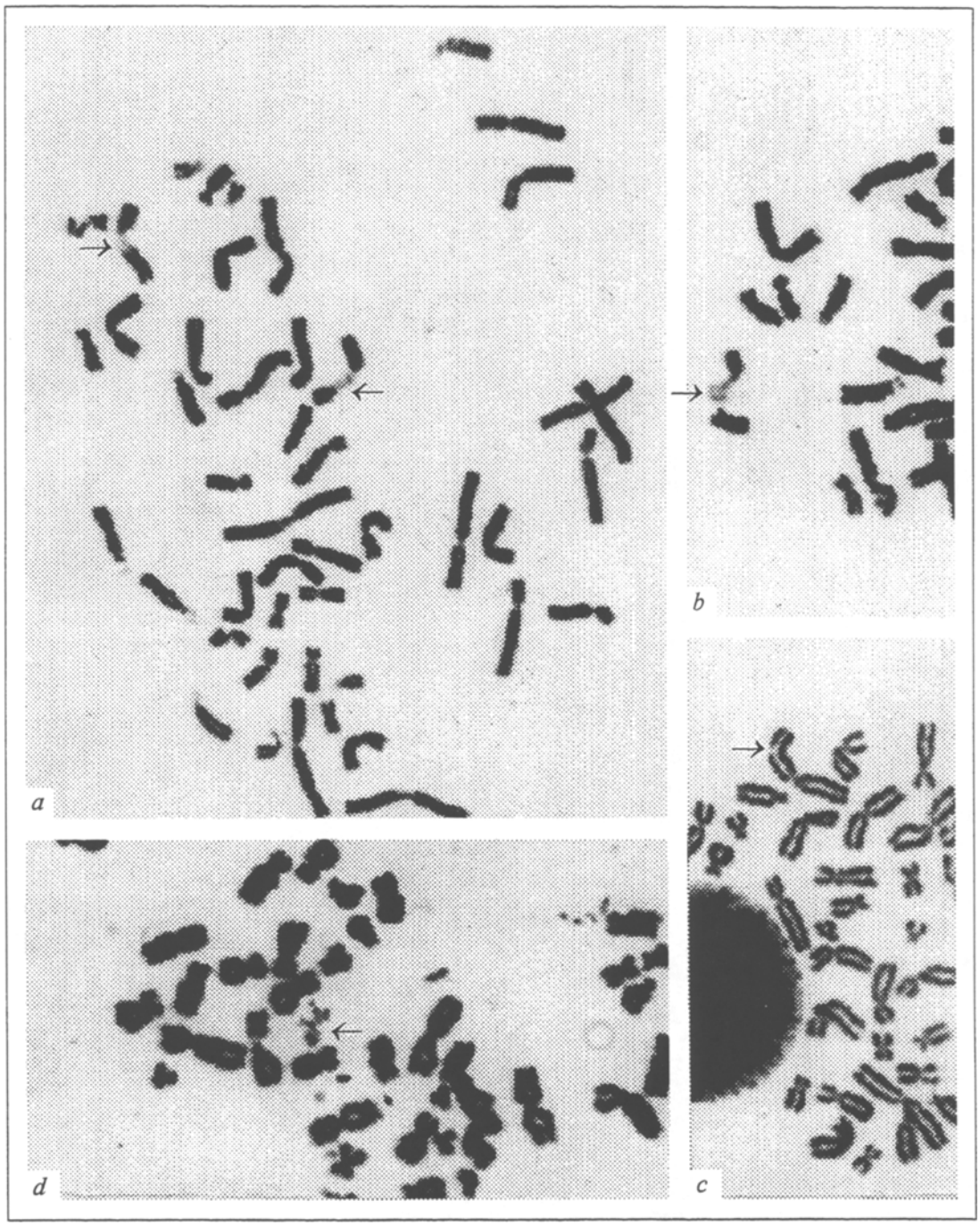

Fig. 1. Chromosomal abnormalities in human lymphocytes exposed to halogen analogs of thymidine. *a*) metaphase plate with delayed spiralization in the heterochromatin region of chromosome 9 in cells treated with 5-IUdR; *b*) fragment of metaphase with "pulverization" of the heterochromatin region of chromosome 9 in 5-IUdR-treated cells; *c*) fragment of metaphase containing a monochromatid break in 5-CUdR-treated cells; *d*) fragment of metaphase with "pulverization" of the entire chromosome in 5-IUdR-treated cells. Azure-eosin staining. ×770.

progressively increases in the following order: 5-IUdR - 5-BUdR - 5-CUdR.

The delay of spiralization in the heterochromatic region of chromosome 9 is probably a locus that is either in early prophase or in the S-phase, i.e., these chromosomes are allocyclic. Such chromosomes have been described in Bloom's syndrome [5]. The authors hypothesized that some regions of chromosomes or even entire chromosomes remain in the S-phase when all the other chromosomes are already in metaphase.

Morphological study of "prematurely condensed chromosomes" showed that a chromosomal locus in the state of DNA synthesis does not respond to

TABLE 1. Frequency of Chromosomal Abnormalities in Human Lymphocytes Exposed to Halogen Analogs of Thymidine

Abnormality	Intact culture	Thymidine analogs		
		5-IUdR	5-BUdR	5-CUdR
Lymphocytes of male				
Metaphases with delayed spiralization or a break in the heterochromatin region of chromosome 9	0	27	5	1
Metaphases with monochromatid break or deletion (except for the heterochromatin regions of chromosomes 1, 9, and 16)	3	4	5	9
Lymphocytes of female				
Metaphases with delayed spiralization or a break in the heterochromatin region of chromosome 9	0	43	12	7
Metaphases with monochromatid break or deletion (except for the heterochromatin regions of chromosomes 1, 9, and 16)	2	1	4	10

the mitosis activator and looks like an achromatic region. The regions in which DNA synthesis has not yet started look like separate fragments, and regions that have already replicated look like paired fragments [6].

Thus, in cultured lymphocytes halogen analogs of thymidine, particularly 5-IUdR, induce a time shift in DNA synthesis in the heterochromatin regions of chromosomes 1, 9, and 16, the reaction being most pronounced in chromosome 9. This assumption is supported by the occasional cases of "pulverization" of the entire chromosome induced by 5-IUdR (Fig. 1, *d*). Experimental evidence indicates that the intensity of this abnormal process directly depends on the molecular weight of the halogen analog.

We believe that when the disruption of telomeric links induced by colcemid or halogen analogs in Chinese hamster cells is delayed, the resulting dicentrics are allocyclic chromosomes, since this disruption generally occurs at the early stages of prophase, while telomeric fusion is characteristic of interphase.

Mitotic rearrangements of chromosomes cannot start without DNA reduplication in all the chromosomes. However, our experimental findings and published data indicate that this postulate has exceptions. Presumably, the end of reduplication in the region of structural chromatin of chromosome 9 is not the determining factor for the start of mitotic rearrangements of this chromosome. In addition, the discovery of such delays in different chromosomal regions in the S-phase in Bloom's syndrome suggests that this mutation is associated with mitotic rearrangements. On the other hand, halogen analogs of thymidine evidently stimulate abnormal mitotic rearrangements of chromosomes in normal cells and may modulate this process. The available experimental evidence indicates that the cell response depends on the type of halogen analog of thymidine, specific features of the cells, and the mode of their treatment.

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