GENETICS

Halogen Analogs of Thymidine Increase the Level of Chromosome Aberrations in Cells of Patients with Fanconi's Anemia

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Lymphocytes of two patients with Fanconi's anemia were cultured with halogen analogs of thymidine. These substances reliably increased the incidence of chromosome aberrations per cell in comparison with the initial level. The effect directly depends on the size of the halogen atom of thymidine.

Key Words: *halogen analogs of thymidine; Fanconi's anemia; chromosome aberrations*

The properties of halogen analogs of thymidine in relation to the mitotic transformation of chromosomes are little known. 5-Iododeoxyuridine (5-IDU), 5-bromodeoxyuridine (5-BDU), and 5-chlorodeoxyuridine (5-CDU) combined with colcemide inducing the formation of cells with micronuclei in heteroploid strains of Chinese hamster cells cause a telomeric fusion of metaphase chromosomes. Dicentric chromosomes joined end-to-end without losing chromosome material are observed in metaphases. The incidence of such dicentrics increases in the series 5-IDU, 5-BDU, and 5-CDU. The temperature also affects the formation of dicentrics. Their maximum formation is observed at 40°C in the presence of 5-CDU, the minimum at 34°C and 5-IDU. Besides the dicentrics, radials are observed in the former case, and if their number is minimal, in the latter case a large number of chromosomes with sites of delayed spiralization are observed [1].

Treating a human lymphocyte culture with halogen analogs of thymidine leads to an increase in the number of allocyclic chromosomes (most often this affects chromosome 9) in the series 5-CDU, 5-BDU, and 5-IDU, that is, when one chromosome in the set or part of a chromosome is in a state of DNA synthesis during metaphase [4]. In our case such a locus was the site of structural heterochromatin of chromosome 9, which looked like an achromatin gap or was in a state of "pulverization" [2]. Such a state corresponds to chromatin during DNA synthesis after experimental "premature condensation of chromosomes" [6]. During exposure of human lymphocytes to halogen analogs of thymidine, the number of single-chromatid breaks increases at chromosome sites other than heterochromatin regions in the gradation from 5-IDU to 5-CDU [2].

Hence, the reaction of cells to halogen analogs of thymidine depends not only on the type of analog and conditions of treatment, but also on the properties of the cells. It is possible that the number of telomeric bonds and chromosome aberrations during exposure to halogen derivatives of thymidine depends on the genotype. We deemed it interesting to test the reaction of mutant cells with an initial repair defect to halogen analogs of thymidine. For this purpose we used the cells of patients with a syndrome of chromosome instability, namely, lymphocytes of patients with Fanconi's anemia — an autosomal recessive disease involving increased fragility of chromosomes.

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TABLE 1. Number of Breaks per Cell in the Lymphocytes of Patients with Fanconi's Anemia Treated with Halogen Analogs of Thymidine

Individual	Intact culture	5-CDU	5-BDU	5-IDU
1	0.36	0.99	1.25	1.92
2	0.31	0.8	0.95	1.27
p	>0.05	>0.05	<0.05	<0.0005

MATERIALS AND METHODS

Lymphocytes from two boys aged 11 and 13 years with clinical symptoms of aplastic anemia and a high level of spontaneous chromosome aberrations were used in the experiments. For culturing, 0.2 ml phytohemagglutinin, 6 ml Eagle's medium, and 1.5 ml bovine serum were added to 0.5 ml heparin-treated blood. The cells were cultured at 37°C. During the 48th hour of culturing, 5-IDU, 5-BDU (Serva), and 5-CDU (Sigma) were added in a final concentration of 20 μg/ml. The cells were fixed for 68 h. Colcemide (0.1 μg/ml, Serva) was added 1.5 h before fixation. The culture was subjected to 11-min hypotonic treatment with 0.65% KCl. The fixing mixture was methanol:acetic acid (3:1). Chromosome preparations were obtained by the routine dry-air method and stained with azure-eosin. One hundred metaphases were analyzed in each variant of the experiment. The basic parameter — number of breaks per cell — was determined as follows: solitary and paired fragments were considered to result from a single break and exchanges were counted as two breaks, because two chromosomes have to be damaged for this.

RESULTS

The studies demonstrated that the incidence of allocyclic chromosomes (chromosomes 9 with "pulverization" at the site of structural heterochromatin) is highest after exposure to 5-IDU, but a complete count is impossible because of the great number of chromosome aberrations. Table 1 shows that halogen analogs of thymidine increased the incidence of chromosome aberrations in the lymphocytes of both patients. The incidence of aberrations increased linearly ($p<0.01$) with an increase of the size of the halogen atom of the thymidine analog: $X=-1.34+2.2Y$, where X is the number of aberrations per cell and Y is the size of the halogen atom in angströms. In contrast to normal cells of healthy donors, in which exposure to 5-CDU results in an increased number of single-chromatid breaks, in patients with Fanconi's anemia a different relationship is observed between the formation of chromosome aberrations and the type of halogen atom. It was shown previously that the cells of patients with Fanconi's anemia are characterized by hypersensitivity to agents causing DNA crosslinks, such as mitomycin C and diepoxybutane, which lead to the death of cells and the formation of chromosome aberrations [5]. Our study showed that such relatively clastogenically "harmless" substances as halogen analogs of thymidine are also capable of raising the initial level of chromosome aberrations in the cells of patients with Fanconi's anemia.

At least 4 complementation groups are known to exist in Fanconi's anemia [3,7]. Our findings indicate that the degree of increase of the number of aberrations per cell for exposure to 5-IDU and 5-BDU appreciably varies in different individuals. This circumstance may be due to the probands' belonging to different complementation groups that probably differ in the degree of repair of the damaged chromosomes.

It is universally believed that the cells start a new phase of the mitotic cycle after the previous stage is over, that is, a sort of a barrier is thought to exist between cycle phases. Our studies of cell reactions to halogen analogs of thymidine showed that incorporation of halogen atoms in the DNA alters the properties of chromosomes to such an extent that they are no longer regulated by the barrier at the interface between the S-period and mitosis. In such a case the cells may enter mitosis with their replication incomplete, chromosomes condensed, and in a state of telomeric fusion, which is characteristic of stages S and G_2. In the case of a mutagenic exposure of the cells of patients with Fanconi's anemia it is possible that not all the cells with DNA damage will reach metaphase. Under the usual conditions some of the cells will remain in interphase. When halogen atoms incorporate into the DNA, the repair systems evidently fail to differentiate between damaged and normal cells. An extra number of metaphases with chromosome aberrations may be observed in such a case.

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