TELOMERIC FUSION OF CHROMOSOMES IN CELLS WITH MICRONUCLEI DURING COMBINED EXPOSURE TO HYPER- OR HYPOTHERMIA AND HALOGEN ANALOGS OF THYMIDINE

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Spontaneous telomeric fusion of chromosomes in human diploid cells, discovered in 1975 [8] and confirmed in 1976 [7], is manifested as the appearance of dicentric chromosomes, joined end-to-end, i.e., without any damage to the integrity of the telomeres. Induced telomeric fusion of chromosomes was discovered in 1976 [1] in cells with micronuclei, obtained after long-term exposure to the action of colcemid accompanied by simultaneous exposure to 5-bromodeoxyuridine (5-BUdR). Induced telomeric fusion of chromosomes is the first experimental model enabling action to be taken on the functioning of telomeres in mammalian cells. This experimental phenomenon is evidently reproduced only in heteroploid cells of the Chinese hamster and human – Chinese hamster cell hybrids [2, 3]. It has also been shown that, instead of 5-BUdR, other halogen analogs of thymidine can be used to induce the phenomenon, namely 5-iododeoxyuridine (5-IUdR) or 5-chlorodeoxyuridine (5-ClUdR) [5, 6]. In experiments with 5-BUdR, hyperthermia (40°C) and hypothermia (34°C) were found to affect the frequency of dicentric chromosomes in cells with micronuclei [4].

The aim of this investigation was to determine the reaction of cells with micronuclei to induction of dicentric chromosomes as a result of combined exposure to hyper- or hypothermia and various thymidine analogs.

EXPERIMENTAL METHOD

Chinese hamster cells of clone 237S of line BIId-ii-FAF28 were used. The cells were grown in Eagle's medium with 10% bovine serum in rectangular flasks with a capacity of 0.5 liter. Hypnotic treatment was applied with 0.65% potassium chloride solution for 5 min. The cells were fixed with ethanol:chloroform:acetic acid mixture (3:1:1) for 1 h, with three changes of fixative. The suspension of fixed cells was applied to slides wetted with water. The experiment was carried out as follows. Immediately after seeding the cell cultures were incubated at 37°C. After 24 h, colcemid (0.1 μg/ml, from "Serva") and one of the thymidine analogs in a dose of 20 μg/ml (5-BUdR and 5-IUdR, from "Serva," 5-ClUdR from "Sigma") were added to the actively growing cell culture. Cultures with 5-IUdR, 5-BUdR, and 5-ClUdR were grown at 34, 37, and 40°C for 42 h, after which they were fixed. In each alternative version of the experiment 100 metaphases were counted.

EXPERIMENTAL RESULTS

The results of the experiments to study induction of dicentrics are given in Table 1. They show that induction of specific dicentric chromosomes in cells with micronuclei by halogen analogs of thymidine is modified by the effect of temperature in a uniform manner. For all halogen analogs of thymidine, hypothermia (34°C) reduced the frequency of dicentrics, whereas hyperthermia (40°C) increased it. The parameters of the number of dicentrics at 34 and 40°C differed

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TABLE 1. Induction of Dicentric Chromosomes in Cells with Micronuclei under Combined Influence of Different Temperatures and Halogen Analogs of Thymidine

Culture conditions	Number of cells with dicentrics	Total number of dicentrics
With 5-IUdR		
at 34°C	7	7
at 37°C	12	12
at 40°C	20	25
With 5-BUdR		
at 34°C	16	17
at 37°C	23	29
at 40°C	38*	65
With 5-ClUdR		
at 34°C	12	16
at 37°C	44**	63
at 40°C	49	85

Legend. Colcemid was added to all cultures in a dose of 0.1 μg/ml. Cells were cultured for 42 h. 5-IUdR, 5-BUdR, and 5-ClUdR were added in a dose of 20 μg/ml. In each case 100 metaphases were analyzed. *) One tricentric and two circular chromosomes were found in 100 metaphases; **) one tricentric was found in 100 metaphases.

significantly: in the case of 5-IUdR $\chi^2 = 10.12$, $p < 0.01$; for 5-BUdR $\chi^2 = 28.01$, $p < 0.001$; and for 5-ClUdR $\chi^2 = 47.6$, $p < 0.001$. Culture at 37°C gave an intermediate number of dicentric chromosomes for each halogen analog of thymidine.

The smallest number of dicentrics was found in the case of hypothermia (34°C) under the influence of 5-IUdR, and the highest frequency in the case of hyperthermia (40°C) and 5-ClUdR. These results demonstrate the apparently extreme possibilities for induction of dicentric chromosomes and they are of special interest. For instance, in the first case (5-IUdR + 34°C) a low frequency of mitotic figures was noted. We had difficulty in finding 100 metaphases for analysis. With an increase of temperature (5-IUdR, +40°C) the frequency of metaphases increased and did not differ from that in the other versions of the experiment. In the second case (5-ClUdR, +40°C) we were unable to find a single chromosome with regions of delayed spiralization, which were systematically found in the other samples, during analysis. In the same version of the experiment there was an unusually large number of cells with quadri- and triradials (19%), whereas in the other tests the fraction of these cells did not exceed 3%. The investigation thus showed that at extreme values of temperature, and depending on the type of halogen analog of thymidine, the following different types of response of the cells with micronuclei to exposure to these factors may take place: in the first case mitotic condensation of the micronuclei is possible, in the second case — the formation of chromosomes with regions of delayed spiralization. The results can be used by research workers studying polykaryocytes consisting of micronuclei. They enable the suitable temperature and halogen analog of thymidine to be chosen for a particular experiment.

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