EFFECT OF DURATION OF EXPOSURE TO PRELIMINARY CONCENTRATION ON PRIMARY MUTAGENIC EFFECT OF SINGLE- AND DOUBLE-SITE MUTAGENS

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UDC 575.224.46.04.08

KEY WORDS: exposure; single-site mutangens; double-site mutagens.

The discovery of the adaptive response (AR) phenomenon in mammalian cells and its subsequent study have shown that as a result of preliminary treatment with a low concentration of a mutagen, certain protective mechanisms are activated in the cells and weaken the following stronger mutagenic action. The molecular mechanism of AR is still largely a matter for conjecture. The nature of the triggering mechanism in the AR phenomenon is not yet known. According to some workers [1, 2], the triggering mechanism of AR is the direct action of the alkylating agent on a repressor protein, the blocking effect of which on specific repair enzymes is abolished by the action of a low concentration of the mutagen, and in that way operation of the AR mechanism is activated; the nature of the triggering mechanism of AR, according to these workers, is thus not directly connected with injury to DNA. To confirm or refute this hypothesis, we studied the effect of different exposures to preliminary concentrations on AR. If the triggering mechanism of AR is not connected with injury to DNA, different exposure schedules ought not to be reflected in the value of the "protective" concentration but, on the other hand, if the triggering mechanism of AR is connected with injury to DNA, the change in the exposure time ought to be reflected inevitably in the value of the "protective" concentration. The results of this investigation are described below.

EXPERIMENTAL METHOD

Experiments were carried out on a culture of human lymphocytes at the G_0 stage before stimulation by phytohemagglutinin (PHA). The principal concentration of the mutagens thiotepa (thiophosphamide; a single-site mutagen) and dipin [tetra(ethyleneimido)piperazine-N,N'-diphosphoric acid; a double-site mutagen] was 20 μg/ml. The preliminary concentrations were 10, 10^2, 10^3, 10^4, and 10^5 times less than the principal concentration. The experimental program was the same for both mutagens and was as follows: 10 ml of cultural mixture (1 part of whole blood, 3 parts of bovine serum, 12 parts of Eagle's medium) was treated with five preliminary concentrations of the mutagen (in the version without preliminary treatment 1 ml of Hanks' solution was added to the cultural mixture) and incubated for different times at 37°C. For different versions of the experiment the following exposure times to dipin and thiotepa were used: 15 and 30 min, and 1 and 4 h. The cells were then washed twice with Eagle's medium, the cultural mixture was changed, and incubation continued without the mutagen at 37°C for 2 h. Treatment with the principal concentration of mutagen was then carried out with an exposure of 1 h at 37°C. The cells were then washed 3 times with Eagle's medium to remove the mutagen, the cultural mixture was changed, and PHA (Difco "P") was added at the rate of 0.015 ml to 10 ml of cultural mixture. Colchicine was added in a dose of 0.5 μg/ml 2 h before fixation. The culture was fixed after 56 h of incubation with a mixture of methanol and glacial acetic acid in a ratio of 3:1. The preparations were stained with azure-eosin. In each version from 100 to 300 metaphases were analyzed. The significance of differences was estimated by the chi-square test.

Laboratory of Mutagenesis, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 477-479, October, 1989. Original article submitted October 20, 1988.

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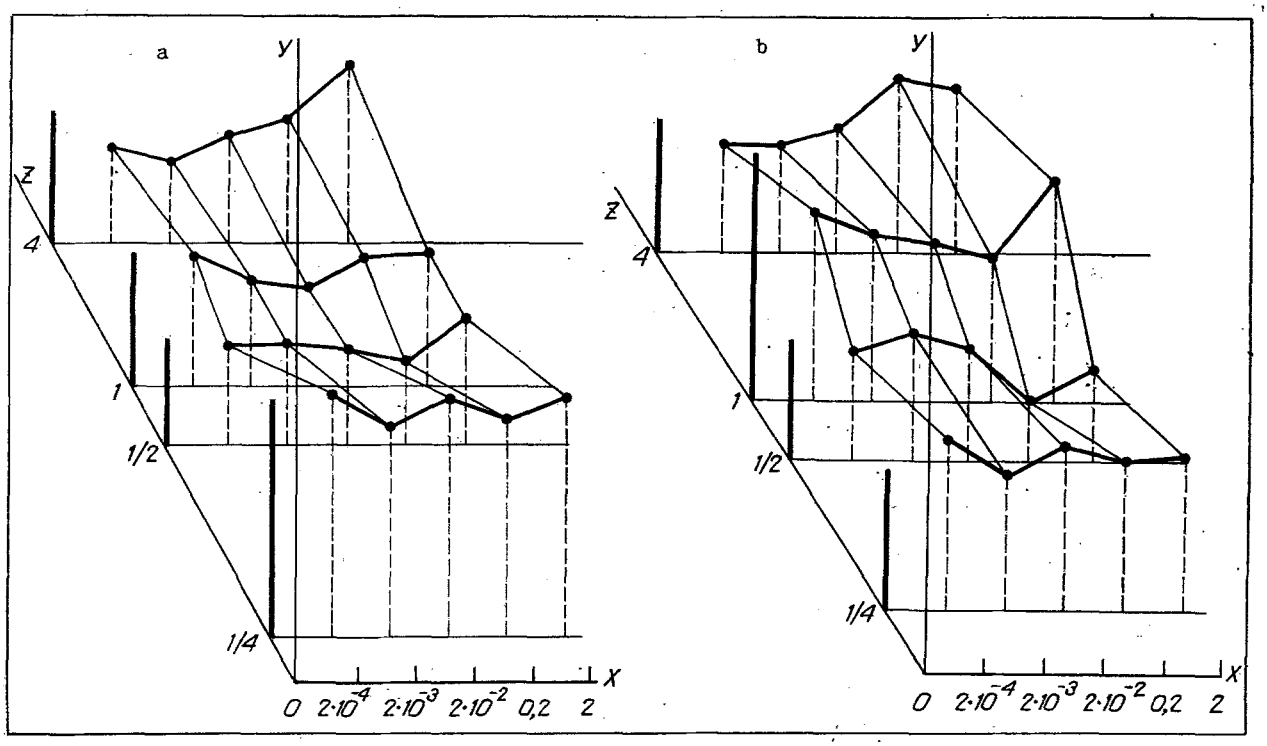

Fig. 1. Frequency of chromosomal aberrations following action of 20 μg/ml of thiotepa (a) and diphin (b) with different exposure schedules to preliminary concentrations. X axis) Concentration of mutagens during preliminary treatment; Y axis) number of chromosomal breaks per 100 cells; Z axis) exposure to preliminary concentration, h.

EXPERIMENTAL RESULTS

The experimental results are illustrated in Fig. 1a for thiotepa and Fig. 1b for dipin. The bold vertical lines correspond to the control version without preliminary treatment. It will be clear from Fig. 1a that fewer chromosomal breaks following exposure to the principal concentration of thiotepa were observed than in the control version without preliminary treatment for exposures of 1 and 4 h to the preliminary concentrations. With an exposure of 1 h the number of chromosomal breaks was significantly reduced for concentrations of 2×10^{-2} and 2×10^{-3} μg/ml ($p < 0.01$), and for an exposure of 4 h, for concentrations of 2×10^{-2} μg/ml ($p < 0.05$), 2×10^{-3} μg/ml ($p < 0.001$), and 2×10^{-4} μg/ml ($p < 0.01$). In addition, for a concentration of 2 μg/ml and an exposure of 4 h, a significant increase was found in the number of chromosomal breaks compared with the control version without preliminary treatment. Figure 1b shows that there were fewer chromosomal breaks through the action of the principal concentration of dipin for exposures of 30 min and 1 h. With an exposure of 30 min a significant decrease in the number of chromosomal breaks was observed for a preliminary concentration of 0.2 μg/ml ($p < 0.001$), and with an exposure of 1 h for concentrations of 0.2 μg/ml ($p < 0.001$), 2×10^{-2} μg/ml ($p < 0.001$), and 2×10^{-3} μg/ml ($p < 0.01$). The results of these experiments provide definite evidence that the value of the "protective" concentration changes with variation of the duration of exposure: for both mutagens, with an increase in exposure time the value of the "protective" concentration shifted toward a decrease, and vice versa. Meanwhile very short (pulsed) exposures to the preliminary concentration were ineffective as regards induction of AR for both mutagens; a significant increase in the number of chromosomal breaks with an exposure of 4 h for a concentration of thiotepa of 2 μg/ml may be connected with the additive nature of the mutagenic effects of the principal and preliminary concentrations. The results suggest that the triggering mechanism of AR is a substance of DNA-like nature (possibly the level of a certain alkylation product of DNA). When the results are analyzed, one aspect merits attention. Whereas optimal conditions of exposure for thiotepa to obtain an AR effect were 1 and 4 h, for dipin an AR appeared after shorter exposure, namely 1 h and 30 min. This may be connected with differences in the chemical structure of the mutagens. The presence of two active centers in the

dipin molecule leads to more rapid alkylation of DNA, and as a result of this the level of alkylated products capable of activating AR is achieved after a shorter exposure time.

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SPECIFICITY OF ARRANGEMENT OF HUMAN CHROMOSOMES IN THE NUCLEUS OF A MOVING CELL

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UDC 612.014.24:476.316

KEY WORDS: interphase nucleus; arrangement of chromosomes; alphoid DNA.

The study of the arrangement of chromosomes in the interphase nucleus is currently being intensively pursued. However, the difficulty of direct demonstration of chromosomes during interphase has hitherto prevented its being proved by direct experiments that they occupy a stable position in the nucleus. Attempts have therefore been made to obtain information about the arrangement of chromosomes in the nucleus by indirect methods [7, 12, 14, 15].

Electron microscopy has enabled information on the chromosomal organization of the interphase nucleus to be obtained directly [1]. The nonrandom nature of the chromosome distribution in the interphase nucleus has been demonstrated in cells of the Indian muntjac [6]. Nevertheless, the fundamental question of specificity of the chromosomes distribution in the interphase nucleus of human cells remains unexplained. Two conditions determine the possibility of obtaining an answer: 1) the availability of a model system for evaluating the localization of chromosomes in a system with assigned coordinates; 2) the use of the most promising approach to determine the position of chromosomes in the interphase nucleus with the aid of highly specific molecular probes in separate regions of the chromosome. The aim of the investigation described below was accordingly to determine the arrangement of the centromere of one chromosome in the interphase nucleus by the use of a molecular probe.

EXPERIMENTAL METHOD

Diploid skin fibroblasts from an 8-12-week male human fetus, obtained at therapeutic abortion, were used. The cells were cultured on Eagle's medium with 10% bovine serum and 5% human umbilical cord serum. DNA was labeled by the nick translation method [11]. In situ hybridization on interphase nuclei was carried out by the method in [8], including denaturation of the preparations in 0.07 N NaOH for 2 min, hybridization for 18 h, washing under standard conditions, and exposure under emulsion for 21 days.

EXPERIMENTAL RESULTS

The problem was tackled by the use of a cloned fragment of human alphasatellite DNA, specific for the centromeric region of the X chromosome. The use of human male fetal

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 479-481. October, 1989. Original article submitted June 20, 1988.

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