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PATHOLOGY OF MITOSIS AFTER RECOVERY OF CELLS FROM METAPHASE BLOCK

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Experiments on fibroblast-like Chinese hamster cells showed that agents inducing c-mitosis (colchicine, colcemid, low temperature) give rise to two distinct effects: stathmokinetic and radiomimetic. Toward the time of reversibility of the first of these effects, the second becomes clearly manifested as bridge formation. The appearance of this pathological form is evidently due to disturbance of cell nucleoprotein metabolism during c-mitosis.

KEY WORDS: pathology of mitosis; stathmokinetic effect; radiomimetic effect; chromosome bridges.

The appearance of colchicine-like mitosis, or c-mitosis, may be caused by several factors. The c-mitosis is connected with disturbance of several mechanisms of normal mitosis [1]. Our previous studies of colchicine-like mitosis were mainly concerned with the study of the character of injury to the microtubules of the division spindle and ways of its repair, depending on the factor causing the c-mitosis. We were also interested in the successive replacement of pathological forms of mitosis during the development of the stathmokinetic response and in the course of its reversibility [5, 6], due to differences in the degree of disorganization of the mitotic spindle [7]. The attainment of the control values of the mitotic index and of the relative proportions of the stages of mitosis demonstrated normalization of the spindle and the ability of the cells to recover from metaphase block and to complete the final stages of mitosis – anaphase and telophase. However, analysis of these last stages of mitosis was not studied at that time; the present investigation was devoted to this problem.

EXPERIMENTAL METHOD

Just as in the previous investigations [2, 4, 5], experiments were carried out on a monolayer culture of Chinese hamster fibroblast-like cells (clone 237). Colchicine (exposure for 30 min, 1 μg/ml), colcemid (exposure for 2 h, 0.03 μg/ml), and cold shock (culture for 2 h at 21°C) served as the inducers of the stathmokinetic reaction. The doses and exposures used were such that, after removal of the alkaloid by washing or after heating (37°C) of the cooled cultures, the latter were able to recover from the metaphase block and to complete mitosis.

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EXPERIMENTAL RESULTS

The previous investigations showed that despite similar morphological pictures of c-mitosis produced by the above-mentioned agents, the mechanisms of recovery of the cells from metaphase block were different. After treatment with colchicine, the destroyed spindle microtubules recovered mainly by the formation of new tubulins; after cooling, recovery took place mainly through repolymerization of tubulins; recovery of the microtubules after treatment of the cells with colcemid, however, occupied an intermediate position in this respect and took place both by protein synthesis de novo and by tubulin repolymerization. The normal mitotic regime was restored most rapidly after cold shock, within 40-60 min. After treatment with colcemid this process took 2-2.5 h. Most time of all was required for recovery of the normal course of mitosis after colchicine, namely 8-10 h.

During recovery of the cells from cold block, besides a regular decrease in the number of c-metaphases and also in the number of metaphases with scattered and deleted chromosomes, the number of final stages of mitosis with single and multiple bridges was increased. In cells of the control cultures the mean number of anaphases and telophases with bridges was usually not more than 0.6% (in all cases 100 dividing cells were counted). In experiments in which recovery of the control level of the mitotic index and of the ratio between the stages was completed 60 min after return of the cultures to normal temperature conditions, the number of bridges (mainly anaphase) reached 3% only 40 min after heating, and after a further 20 min it was about 6%. On recovery of the cells from metaphase block induced by colcemid the number of pathological bridges also increased gradually. The percentage of bridges 1 and 2 h after rinsing out the alkaloid was 3 and 4 respectively, and remained at this level until complete restoration of the control values of the mitotic index and ratio between the stages of mitosis.

A gradual increase in the number of bridges also was observed at the end of reversibility of the stathmokinetic reaction to colchicine. In this case the number of bridges reached 8%.

The results thus show that during the period of reversibility of the stathmokinetic reaction the decrease in mitotic index and increase in the number of anaphases and telophases are accompanied by the accumulation of bridges; this phenomenon was observed during reversibility of c-mitoses induced by all three agents. However, the phenotypical reaction of the cells (in this case bridge formation) may have been due to similarity of the final stages of a long chain of cell changes based on different causes. In particular, bridges are known to be formed as a result of disturbance of the integrity of chromosomes, during their fragmentation and subsequent combination of the fragments with the formation of dicentric chromosomes. There is evidence of the effect of colchicine and colcemid [8, 11], and also of a lowered temperature [9] on synthesis of structural proteins and enzymes in the cell. Furthermore, under the influence of these alkaloids, in cells of the Chinese hamster culture disintegration of polysomes and an increase in the number of single ribosomes were observed. These features point to a decrease of protein synthesis in the cells and, consequently, a decrease in their powers of repair. This last fact evidently also explains the increased fragmentation of the chromosomes and disturbance of the formation of normal "reunions" of the fragments under the conditions investigated.

On the other hand, bridge formation may be connected with conformational changes in DNA structure, when metaphase chromosomes appear to be glued together by chromatin fibrils, and as a result, they remain connected during separation to the poles. Fibrils joining sister chromatids and also different chromosomes have been observed in HeLa cells after prolonged exposure to colchicine [10] and in cells of various Chinese hamster strains treated with ethidium bromide [12]. Interchromosomal fibrils of different types have also been observed after similar treatment in mouse bone marrow cells [15]; end-to-end connections were observed most frequently, although connections between sister chromatids also were found. The possibility cannot be ruled out that this phenomenon is due either to direct binding of the colchicine to DNA or to its indirect action through disturbance of the normal order of DNA replication (increased asynchrony of DNA synthesis) [1], or through inhibition of synthesis of the protein responsible for maintaining the normal conformation of DNA strands. Indirect confirmation of this view is given by data on induction of adhesion by other substances (amikhellin, for example) of chromosomes at certain points during anaphase. It has been suggested that amikhellin, if incorporated into the DNA molecule, disturbs relations of the DNA molecules with histones and with each other [14].

Finally, bridge formation in the late stages of recovery of the cells from stathmokinetic block may be connected with disturbance of synthesis of the RNA concerned in maintenance of normal chromosomal structure [4]. An increase in the number of chromosomal anomalies (mainly bridges) to up to 8% of all cell divisions (compared with 1% in the control) was observed in a culture of human T lymphocytes after treatment with

olivetol – a substance disturbing RNA transcription [13]. On the basis of the results it is impossible to express preference for any one of the three possible mechanisms of bridge induction at the end of reversibility of the stathmokinetic effect. The possibility cannot be ruled out that these mechanisms may be different in c-mitoses caused by different factors or they may be due to different combinations of disturbances to individual stages of cell metabolism.

The data so far available indicate that all the agents used to induce c-mitosis bring about two distinct effects: stathmokinetic and radiomimetic. The first is expressed as the development of metaphase block and induction of colchicine-like mitoses, the second as pathology of the final stages of mitosis, specifically as bridge formation. The observations described above show that these effects differ in their stability. The stathmokinetic effect is more labile and less resistant.

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