IMPORTANCE OF PROTEIN THIOL GROUPS FOR THE APPEARANCE OF PATHOLOGICAL MITOSES IN TUMOR CELLS

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The importance of sulfhydril and disulfide groups of protein for the formation of the mitotic apparatus and, consequently, for the movement of the chromosomes during mitosis has frequently been confirmed by cytochemical [5-8, 11-13] and experimental [2-4, 7, 9-11] observations. In a previous investigation [2] the authors showed that disturbance of the equilibrium between the sulfhydril and disulfide groups of proteins by inhibition of the SH groups or reduction of the S–S groups in a culture of human amnion cells leads to arrest of mitosis at the metaphase stage and to the appearance of numerous pathological mitoses. Similar changes in mitotic division are observed usually in tumors without any external intervention [1]. The similarity between these changes (the high percentage of pathological mitoses and delay in the metaphase) and those reproduced experimentally in tissue culture suggests that the disturbance of the normal course of mitosis in tumor cells is associated with a shift of the physiological equilibrium between the protein thiol groups in these cells.

The object of the present investigation was to verify the hypothesis that blocking SH groups or excessive reduction of S–S groups leads to restoration of the normal course of mitosis, i.e., to a decrease in the number of pathological mitoses and to shortening of the delay of division in the metaphase.

EXPERIMENTAL METHOD

Two series of experiments were carried out, all variants of which were repeated twice. The experiments of series I were carried out on a primary culture of the cells of a malignant glioma of mice (strain No. 51/11 Yablonovskaya), and those of series II on the cells of an ascites strain of Ehrlich's carcinoma).

In the experiments of series I cells of a tumor transplanted into the mouse brain were cultivated. To obtain monolayer cell cultures, tumors aged 8-10 or 14-16 days were used. On the 3rd day of cultivation of the cells, either p-chloromercuribenzoate (CMB, 5×10^{-6}, 10^{-5} M) – an inhibitor of SH groups, – or cysteine hydrochloride (CH, 25×10^{-6}, 125×10^{-6} M) – a reducing agent for S–S groups – was added to the medium. One hour later the cultures were fixed and the mitotic activity, the percentage ratio between the phases of mitosis, the percentage of pathological mitoses, and of their individual forms were determined. In the two groups of experiments, CMB was added twice to the culture: 2h and 1 h before fixation. Each experimental and control group consisted of 5-8 cultures.

In the experiments of series II, conducted on mice with Ehrlich's ascites carcinoma (tumor cells were transplanted 4-5 days before the beginning of the experiment), the animals received CMB (5×10^{-6}, 10^{-5} M) or CH (25×10^{-6}, 50×10^{-6} M) by intraperitoneal injection. Both preparations were injected either at the same time, 1 h before sacrifice, or in one group of experiments, they were injected twice, 2 h and 1 h before sacrifice. Control animals received the corresponding volumes of physiological saline. Each experimental and control group contained 8-10 mice.

EXPERIMENTAL RESULTS

The study of the primary cultures of the malignant glioma showed that a very large number of pathological mitoses was present, with a relatively high percentage of mitoses at the metaphase stage. Whereas in transplanted cultures of amnion cells [2] the mean number of pathological mitoses is only 3-5% and the relative proportion of metaphases is 59%, in the primary culture of the malignant glioma the corresponding values in the different series were 20-51% and 60-95%.

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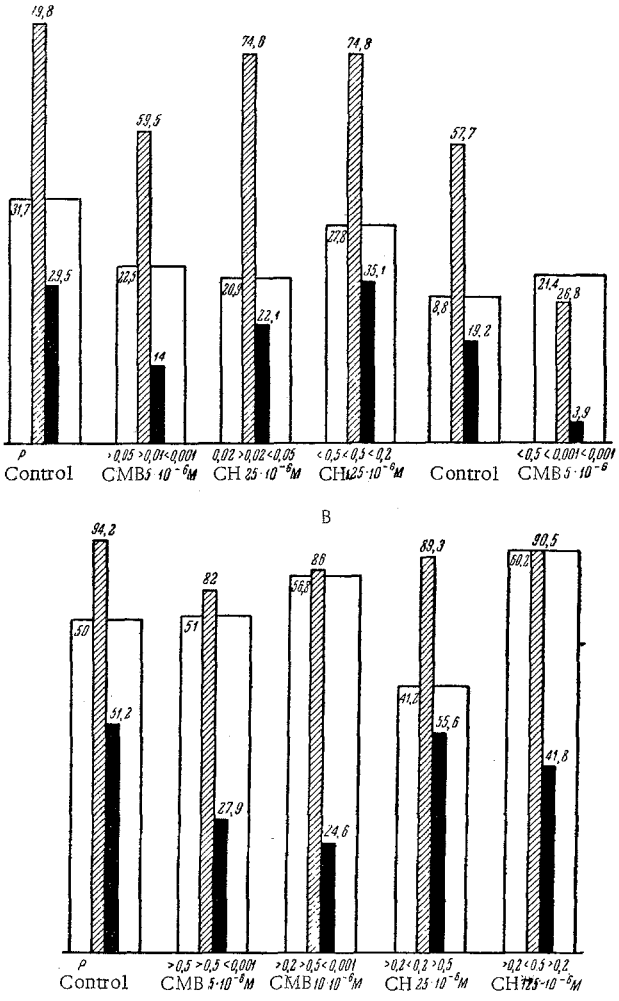

Fig. 1. Changes in the mitotic pattern in cells of a culture from a malignant glioma growing in vivo for 8-10 (A) and 14 (B) days. Unshaded columns) Mitotic activity (in %); cross-hatched) metaphases (in %); black columns) pathological mitoses (in %).

The results of the experiments on the malignant glioma cells showed that blocking the SH groups caused a sharp fall in the percentage of pathological mitoses and a decrease in the relative number of metaphases. Administration of cysteine hydrochloride caused no significant change in these indices. The effect of restoration of the normal mitotic pattern was dependent on the age of the tumor, i.e., on the length of time it had grown in the body. In experiments on cultures of cells from young tumors, growing in the body for 8-10 days, administration of CMB lowered the proportion of pathological mitoses by 50-80% and the number of metaphases by 20-30% (Fig. 1A). In one group of experiments, after the repeated administration of CMB, the number of pathological mitoses fell from 20 to 3.9%, i.e., it reached figures characteristic of a culture of normal cells.

Hence, inhibition of the sulfhydril groups almost completely restored the normal course of mitosis. These observations confirmed the original hypothesis and suggested that the onset of pathological mitoses and the delays of division in metaphase are associated with disturbance of the sulfhydryldisulfide equilibrium. This can be represented schematically by assuming that in the tumor cells of this type during the process of carcinogenesis, this equilibrium is displaced toward predominance of the SH groups, and their blocking therefore leads to restoration of the normal course of mitosis.

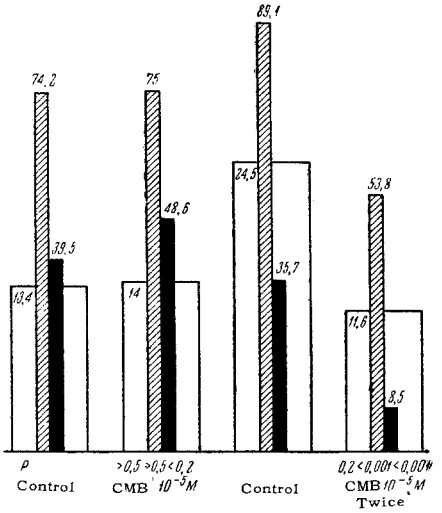

Fig. 2. Changes in mitotic pattern in cells of a culture from a malignant glioma grown in vivo for 16 days. Legend as in Fig. 1.

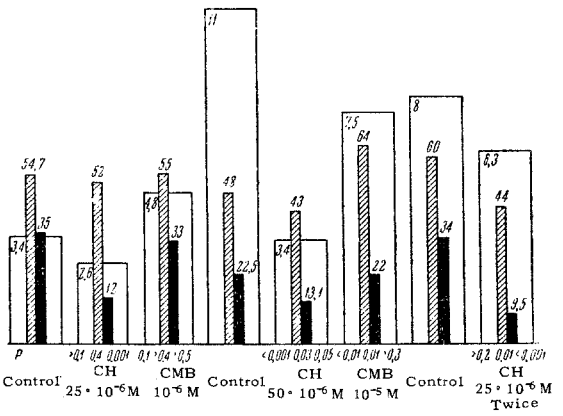

Fig. 3. Changes in the mitotic pattern in cells of an Ehrlich's ascites carcinoma. Legend as in Fig. 1.

In the cause of cells from tumors given for long periods in vivo, the "therapeutic" effect of CMB was reduced. In cultures from tumors grown in animals for 14 days, administration of CMB also reduced the proportion of pathological mitoses almost by 50%, but the relative number of metaphases changed only very slightly (Fig. 1B). In cultures from even older tumors (growing in the body for 16 days) a single dose of CMB caused no significant change in the number of metaphases or in the number of pathological mitoses (Fig. 2). Probably during growth of the tumor in the body, the disturbance of the sulfhydryl–disulfide equilibrium becomes steadily less reversible. In these circumstances the delay in metaphase becomes stabilized first, and the pathological course of the mitosis later. However, this stability is not absolutely irreversible. Even in cultures from tumors grown for a long time in the animal, the normal course of mitosis could be restored in most of the cells. After two applications of CMB the number of pathological mitoses fell by almost 80% and the number of metaphases by 35% (Fig. 2).

The disturbances of the sulfhydryl–disulfide mechanism leading to pathological changes in mitosis may probably differ in character in different types of tumor cells. By acting on the thiol groups of cells of an Ehrlich's carcinoma, in a large proportion of cells it was also possible to abolish the pathological course of mitosis. However, in this case it was cysteine hydrochloride which had a "therapeutic" effect on the tumor cells, while CMB caused no significant change in the mitotic pattern (Fig. 3). A single administration of cysteine depressed mitotic activity slightly and reduced the number of pathological mitoses by 50-67%. The relative number of metaphases was not significantly changed. The clearest results were obtained after the repeated administration of cysteine. In these experiments the number of pathological mitoses was reduced by 75% and the relative number of metaphases by 16%.

The results of the two series of experiments thus show that pathological mitoses in tumor cells arise in connection with disturbance of the sulfhydryl–disulfide equilbrium. The development of pathological mitoses in tumor cells of different types may be associated with different disturbances of this equilibrium In some cells it is displaced so that it can be restored by inhibition of the SH groups, while in other tumor cells the shift of this equilibrium takes place in the opposite direction, when the normal course of mitosis is restored by conversion of disulfide groups into sulfhydryl. During growth of a tumor in the body, the pathological mitoses become more stable. The length of growth of the tumor limits the possibility of restoring the normal course of mitosis.

Since no methods are yet available for the direct biochemical investigation of the compounds containing sulfhydryl and disulfide groups in the dividing cell, the problem of the intimate mechanisms of the shift in sulfhydryl–disulfide equilbrium cannot be answered at this stage. Two possibilities may be considered: either CMB and CH, when restoring the normal course of mitosis, act directly on the contractile

proteins of the mitotic apparatus, or this effect is achieved by indirect methods – through enzyme systems containing thiol groups. In any case, the results obtained point the way to the study of direct interference with pathological mitoses, the appearance of which is one of the essential mechanisms of carcinogenesis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
