SYNCHRONIZING ACTION OF CYCLIC AMP ON THE MITOTIC CYCLE OF EHRLICH'S ASCITES CARCINOMA CELLS

I. N. Volkov

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The number of mitoses and of DNA-synthesizing cells in an Ehrlich's ascites carcinoma was studied during the 24 h after injection of dibutyryl cyclic 3',5'-adenosine monophosphate. As a result of preprophase inhibition and, probably, of stimulation of entry of the cells into the synthetic period, a large number of cells commences mitosis simultaneously 8 h after the injection. The resulting synchronization of mitosis in the cell population of the tumor is evaluated.

KEY WORDS: cyclic 3',5'-adenosine monophosphate; cell division; Ehrlich's ascites carcinoma; DNA-synthesizing cells.

Investigations have shown that the efficacy of cytostatic preparations is increased if allowance is made for the diurnal rhythm of mitosis and the number of DNA-synthesizing cells in tumors [2]. Attempts to use cytostatic preparations in conjunction with experimental synchronization of mitotic cycles in tumors have proved even more effective [7].

Recent investigations have also shown a connection between the processes of malignant change and disturbance of the function of the adenylate-cyclase system [1, 6, 10]. Cyclic adenosine monophosphate (cAMP) has been shown to delay tumor cells in the G_2 phase [3, 7, 8].

In the investigation described below cyclic AMP was used as an agent to synchronize cell divisions in tumors.

EXPERIMENTAL METHOD

Experiments were carried out on 140 sexually mature male albino mice weighing 20-25 g, inoculated intraperitoneally 4 days before sacrifice with a dose of 10^6 cells of a diploid strain of Ehrlich's ascites carcinoma. The animals were divided into two groups. The mice of group 1 were given an injection of dibutyryl cyclic 3',5'-AMP (DBcAMP) in a dose of 20 μg/g body weight at 10 a.m., while the second group (control) consisted of intact animals.

The experimental mice were killed 30 min and 1, 2, and 4 h after injection of the compound and the control mice after 1 and 4 h; subsequently mice of both groups were killed at intervals of 2 h in the course of 24 h (11 mice at each time).

All the mice received an injection of thymidine-H^3 in a dose of 0.5 μCi/g 1 h before sacrifice.

Ascites fluid was aspirated from the peritoneal cavity of the mice, and films prepared from it were fixed with methanol. Preparations for counting the mitotic index (MI) were hydrolyzed in 1 N HCl at 56°C for 4 min and stained with Methylene Blue. Mitoses were counted by phases in 4000 cells in each case. The mitotic index was expressed in promille.

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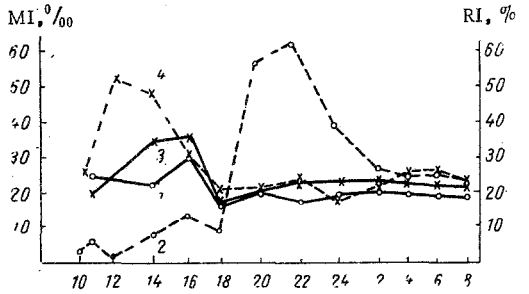

Fig. 1

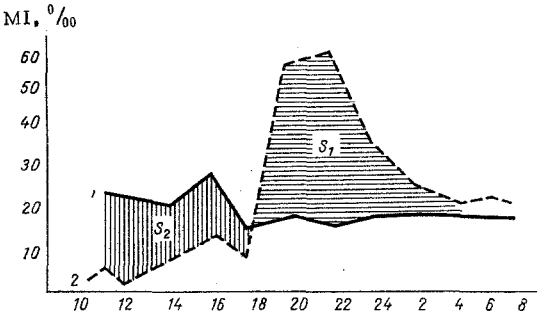

Fig. 2

Fig. 1. Changes in values of MI and RI during the 24-h period in tumors of control mice and mice receiving DBcAMP at 10 a.m. Here and in Figs. 2 and 3: 1) MI_c; 2) MI_e; 3) RI_c; 4) RI_e. Abscissa, time (in h); ordinate: on the left MI (in ‰); on the right RI (in %).

Fig. 2. Number of cells in various phases of the mitotic cycle in experimental animals. S_1) Area corresponding to number of cells causing the wave of mitosis from 6 p.m. to 4 a.m.; S_2) area corresponding to number of cells delayed in the G_2 phase.

Preparations for studying the number of DNA-synthesizing cells were coated with type M liquid photographic emulsion. After development of the autoradiographs, DNA-synthesizing cells were counted in 1000 cells and the radioactive index (RI) was expressed in per cent.

The significance of the results was assessed statistically. The MI curves were analyzed by the method of comparison of areas using the ÉVM-220 computer.

EXPERIMENTAL RESULTS

The MI curve of the control mice reflected the diurnal rhythm of mitosis in the tumor, with a maximum at 11 a.m.–4 p.m. and a minimum during the evening and night (Fig. 1). The mean diurnal MI of the control animals was 19.5 ± 0.72 ‰.

The total MI for the experimental mice 1 h after injection of DBcAMP was 4.9 ‰ and no prophases were found, whereas in the animals of the control group the prophase index was 4.8 ‰ and the total MI 23.5 ‰. Starting from 2-4 p.m., the relative proportions of the various phases of mitosis returned to the characteristic values for the control animals, in which prophases accounted for 19% and anaphases and telophases for 33% of all mitoses. These figures indicate that changes in MI during the 24-h period in both groups of animals were caused by differences in the rates at which the cells started mitosis and not by changes in the duration of cell division.

By 8-10 p.m. the mean values of MI increased to 56.35–60.57 ‰. By 2 a.m. MI had fallen to the level observed in the control animals. The mean diurnal MI in tumors of the experimental animals was 24.4 ± 2.7 ‰. The differences between the mean diurnal values of MI in the experimental and control groups were not significant. The character of the change in the RI curves (Fig. 1) in the two groups of animals was in general similar. In the control mice RI reached a maximum at 4 p.m. and the decrease in RI by 6 p.m. was significant ($P = 0.01$). At other times the changes in RI were not significant. The mean diurnal RI was 31.6 ± 1.2%. In the experimental animals RI reached a maximum at noon, and by 4-6 p.m. and later it was lower and indistinguishable from its value in the control. The mean diurnal RI in the experimental group was 34.1 ± 1.2% and did not differ significantly from the mean diurnal RI of the control animals.

The earlier maximum of RI in the experimental group than in the control could be evidence of stimulation of DNA synthesis in the first hours after administration of DBcAMP. However, the absence of data for the value of RI in the control animals at noon prevents the confirmation of this view.

The absence of significant differences between the mean diurnal values of MI and RI in the control and experimental mice indicates that during the 24 h approximately equal numbers of cells pass through the mitotic cycle in both groups and that the changes in MI and RI in the course of the 24 h are attributable mainly to quantitative redistribution of the cells with respect to the time in the mitotic cycle.

The decrease in MI during the first hours after administration of DBcAMP mainly at the expense of the early phases of mitosis can be regarded as the result of blocking of cells ready to start mitosis in the G_2

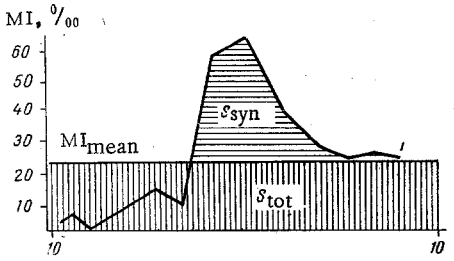

Fig. 3. Number of cells dividing in the 24-h period in the experimental animals (S_{tot}) and number of cells starting mitosis at 6 p.m. and 4 a.m. as a result of synchronizing treatment (S_{syn}).

phase, whereas the high values of MI 8 h after the beginning of the experiment can be explained by the onset of mitosis in these cells at the end of the blocking action.

Analysis of the areas beneath the curves (Fig. 2) showed that in the experimental mice more cells started mitosis in the period of maximal values of MI from 6 p.m. to 4 a.m. than were held up in the G_2 phase. In fact, the area S_1 (Fig. 2), reflecting the number of cells producing the high values of MI 8 h after injection of DBcAMP was 386 conventional units, which was greater than the area S_2 corresponding to the number of cells held up before starting mitosis, which was 184.5 conventional units.

The conflicting data in the literature on the duration of the synthetic phase on the fifth to sixth day after inoculation with the experimental tumor, ranging from 6.5 [4] to 20 h [9] and on the duration of mitosis ranging from 0.5 [9] to 5 h [5] make any quantitative comparison of the values of MI and RI difficult. However, if it is accepted that the entry of the cells into DNA synthesis was stimulated in the experimental group, it can be postulated that the same cells also participate in the formation of the wave of high MI values at 8-10 p.m.

The high MI values 8 h after the beginning of the experiment in the experimental animals could therefore be explained by the double synchronizing mechanism of DBcAMP: by the accumulation of cells in the G_2 phase and, probably, by simultaneous stimulation of the entry of the cells into the synthetic phase of the mitotic cycle, followed by the more or less synchronized commencement of mitosis in the two groups of cells.

To assess the effects of synchronization the number of cells starting mitosis as a result of the action of the compound must be compared with the total number of cells dividing during that same 24-h period (Fig. 3). The number of cells of the experimental animals dividing in the course of 24 h is reflected by the area of the rectangle S_{tot}, namely 965 units. A wave of mitosis induced by DBcAMP exceeded the limits of the mean diurnal MI (MI_{mean}) by an amount equal to the area of the shape S_{syn} (315 units). The relative index of synchronization is given by the ratio between the two areas:

$$\frac{S_{syn}}{S_{tot}} = \frac{315 \text{ units}}{965 \text{ units}} = 0.326 \text{ or } 32.6\%.$$

The synchronizing effect of DBcAMP is strong enough to produce a more marked antitumor action of a specific antimitotic preparation.

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