DIURNAL FLUCTUATIONS IN RESPONSES OF MOUSE CORNEAL EPITHELIAL CELLS TO VINBLASTIN

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A drop of 0.2% vinblastin solution was applied to the mouse cornea at 7 a.m. and 7 p.m. The cornea of the opposite eye of the same animal was used as the control (physiological saline was applied). The animals were killed 3 h after each application. The results of two analogous experiments showed that application of vinblastin in the evening induced a more marked stathmokinetic reaction in the corneal epithelium than its application in the morning. The percentage of C mitoses also was higher in the evening.

KEY WORDS: vinblastin; cornea; stathmokinetic reaction; diurnal fluctuations in response.

Investigations have shown that the response of the body to the same factor differs at different times of day and night. As an example, the results of investigations to study the action of a number of physical factors [18], chemical preparations [2-6, 9, 15-17], hormones [8, 10, 19], certain metabolites [14], x rays [12], and general anesthetics [7] can be cited.

The writer studied the action of colchicine on the epithelium of the small intestine and cornea in mice [11]. The stathmokinetic action of the alkaloid on the intestinal epithelium was stronger in the evening than in the morning.

This paper describes the results of a study of the action of vinblastin on the corneal epithelium in mice. Like colchicine, vinblastin is a stathmokinetic agent, causing delay of division in prometaphase [13]. The study of the response to vinblastin is particularly important because this substance is widely used in the chemotherapy of neoplastic diseases (leukemias).

EXPERIMENTAL METHOD

Two analogous series of experiments were carried out on 24 albino mice aged 2-2.5 months, using a method of application of the test substance to the corneal epithelium. A drop of 0.2% vinblastin solution, made up in physiological saline, was applied to the cornea of the left eye. The cornea of the right eye of the same animal acted as the control (physiological saline was applied). The drop was left on the cornea for 10 sec. The solutions were applied at 7 a.m. and 7 p.m. The animals were killed 3 h after application. Total preparations of the cornea were stained with trioxyhematein. Mitoses were counted in 100 fields of vision of each cornea. The total number of mitoses was expressed in absolute figures and the ratio between the phases of mitosis and the number of pathological forms of mitosis as percentages. The results were subjected to statistical analysis by the Fisher–Student method. Pathological mitoses were classified according to Alov [1].

EXPERIMENTAL RESULTS

Vinblastin caused an increase in the number of dividing cells in the corneal epithelium through a sharp delay in mitosis at the metaphase stage. However, the intensity of this response of the cells to vinblastin

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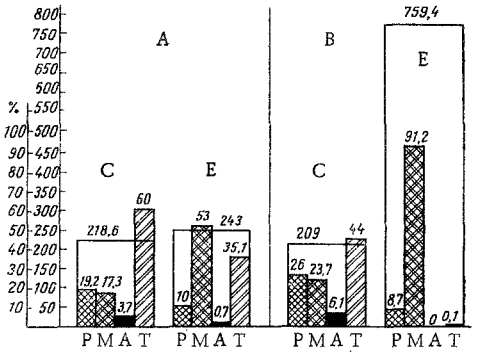

Fig. 1

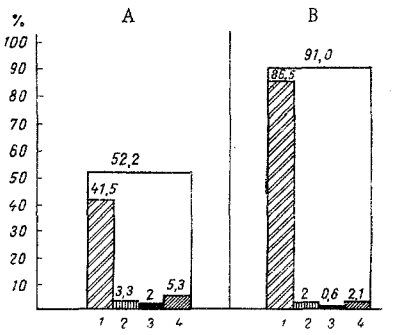

Fig. 2

Fig. 1. Diurnal changes in mitotic activity (wide columns) and relative percentages of phases of mitosis (narrow columns) following application of vinblastin to corneal epithelium in morning (A) and evening (B): C) control; E) after application of vinblastin (experiment); P) prophase, M) metaphase, A) anaphase, T) telophase. Ordinate: on left, relative percentage of phases of mitosis; on right, mitotic activity.

Fig. 2. Diurnal changes in number of C mitoses in corneal epithelium after application of vinblastin in morning (A) and evening (B). Unshaded columns – C mitoses (in %); 1) metaphases with scatter of supercoiled chromosomes (in %); 2) metaphases with fusion of chromosomes (in %); 3) "pseudoanaphases" (in %); 4) "spherical" metaphases (in %).

differed in the morning and evening (Fig. 1). In the evening the increase in the number of dividing cells was not significant and did not exceed 11% ($P = 0.1$), whereas in the evening in the experimental corneas compared with the control it was 268% ($P = 0.01$). Under the influence of vinblastin applied in the morning the increase in the number of metaphases compared with the control was 3.5 times greater, i.e., 35.7% ($P = 0.001$). Judging from the relatively high percentage of telophases after administration of vinblastin in the morning, only metaphase delay of cell division was observed at this time and there was no block at this stage of mitosis. In the evening the stathmokinetic effect was stronger. The number of metaphases was almost 15 times greater than in the control, i.e., an increase of 67.5% ($P < 0.0001$). Complete absence of anaphases and a sharp decrease in the number of telophases were observed. Consequently, the metaphase block effect was observed only in the evening. Vinblastin also caused the appearance of many colchicine mitoses (C mitoses), which were practically never seen in the control corneas. The degree of increase in the number of C mitoses differed in the morning and evening (Fig. 2). They were most numerous in the evening (52.2% in the morning, 91% in the evening; $P = 0.004$). The predominant forms of C mitoses were metaphases with scattered supercoiled chromosomes (41.5% in the morning, 85.5% in the evening). Metaphases with fusion of swollen chromosomes, "spherical" metaphases, and "pseudoanaphases" were less common.

Closely similar results were obtained in the experiments of series II. As in series I, after application of vinblastin in the evening the number of dividing cells was greater than in the morning compared with the control. The relative number of metaphases in the evening was increased more compared with the control than in the morning. A greater increase in the number of C mitoses also was observed after application of vinblastin in the evening than in the morning. The predominant forms of C mitoses also were metaphases with scattering of supercoiled chromosomes.

The results of these experiments thus confirm once again that the action of many substances, including vinblastin, depends on the time of their administration. The stathmokinetic effect of vinblastin on the corneal epithelium is stronger in the evening than in the morning.

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