EFFECT OF CYCLOPHOSPHAMIDE ON DIURNAL RHYTHM OF MITOTIC ACTIVITY IN SPLEEN CELLS OF MICE WITH LEUKEMIA

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A study of mitotic activity in the spleen cells of mice with leukemia showed that mitoses are more frequent by day and less frequent by night. Injection of cyclophosphamide at 10 A.M. and 10 P.M. reduced the number of mitoses by an equal degree.

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The principles governing the diurnal rhythm of mitotic activity in tumor cells have not been adequately investigated, and the published findings are conflicting. Some workers found no diurnal changes in the number of mitoses [1, 7], but most [4, 6, 9, 11] have observed fluctuations in mitotic activity during the 24-h period. Differing results have also been obtained regarding the character of the diurnal mitotic rhythm. Nevertheless, quantitative data for the rates of cell multiplication in tumor tissues during the 24-h period are important when assessing the action of mitotic inhibitors. It has recently been shown that the action of compounds and other factors inhibiting cell division in normal [3] and tumor tissues [5, 6, 10] may vary depending on the phase of the diurnal rhythm of mitosis during which the test procedure was applied.

The object of this investigation was to study the principles governing diurnal changes in the number of mitoses in the spleen of mice with leukemia and to examine the effect of cyclophosphamide, when injected at different times of the day or night, on the mitotic activity of leukemic cells.

EXPERIMENTAL METHOD

Young sexually mature male $C_{57}BL$ mice were used in the experiment. The animals were injected intraperitoneally with 2 million spleen cells from a mouse with La leukemia, suspended in 0.2 ml physiological saline, between 11 A.M. and noon. Altogether three series of experiments were carried out. The experimental animals of each series were divided into 8 groups, depending on the time of sacrifice, with 8-10 mice in each group. In the late stage of leukemia development (on the 7th day after injection), when the spleen consisted almost entirely of blast cells, the animals were sacrificed in groups in the course of the 24-h period, at intervals of 3 h. Pieces of spleen were fixed in Carnoy's fluid and sections were stained with Carazzi's hematoxylin and eosin. The number of mitoses in 7000-10,000 cells in each case was counted in uniform fields of vision in the sections. The mitotic index (MI) was calculated in promille relative to the total number of cells counted. Statistical analysis of the results was by the Fisher – Student method.

In series I, the mitotic activity (MA) of leukemic cells was investigated in mice receiving no treatment. In series II and III, on the 6th day of development of leukemia, the mice received a single injection of cyclophosphamide in a dose of 100 mg/kg: in series II at 10 P.M., in series III at 10 A.M. The animals were sacrificed 6 h after injection of the compound, as in series I, at intervals of 3 h during the 24-h period.

EXPERIMENTAL RESULTS

The results given in Table 1 show that the number of divided cells in the spleen of mice with leukemia varies during the 24-h period. By day (10 A.M.-4 P.M.) MI was high and showed little change. By 7 P.M.

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TABLE 1. Mean Values of MI (in %) in Spleen Cells of Mice with Leukemia

Time of sacrifice	Series I		Series II		Series III	
	MI	P	MI	P	MI	P
10 A.M.	13.9*		3.6		7.4	
1 P.M.	14.1		3.8	0.044	6.4	0.128
4 P.M.	14.8		5.8	0.063	3.8*	0.001
7 P.M.	15.9	0.0001	7.4	0.0001	3.4	
10 P.M.	11.5		5.3		3.4	
1 A.M.	11.5		4.9		3.6	
4 A.M.	10.8		3.4*	0.09	6.0	0.01
7 A.M.	12.2		2.7	0.091	6.8	
Note.	7 P.M.-4 A.M. P = 0.0001 1 P.M.-10 P.M. P = 0.042; 10 A.M.+1 P.M.-7 P.M. P = 0.03; 4-10 A.M. P = 0.02;		7 A.M.-7 P.M. P = 0.0001; 1-10 P.M. P = 0.0001; 1-7 A.M. P = 0.0001; 7 A.M.-1 P.M. P = 0.016; 1-7 P.M. P = 0.0001;		1-10 A.M. P = 0.0001; 0.0001; 10 P.M.-4 A.M. P = 0.006; 10 P.M.-7 A.M. P = 0.0001; 10 A.M.-4 P.M. P = 0.0001; 10 A.M.-7 P.M. P = 0.0001	

*Time when sacrifice of mice in series began.

the number of dividing cells had increased (P = 0.03). By 10 P.M. a marked decrease in MI was observed (P = 0.0001), and this continued until 4 A.M. (P = 0.0001). Toward 7 A.M. MI began to increase (for the interval 4-10 A.M., P = 0.02). Consequently, the lowest MA was observed by night (10 P.M.-4 A.M.), and by day it gradually increased to reach a maximum at 7 P.M. The mean MA for the 24 h period was 13.1%.

Results obtained in the experiments of series I for diurnal changes in the number of mitoses made it possible to investigate the action of cyclophosphamide on division of leukemic cells in the mouse spleen following administration of the compound at different times of day or night. According to data in the literature, cyclophosphamide acts on cells in the S- and G_2-periods of the mitotic cycle [8], and the total duration of the S + G_2 -periods varies from 10.8 to 13 h for different generations of leukemic cells [2]. Consequently, afetr administration of cyclophosphamide at 10 P.M. (series II), i.e., at a time when MA was beginning to fall, a different effect could be expected from it than after its administration at 10 A.M. (series III), i.e., in a period of gradual increase of MA. In both series (Table 1), cyclophosphamide produced a marked decrease in the mean MA for the 24-hour period: in series II to 4.6% (by 2.8 times compared with MA in series I), and series III to 5.1% (by 2.5 times). However, the expected differences were not found in the last two series. This can provisionally be explained by assuming that the dose of cyclophosphamide used was too high to produce different effects depending on the time of administration of the compound. Analogous experiments must be carried out with the use of smaller doses of the compound and its repeated administration.

In the experiments of series II at 4, 7, and 10 A. M. and 1 P.M., i.e., 6-15 h after injection of cyclophosphamide, the number of cell divisions detected was extremely small, and did not exceed 3.8%. The number then increased to reach a maximum (7.4%) at 7 P.M., i.e., 21 h after injection of the compound. This increase in MA in the period from 1 to 10 P.M. is significant (P = 0.0001). The character of diurnal changes in MA after administration of cyclophosphamide at night was thus similar to that of the changes observed in the animals of series I.

In series III, at 4, 7, and 10 P.M. and at 1 A.M., i.e., 6-15 h after injection of the compound, MA likewise did not exceed 3.8%. Thereafter it increased to reach a maximum (7.4%) at 10 A.M., i.e., 24 h after injection of cyclophosphamide (in the interval from 1 to 10 A.M., P = 0.0001). Consequently, after injection of the compound during the morning hours, the diurnal changes in MA also persisted, but in this case the periods of high and low MA did not coincide with the corresponding period observed for the animals of series I and II.

TABLE 2. Degree of Decrease of MI in Spleen of Mice with Leukemia after Injection of Cyclophosphamide Compared with MI for Mice of Series I Not Receiving Cyclophosphamide

Series	Time after injection of cyclophosphamide (in h)							
	6	9	12	15	18	21	24	27
II	3,1	4,5	3,9	3,7	2,5	2,1	2,2	2,3
III	3,6	4,6	3,4	3,1	1,8	1,7	1,9	2,2

Comparison of MA observed at different times after injection of cyclophosphamide showed that the inhibitory effect was greatest during the first 6-15 h. This is clear from the results in Table 2, which show by how many times MA was reduced after injection of cyclophosphamide compared with MA for spleen cells of mice with leukemia not receiving this treatment, at the same times of day and night. The ratio between the maximal and minimal numbers of mitoses was 1.47 (15.9:10.8) for series I, 2.74 (7.4:2.7) for series II, and 2.1 (7.4:3.4) for series III. This increase in the ratio in the last series indicates that cyclophosphamide has a certain synchronizing action on the entry of the cells into mitosis.

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