EFFECT OF CYCLOPHOSPHAMIDE ON DIURNAL RHYTHM OF MITOSIS AND RATE OF GROWTH OF A TRANSPLANTABLE TUMOR OF THE MOUSE FORESTOMACH WHEN ADMINISTERED AT DIFFERENT TIMES OF DAY

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After administration of cyclophosphamide to mice the mean mitotic activity for the 24-h period in a transplantable carcinoma of the forestomach was depressed. The greatest degree of inhibition of tumor growth was observed after administration of the compound during the morning.

Recent investigations have shown that inhibitors of cell division differ in their effects on the diurnal rhythm of mitosis both in normal tissues [4, 5] and in tumor tissues [3, 8] when these inhibitors are administered at different times of day.

Some workers [6, 7, 11] have observed that antitumor compounds, when administered at times of maximal and minimal mitotic activity (MA), differ in their effects on the growth of tumors and on the survival of animals affected by malignant tumors.

The object of the present investigation was to study changes in the character of the diurnal rhythm of mitosis and the rate of growth of a transplantable carcinoma of the forestomach (strain OZh-5) in mice after administration of cyclophosphamide to the animals at different times of day.

EXPERIMENTAL METHOD

Two series of experiments were carried out on sexually mature male C_3HA mice.

In series I the diurnal rhythm of mitosis was studied in tumors of two groups of mice receiving cyclophosphamide at different times of day. The time of administration of the compound was chosen on the basis of previous descriptions [1, 2] of the diurnal rhythm of mitosis in this tumor (the number of mitoses reaches a maximum at 10 A.M. and a minimum at 10 P.M.). Cyclophosphamide was given 5 h before the maximum or minimum of the number of mitoses was reached (at 5 A.M. and 5 P.M. respectively). On the 9th day of tumor growth the animals received cyclophosphamide by intraperitoneal injection in a dose of 50 mg/kg body weight. The first animal in all the groups was sacrificed 2 h after the injection, and other animals were sacrificed subsequently at intervals of 3 h for 24 h. At each time 6-7 animals were killed.

Pieces of carcinoma were fixed in Carnoy's fluid and embedded in paraffin wax and sections cut to a thickness of 7-8 μ were stained with hematoxylin. The number of mitoses in the various phases was counted (binocular microscope, 900×) in 10,000 cells in parts of the tumor free from necrosis. The mitotic index (MI) was calculated inpro mille. In the experiments of series II the rate of growth of the tumor was studied after administration of cyclophosphamide at 5 A.M. and 5 P.M. The 60 mice were divided into 3

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TABLE 1. Diurnal Changes in Number of Mitoses in Carcinoma of Forestomach after Administration of Cyclophosphamide at 5 A.M. and 5 P.M.

Time of day	Without cyclophosphamide (control)		Cyclophosphamide injected at 5 AM		Cyclophosphamide injected at 5 PM	
	MI	P	MI	P	MI	P
10	20,6	0,08	7,6	—	11,0	—
13	16,7	0,09	7,6	0,138	10,8	—
16	13,7	0,06	9,9	—	9,6	—
19	11,4	0,02	8,7	0,04	8,1*	0,07
22	8,7	—	6,4	—	6,5	0,389
1	8,5	0,004	6,4	0,006	5,8	0,02
4	12,0	0,13	9,9	—	8,8	—
7	14,2	0,004	5,7*	0,08	9,0	0,09
Mean	13,2		7,8		8,6	
10—16h		0,0001	7—16 h	0,005	19—1 h	0,017
10—1 h		0,0001	16—22 h	0,01	1—7 h	0,008
1—7 h		0,0001	16—1 h	0,02	1—10 h	0,0001

*1st animal sacrificed 2 h after injection.

groups, with 20 animals in each group. In group 1, starting from the 4th day after transplantation of the tumor, the animals received cyclophosphamide daily for 4 days by intraperitoneal injection in a dose of 50 mg/kg at 5 A.M. In group 2 the cyclophosphamide was injected at 5 P.M. in the same dose for 4 days starting from the 4th day after transplantation. The total dose of cyclophosphamide in each experimental group was thus 200 mg/kg. The animals of group 3 acted as the control. On the 12th day of growth of the carcinoma, i.e., 4 days after the last injection, the animals of all 3 groups were sacrificed and the tumor was removed, freed from surrounding tissues, and weighed. The mean weight of the tumor in each group was calculated and the percentage inhibition determined as the ratio of the difference in mean weight of the tumor in the control and the experimental groups to the mean weight of the tumor in the control group and expressed in percent.

Statistical analysis of the results was carried out by the Fisher-Student method.

EXPERIMENTAL RESULTS

As Table 1 shows, when cyclophosphamide was given in the morning or in the evening there was a decrease in the mean MA of the carcinoma of the forestomach over the 24-h period. The differences between the mean daily values of MI in the carcinoma of the control mice and after injection of cyclophosphamide at 5 A.M. and 5 P.M. were significant ($P = 0.003$ and 0.01 respectively), but differences between the mean daily MI after administration of the compound in the morning and evening were not significant ($P = 0.383$).

After administration of cyclophosphamide in the evening the character of the diurnal rhythm of cell division in the carcinoma was the same as in animals not receiving the compound. The number of mitoses reached a minimum between 10 P.M. and 1 A.M., after which the number of dividing cells showed an increase which became significant by 7 A.M. and reached its maximum between 10 A.M. and 1 P.M.

It is interesting to note that although 2 h after injection (at 7 P.M.) MA was depressed compared with its level in the untreated carcinoma ($P = 0.008$), the greatest effect was not observed until after 17 h (a decrease of 1.9 times), i.e., at 10 A.M.

A different picture was found when the cyclophosphamide was given in the morning: 2 h after injection (at 7 A.M.) inhibition of cell divisions reached a maximum, and the subsequent increase in the values of MI did not become significant until 4 P.M., after which a decrease in the number of dividing cells was observed as is characteristic of this tumor during the evening and night, and this was again followed by an increase in the number of mitoses. The effect of cyclophosphamide reached its maximum 5 h after injection (a decrease by 2.7 times), i.e., at 10 A.M.

After administration of cyclophosphamide in the morning, the values of MI at the times of maximal inhibition of cell division (7 and 10 A.M., 1 P.M.) were significantly lower than after its administration in the evening (P = 0.007, 0.01, and 0.008 respectively). Accordingly, the character of the diurnal rhythm of mitosis was altered.

Whereas in the control group and after administration of the compound in the evening the number of mitoses reached a maximum at 10 A.M., after administration in the evening the maximum was reached at 4 P.M.

It can therefore be concluded that the greatest depression of MA after administration of cyclophosphamide is observed at the times of maximal cell proliferation in the tumors, and if the compound is given at times before the number of mitoses reaches a maximum, this inhibition is greater in degree.

In the experiments of series II to study the action of cyclophosphamide on the rate of growth of carcinoma of the forestomach the following results were obtained: the mean weight of the tumor in the control series was 4.2 g, after administration of cyclophosphamide at 5 A.M. it was 3.1 g, and after its administration at 5 P.M. it was 3.6 g. The difference between the mean weight of the tumor in the control animals and after administration of cyclophosphamide at 5 A.M. and 5 P.M. respectively was significant (P = 0.0001 and 0.01 respectively). Differences between the weight of the tumor after administration of cyclophosphamide at the different times of day also were significant (P = 0.005). The percentage inhibition after administration in the morning was 26.2, and in the evening 13.9.

These results show that the smallest weight of the tumor was observed after administration of cyclophosphamide at 5 A.M., i.e., when it was given before the time of maximal MA.

These results show that the inhibitory effect of cyclophosphamide is exhibited soon after its administration, and that this effect is greatest if the compound is given at times when most cells of the given population are preparing for mitosis. This suggests that the compound acts preferentially on cells in the premitotic phase of the cell cycle.

These results are in agreement with those obtained by other workers [6, 7] who found maximal inhibition of tumor growth and maximal survival of animals with tumors after administration of cyclophosphamide (Endoxan) either at times before the number of mitoses reached a maximum or actually at the time of the maximum. The results of the present investigation also support the view that cyclophosphamide, a compound with alkylating action, affects the premitotic phase G_2 of the cell cycle in small doses (30-60 mg/kg) [9, 10].

The results of the present investigation indicate that the effect of cyclophosphamide, both on the diurnal rhythm of mitosis and on the rate of growth of carcinoma of the forestomach, differs when the compound is administered at different times of day.

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