EFFECT OF CYCLOPHOSPHAMIDE ON MITOTIC ACTIVITY OF THE ESOPHAGEAL AND CORNEAL EPITHELIA OF LEUKEMIC MICE

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UDC 616.155.392-092.9-085.277.3-07 : 616-018.7-018.15-076.5

Studies of the esophageal and corneal epithelia have shown that cyclophosphamide differs in its action on mitotic activity in various normal tissues of leukemic mice. The action of cyclophosphamide is exhibited at the peak of mitotic activity, approximately 9 h after the morning injection and 21 h after the evening injection. Changes in the mean mitotic activity over the 24-h period are independent of the time of injection of cyclophosphamide during that period.

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A diurnal rhythm of mitosis has been demonstrated in many normal tissues of the body and in tumors. It has also been shown that antimitotic agents differ in their effect depending on the time of day or night when they are given. However, this problem has been studied inadequately. Some investigations have dealt with the effect of time of administration of cytostatics on the level and diurnal rhythm of mitotic activity (MA) in some animal tissues [2, 3, 5]. Other workers have demonstrated the unequal antitumor effectiveness of compounds when given at different times of day [4, 6-8], the criteria of effectiveness in these investigations being the survival rate of the animals and the decrease in size of the tumors. In a previous investigation by one of the writers [1], results were obtained reflecting the effect of time of administration of cyclophosphamide on the level and diurnal rhythm of MA in the leukemic mouse spleen. It was also deemed necessary to study the effect of time of administration of cytostatics on the level and diurnal rhythm of MA in normal tissues of an animal affected by a tumor. In this case, besides determining the degree of toxicity of antitumor compounds relative to cell multiplication in normal tissues, it was also decided to determine the time of day at which cytostatics should be administered in order to increase the specificity of their action. For this purpose, the differences between rhythms of MA in tumors and normal tissues must be taken into account.

The object of this investigation was to study the effect of time of injection of cyclophosphamide on the level and diurnal rhythm of MA in the esophageal and corneal epithelia of leukemic mice.

EXPERIMENTAL METHOD

Young male adult mice of line C57BL were used in the experiments. All the mice were inoculated with La leukemia between 11 A. M. and 12 noon by intraperitoneal injection of $2 \cdot 10^6$ leukemic spleen cells. The animals were sacrificed on the 7th day after inoculation in groups of 8-10 mice at each time of investigation throughout the 24-h period at intervals of 3 h.

The number of mitoses in 10,000 cells was counted in histological sections through the lower third of the esophagus, while mitoses in the corneal epithelium were counted in total preparations, among 15,000-20,000 cells in each case. The mitotic index (MI) was expressed in promille.

Department of General Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 7, pp. 82-85, July, 1970. Original article submitted December 31, 1969.

TABLE 1. Diurnal Changes in MI in Esophageal and Corneal Epithelia of Leukemic Mice (in %)

Time of day	Without injection of cyclophosphamide		Injection of cyclophosphamide at 10 P. M.		Injection of cyclophosphamide at 10 A. M.	
	esophagus	cornea	esophagus	cornea	esophagus	cornea
10 A. M.	5.9	19.2	10.1	10.2	8.0	9.1
1 P. M.	4.1	13.3	7.0	8.9	4.7	7.7
4 P. M.	2.1	6.4	3.9	6.5	1.5	3.1
7 P. M.	2.6	3.1	2.8	3.7	2.1	1.3
10 P. M.	1.3	4.2	2.2	1.8	1.4	3.0
1 A. M.	2.3	4.4	2.4	1.4	3.6	3.9
4 A. M.	6.3	5.4	6.3	1.5	10.3	9.1
7 A. M.	5.3	16.8	8.9	10.3	9.6	11.0
Mean value of MI in 24-h period	3.7	9.1	5.4	5.5	5.1	5.9

The experiment consisted of three series. Series I included leukemic mice receiving no form of treatment. In series II, on the 6th day after inoculation with leukemia, the mice received an intraperitoneal injection of cyclophosphamide in a dose of 100 mg/kg body weight at 10 P. M. In series III the experimental conditions were the same as in series II, except that cyclophosphamide was injected at 10 A. M. In the last two series, the animals began to be sacrificed 6 h after injection of cyclophosphamide, at the same times of day and night as in series I.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

It is clear from Table 1 that diurnal differences in MA were clearly present in the esophageal epithelium of the leukemic mice (series I). The largest number of mitoses was found between 4 A. M. and 1 P. M., and the smallest between 4 P. M. and 1 A. M.

After injection of cyclophosphamide at 10 P. M. and 10 A. M. the character of the diurnal rhythm of mitosis was not substantially changed. At the same time, an increase in the mean daily MI compared with MI of leukemic mice not receiving cyclophosphamide was observed. Comparison of the mean MI for the 24-h period in the animals of series I and II showed that the differences exceeded the mean error by 3-4 times, and when the results of series I and III were compared, the mean error was exceeded by 2.6 times.

Additional experiments with colchicine did not confirm the view that the observed increase in the number of mitoses was due to an increase in the duration of mitosis.

Comparison of MI in the animals of series I and II at the same times of day and night (Fig. 1A) showed a marked increase in the number of cell divisions in the esophageal epithelium ($P = 0.003$) in series I 9 h after injection of the compound, i.e., at 7 A. M. The increase was still more marked 12 h after injection, i.e., at 10 A. M. ($P = 0.001$).

A different picture was found after injection of cyclophosphamide at 10 A. M. (Fig. 1B). During the first 15 h the compound had no effect on the number of mitoses, only after 18 h, i.e., at 4 A. M., was a slight increase in the number of mitoses found ($P = 0.06$), and the increase was more marked in degree at 7 A. M., i.e., 21 h after injection ($P = 0.0001$).

Consequently, the action of cyclophosphamide, regardless of the time of its administration, was exhibited during the period of maximal MA observed in the esophageal epithelium of leukemic mice not receiving the compound. Depending on the time of injection of cyclophosphamide, its stimulant action on cell division was exhibited at different times after administration.

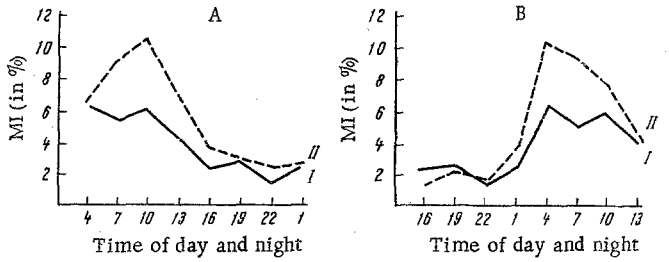

Fig. 1. Change in MA in esophageal epithelium of mice with leukemia after injection of cyclophosphamide at 10 P. M. (A) and at 10 A. M. (B). I) Without injection of cyclophosphamide; II) with injection of cyclophosphamide.

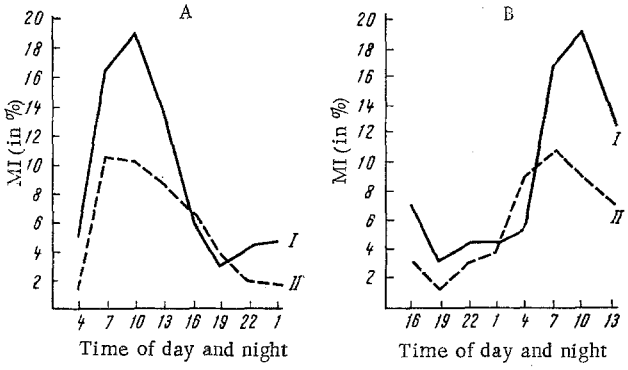

Fig. 2. Changes in MA in corneal epithelium of mice with leukemia after injection of cyclophosphamide at 10 P. M. (A) and at 10 A. M. (B). I) without injection of cyclophosphamide; II) with injection of cyclophosphamide.

In the corneal epithelium of leukemic mice (series I), considerable changes in the number of mitoses also were observed in the course of the 24-h period. The largest number of mitoses was found in the period from 7 A. M. to 1 P. M. and the smallest from 7 P. M. to 4 A. M.

The character of the diurnal rhythm of mitosis in the cornea of leukemic mice receiving cyclophosphamide at 10 P. M. (series II) or at 10 A. M. (series III) remained unchanged, although injection of the compound caused a decrease in the mean value of MI for the 24-h period (Table 1).

Comparison of MI for the animals of series I and II at the same times of day and night (Fig. 2A) shows that 6-9 h after injection of the compound the number of mitoses in the cornea of the animals of series II was slightly reduced (at 7 A. M. $P = 0.063$), while 12-15 h after injection, the inhibitory action of the compound was distinctly observable (at 10 A. M. and 1 P. M., $P = 0.0001$ and $P = 0.019$, respectively).

When cyclophosphamide was injected in the morning, the pattern of subsequent changes in MA was different (Fig. 2B). Throughout the first 18 h the compound had no significant effect of MA, and not until 21 h after its administration (at 7 A. M.) was a decrease in the number of cell divisions found ($P = 0.077$). The value of MA still remained lower at later times (at 10 A. M. and 1 P. M. $P = 0.0001$).

The inhibitory action of cyclophosphamide was thus exhibited in this series, as in the previous series, at the time of maximal MA.

Although there were no significant differences between the mean value of MI for the 24-h period in the corneal and esophageal epithelium depending on whether cyclophosphamide was given in the morning or in the evening, it would evidently be wrong to conclude that the compound had an identical action regardless of the time when it was given. The action of cyclophosphamide was exhibited in the early stages after its injection at the minimum of MA (at 10 P.M.), but at much later stages after its injection during the morning (at 10 A.M.), during a time of considerable MA.

The effect of cyclophosphamide in both cases was exhibited at the maximum of MA. This corresponds to the familiar view that cyclophosphamide acts not on mitosis itself, but on the processes preceding it.

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