EFFECT OF TIME OF CYCLOPHOSPHAMIDE ADMINISTRATION ON LIFESPAN OF MICE WITH La LEUKEMIA

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UDC 615.277.3.036.8;616.155.392-092.9

After administration of cyclophosphamide to mice with transplanted La hemocytoblastosis at 10 A.M., their mean lifespan was significantly longer than if the cyclophosphamide was injected at 10 P. M.

Investigations have shown that cytostatic agents differ in their action on the intensity of cell division in normal tissues [4, 7, 10] and on the lifespan of animals with malignant tumors [8, 9, 15] when administered at different times of day or night.

The object of this investigation was to study the effect of the time of administration of the alkylating compound cyclophosphamide, which is widely used for the treatment of leukemia, on the lifespan of mice with transplanted leukemia (La hemocytoblastosis). This problem was investigated after administration of single large doses of cyclophosphamide at the height of leukemia development and in the initial periods after transplantation (series I). The effect of small doses of the compound, injected repeatedly at different times of day, on the lifespan of the mice also was investigated (series II).

EXPERIMENTAL METHOD

Leukemia was transplanted into C57Bl mice by intraperitoneal injection of $2 \cdot 10^6$ cells obtained from the spleen of a mouse with La hemocytoblastosis.

In series I the effect of cyclophosphamide, injected as a single dose of 100 mg/kg at different times of day, on the lifespan of mice with leukemia was studied. The experimental animals were divided into 5 groups. Group 1, the control, included mice with leukemia and not receiving cyclophosphamide. The animals of group 2 received cyclophosphamide at 11 A. M., which was 5 days 23 h after transplantation of the leukemia, and the animals of group 3 received cyclophosphamide at 11 P. M., or 6 days 11 h after transplantation of the leukemia.

The difference in the duration of leukemia development up to the time of injection of the compound occurred because leukemia was transplanted at the same time in all 3 groups, while cyclophosphamide was injected at different times of day. The effect would also have been different if the leukemia had been transplanted at different times so as to equalize the duration of its development up to the time of injection of the cyclophosphamide, because the time of carcinogenic action also affects the intensity of development of tumors [11, 12].

In group 4 the leukemia was transplanted at 9 A.M., and cyclophosphamide was injected 2 h later. In group 5 the leukemia was transplanted at 9 P.M., and cyclophosphamide also injected 2 h later. The role of the last 2 groups is largely one of a technical control, because the early injection of antitumor substances is frequently used to study the activity of these compounds in experimental chemotherapy. The results of the experiments of series I are given in Table 1.

Department of General Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. F. Bilibin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 2, pp. 76-78, February, 1971. Original article submitted July 1, 1970.

TABLE 1. Effect of Cyclophosphamide, Injected at Different Times of Day, on Lifespan of Mice with La Hemocytoblastosis

Group	Number of mice in group	Time of transplantation of leukemia (in h)	Time of injection of cyclophosphamide (in h)	Time elapsing after transplantation of leukemia	Mean lifespan (in days)	Lifespan (in % of control)	P relative to control	P relative to other group
1-я	12	12	—	—	9,4	100	—	—
2-я	11	12	11	5 суток 23 часа	19,5	207	0,0001	
3-я	14	12	23	6 суток 11 часов	16,9	169	0,0001	0,002
4-я	15	9	11	2 часа	14,6	155	0,0001	0,01
5-я	15	21	23	2 »	14,5	154	0,0001	

TABLE 2. Effect of Cyclophosphamide, Injected in Small Doses at Different Times for 5 days, on Lifespan of Mice with La Hemocytoblastosis

Group	Number of mice in group	Beginning of administration of compound after transplantation of leukemia (in days)	Time of injection of cyclophosphamide (in h)	Sessional dose (in mg/kg)	Total dose (in mg/kg)	Mean lifespan (in days)	Lifespan (in % of control)	P relative to control	P relative to other group
1-я	10	—	—	—	—	7,9	100	—	—
2-я	17	4	10	20	100	12,6	158	0,0001	
3-я	18	4	22	20	100	10,6	133	0,0001	0,0001
4-я	18	4	10	7	35	8	100	—	—
5-я	18	4	22	7	35	8,2	103	—	—

EXPERIMENTAL RESULTS

A single injection of cyclophosphamide in a dose of 100 mg/kg body weight on the 6th-7th day of leukemia development led to a significant increase in the lifespan of the mice. However, the degree of increase in the lifespan depended on the time of injection of the compound: it was greater if the injection was given in the morning than in the evening. The difference between the mean lifespans of the mice was 2.6 days, and was statistically significant.

Injection of the same dose of cyclophosphamide 2 h after transplantation of the leukemia also led to an increase in lifespan of the animals ($P = 0.0001$), but there was no difference depending on the time of injection of the compound.

In series II the effect of cyclophosphamide, when injected in small doses, on the lifespan of the mice with leukemia was studied if the injections were given repeatedly at different times of day. The animals also were subdivided into 5 groups. Group 1 consisted of control mice with leukemia and not receiving cyclophosphamide. The rats of group 2 received cyclophosphamide for 5 days, starting from the 4th day after transplantation of the leukemia, in a dose of 20 mg/kg at 10 A.M. each day; in group 3 the experimental conditions were the same but the injections were given at 10 P.M. each day; in group 4 cyclophosphamide was given for 5 days, starting on the 4th day after transplantation of the leukemia, in a dose of 7 mg/kg body weight at 10 A.M. each day; and in group 5 the conditions were the same but the injections were given at 10 P. M. each day. The results are given in Table 2.

As a result of 5 injections of the compound in a total dose of 100 mg/kg, a significant increase in mean lifespan of the mice compared with the control was found. The lifespan of mice receiving cyclophosphamide at 10 A.M. was longest. Its difference (2 days) from the lifespan of mice receiving cyclophosphamide at 10 P.M. was statistically significant. A dose of 7 mg/kg, injected daily for 5 days, was ineffective.

Regular diurnal changes in the number of mitoses have now been found in several spontaneous, induced, and transplanted tumors. In recent investigations of some normal tissues of laboratory animals it has also been shown that the number of cells in various phases of the mitotic cycle also changes during the 24-h period. The number of cells synthesizing DNA is greater at some times of day than at others [5, 6, 14]. Although there is evidently no strictly definite interval between the time of day when the number of cells synthesizing DNA is at its maximum and the time of the maximum number of mitoses, the possibility nevertheless is not ruled out that these two periodic processes may be linked in some definite manner.

The writer has previously demonstrated [1] a diurnal rhythm of mitosis in the spleen cells of mice with La hemocytoblastosis. The largest number of mitoses was found at 7 P.M. and the smallest between 10 P.M. and 4 A.M. On the basis of figures for the duration of the mitotic cycle and of its individual periods in leukemic cells [3], and also of the fact that cyclophosphamide exerts its antitumor action through its blocking effect on processes taking place in the premitotic and synthetic periods of the cycle [13], it was decided to inject cyclophosphamide at different times of day, 6-9 h before the minimum and maximum of the number of mitoses, with the object of ensuring that it acted on interkinetic cells at the periods of the mitotic cycle when they were most sensitive to it.

The results of an earlier investigation [2], when no differences in the mean daily level of mitotic activity depending on the time of injection of the compound could be discovered after a single injection of cyclophosphamide into mice in a dose of 100 mg/kg, cannot be regarded as contradicting the results of the present investigation. On the contrary, the results now obtained, confirming the role of the time of injection of compounds in increasing their effectiveness, call for further attempts to be made to discover the causes of this phenomenon.

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