EFFECT OF COLCHAMINE (DEMECOLCINE) ON MITOTIC ACTIVITY OF MOUSE LEUKEMIC SPLEEN CELLS

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KEY WORDS: mitotic activity; La hemocytoblastosis; colchamine (demecolcine); preprophase inhbition of mitosis.

To determine the rates of cell division in tissues from normal animals or after various procedures, the colchicine method is widely used. It is based on the fact that colchicine and its derivatives, especially colchamine (demecolcine, colcemid), block cell division in metaphase. The intensity of cell proliferation is judged from the rate of accumulation of blocked metaphases in the tissues. It is usually assumed that colchicine does not cause preprophase inhibition and, consequently that it does not affect the rate at which cells enter into mitosis. However, there are insufficient grounds for this assumption, for only a few investigations of this problem have been published and the results are contradictory [1, 6-10].

The problem of the effect of colchicine on the rate of entry of cells into mitosis can be solved only by comparing the number of prophases in the tissues of intact animals and of animals treated with colchicine. If the number of prophases in the tissues of animals treated with colchicine is statistically significantly lower, it can be concluded that colchicine inhibits the entry of cells into mitosis and, consequently, the suitability of this method for judging the rates of cell proliferation must be questioned.

The object of this investigation was to study the effect of colchamine on diurnal changes in mitotic activity of mouse leukemic spleen cells.

EXPERIMENTAL METHOD

Adult C57BL mice were used. All the animals were injected intraperitoneally with 2,000,000 leukemic cells from the minced spleen of a mouse with La hemocytoblastosis.

On the 6th day, at the stage of developed acute leukemia, the animals were killed and pieces of spleen were fixed for histological investigation. The animals of the experimental groups (three or four mice in each group, total number of mice 21) were given an intraperitoneal injection of colchamine 4 h before sacrifice in a dose of 5 mg/kg body weight. Parallel with the experimental series, control animals were killed at intervals of 2 h for a period of 24 h, three or four mice at each time (total number of animals 45).

The number of mitoses in histological sections through the spleen was counted in phases, in each case in 8000-10,000 cells, in areas uniformly filled with leukemic cells.

Mitotic indices in the spleens of the control mice (MI), mitotic indices for the number of colchamine-blocked mitoses (MI_C), and prosphase indices (PI) were determined and the duration of mitosis (tm) was calculated by the usual equation:

$$tm = \frac{MI \cdot A}{MI_C},$$

where MI is the mean mitotic index of leukemic spleen cells from control mice, MI_C the mean mitotic index of leukemic spleen cells from mice receiving colchamine, and A the duration of action of colchamine (in h).

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TABLE 1. Diurnal Changes in Mitotic and Prophase Indices and in tm in Mouse Leukemic Spleen Cells

Periods of day or night, h	MI ‰	MI_c ‰	*tm*, h	PI, ‰ control	PI, ‰ experiment	*P*	$PI_{colchamine}/PI_{control}$, %
8 a.m-noon	15.2	111,9	0,54±0,059	1,78	0,74	0,042	41,5
Noon-8 p.m	15,9	153,1	0,41±0,075	2,30	1,34	0,009	57,8
8 p.m.-midnight	17,3	140,3	0,49±0,039	3,07	1,16	∞	37,7
Midnight-4 a.m	14,8	122,5	0,48±0,060	2,30	1,03	0,055	44,7
4 a.m.-8 a.m.	15,9	129,1	0,46±0,045	2,37	0,70	0,001	29,1
Mean value for 24-h period	15,8	136,7	0,46±0,043	2,29	0,99	∞	43,2

The error of the duration of mitosis was calculated by the equation for the error of the quotient obtained by dividing arithmetic means with their errors [4]. All numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Since in some of the experimental mice of two of the time groups studied (noon-4 p.m. and 4-8 p.m.) the leukemia was independently developed and the spleens of those animals were not studied histologically, the data for those experimental mice are pooled into one time group (noon-8 p.m.).

Diurnal fluctuations in MI in the control animals were small, but within the 8 p.m.-midnight period a significant increase ($P = 0.045$) in the number of mitoses was observed, in agreement with earlier observations [2] showing that values of MI in the leukemic mouse spleen reach a maximum at 7 p.m.

The value of MI_c fluctuated a little during the 24-h period, but its values for neighboring 4-h intervals never differed significantly. Differences between maximal and minimal values of MI_c likewise were not significant.

Values of PI in the control and experimental mice also did not differ significantly during the 24-h period. Comparison of the values of PI showed that in all time intervals of day and night their values in the experimental animals were significantly lower than in the controls. Mean differences between PI for the control and experiment mice for the whole 24-h period likewise were significant to the highest degree (∞).

These results indicate that colchamine causes marked preprophase inhibition of the entry of cells into mitosis.

Fluctuations in the value of tm during the 24-h period were small and not significant. It must be emphasized that values of tm obtained in experiments using the colchicine technique do not reflect true values, for, as the data given above show, colchamine sharply inhibits the rate of entry of cells into mitosis.

The effect of colchamine on mitotic activity of leukemic spleen cells has certain unique features. It was shown recently [3] that colchamine also evokes marked preprophase inhibition in normal tissues of adult rats, but the effect is manifested only at night. At the same time it has been found [1] that colchamine causes a significant decrease in the early prophase index in carcinoma of the mouse forestomach between 10 p.m. and 2 a.m., but does not reduce the values of PI.

It can be concluded from these results that the colchicine technique cannot be used to determine the duration of mitosis or the rates of cell proliferation in tissues. The method determining the rates of cell renewal of tissues suggested previously [5, 7], which is based on counting the total number of mitoses blocked by colchicine in the 24-h period, is likewise unsuitable for these purposes.

LITERATURE CITED

1. M. V. Berezkin, Byull. Éksp. Biol. Med., No. 11, 1363 (1976).
2. V. I. Vasil'eva, Byull. Éksp. Biol. Med., No. 2, 85 (1970).
3. V. N. Dobrokhotov, V. S. Valvas, V. I. Vasil'eva, et al., in: Methods of Regeneration and Cell Division [in Russian], Moscow (1979).
4. G. F. Lakin, Biometrics [in Russian], Moscow (1973), p. 95.

5. F. D. Bertalanffy, Acta Anal. (Basel), 40, 130 (1960).
6. J. L. Henry, J. Meyer, J. P. Weinmann, et al., Arch. Path., 54, (1952).
7. C. R. Leblond and C. E. Stevens, Anat. Rec., 100, 357 (1948).
8. H. R. Mühlemann, T. M. Marthaler, et al., Bull. Schweiz. Akad. Med. Wiss., 12, 162 (1956).
9. C. E. Stevens-Hooper, Am. J. Anat., 108, 231 (1961).
10. C. E. Stevens and C. R. Leblond, Anat. Rec., 115, 231 (1953).

TRANSFER OF THE HEMATOPOIETIC MICROENVIRONMENT DURING HETEROTOPIC TRANSPLANTATION OF A BONE MARROW CELL SUSPENSION

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Heterotopic (i.e., extraskeletal) bone marrow transplantation has been widely used in recent years to study the hematopoietic microenvironment and, in particular, the cells responsible for its transfer [1-3, 6]. Meanwhile the cellular mechanisms of formation of the new hematopoietic organ during transplantation, including the formation of the hematopoietic microenvironment, have not yet been explained. We do not know the minimal tissue structure capable of the transplantation effect. Heterotopic transplantation of bone marrow with the formation of a new hematopoietic organ by transfer of a suspension of hematopoietic cells has not yet proved successful. Conversely, many workers have specially stated that this would be impossible, i.e., that self-assembly of stromal structures from dissociated cells cannot take place [5] and that transfer of certain undisturbed stromal structures is essential for the formation of new hematopoietic territories.

The investigation described below showed that successful heterotopic transplantation of bone marrow may be achieved by the transfer of initially isolated bone marrow cells, and the conditions necessary for such transplantation and the subsequent stages of self-assembly of the hematopoietic tissue also are described.

EXPERIMENTAL METHOD

CBA and (CBA × C57BL)F_1 mice aged 8 weeks were used as donors of bone marrow. The bone marrow was flushed out of the femora and tibiae by means of a syringe with a needle, and was suspended by means of a syringe and needles of diminishing diameter in medium 199 or an MEM spinner and filtered through two to four layers of Kapron gauze. The filtered suspension was centrifuged so that (4-6) × 10^6 bone marrow cells were deposited on a millipore filter (of HA, AUFS, or RA type) with an area of 20-25 mm^2. The filter was then folded in half with the cells inside and transplanted syngeneically beneath the kidney capsule. Filters without bone marrow cells and filters with a suspension of bone marrow cells irradiated in a dose of 5000 R served as the control.

After 1, 2, 3, 4, and 8 weeks the grafts were fixed in alcohol-formol, decalcified, and embedded in paraffin wax; the PAS reaction was carried out on sections, which were counterstained with hematoxylin.

To determine whether the bone marrow cells responsible for transfer of the hematopoietic microenvironment are phagocytes, phagocytic cells were removed beforehand from the cell sus-

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