DIURNAL VARIATIONS IN THE RESPONSE OF INTESTINAL AND CORNEAL EPITHELIAL CELLS OF MICE TO COLCHICINE

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The response of epithelial cells of the small intestine and cornea to administration of colchicine at different times of day and night was studied in mice. Colchicine, made up in physiological saline, was injected subcutaneously into the animals in a dose of 1 mg/kg at 7 a.m. and 7 p.m. Mice of the control group received physiological saline. The animals were killed 2 h after each injection. After injection of the alkaloid in the evening the stathmokinetic effect in the intestinal epithelium was more marked than after the morning injection. In the corneal epithelium the diurnal variations in the stathmokinetic reaction were less marked and were classed chiefly as metaphase delay.

KEY WORDS: colchicine; intestinal epithelium; corneal epithelium; c-mitoses.

Diurnal variations in the response of cells to various physiological factors and chemical substances discovered at the end of the 1950's[13, 14] have recently attracted increasing attention. Investigations have been carried out to study diurnal variations in the response to sarcolysin [5], antimetabolites (actinomycin D, 5-fluorouracil) influencing RNA and DNA synthesis [11], hydroquinone [6], adrenalin [9], hydrocortisone [7], chemotherapeutic preparations [2-4, 8], x rays [10], and colcemid [12]. Considering the importance of these observations for the development of rational therapeutic schemes, it was decided to study diurnal variations in the response of the intestinal and corneal epithelium to the stathmokinetic poison colchicine.

EXPERIMENTAL

Experiments were carried out on mice (65 animals in two analogous series) aged 2-2.5 months. Colchicine, made up physiological saline, was injected subcutaneously into the animals at 7 a.m. and 7 p.m. Mice of the control group received physiological saline. The animals were killed 2 h after each injection. Mitoses were counted in sections through the epithelium of the small intestine stained with hematoxylin and eosin and in total preparations of the cornea stained with trioxyhematein (in 100 fields of vision of each preparation). The total number of mitoses was expressed in absolute numbers, and the phases of mitosis and the number of c-mitoses as percentages. Statistical analysis of the results was carried out by the method of Fisher and Student. Pathological mitoses were defined by Alov's classification [1].

RESULTS

Counting the number of dividing cells in the intestinal epithelium of the control groups revealed an almost identical level of mitotic activity in the morning and evening. In the experimental group of animals colchicine produced a sharp rise in the number of dividing cells and inhibited division at the metaphase stage. However, the stathmokinetic reaction of the intestinal epithelium to administration of colchicine at the different times differed. In the morning (Fig. 1) the number of dividing cells was increased by 2.7 times,

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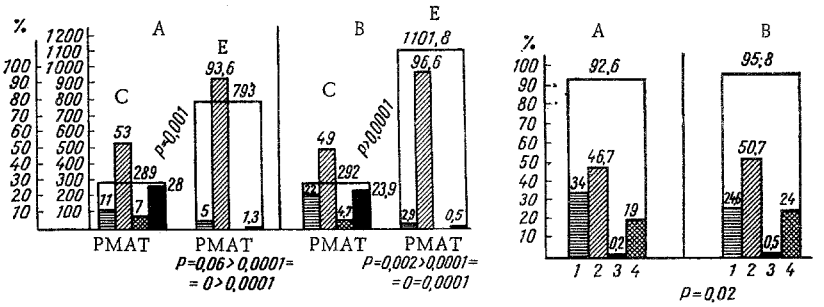

Fig. 1. Diurnal changes in mitotic activity and relative percentages of the phases of mitosis in the intestinal epithelium in response to colchicine injected in the morning (A) and evening (B): C) control; E) after injection of colchicine (experimental); P) prophase, M) metaphase, A) anaphase, T) telophase.

Fig. 2. Diurnal variation in number of pathological mitoses in intestinal epilium after morning (A) and evening (B) administration of colchicine. Unshaded columns represent pathological mitoses (in %). 1) Scattered metaphases with shortened, condensed chromosomes (in %); 2) metaphases with fusion of swollen chromosomes (in %); 3) spherical metaphases (in %); 4) pseudoanaphases (in %).

TABLE 1. Changes in Mitotic Activity in Epithelium of Small Intestine under the Influence of Colchicine

Time of injection	Group of animals	Number of animals	Mitotic activity	Relative percentages of phases of mitosis				c-mitoses (in %)	Relative percentages of different forms of c-mitosis (%)			
				prophase	metaphase	anaphase	telophase		scattered metaphase	fusion of chromosomes	spherical metaph.	pseudoanaphase
7 a.m.	Control	7	358,7	22,7	37,2	4,4	35,7	—	—	—	—	—
	Exprt.	7	993,1	11,7	86,5	0	1,3	86,6	38,5	36,1	1,5	23,8
7 p.m.	Control	7	366,0	18,0	39,8	5,4	36,7	—	—	—	—	—
	Exprt.	8	1196,5	8,7	90,2	0	1,4	90,0	25,6	37,0	0,9	36,4

i.e., by 174.3% ($P < 0.001$). After administration of colchicine at 7 p.m. the number of cell divisions increased by 3.7 times, i.e., by 279.8% ($P < 0.0001$). After the morning injection of colchicine the number of metaphases was increased to almost 5 times the control value, i.e., by 381.3% ($P < 0.0001$). In the evening the effect of delayed division was more marked. The number of metaphases in the experimental group was increased by 7.4 times, i.e., by 642.7% ($P < 0.0001$). After both morning and evening injections the complete absence of anaphases and the sharp decrease in the number of telophases (by 8 times in the morning and by 12 times in the evening) were observed in the animals of the experimental groups. Pathological mitoses in the intestinal epithelium of the control groups of mice were virtually absent. Under the influence of colchicine their number rose sharply. The degree of increase in the number of c-mitoses also differed in the morning and evening (Fig. 2). They were more numerous in the evening (92.6% compared with 95.8% of c-mitoses in the evening; $P = 0.02$). The pathological forms included severe swelling and fusion of the chromosomes, together with clumped metaphases. The highest percentage of pathological forms of c-mitoses was accounted for by scattered metaphases with shortened, highly condensed chromosomes, metaphases with fusion of swollen chromosomes, and spherical metaphases (99.8% in the morning and 99.4% in the evening). Pseudoanaphases were rare (0.2% in the morning and 0.6% in the evening).

Similar results were obtained in the second series of experiments (Table 1).

Less clear results were found in the experimental animals when the response of the corneal epithelium to injection of colchicine in the morning and evening was studied. High indices of mitotic activity were found in these cells in the morning, and low in the evening. Colchicine induced changes in the cornea similar to those observed in the intestinal epithelium. However, the response of the cells to colchicine in the dose used (1 mg/kg) was less clear. The increase in the level of mitotic activity (Fig. 3) in the experimental

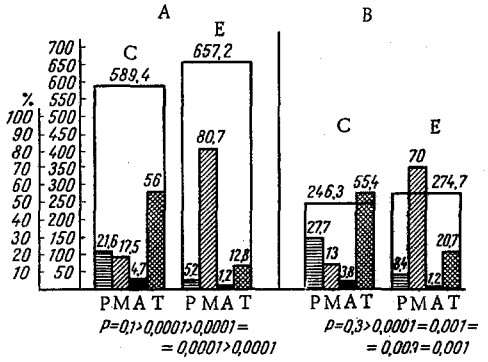

Fig. 3. Diurnal changes in mitotic activity and relative percentages of the phases of mitosis in the corneal epithelium produced by morning (A) and evening (B) injection of colchicine. Legend as in Fig. 1.

groups was not significant (P = 0.1) both in the morning and in the evening. A larger increase in the number of c-mitoses was observed in the morning (80%) than in the evening (68.3%; P < 0.0001), with a predominance of metaphases with scattering of shortened, condensed chromosomes (83.4% in the morning, 89.2% in the evening). Metaphase delay was most marked after injection of the alkaloid in the evening (Fig. 3). In the morning, the number of metaphases was increased by 4.6 times compared with the control group under the influence of colchicine, i.e., by 413% (P 0.0001). In the evening the number of metaphases in the experimental group was increased by 5.4 times, i.e., by 493%, suggesting that colchicine acts more strongly in the evening.

The results of these experiments thus showed that the response of the cells to colchicine varies with the time of day. The stathmokinetic effect was more marked in the intestinal epithelium after evening injection of the alkaloid than after its morning injection. In the corneal epithelium diurnal differences in the stathmokinetic response also were observed, but they related chiefly to metaphase delay and they were less marked. The reason for this may probably be the poorer blood supply to the cornea, resulting in penetration of less of the colchicine into it, and also differences in the sensitivity of different tissues to this alkaloid.

LITERATURE CITED

1. I. A. Alov, Vestn. Akad. Med. Nauk SSSR, No. 11, 58 (1965).
2. M. V. Berezin and S. P. Arbuzov, in: Diurnal Rhythms of Physiological Processes [in Russian], Moscow (1972), p. 29.
3. M. V. Berezkin, Effect of Cyclophosphamide on Cell Division in Tumors and in Normal Mouse Tissue after Its Administration at Different Times of Day and Night. Author's Abstract of Candidate's Dissertation, Moscow (1973).
4. V. I. Vasil'eva, Principles Governing the Diurnal Rhythm of Cell Multiplication in Mice with Leukemia. Author's Abstract of Candidate's Dissertation, Moscow (1970).
5. V. N. Dobrokhotova, Vestn. Akad. Med. Nauk SSSR, No. 7, 50 (1963).
6. A. A. Zhirnova, Byull. Éksperim. Biol. i Med., No. 1, 71 (1971).
7. É. T. Ostroushko and A. G. Mustafin, in: Diurnal Rhythms of Physiological Processes [in Russian], Moscow (1972), p. 36.
8. V. V. Sinel'shchikov, in: Diurnal Rhythms of Physiological Processes [in Russian], Moscow (1972), p. 33.
9. N. F. Semenova, Byull. Éksperim. Biol. i Med., No. 2, 91 (1971).
10. N. V. Uryupina, Byull. Éksperim. Biol. i Med., No. 11, 90 (1972).
11. S. S. Cardoso and J. R. Carter, Proc. Soc. Exp. Biol. (New York), 131, 1403 (1969).
12. K. Hapler Pater, J. Cell. Biol., 55, 112 (1972).
13. F. Halberg, E. Haiss, and A. Stephens, Fed. Proc., 18, 63 (1959).
14. F. Halberg, E. Johnson, W. Brown, et al., Proc. Soc. Exp. Biol. (New York), 103, 142 (1960).