EFFECT OF TIME OF MYLERAN ADMINISTRATION ON DIURNAL MITOTIC RHYTHM OF EPITHELIUM OF THE RAT SMALL INTESTINE

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Administration of myleran to rats reduces the number of mitotically dividing cells in the epithelium of the small intestine. This effect is observed whether the myleran is given at 6 A. M. (the maximum of mitoses under normal conditions) or at 10 P. M. (minimum of mitoses). Myleran completely abolishes diurnal changes in mitotic activity observed in control animals.

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Several investigations have shown that the action of various factors on the living organism varies depending on the time of day. This has been demonstrated, in particular, by the study of the pattern of cell division in animals [5-8, 10, 11, 14, 15] and plants [9, 13].

In this connection V. N. Dobrokhotov [6, 7] has suggested that differences in the response of the organism may depend on selectivity of the action of a particular factor on one of the periods of the mitotic cycle of the cell (postmitotic period, period of DNA synthesis, premitotic period, mitosis), and also on the number of cells in a given period of the cycle at a given moment.

In the present investigation the importance of the time of administration of myleran, an alkylating compound, for its action on the diurnal mitotic rhythm was studied in the crypt epithelium of the rat small intestine.

Myleran is an effective cytostatic drug in the treatment of chronic myeloid leukemia [2, 3, 12]. It is essential to study this antimitotic agent from this aspect because of the risk that normal diurnal fluctuations in the number of mitotically dividing cells may be erroneously taken as indicating the action of myleran.

EXPERIMENTAL METHOD

Experiments were carried out on 173 noninbred male albino rats with a mean weight of 180-200 g. Myleran was dissolved in peach oil and injected subcutaneously into the experimental animals in a single dose of 1.8 mg/100 g body weight in 0.2 ml oil. The control animals received 0.4 ml oil only.

In the experiment of series I myleran was injected at 6 A. M., i.e. at a time when in most tissues the number of mitotically dividing cells reaches a maximum. Myleran was injected into the experimental animals of series II at 10 P. M. i. e., when the number of mitoses is at minimum. The animals were sacrificed at 4-hourly intervals during the 24 hours, 7 experimental and 5 or 6 control rats being sacrificed at each time. The first time of sacrifices was 4 h after injection of myleran.

Mitoses were counted in 50 longitudinally divided crypts of the small intestine epithelium. The mitotic index (MI) was calculated for the total number of cells (5 000) in promille. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

In the experiments of series I diurnal fluctuation in the number of mitotically dividing cells were found in the epithelium of the crypts of the small intestine in the control animals. As Fig. 1 shows, the

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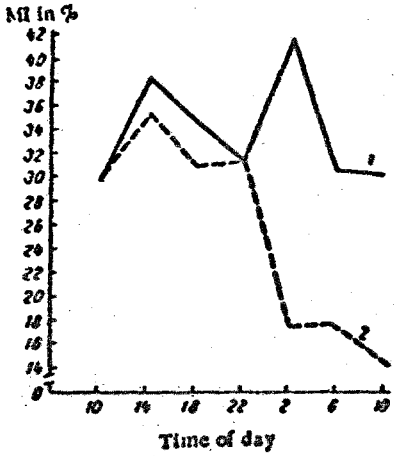

Fig. 1. Changes in mean mitotic indices in epithelium of rat small intestine after administration of myleran at 6 A. M. 1) Control; 2) experiment.

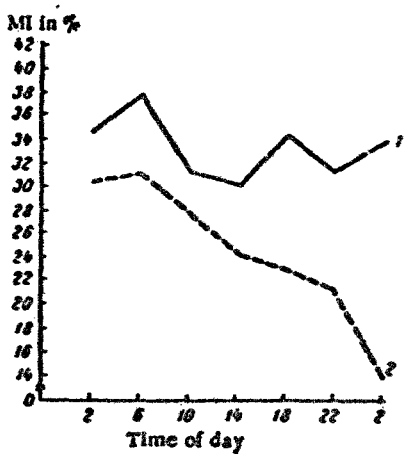

Fig. 2. Changes in mean mitotic indices in epithelium of rat small intestine after administration of myleran at 10 P. M. 1) Control; 2) experiment.

changes in MI during the 24 h followed a bimodal curve. The greatest and statistically significant differences between the MI values were found in the early morning (at 2-6 A. M. (for the interval 10 P. M.-2 A. M. P=0.005; 2-6 A. M. P=0.017). The second increase in number of mitoses at 2-6 P. M. was not statistically significant (for the interval 10 A. M.-2 P. M. P=0.043, 2-6 P. M. P=0.027). In the experimental animals 4 h after administration of myleran the number of mitoses was the same as in the controls. The curve showing the changes in MI 8, 12, and 16 h after administration almost precisely repeated the corresponding changes in the control animals. However, 20 h after administration of myleran the number of mitoses fell sharply (for the interval 10 P. M.-2 A. M. P=0.001) and it remained low 24 and 28 h after administration. Consequently, after administration of myleran at 6 A. M. we observed a sharp decrease in the mean values of MI in the experimental rats 20 h after administration of this inhibitor of cell division. The differences between the values of MI in the control and experimental series 20, 24, and 28 h after administration of myleran were statistically significant (at all times P=0.0001). The mean mitotic index over the 24 h period in the control series was 33.7% and in the experiment 25.2%.

In the experiments of series II the mitotic activity of the crypt epithelial cells of the small intestine reached a maximum at 6 A. M. (for the interval 2-6 A. M. P=0.01, 6-10 A. M. P=0.02), whereas the increase in the number of cell divisions at 6 P. M. was not statistically significant (from 2-6 P. M. P=0.16, from 6 to 10 P. M. P=0.22). Low mitotic activity was found during the afternoon and late evening (Fig. 2). The value of MI in the experimental animals 4 and 8 h after administration of myleran was actually lower than in the control animals. However, the differences between control and experiment were still not statistically significant (P=0.06). With an increase in the duration of action of myleran the level of mitotic activity in the experimental rats steadily declined, so that the differences in the interval between 6 A. M. and 2 P. M. were no longer statistically significant (P=0.009). The probability of a random difference in the interval between 2 P. M. and 2 A. M. likewise was very small (P=0.002). However, despite the slightly lower mean values of MI in the experiments of series II, significant differences between control and experiment, as in series I, were found only 20 h after administration of myleran (P=0.0001). The mean mitotic activity for the 24 h in the controls was 33.1% and for the experiment 24.3%.

The results of these experiments thus showed that after administration of myleran both at 6 A. M. and at 10 P. M., a distinct decrease in the number of mitotically dividing cells was found in the crypt epithelium of the small intestine of the experimental animals compared with the controls. A common factor to both series of experiments was that myleran completely abolished diurnal fluctuations in mitotic indices observed in the control animals. In addition, statistically significant differences between control and experiment were found 20 h after administration of myleran in both series I and series II. It may be concluded from the similar pattern of these changes that the time of administration of myleran has no effect on the subsequent rate of cell multiplication in the crypt epithelium of the small intestine.

In my opinion, the absence of differences in the response of the body to administration of myleran at different times of the day may be attributed to the specific pattern of cell division in the crypts of the small intestine. Published data and our own observations [1, 4] indicate that to obtain a clearly defined unimodal rhythm of mitotically dividing cells in the crypts of the small intestine the animals must be fed at strictly definite times (in the evening). Since our experimental animals were kept under ordinary vivarium conditions with free access to food throughout the 24 h, in the control animals we did not always obtain a definite unimodal diurnal mitotic rhythm in the crypt epithelium of the small intestine. In addition, the differences between maximal and minimal values of the mitotic indices often disappeared. That is evidently why, with certain factors acting at different times of day, but with insignificant differences in the initial background, i.e., in the level of mitotic activity in the epithelium of the crypts of the small intestine, we found that the result was independent of the time of injection of myleran.

Further evidence in support of this view is that our results were in full agreement with those obtained by V. M. Dobrokhotov and co-workers [6, 7], who found that when sarcolysin was administered during the morning and evening, a significant decrease also took place in the mean number of mitotically dividing cells during the 24 h in the crypt epithelium of the small intestine. Moreover, just as in our experiments, these workers found no difference depending on the time when the sarcolysin was injected.

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