STIMULATION OF FIBROBLAST PROLIFERATION DURING EXPERIMENTAL WOUND HEALING

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The dynamics of DNA synthesis by connective-tissue fibroblasts of intact mice and in the wounds of experimentally wounded mice was studied after administration of potassium orotate under different conditions. Potassium orotate was shown to increase the number of DNA-synthesizing fibroblasts equally in intact loose connective tissue and during wound healing. Stimulation of DNA synthesis is also possible within the strictly defined limits of the genetically controlled reserve capacity of the cells.

KEY WORDS: fibroblasts; stimulation; DNA synthesis; proliferation.

The intensity of wound healing largely depends on the number of functionally active fibroblasts, and for that reason the study of the rate of proliferation and the mitotic activity of these cells during stimulation of wound healing is important from the practical as well as the theoretical point of view. Knowledge of the optimal conditions for stimulation of wound healing, to ensure the largest number of these cells, is particularly important.

Previous investigations [2] showed that the use of potassium orotate in the postoperative period led to more rapid healing of wounds. This was accompanied by an increase in the number of DNA-synthesizing fibroblasts and by modification of the rhythm of DNA synthesis in these cells.

The object of the present investigation was to study the intensity of proliferation of fibroblasts and the rhythm of DNA synthesis by these cells during healing of soft tissue wounds under the influence of potassium orotate administered in accordance with different therapeutic programs.

EXPERIMENTAL METHODS

Experiments were carried out on 144 noninbred albino mice weighing 20-25 g. Standard incised wounds of skin and muscles, 1 cm long, were inflicted on 120 of these mice. The wounded mice were divided into four groups, with 30 mice in each group. The mice of group 1 served as the control. The mice of group 2 received potassium orotate by mouth in a dose of 0.2 ml of a 2% solution once a day from the first day after the operation. The mice of group 3 received potassium orotate by the same scheme for five days before wounding. The animals of group 4 received potassium orotate in a dose of 0.2 ml of a 2% solution twice a day, at an interval of 4 h, from the first day after the operation. Pieces of wound tissue were fixed daily at 11 a.m. in 10% formalin solution 2 h after intraperitoneal injection of [^{3}H]thymidine with a specific activity of 16 Ci/mmole and in a dose of 1 μCi/g body weight. Autoradiographs were obtained on paraffin sections in the usual way with the use of type M photographic emulsion. After development and staining of the autoradiographs, the number of fibroblasts labeled with [^{3}H]thymidine was counted and their ratio to the total number of these cells determined. The density of distribution of fibroblasts in the wound of the animals belonging to the different groups was estimated from their number in 20 fields of vision under a magnification of the microscope of 280 times. Similar counts were made in the skin and subcutaneous cellular tissue of eight intact mice, and also of 16 intact mice which received potassium orotate once and twice a day. The results were subjected to statistical analysis by Wilcoxon's method.

EXPERIMENTAL RESULTS

Autoradiographic analysis of incorporation of [^{3}H]thymidine into fibroblasts of the skin and subcutaneous cellular tissue of the intact mice showed that the number of DNA-synthesizing cells increased under the influ-

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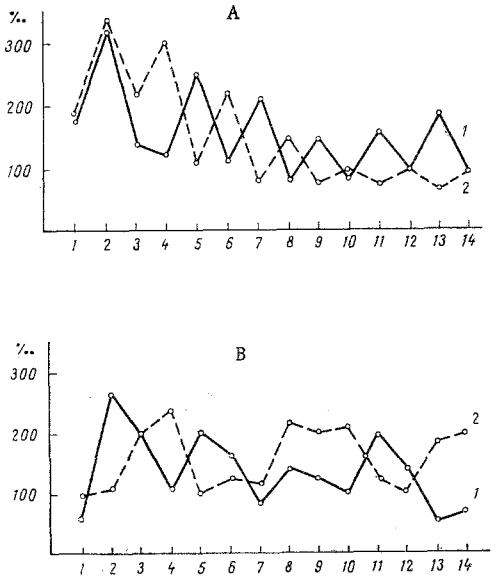

Fig. 1. Dynamics of DNA synthesis by fibroblasts during wound healing. Ordinate, index of labeled nuclei (ILN) of fibroblasts (in ‰); abscissa, time after operation (in days). A) DNA synthesis by wound fibroblasts in animals of groups 2 and 4: 1) ILN of fibroblasts in group 2; 2) ILN of fibroblasts in group 4. B) DNA synthesis by wound fibroblasts of animals of groups 1 and 3: 1) control, 2) in wound of animals of group 3.

ence of potassium orotate by 31% compared with the control (from 95 to 125‰). No difference was found between the animals receiving the stimulator once or twice a day. However, administration of potassium orotate once a day caused an increase in the number of fibroblasts in 20 fields of vision by 15%, whereas its administration twice a day led to an increase of 22%. The differences are statistically significant.

Comparison of the number of DNA-synthesizing fibroblasts in the wounds showed a statistically significant increase in their number in the mice of groups 2 and 4 by 31% and in group 3 by 23% compared with the control. Administration of potassium orotate twice a day to the wounded animals led to the same increase in the number of DNA-synthesizing cells as in the animals treated once a day. The maximal number of these cells in the control wounds and in the wounds of the mice receiving potassium orotate from the first postoperative day occurred on the second day after wounding (Fig. 1A). In mice receiving the stimulator before the operation, the number of fibroblasts synthesizing DNA reached a maximum on the fourth day after wounding (Fig. 1B). Counting the number of fibroblasts in 20 fields of vision showed that the density of distribution of these cells increased more rapidly in mice receiving potassium orotate twice a day than in those receiving it once a day in the postoperative period. During the first six days after wounding, the density of distribution of the fibroblasts in the wounds of mice receiving the stimulator before the operation was a little higher than in the control animals, but on the following days it was below the control level (Fig. 2). In all groups of wounded mice a greater or smaller increase in the density of distribution of the fibroblasts was observed later in all groups of wounded mice during the period of completion of granulation tissue formation.

The results of these experiments thus show that potassium orotate led to an equal increase in the number of DNA-synthesizing fibroblasts both in intact skin and subcutaneous cellular tissue and during wound healing. However, the density of distribution of the fibroblasts in the wounds increased at different rates in animals of different groups.

DNA synthesis by the fibroblasts took place in accordance with a regular rhythm. In the wounds of the control mice the largest number of DNA-synthesizing fibroblasts was observed every third day. In the wounds of mice receiving potassium orotate from the first day after the operation the numbers of these cells reached

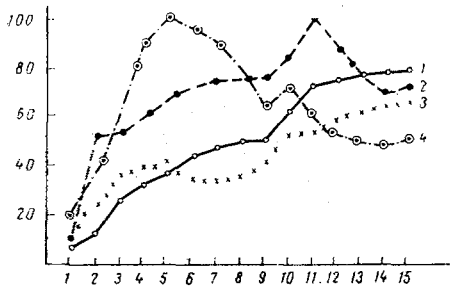

Fig. 2. Intensity of proliferation of fibroblasts in wounds. Ordinate, number of fibroblasts in 20 fields of vision; abscissa, time after operation (in days). 1) Control; 2) in wounds of animals of group 2; 3) group 3; 4) group 4.

a maximum every second day. However, after administration of the stimulator once a day the change in the rhythm of entry of the cells into the phase of DNA synthesis was observed from the 5th day after the operation, whereas in the wounds of the mice receiving the stimulator twice a day it was observed after the 2nd day. This suggests that administration of potassium orotate twice a day led to more rapid synchronization of the cells in the new rhythm of DNA synthesis. The increase by one-third in the number of DNA-synthesizing fibroblasts in the wounds of the mice of groups 2 and 4 was thus accompanied by shortening of the intervals between the time of entry of the fibroblasts into consecutive phases of DNA synthesis also by one-third compared with the control (Fig. 1, A and B). Administration of the stimulator to the animals in the preoperative period caused lengthening of the time intervals between the phases of DNA synthesis in most wound fibroblasts to four days, i.e., by one-third compared with the control. This less frequent entry of the cells into the synthetic phase of the mitotic cycle can be explained as follows: In the animals receiving potassium orotate for the five days before the operation not only did the number of fibroblasts in the skin and subcutaneous cellular tissue increase, but also, as electron-microscopic investigations showed [3], more rapid differentiation of these cells took place. As a result, at the time of wounding the loose connective tissue of the animals of group 3 had a high density of distribution of its fibroblasts, the mitotic activity of which was lower, because of their more advanced differentiation, than in the wounds of the other groups of animals.

The results confirm that the mitotic activity of fibroblasts can be not only depressed, but also increased. One cause of this may be the length of time taken by these cells to pass through the mitotic cycle. A change in this time has been observed under the influence of certain hormones [1, 4]. The results of autoradiographic analysis showed that stimulation of DNA synthesis aimed at obtaining reproduction of fibroblasts reached the same limits in intact loose connective tissue as in healing wounds. The fact that at times of most intensive stimulation of wound healing there was a further increase in the number of fibroblasts in the granulation tissue, but no appreciable changes in the rhythm of DNA synthesis by these cells, suggests that this was due to the more intensive transformation of undifferentiated cells into fibroblasts.

LITERATURE CITED

1. V. P. Vunder, Byull. Éksp. Biol. Med., No. 2, 83 (1974).
2. G. N. Dudnikova, Byull. Éksp. Biol. Med., No. 9, 352 (1977).
3. G. N. Dudnikova and V. V. Ryvnyak, Arkh. Patol., No. 4, 13 (1976).
4. S. S. Laguchev, Hormones and the Mitotic Cell Cycle [in Russian], Moscow (1975).