EFFECT OF PREDNISOLONE ON INTENSITY OF NUCLEIC ACID SYNTHESIS BY GRANULATION TISSUE FIBROBLASTS

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The effect of hormones on the course of experimental wound healing has long attracted the attention of research workers in the USSR and elsewhere [1-7]. However, their attention has been concentrated on intertissue and intercellular correlations. The results of the study of repair processes in loose connective tissue at the cellular and intracellular levels under the influence of hormonal induction find little space in the literature. A fuller understanding of this process would help with the development of the most rational methods of stimulating wound healing. Since corticosteroids occupy an important place in the arsenal of modern hormone therapy, the aim of this investigation was to study, by light-optical autoradiography, the particular features of nucleic acid synthesis by granulation tissue fibroblasts of experimental wounds under the influence of prednisolone.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred mice weighing 20-25 g. Standard incised wounds of skin and muscles, 1 cm long, were inflicted on the thigh of all the animals. The wounds healed under a scab. After the operation the mice were divided into control and experimental groups. The experimental animals received prednisolone per os daily after the operation in a dose of 4 mg/kg body weight. Pieces of wound tissue were fixed in 10% neutral formalin solution. Autoradiographic analysis of DNA synthesis was carried out with the aid of ^{3}H-thymidine, which was injected intraperitoneally 2 h before fixation in a dose of 1 μCi/g body weight. The intensity of RNA synthesis was studied with the aid of 5-^{3}H-uridine, which was injected intraperitoneally in a dose of 20 μCi/g body weight 6 h before fixation. Autoradiographs were obtained on paraffin sections 3-4 μ thick by the usual method with type M photographic emulsion. The exposure was 4 weeks at 4°C. After development and staining of the autoradiographs with hematoxylin and eosin the index of labeled nuclei (ILN) of fibroblasts and the number of uridine tags above the nuclei and cytoplasm of 200 fibroblasts in the region of the wound defect were determined. The density of distribution of these cells was judged from their number in 20 fields of vision under a magnification of the microscope of 280 times. The numerical data was subjected to statistical analysis by Wilcoxon's method. The intensity of fibrillogenesis was assessed in histological sections stained with picrofuchsine and by Masson's method.

EXPERIMENTAL RESULTS

Healing of the wounds in the control mice took place on the 10th-11th day after the operation, compared with on the 7th or 8th day in animals receiving prednisolone. There was no change in the character of the phases of wound healing. Under the influence of prednisolone the duration of the phase of inflammation and cleansing of the wound surface was shortened, and formation and maturation of the granulation tissue took place faster than in the control. For instance, on the 3rd day after the operation the wounds in the experimental animals were filled with well-developed granulations, the vertically arranged capillaries of which were surrounded not only by undifferentiated cells, but also by large, juicy fibroblasts with oval nuclei, containing two or three nucleoli, and long processes of basophilic cytoplasm. The upper layers of granulations contained

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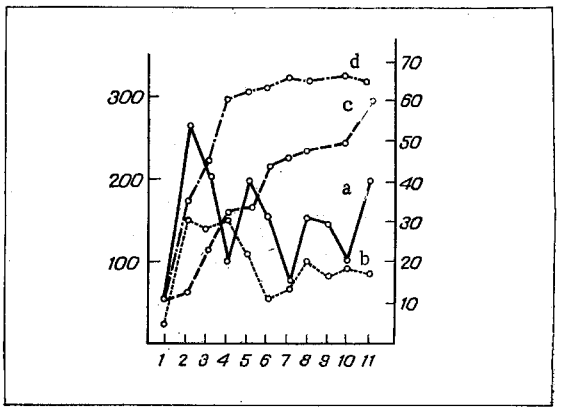

Fig. 1. Proliferation of fibroblasts in wounds of experimental and control mice. Ordinate, on left: ILN of fibroblasts, %; on right: number of fibroblasts in 20 fields of vision; abscissa, time after operation, days. a) ILN of fibroblasts in control wounds, b) in wounds of experimental animals; c) number of fibroblasts in 20 fields of vision in wounds of control animals, d) in wounds of experimental animals.

very few neutrophils and macrophages. Phagocytosed neutrophils could be seen in the cytoplasm of the macrophages. Solitary mast cells were present in the deep layers. On the following days intensive epithelization of the wound surface took place with the formation of granulation tissue. By the 7th-8th days, at the site of the former wound defect, young connective tissue was present in the form of a narrow strip and consisted of thick, short collagen fibers and fibroblast-fibrocytes, which were small, elongated cells with a long nucleus and a narrow border of dense cytoplasm.

Signs of edema and hemorrhages and leukocytic infiltration still remained in the wounds of the control mice by the 3rd day. Islands of granulations were covered with a fairly thick barrier of leukocytes and necrotic tissue, and consisted of capillaries surrounded as a rule by numerous undifferentiated cells of the polyblast type. Large fibroblasts with oval nuclei and long processes of cytoplasm were distributed mainly at the edges of the wound in the intermuscular spaces in the floor of the wound. On the following days, development of granulation tissue and epithelization of the wound surface were observed. However, the intensity of these processes was less than in the wounds of the experimental mice. Completely epithelized wounds in the control mice differed from those in mice receiving prednisolone in the fact that connective tissue at the site of the previous wound defect was represented in the control mice by a much thicker layer and it contained numerous capillaries and fibroblasts with features of higher functional activity. The collagen fibers in it were more loosely arranged than those in the experimental mice. The newly formed epithelium in the control animals consisted of 6 to 8 rows. In the wounds of the experimental mice it was formed by 3 or 4 rows of regularly oriented and compactly arranged cells. Granulation of the wounds in the experimental mice, incidentally, took place against the background of a denser arrangement of the fibroblasts than in the control (Fig. 1).

The results of counting thymidine-labeled fibroblast nuclei showed that DNA synthesis in these cells was at a lower level in the wounds in the experimental animals than in the control (Fig. 1). When an attempt is made to explain this disparity between the low level of DNA synthesis by fibroblasts and the considerable density of distribution of these cells in the wounds of the experimental mice, it must evidently be recalled that not only do cells mature more rapidly under the influence of prednisolone, but also that they may migrate more intensively into the focus of injury from surrounding areas of connective tissue.

The level of incorporation of 5-^{3}H-uridine by fibroblasts in wounds of the experimental mice was significantly higher than in the control mice. It reached a maximum on the 3rd day after the operation. In the control a stable level of incorporation of this isotope began with the 4th day after the operation. It remained stable until epithelization of the wound surface was complete. A sharp decline in the intensity of incorporation of labeled uridine took place in the wounds of the experimental mice with effect from the 8th day after the

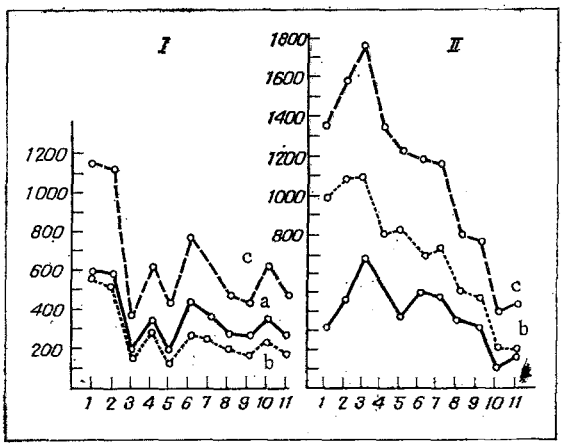

Fig. 2. Intensity of incorporation of 5-^{3}H-uridine by fibroblasts. Ordinate, number of tags per 100 fibroblasts; abscissa, time after operation, days. I) Control; II) experiment. a) Above nuclei; b) above cytoplasm; c) above fibroblasts as a whole.

operation, compared with the 15th day in the control, i.e., when healing of the wounds was ending and fibroblasts were turning into an inactive state. From this time a tendency was noted for the number of fibroblasts in the young connective tissue to decrease. Separate counting of tags above the nuclei and cytoplasm of the fibroblasts showed that most of them were located above the cytoplasm of the cells in the experimental mice, and in conjunction with the higher over-all level of labeling of these cells this indicated an increase in the velocity of nucleocytoplasmic transport of newly formed RNA to sites of protein synthesis (Fig. 2).

The results of this investigation thus demonstrate shortening of the course of wound healing under the influence of prednisolone: Inflammatory changes subsided more rapidly, and the rate of formation and maturation of granulation tissue and regenerating epithelium was increased. Under the influence of prednisolone, granulation of the wounds was accompanied by increased density of distribution of the fibroblasts in the region of the wound defect and by an increase in the rate of synthesis and migration of the newly formed RNA from the cell nucleus to the cytoplasm.

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