AUTORADIOGRAPHIC EVALUATION OF THE EFFECTS OF DRESSINGS ON HEALING OF EXPERIMENTAL WOUNDS

G. N. Dudnikova and T. T. Daurova

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Production of modern dressings involves the choice of materials and evaluation of their functional properties by various methods. The technique of histoautoradiography, which has proved itself well for the study of wound healing under ordinary conditions and during stimulation [1, 2, 4-6], has not hitherto been used for evaluating the course of wound healing under dressings.

The object of this investigation was to study the usefulness of this method in evaluating functional properties of some dressings.

EXPERIMENTAL METHOD

Experiments were carried out on 20 noninbred albino rats weighing 150-170 g, on which full-thickness excised wounds measuring 1.5 × 1.5 cm were inflicted in the dorsal region. The animals were divided into four groups: in group 1 (control) traditional cotton and gauze dressings were used during healing, in group 2 a dressing of "Branolind" type (specimen No. 1), in group 3 a dressing of the burn mask type produced in the USA (specimen No. 2) was used, and in group 4, a burn dressing produced in West Germany (specimen No. 3). Considering the aims of the investigation, selection of the dressings was random in character. The dressings were changed on the 2nd, 4th, and 6th days after the operation. During dressings planimetry of the wounds was carried out and squash preparations taken from the wound surface by the method of Pokrovskaya and Makarov [3]. Pieces of wound tissue measuring 2 × 2 × 2 mm for autoradiographic study was incubated in media containing [5-^{3}H]uridine (specific activity 18 Ci/mmole) and [^{3}H]proline (specific activity 5 Ci/mmole) for 3.5 h at 37°C. Biopsy specimens were fixed in 15% formalin solution and embedded in paraffin wax. Sections 2-5 μ thick were cooled with type M photographic emulsion and exposed at 4°C for 4 weeks. After development the autoradiographs were stained with hematoxylin and eosin. The number of grains of silver was counted above the fibroblasts and adventitial cells, and also in their immediate neighborhood. Comparative analysis was based on determination of the number of grains per 100 cells. All numerical results were subjected to statistical analysis by Wilcoxon's test.

EXPERIMENTAL RESULTS

On the 8th day after the operation the wounds on all groups of animals were filled with granulation tissue, and the wound edges were covered with newly formed epidermis. In wounds healing under cotton and gauze dressings the granulation tissue was covered with a thick leukocytic-necrotic barrier layer. The commonest cells in the granulations were young forms of fibroblasts. Mature fibroblasts and macrophages were present in small numbers. Many leukocytes and macrophages were concentrated in the upper layers of the granulation tissue and immediately beneath the dressings.

Signs of marked inflammation were still present on the 8th day after the operation in wounds under dressings of the "Branolind" type. Epithelization of the wound edges was sluggish. The granulation tissue was edematous, with areas of hemorrhages, infiltration by leukocytes, and microabscesses; it was poorer in cells than the granulation tissue in the control.

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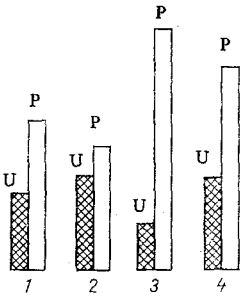

Fig. 1

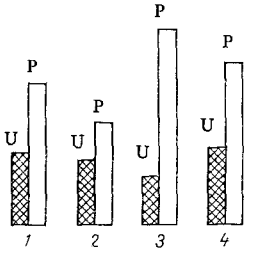

Fig. 2

Fig. 1. Intensity of incorporation of [5-^{3}H] uridine (U) and [^{3}H]proline (P) into fibroblasts of wounds covered with: 1) cotton and gauze dressing (control), 2) dressing No. 1, 3) dressing No. 2, 4) dressing No. 3.

Fig. 2. Intensity of incorporation of [5-^{3}H] uridine (U) and [^{3}H]proline (P) into adventitial cells of wounds covered by: 1) cotton and gauze dressing (control), 2) dressing No. 1, 3) dressing No. 2, 4) dressing No. 3.

In wounds covered by specimens of dressings Nos. 2 and 3, the well formed granulation tissue completely filled the wound defects and there were no signs of inflammation in it. The granulations contained many mature fibroblasts, processes of which were interwoven with collagen fibers. A very few leukocytes and solitary macrophages were found only in the uppermost layers of the wounds. The regenerating epithelium spread over the granulation tissue in a thick wedge consisting of seven or eight rows of cells. The degree of their differentiation and the regularity of their arrangement increased with increasing distance from the center of the wounds to their edges. Histological investigation thus revealed no significant differences in the course of wound healing in the animals of groups 3 and 4.

Counting the number of grains showed that incorporation of labeled precursors of RNA and proteins into fibroblasts and adventitial cells in the wounds of animals of all four groups took place with different intensities (Figs. 1 and 2). In wounds covered with dressing No. 1 the number of uridine labels above fibroblasts was 20% greater than in the control whereas the number of labels formed after incubation with [^{3}H]proline under these circumstances was 20-25% less than in the control. Counting labels above adventitial cells in the animals of this group showed that their number after incubation with [5-^{3}H]uridine was practically the same as in the control, whereas the number of labels due to incorporation of [^{3}H]proline was 20-25% less than in the control. In wounds under dressing No. 2 fibroblasts and adventitial cells contained 50% fewer uridine labels than the control, but 50% more [^{3}H]proline labels than in the control. The number of labels above fibroblasts of wound in the rats of group 4 was 20% greater after incorporation of [5-^{3}H]uridine and 30% greater after incubation with [^{3}H]proline than in the control. Adventitial cells under these circumstances contained 25 and 20% fewer labels respectively than in the control.

The results are thus evidence that dressings of the "Branolind" type, applied to a clean excised wound immediately after the operation, inhibited wound healing in all the animals of this group. The biosynthetic activity of the fibroblasts and adventitial cells was much lower than in the control.

Analysis of the label counts above cells in the animals of groups 3 and 4 showed that wound healing under dressings Nos. 2 and 3 took place at different rates, although no significant differences in the course of wound healing were revealed histologically. For instance, in rats of group 3 adventitial cells and fibroblasts incorporated the RNA precursor less intensively than in animals of group 4, but at the same time, they incorporated labeled proline more intensively, and this could only be due to more intensive protein synthesis. This disparity between the low level of RNA synthesis and the high level of protein synthesis in these cells is evidently only apparent. Undifferentiated cells and young forms of cells are known to synthesize large quantities of simple proteins, with only

small amounts of proline in their composition. These proteins are utilized by the cells in their structural organization. Young and mature fibroblasts produce polysaccharides, collagen, and other proteins essential for collagen formation and with a high proline content, which find their way into the extracellular space. It has also been shown [4] that young forms of fibroblasts have a higher level of RNA synthesis than mature forms. All these considerations thus suggest that wound healing took place more rapidly in the rats of group 3 than in those of group 4. Meanwhile, RNA and protein synthesis in fibroblasts in the wounds of animals of group 4 exceeded the control level. The level of RNA synthesis in the adventitial cells did not differ significantly from the control. Cell differentiation and protein synthesis by cells in the control wounds and under dressing No. 3 took place at about the same speed. However, since the number of functionally active fibroblasts in the wounds under dressing No. 3 was greater than in the control, the formation of proteins and of RNA controlling this process took place more rapidly.

The results of the histoautoradiographic study correlate with planimetric data. For instance, the area of the wounds on the 8th day after the operation under a dressing of "Branolind" type was greater on the 8th day of the operation by 40%, whereas under dressing No. 2 it was 40% less, and under dressing No. 3 it was 20% less than in the control.

Quantitative characteristics of biosynthesis in wounds healing under different dressings, which determines to some degree the functional properties of these dressings, can thus be obtained by histoautoradiographic analysis.

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