ELECTRON-AUTORADIOGRAPHIC STUDY OF THE EFFECT OF POTASSIUM OROTATE ON RNA AND PROTEIN SYNTHESIS IN FIBROBLASTS DURING EXPERIMENTAL WOUND HEALING

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UDC 617-001.4-003.9-07:616-008.939.6-02:615.356

KEY WORDS: potassium orotate; RNA and protein synthesis; wound healing; autoradiography.

Orotic acid and its derivative potassium orotate are pyrimidines which participate in nucleic acid metabolism and are well known as biogenic stimulators [7]. The effect of these pyrimidines on regeneration of various organs and tissues has been studied [1, 5, 10] and they have been used in the treatment of certain diseases, both experimentally [2, 11, 12] and clinically [6, 8]. As a rule in every case the compounds were found to have a stimulating effect on repair processes. In recent years an attempt has been made to discover the intracellular mechanisms of the action of potassium orotate *in vivo*. For this purpose the method of light autoradiography has been used to study the effect of this compound on DNA and RNA synthesis in fibroblasts during experimental wound healing [3, 4]. The stimulating effect of potassium orotate also was demonstrated in these investigations.

The object of the present investigation was an electron-autoradiographic study of RNA and protein synthesis in fibroblasts during wound healing under the influence of potassium orotate.

EXPERIMENTAL METHOD

Fibroblasts from intact mouse skin and also granulation tissue on the 3rd and 7th days after infliction of a wound of skin and muscle in the leg region with a tissue defect measuring about 27-30 mm^3 were studied. Animals of each of three groups (intact, on the 3rd and 7th days after wounding) were divided into two subgroups: control, in which mice were not given potassium orotate, and experimental, in which the animals were given potassium orotate daily per os in a dose of 0.2 g/kg as a 2% solution. Altogether 30 animals were used, five in each subgroup. Experimental intact animals were given the compound for three days before material was taken. The wounded animals began to receive potassium orotate on the day of the operation. RNA and protein synthesis in the fibroblasts were studied by the double labeling method [9]. For this purpose, 1 h before material was taken the animals were given a mixture of precursors: [5-^{3}H]uridine in a dose of 4 μCi/g (specific activity 22.5 Ci/mmole) and [3,4,5-^{3}H]proline in a dose of 20 μCi/g (specific activity 115 mCi/mmole). The material was fixed by the usual method and embedded in Epon. Electron-microscopic autoradiographs were prepared by the method described previously [13] and studied in the JEM-100B electron microscope.

Quantitative analysis of the autoradiographs was carried out as follows. The number of grains of silver above the nucleus and cytoplasm of the cell was counted on negatives and the area of cross section of the nucleus and cytoplasm determined in conventional units (the product of length and width in millimeters). The labeling density was calculated as the ratio of the number of grains above the given part of the cell and its area of cross section. Values of the ratio of labeling density in the cytoplasm to labeling density in the nucleus were compared in the experimental and control subgroups of the three groups of animals. The results were subjected to statistical analysis by Student's method.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 115-118, August, 1983. Original article submitted January 31, 1983.

0007-4888/83/9608-1173$07.50

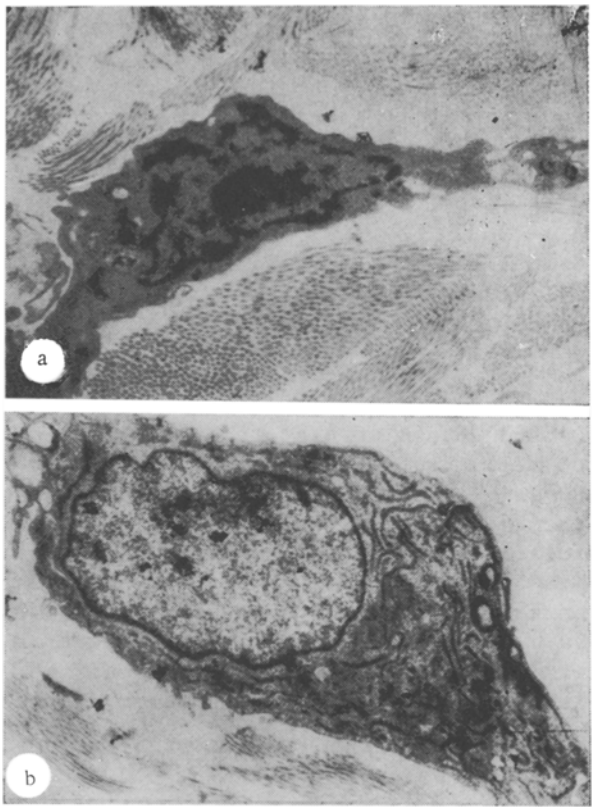

Fig. 1. Fibroblasts of intact skin of mice not receiving (a) and receiving (b) potassium orotate. Grains of silver located above nucleus and cytoplasm of cells. Bundles of collagen fibers can be seen around the cells. 5000 ×.

EXPERIMENTAL RESULTS

Fibroblasts of intact skin of the experimental animals differed from the corresponding cells of the control animals (Fig. 1). First, they were larger, and second, in their structure they were more reminiscent of fibroblasts of granulation tissue: They had a fairly large nucleus with chromatin which lay next to the nuclear membrane in the form of a thin band; the rough endoplasmic reticulum was well developed, and most frequently consisted of narrow tubules. Fibroblasts with an ill-defined structure of their cytoplasm also were found. The label was distributed more or less uniformly between nucleus and cytoplasm. Quantitative analysis showed that the fibroblasts of the experimental animals were almost twice as large on average as the fibroblasts of the control animals (Table 1). The labeling density in the nucleus and cytoplasm of these fibroblasts (0.008 ± 0.001 and 0.004 ± 0.0003 respectively) was only half that in the control (0.020 ± 0.001 and 0.0090 ± 0.0004). Consequently, whereas the area of the cell was almost doubled and the labeling density was reduced by half, the intensity of incorporation of label into these fibroblasts was the same as in the control, i.e., the rate of RNA and protein synthesis was unchanged in the experiment. This is clearly reflected by the ratios of labeling density in the cytoplasm to labeling density in the nucleus in the experimental and control subgroups (Fig. 2).

The morphology and function of the fibroblasts changed abruptly after wounding. On the 3rd day after wounding the fibroblast was converted into a cell actively synthesizing protein: it was almost doubled in size compared with the fibroblast in the skin (Table 1); its structure changed and, in particular, it acquired a well-developed rough endoplasmic reticulum, the structure which produces protein. The ratio of the mean labeling density in the cytoplasm to the mean labeling density in the nucleus rose sharply (Fig. 2). On the third day of wound healing the fibroblasts of animals of the experimental and control subgroups did not differ in structure. The dimensions of the cells also were similar. The labeling density in the nucleus of the experimental and control animals was equal (0.008 ± 0.001), but the labeling density in the cytoplasm in the experiment was lower than in the control (0.006 ± 0.001 and 0.009 ± 0.001 respectively), as a result of which the ratio of labeling density in the cyto-

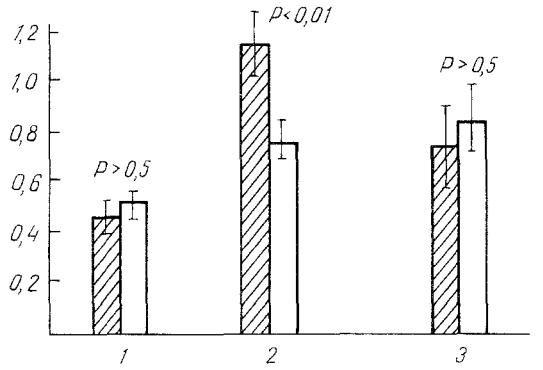

Fig. 2. Changes in ratio of labeling density in cytoplasm to labeling density in nucleus in fibroblasts during wound healing and stimulation by potassium orotate. 1) intact skin, 2) 3rd day of wound healing, 3) 7th day of wound healing. Shaded columns — without stimulation, unshaded — with stimulation.

TABLE 1. Changes in Area of Cross Section of Fibroblast (magnification 5000) during Wound Healing and Stimulation by Potassium Orotate (M ± m)

Experimental conditions	Total number of cells investigated	Area of cross section of nucleus, mm^2		P	Area of cross section of cytoplasm, mm^2		P
		without stimulation	with stimulation		without stimulation	with stimulation	
Intact skin	502	389,00±9,06	608,65±26,36	<0,001	618,76±19,97	1167,29±52,37	<0,001
Third day of wound healing	605	697,00±18,66	792,62±25,49	<0,01	1584,00±39,19	1414,69±44,44	<0,01
Seventh day of wound healing	508	608,00±17,53	610,54±15,32	>0,5	1265,20±38,19	1413,52±41,62	<0,01

plasm to the labeling density in the nucleus was reduced (Fig. 2). In other words, protein synthesis in the fibroblasts of the experimental animals was depressed compared with the control.

Electron-microscopic analysis of the fibroblasts on the 7th day of healing showed that these cells were on average smaller than on the 3rd day (Table 1). However, they did not differ structurally. The reduction in average size of the fibroblast and also in the value of the ratio of labeling density in the cytoplasm to labeling density in the nucleus is evidence that the intensity of protein synthesis at this stage of wound healing was lower than on the 3rd day. After administration of potassium orotate to the animals, there was no significant change in the size of the fibroblast, but the ratio of labeling density in the cytoplasm to that in the nucleus was increased somewhat.

The action of potassium orotate on fibroblasts of intact skin was thus manifested only as in increase in size of the cells, for RNA and protein synthesis in these cells was unchanged. It can be tentatively suggested that potassium orotate affected certain other plastic processes in the cell, as a result of which the cell increased in size. Depression of protein synthesis in the fibroblasts of the experimental animals on the 3rd day of wound healing appears at first glance to be paradoxical. However, the following suggestion can be made. At this stage of wound healing the rate of protein synthesis in fibroblasts is sufficiently high even so, for it is much higher than in fibroblasts of intact skin (Fig. 2). In other words, after wounding, the fibroblast sets to work at "full power." The action of the stimulator under these conditions of maximal mobilization of the potential of the cell can thus evidently alter its work only a little. However, the stimulator can increase proliferation of fibroblasts, as Dudnikova [3] found, and thus reduce the load on a single cell. In the same way we can explain the depression of protein-synthesizing activity of fibroblasts in the control on the 7th day of wound healing, when the number of fibroblasts was much greater than on the 3rd day. The explanation of the lower level of protein synthesis in fibroblasts on the 3rd day compared with the control by the inhibitory action of potassium orotate seems unlikely. If it did in fact inhibit the protein-synthesizing activity of the fibroblast, this ought to be revealed by investigation of fibroblasts of normal skin and on the 7th day of wound healing. However, this was not observed. The level of protein synthesis in the

fibroblasts on the 3rd day in the experimental subgroup and on the 7th day in the control subgroup was equal. Does this not reflect the stimulating action of potassium orotate? Wound healing is accelerated: on the 3rd day, during the action of potassium orotate the fibroblast functions just as it does on the 7th day in the control. Our investigation thus suggests that the action of potassium orotate as a biological stimulator is manifested as activation of the proliferative ability of wound fibroblasts and not as stimulation of the protein-synthesizing function of each single cell.

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EFFECT OF AN INCREASED FUNCTIONAL LOAD ON STRIATED MUSCLE ULTRASTRUCTURE OF THE RAT ESOPHAGUS

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UDC 612.315:612.741.9.014.2-086.3

KEY WORDS: esophagus; striated muscle; ultrastructure; hypertrophy.

The morphological and functional properties of esophageal striated muscle (ESM) have been inadequately studied [1, 5, 7]. Their ultrastructural features, innervation, and plastic transformations in response to an increased functional load require further investigation.

The object of this investigation was a microscopic and ultramicroscopic study of ESM in control animals and during the development of experimental hypertrophy.

EXPERIMENTAL METHOD

Experiments were carried out on 30 albino rats. Under aseptic conditions and ether anesthesia the abdominal portion of the esophagus was enclosed in a longitudinally cut elastic tube 10 mm long with an internal diameter of 3.5 mm, which led to disturbance of patency. The experiments lasted 1, 3, 7, 10, and 20 days. For morphological investigation the animals were

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 118-120, August, 1983. Original article submitted February 18, 1983.

0007-4888/83/9608-1176$07.50