METABOLISM OF ARTICULAR CARTILAGE IN THE PRESENCE OF INTERLEUKIN-1-ALPHA, ITS INHIBITOR, AND BLOOD SERUM

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Interleukin-1 (IL-1), a polypeptide with mol. wt. of 17.5 kD, is an immunoregulator which was isolated originally from mononuclear phagocytes, but later was discovered in fibroblasts, synovial cells, and chondrocytes. Two forms of interleukin-1 are known: interleukin-1α (IL-1a) and interleukin-1β (IL-1β), which differ in their isoelectric points [4, 8, 11]. IL-1 stimulates secretion of enzymes which degrade proteoglycans (PG) and inhibits the production of these molecules by chondrocytes, so that it plays the role of triggering factor in arthritis for cartilage destruction [1, 6, 9]. Activity of IL-1a is regulated by various different biological factors [3, 4]. In particular, one such factor is interleukin receptor antagonist protein (IRAP), isolated by American research workers from leukocytes (mol. wt. = 25 kD) [11].

The aim of this investigation was to study the effect of IRAP and of native bovine blood serum on activity of IL-1 in a culture of articular cartilage tissue.

EXPERIMENTAL METHOD

Pieces of tissue approximately square in shape and measuring 50-70 mm^2, as thick as the total depth of the cartilage, and weighing 40-50 mg were taken for investigation from the metacarpal joints of year-old bulls. The tissue was cultured in plastic cuvettes with 24 wells, each with a capacity of 2 ml, for 9 days in nutrient medium F12:DME = 50:50, with 10% bovine serum, under atmospheric pressure of air with a CO_2 concentration of 5%. An experimental series consisted of four wells. On the 7th day the experiment began with incubation of cartilage in the presence or absence of IL-1a in a dose of 0.005, 0.01, 0.2, and 1.0 ng/ml, and IRAP in a dose of 10, 100, 500, and 1000 ng/ml. The action of the test preparations on cartilage metabolism was assessed by measuring the change in content of sulfated glucosaminoglycans (sGAG), which was determined by the dimethylmethylene reaction [2], and the concentration of bound radioactive sulfate (35S) with proteoglycan [6] in tissue extract and culture medium. The results obtained in the control wells were taken to be 100%.

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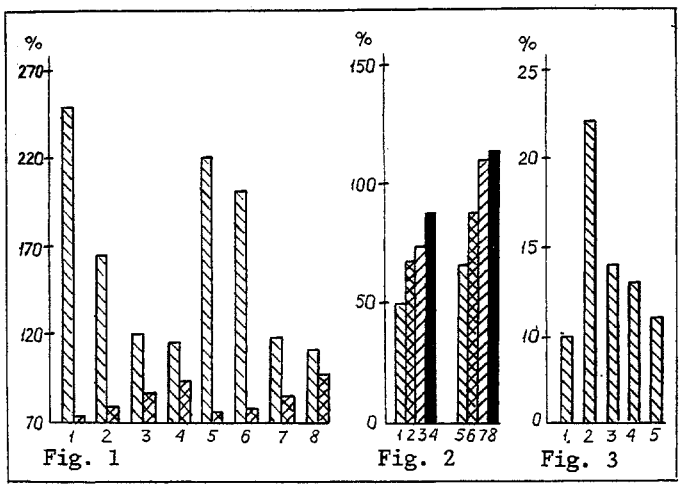

Fig. 1. Content of sGAG, in percent (control 100%) in nutrient medium (left) and in cartilage tissue (right) during incubation with IL-1a (1-4) and IL-1a + IRAP (5-8). Concentration of IL-1a, ng/ml: 1) 1; 2) 0.2; 3) 0.05; 4) 0.01; concentration of IL-1a and IRAP: 5) 1 ng/ml; 6) 10 ng/ml; 7) 100 ng/ml; 8) 1000 ng/ml.

Fig. 2. Total radioactivity of 35S-PG in nutrient medium and tissue, in percent (control 100%). Legend as to Fig. 1.

Fig. 3. Total radioactivity of 35S-PG in nutrient medium without blood serum and of cartilage tissue in percent (control – incubation with serum, 100%). 1) Incubation of cartilage without blood serum. Incubation of cartilage without blood serum in presence of IL-1a in concentration of, ng/ml: 2) 1; 3) 0.2; 4) 0.05; 5) 0.01.

EXPERIMENTAL RESULTS

The dimethylmethylene reaction for sGAG revealed elevation of the polysaccharide level during incubation of the tissue with IL-1a compared with the control, at a cytokine concentration of 1 ng/ml to 249% ($p < 0.01$) and, correspondingly, to 165% ($p < 0.01$) at 0.2 ng/ml, to 120% ($p < 0.05$) at 0.05 ng/ml, and to 115% at 0.01 ng/ml. Meanwhile, a decrease in the sGAG concentration was observed in the tissue extracts (Fig. 1). In experiments in which cartilage was incubated with IRAP and with IL-1a in a concentration of 1 ng/ml, an increase in sGAG in the nutrient medium to 200% ($p < 0.05$) was observed at a concentration of the inhibitor of 1 ng/ml, and to 183% ($p < 0.05$) at 10 ng/ml, but no difference was found from the control values with IRAP in a concentration of 100 and 1000 ng/ml (Fig. 2). In the tissue extracts sGAG fell to 75% ($p < 0.05$) with IRAP in a concentration of 1 ng/ml. No significant decrease was observed in polysaccharides with IRAP in concentrations of 10 and 100 ng/ml, and their level actually rose in response to a concentration of 1000 ng/ml. In a study of newly synthesized proteoglycans (PG), labeled with radioactive sulfate (35S-PG) (the level of radioactivity in the nutrient medium and in the tissue extracts was added together), a decrease in glycan production by chondrocytes to 50% ($p < 0.01$) was observed in experiments in which the culture was incubated with IL-1a (at a concentration of 1 ng/ml; Fig. 2). Meanwhile, during culture with IRAP (in the presence of a smaller or equal concentration, namely 100 ng/ml) normal values of 35S-PG were observed, and synthesis was actually increased to 130% ($p < 0.05$) with the inhibitor in a concentration of 1000 ng/ml. Some interesting results were obtained when cartilage was cultured in serum-free medium (Fig. 3). A signifi-

cant decrease in activity of the chondrocytes with respect to production of 35S-PG (<10%) was observed, but addition of IL-1a led to an increase of their activity (over 20%). IRAP did not affect IL-1a activity in serum-free medium.

Analysis of our results confirms previous information on the destructive action of IL-1a on cartilage tissue: in culture medium or synovial fluid the content of sGAG is increased, whereas in cartilage, their level is low [5, 9, 10, 12]. Native bovine serum leads to a marked increase in viability of cellular and tissue cultures [7], and it is natural, therefore, that a decrease in the synthetic capacity of the chondrocytes would be observed in serum-free medium. However, the action of IL-1a in this case was manifested in the opposite direction, for it stimulated the cells to synthesize PG. It can be postulated that certain growth factors in blood serum, and also, perhaps, as yet unknown substances modify the properties of IL-1a.

The investigations thus confirm existing data on the degrading effect of IL-1a on cartilage, and they also reveal new facts concerning the possibility of regulating the activity of this cytokine with the aid of an inhibitor from leukocytes (IRAP) and of certain as yet unknown serum factors.

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