GLYCOPROTEIN AND FIBRONECTIN METABOLISM IN CUTANEOUS FIBROBLASTS OF PATIENTS WITH RHEUMATOID ARTHRITIS

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UDC 616.72-002.77-039-07:
616.5-008.93:577.112.853

KEY WORDS: glycoproteins; fibronectin; rheumatoid arthritis.

The fibronectins are high-molecular-weight glycoproteins found in blood plasma and the extracellular matrix and basal and cytoplasmic membranes of various types of cells [11]. Fibronectin plays an active role in the regulation of various processes during the formation of connective tissue [8, 13], it is a protein secreted by the cells of this tissue, and is capable of interacting with several of its components (fibrin, collagen, glycosaminoglycans), connected with the organization and repair of injured or inflamed tissue [9, 11]. In rheumatoid arthritis (RA) the fibronectin concentration in a cell monolayer of cutaneous fibroblasts from patients in culture is higher than normal [2]. Fibronectin is more sensitive to the action of various proteinases than basement membrane proteins of cells such as laminin and type IV collagen [4]; removal of fibronectin from the surface of cells by means of proteinases, moreover, leads to stimulation of their proliferation [2, 5].

The aim of this investigation was to study metabolism of pre-labeled glycoproteins, in particular fibronectin, in cutaneous fibroblasts of patients with RA, and activity of proteolytic enzymes secreted by these fibroblasts into the culture medium, for we postulated an active role for proteinases in glycoprotein and, in particular, fibronectin metabolism.

EXPERIMENTAL METHOD

The cell cultures and method of culture were described previously [1]. The cultures were studied at the 3rd-10th subcultures in the stationary phase of growth. The glycoproteins and fibronectin were labeled with ^{14}C-fucose (Amersham International, England). Before the experiment the cells were synchronized in serum-free medium for 24 h. ^{14}C-fucose in a concentration of 1.6 μCi/ml was added to DMEM medium or medium 199 with 1% human serum (HS) and cultured for 19-20 h. The cell layer was then washed with Earle's medium and the cells were cultured either in the same medium or (in individual cases) in medium 199 with 20% bovine serum (BS), changing the medium every 2 h. The content of labeled glycoproteins and fibronectin in the cell monolayer and in the culture medium was determined immediately at the end of labeling and thereafter every 2 h of culture in medium without ^{14}C-fucose, by a modified method [15]. The number of cells and the radioactivity of the material were determined as described previously [1]. Pepstatin, EDTA, monoiodoacetic acid, and phenylmethylsulfonyl fluoride (PMSF) were used as proteinase inhibitors. Protein synthesis and glycoprotein labeling were measured as described previously [1, 3] after the cells had been cultured for 20 h in the presence of inhibitors in DMEM medium with added vitamins (Serva, West Germany) and 1% HS. The caseinolytic and collagenolytic activity in the culture medium was determined by the method [3].

EXPERIMENTAL RESULTS

The quantity of fibronectin in the culture medium collected after 24 h of culture and in cutaneous fibroblasts from patients with RA, cultured under ordinary conditions, was a little higher than that in medium and cutaneous fibroblasts from healthy blood donors (HBD): in the medium – 18.1 ± 1.5 and 20.4 ± 3.06 μg/10^6 cells respectively; in the cell monolayer – 9.1 ± 1.35 and 13.2 ± 2.84 μg/10^6 cells respectively. After culture of the fibroblasts in medium with a low concentration (0.5-1%) of serum for 24 h the quantity of labeled glyco-

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 105, No. 1, pp. 30-33, January, 1988. Original article submitted February 16, 1987.

0007-4888/88/0001-0036$12.50

TABLE 1. Distribution of ^{14}C-Fucose-Labeled Glycoproteins and Fibronectin in Culture Medium and Cell Monolayer of Cutaneous Fibroblasts from HBD and Patients with RA

Fibroblasts	Medium	Surface	Cells	Extracellular matrix
	Quantity of glycoproteins, %			
HBD	51	11	21	17
RA	46	21	26	7
	Quantity of fibronectin, %			
HBD	39	28	13	20
RA	41	12	8	39

Legend. Total quantity of glycoproteins and fibronectin taken as 100%. Mean results of three independent experiments are shown.

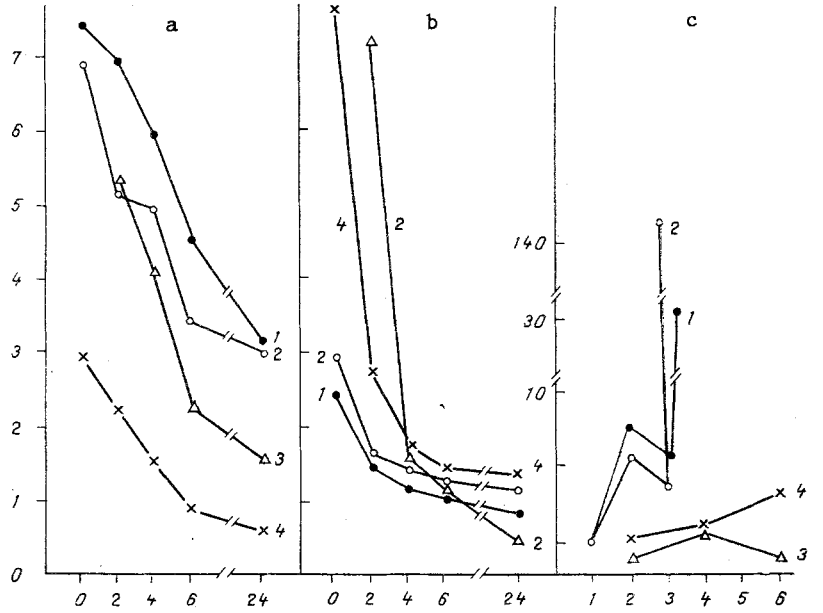

Fig. 1. Metabolism of fibronectin, prelabeled with ^{14}C-fucose, in cutaneous fibroblasts of HBD (a) and RA patients (b) and proteolytic activity in culture medium of these fibroblasts (c). a, b) Labeling and culture in DMEM medium with 1% HS. 1) Content of labeled glycoproteins in cells, 2) on cell surface, 3) in culture medium, 4) in extracellular matrix. Abscissa, time (in h); ordinate, incorporation of ^{14}C-fucose ($\times 10^3$, in cpm/10^6 cells). c) Caseinolytic (1, 2) and collagenolytic (3, 4) activity in culture medium of cutaneous fibroblasts of HBD (1, 3) and RA patients (2, 4). Abscissa, time (in days); ordinate, proteolytic activity ($\times 10^2$, in cpm hydrolyzed ^{14}C-collagen or ^{14}C-casein/ml of culture medium).

proteins and fibronectin, synthesized de novo, in the medium was virtually identical in cutaneous fibroblasts from patients with RA and from HBD, but their distribution in the cell monolayer differed considerably (Table 1). The total glycoprotein content was higher on the surface of and within the cutaneous fibroblasts from patients with RA, but cutaneous HBD fibroblasts contained more glycoproteins in the extracellular matrix. There was much less fibronectin on the surface of the cells from RA patients, but far more of it than normally in the extracellular matrix of these cells. This can be explained by the more rapid

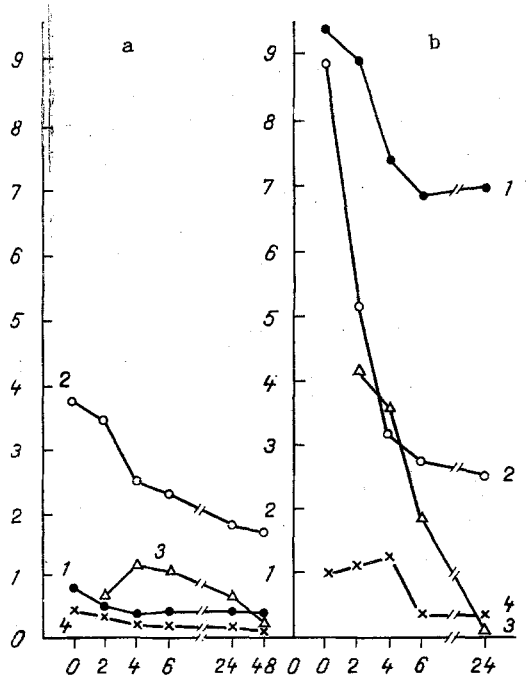

Fig. 2. Metabolism of glycoproteins, prelabeled with ^{14}C-fucose, in cutaneous fibroblasts of HBD (a) and RA patients (b) when cultured in DMEM medium with 1% HS. Legend as to Fig. 1a, b.

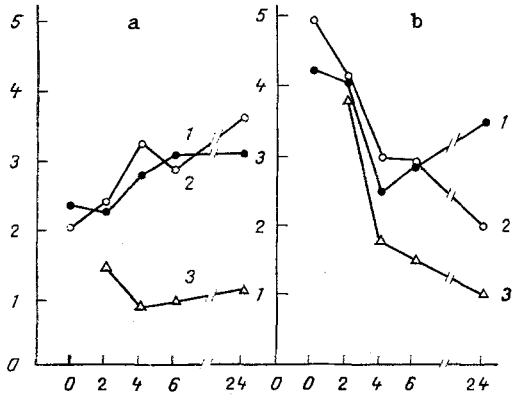

Fig. 3. Analysis of metabolism of ^{14}C-fucose prelabeled glycoproteins in cutaneous fibroblasts of HBD (a) and RA patients (b) when labeled in medium 199 with 1% HS and subsequently cultured in medium 199 with 20% BS. Legend as to Fig. 1a, b.

fibronectin turnover on the surface of fibroblasts from RA patients and its more rapid degradation by proteinases secreted by cells into the culture medium. Evidence in support of this view is given by the more rapid shedding of fibronectin from the extracellular matrix of cutaneous fibroblasts from RA patients during the first 2 h of a "chase" experiment (Fig. 1b). The higher level of accumulation of labeled fibronectin in the extracellular matrix may be evidence of a higher level of its synthesis by cutaneous fibroblasts of RA patients. The subsequent relatively slow decrease in the quantity of prelabeled fibronectin in the cells may probably be evidence of the presence of a certain quantity of fibronectin firmly bound with the cell membranes and somehow protected against proteinase attack. A virtually uniform decrease in the quantity of prelabeled fibronectin in the cell monolayer was observed in the case of cutaneous fibroblasts from HBD (Fig. 1a).

The rate of decline of the quantity of prelabeled glycoproteins on the surface of cutaneous fibroblasts from RA patients was much lower during the first few hours of the "chase"

TABLE 2. Effect of Proteinase Inhibitors on Distribution of ^{14}C-Fucose-Labeled Glycoproteins in Culture Medium and Cell Monolayer of Human Cutaneous Fibroblasts (total quantity of glycoproteins taken as 100%)

Initiator	Quantity of glycoproteins, %		
	in culture medium	in cell monolayer	in extracellular matrix
Without inhibitor	87	11,6	1,4
Pepstatin, 1 μg/ml	54	44	2
EDTA, 10^{-3} M	62	36	2
PMSF, 10^{-3} M	68	31	2

experiment than under normal conditions (Fig. 2) when the cells were cultured in medium with 1% HS. This may be connected with the lower stability of the cell membranes during culture under these conditions. When the cells were cultured in medium with 20% BS a marked decrease was observed in the rate of loss of labeled glycoproteins by the cells, and in cutaneous HBD fibroblasts their content in the cell monolayer actually increased (Fig. 3), possibly due to weakening of activity of destructive processes in the cell membranes and an increase in their stability.

A phenomenon of activation of proteolysis in fibroblasts after the culture has reached the confluent state has been described in the literature [7]. We found that caseinolytic activity is present in the culture medium of fibroblasts as early as during the first day of culture (Fig. 1b). It reached a maximum on the 4th-6th days; in the case of RA its values were 2-4 times higher than normally. Collagenolytic activity during the first 2 days was detected in trace amounts and reached a peak also on the 4th-6th days.

During culture of fibroblasts in the presence of proteinase inhibitors pepstatin, EDTA, monoiodoacetic acid, and PMSF protein synthesis in the cells was reduced by 60, 71, 98.2, and 67% respectively. Evidence of the limiting role of proteinase in glycoprotein metabolism is given by the three-fourfold increase in the content of labeled glycoproteins in the cellular layer and the decrease in outflow of glycoproteins into the medium in the presence of inhibitors (Table 2).

Changes in glycoprotein, especially fibronectin, metabolism in connective-tissue cells in RA may therefore be connected not only with their increased synthesis, but also with a change in proteolytic enzyme activity in this tissue in this disease.

The authors are grateful to L. A. Kuznetsova and T. V. Usova for technical help with the work.

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CYCLIC NUCLEOTIDE CONCENTRATION IN THE RENAL CORTEX OF VITAMIN D DEFICIENT RATS

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UDC 616.391:577.161.2-]-008.64-07:616.61-008.93:577.123.3

KEY WORDS: cyclic nucleotides; kidneys; vitamin D; calcium-phosphorus metabolism.

A deficiency of vitamin D, a powerful factor controlling mineral metabolism, leads to considerable changes in biological systems responsible for maintenance of calcium and phosphorus homeostasis in the body [5]. The development of the diverse pathological manifestations of vitamin D deprivation is a problem many aspects of which remain unsolved, and this may to some extent account for the sometimes delayed normalization of biochemical and clinical parameters when the deficient vitamin alone is given therapeutically.

An important role in the regulation of calcium and phosphorus homeostasis in the body is played by the kidneys, which serve as the target for calcium-regulating hormones, synthesize active vitamin D metabolites, and are also the site of intensive formation of cyclic nucleotides, which are directly involved in the mechanism of the biological effect of many hormones and, perhaps, also of active forms of vitamin D [8].

To clarify the causes of disturbances of calcium and phosphorus metabolism in vitamin D deficiency, the concentrations of cyclic nucleotides in the renal cortex were studied and parameters of calcium and phosphorus metabolism in the blood were studied in experiments on rats with vitamin D deficiency.

EXPERIMENTAL METHOD

Experiments were carried out on 35 Wistar albino rats of both sexes, weighing 40-50 g, divided into two groups: control, consisting of 10 animals, and experimental, consisting of 25 rats, in which vitamin D deficiency was created at the level of State Standard 11222-65. Four weeks after the rats had developed signs of rickets, due to vitamin D deficiency, the animals were decapitated and the kidney tissue quickly frozen.

Concentrations of cAMP and cGMP in the renal cortex were studied by radioimmunoassay using standard commercial kits (Amersham International, England). Radioactivity was counted on a Mark III liquid scintillation system (Tracor Europa, USA).

The calcium concentration in the blood serum and erythrocytes was determined by a complexometric method [4]; the phosphorus concentration in the blood serum and erythrocytes and the concentration of 2,3-diphosphoglycerate (2,3-DPG) was determined by the method in [6], and alkaline phosphatase activity as in [7], and expressed in Bodansky units.

EXPERIMENTAL RESULTS

In experimental vitamin D deficiency a marked increase (more than twofold) in the cAMP concentration in the renal cortex of the rats was observed (Fig. 1). This was accompanied by a less marked but significant fall in the cGMP concentration. In animals deficient in vitamin D significant deviations were observed in the parameters of calcium and phosphorus metabolism, as reflected in the data for blood serum and erythrocytes (Table 1). The calcium

Kiev Research Institute of Pediatrics, Obstetrics, and Gynecology, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR, E. M. Luk'yanova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 1, pp. 33-35, January, 1988. Original article submitted June 23, 1986.

 0007-4888/88/0001-0040$12.50