ONCOLOGY

Inhibitory Effect of Human Blood Granulocyte Glycosaminoglycans on Colony-Forming Activity of Bone Marrow Fibroblasts

O. D. Golenko, E. E. Batrak, and M. F. Kharchenko

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Inhibitory effect of glycosaminoglycans isolated from peripheral blood granulocytes of patients with chronic myeloleukemia and normal subjects on increment in colony-forming units of fibroblasts in monolayer cultures of rat bone marrow is determined by the degree of sulfation of their main component chondroitin-4-sulfate and by increased content of heparan sulfate.

Key Words: *glycosaminoglycans; increase in the number of colony-forming units of bone marrow fibroblasts*

Glycosaminoglycans (GAG) and their conjugates with proteins proteoglycans are the major components of the extracellular matrix of bone marrow. Human bone marrow stromal cells produce chondroitin-6-sulfate (C-6-S), dermatan sulfate, and heparan sulfate that participate in cell-to-cell reactions with stem cells and hemopoiesis precursor cells, determining their adhesion, migration, proliferation, and differentiation [4,6]. On the other hand, bone marrow and blood hemopoietic cells contain GAG in the form of proteoglycans and free polysaccharide chains. The major component of granulocytic GAG is polydispersed C-4-S, while heparan and dermatane sulfates are minor components [5]. Activation of hemopoietic cells is associated with intense production and secretion of GAG functioning as mediators, reacting with bone marrow stromal elements [4,5]. However, the mediator role of hemopoietic cell GAGs has not been evaluated. We revealed the ability of GAG isolated from human blood granulocytes to modify proliferative activity of precursor cells of stromal fibroblast colony-forming units (CFU) in rat bone marrow monolayer cultures [1]. The purpose of this study was to compare the effects of GAG of different degree of sulfation isolated from peripheral blood granulocytes of patients with chronic myeloleukemia (CML) and donors and of commercial chondroitin sulfates on colony-forming activity of bone marrow fibroblasts.

Department of Endogenous Modifiers of Carcinogenesis, Institute of Carcinogenesis, Oncology Research Center, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out in rat bone marrow monolayer cultures [2]. Bone marrow cells were isolated from femoral bone of Wistar rats weighing 200-250 g. The cells ($0.5\text{-}5.0\times10^6$) were expanded in Carrel's flasks (25 cm^2) in RPMI-1640 (Sigma) with 15% fetal calf serum in an atmosphere with 5% CO_2. Nonadherent bone marrow cells were removed after 48 h, then GAG in a concentration of 1-5 μg uronic acids/ml and chondroitin sulfates (0.5, 1, 10, and 30 μg/ml) were added. Two fractions of low- and sulfated GAGs were used They were isolated by the papain method [3] from acetone-dried blood granulocytes from patients with CML during the chronic

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stage of disease (n=2), with myeloblastic crisis (n=1), and from donors (n=2) and with C-4-S (Sigma) and C-6-S (Seikagaku). The efficacy of cloning of rat bone marrow fibroblasts after addition of GAG and chondroitin sulfates was assessed after 10-12 days in cultures fixed with 96° ethanol and stained with azur II-eosin by the method of Romanovskii. Colonies containing at least 50 fibroblasts per 10^5 explanted bone marrow cells were counted under an inverted microscope; when adding GAG and chondroitin sulfates, fibroblast CFU growth stimulation or inhibition indices calculated from the ratio to control cultures (%) were used. A total of 150 monolayer cultures of bone marrow were analyzed. The data were processed using Student's t test.

RESULTS

Efficacy of fibroblast cloning in rat bone marrow monolayer cultures was $10.3\pm0.9\times10^5$ cells after explantation of no more than 5×10^6 bone marrow cells without feeder cells. Fibroblasts in large and small colonies had typical morphological structure (Fig. 1). Addition to monolayer cultures of GAG fractions of different degree of sulfation decreased the efficacy of fibroblast cloning. The greatest inhibitory effect toward fibroblast CFU was observed after addition of sulfated GAG fractions isolated from blood granulocytes of CML patient with myeloblastic crisis in the presence of high count of blast cells and promyelocytes (50%) with marked basophilia (15%) of the blood. After their addition in a concentration of 1 μg uronic acids/ml, the fibroblast CFU growth inhibition was 60.1±2.0% (Fig. 2). The inhibitory effect of sulfated GAG from blood granulocytes of CML patients with chronic stage of the disease and from donor granulocytes was much lower. Inhibition of the growth of bone marrow fibroblast CFU added in a concentration of 1 μg uronic acids/ml was no higher than 41.2±4.0 and 36.7±5.0%, respectively (Fig. 2, $p<0.05$). A lesser inhibitory effect on the growth of rat bone marrow fibroblast CFU was observed after the addition of low-sulfated GAG fractions isolated from granulocytes of patients with CML and donors and represented by the C-4-S fraction with a low negative charge. Inhibition of fibroblast CFU growth after their addition in a concentration of 1 μg uronic acids/ml was no higher than 38.4±7.9% (Fig. 2).

Fractions of low- and sulfated GAG extracted from the GAG-cetavlone complexes in 0.5, 1, and 2 M NaCl solutions varied by their anionic properties and fraction composition [4]. The main structural components (uronic acids and hexosamines) were represented in equimolar amounts in both fractions of GAG isolated from blood granulocytes of patients with CML and donors. However, in sulfated GAG fraction not all hexosamine residues were sulfated completely, and the content of sulfate was 5.5 times higher than in the low-sulfated GAG fraction. Identification of GAG by their degradation products after treatment with hyaluronidase (Serva), chondroitinase (Seikagaku), and electrophoresis on cellulose acetate films in 0.1 M Ba-acetate buffer (pH 5.5) showed that C-4-S is the main component of sulfated GAG of donor blood granulocytes; its most homogeneous fraction corresponds to donor blood

Fig. 1. Fibroblast colony-forming units in rat bone marrow monolayer cultures on day 14 of growth. Azur II-Eosin staining by the method of Romanovskii.

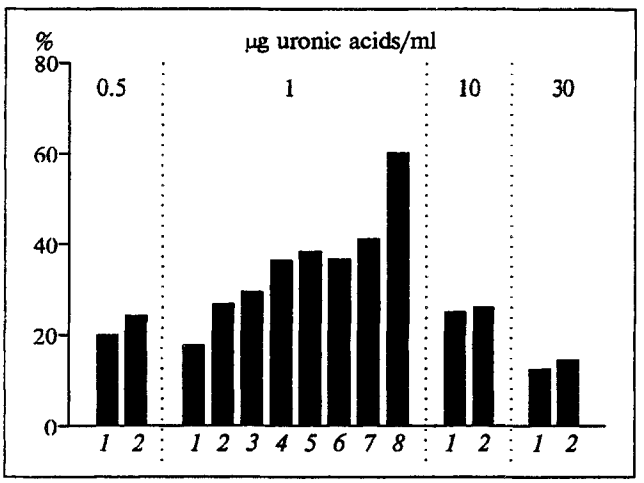

Fig. 2. Modulating effects of low- and sulfated glycosaminoglycans (n=70) and peripheral blood granulocytes of patients with chronic myeloleukemia and of donors and of chondroitin sulfates (n=80) on colony-forming activity of rat bone marrow fibroblasts (% of control level). *1*) chondroitin-4-sulfate; *2*) chondroitin-6-sulfate; *3-5*) low-sulfated; *6-8*) sulfated glycosaminoglycans isolated from blood granulocytes of donors (*3*, *6*) and patients with chronic myeloleukemia during the chronic phase of disease (*4*, *7*) and myeloblastic crisis (*5*, *8*).

platelet and reference C-4-S (Fig. 3). Heparan sulfate was a minor component of donor blood granulocytes, its content being no more than 9% of total amount of sulfated GAG. C-4-S and heparan sulfate were present in granulocyte GAG of CML patients, but only in low-sulfated GAG, the content of heparan sulfate being at least 3-4 times higher than in donors (Fig. 3). Moreover, after extraction of sulfated GAG fractions from blood granulocytes of CML patients in 0.7 M NaCl solution, electrophoregram showed a band corresponding to C-6-S by

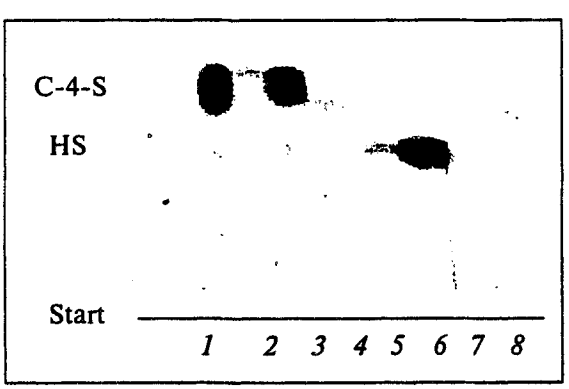

Fig. 3. Electrophoregram of peripheral blood granulocyte glycosaminoglycans of patients with chronic myeloleukemia and donors on a cellulose acetate film in 0.1 M Ba-acetate buffer (pH 5.5). Fractions of sulfated glycosaminoglycans from blood granulocytes of donors (*1*, *3*) and patients with chronic myeloleukemia (*5*, *6*); reference: chondroitin-4-sulfate from donor blood platelets (*2*); fractions of low-sulfated glycosaminoglycans from blood granulocytes of donors (*4*, *8*) and patients (*7*). HS: heparan sulfate.

mobility, which was absent from donor blood granulocytes.

Efficacies of fibroblast cloning in rat bone marrow monolayer cultures with commercial C-6-S and C-4-S were compared. C-6-S is a component of the extracellular matrix proteoglycans, produced predominantly by bone marrow stromal cells. Addition of C-6-S to rat bone marrow monolayer cultures slightly inhibited the growth of fibroblast CFU. At 0.5-1-10 μg/ml C-6-S, inhibition of fibroblast CFU growth was no more than 26.7±3.8% in comparison with 41.2±4.0% in exposure to sulfated GAG from granulocytes of CML patients (Fig. 2). However, the inhibitory effect of C-6-S on fibroblast colony-forming activity was not universal. Addition of C-6-S in the maximum concentration of 30 μg/ml inhibited fibroblast CFU growth and had a slight (no more than 12.5±2.9%) or none at all growth-modifying effect on colony formation. Addition of C-4-S to rat bone marrow monolayer cultures as the main component of sulfated GAG of blood granulocytes had a similar effect. Fibroblast CFU growth upon its addition in concentrations of 0.5-1-10 μg/ml was inhibited by no more than 25.1±3.4% (Fig. 2).

Thus, the modifying effect of GAG on colony-forming activity of rat bone marrow fibroblasts is largely determined by their fraction composition and anion characteristics. The growth of rat bone marrow fibroblast CFU was inhibited only by sulfated GAG with a high content of heparan sulfate, which were isolated from blood granulocytes of a CML patient with myeloblastic crisis. The inhibitory effect of low-sulfated GAG fractions of commercial chondroitin sulfates on fibroblast colony-forming activity was much lower. Our studies confirmed the *in vitro* growth-inhibitory effect of heparan sulfate proteoglycans isolated in other cell systems [9]. In contrast to chondroitin sulfates, high biological activity of heparan sulfate is determined by its structure: high density of negative charge and unstable configuration caused by L-iduronic acid residues [4]. Antiproliferative effect of heparan sulfate may be determined by its sulfate-rich domains containing uronic acid [9]. Due to their anion properties, proteoglycans react with many growth factors and cytokines (the main fibroblast growth factor, nerve growth factor, granulocyte-macrophage colony-stimulating factor, interleukin-3, and growth-transforming factor-β) characterized by high affinity for GAG or for their protein moiety, and modulate their functional activity [9]. Only after preliminary reaction with heparan sulfate/heparin of the plasma membrane or with heparan sulfate (the extracellular matrix proteoglycan, the multifunctional principal factor of fibroblast growth is capable of binding to the high-affinity receptor and induce the

mitogenic response of effector stromal and hemopoietic cells [8,10]. β-Glycan containing heparan and chondroitin sulfate chains is one of receptors for growth-transforming factor-β required for the function of its two other receptors participating in transmembrane transfer of mitogenic signal [9]. These mechanisms of modulating action of proteoglycans may determine the regulation of hemopoietic and stromal cell proliferation and differentiation.

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