Role of Hyaluronidase in the Regulation of Functions of Mesenchymal Precursor Cells

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The possibility of using hyaluronidase for the regulation of the state of different pools of mesenchymal precursors *in vivo* was demonstrated. The reaction of mesenchymal precursor cells depended on the dose of the enzyme. Administration of hyaluronidase in a dose of 20 U/mouse increased the content of stromal precursors and mesenchymal stem cells in the bone marrow and promotes mobilization of precursor cells induced by granulocyte CSF. Administration of high dose of hyaluronidase (100 U/mouse) reduced the content of mesenchymal precursors in hemopoietic tissue and abolished granulocyte CSF-induced mobilization of mesenchymal precursor cells of different maturity.

Key Words: *Mesenchymal stem cell; hyaluronic acid; hyaluronidase; granulocytic colony-stimulating factor; mobilization*

Hyaluronic acid (HA) is the most abundant glycosaminoglycan, a component of extracellular matrix in different tissues, including bone marrow (up to 40% of all glycosaminoglycans) [5,11,12]. An important role in the metabolism of HA is played by hyaluronidase, an enzyme stepwise cleaving HA molecule with generation of polymers affecting various biological processes and functional activity of cells [11,12]. It is known that activation of endogenous hyaluronidases can led to hydrolytic degradation of HA to low- and intermediate-molecular-weight forms [7] stimulating angiogenesis, proliferation, and differentiation of various cell elements [12]. Moreover, HA *in situ* in the bone marrow binds precursor cells of different degree of maturity via surface CD44 and RHAMM receptors on these cells [5,10]. Disturbances in the engrafting of exogenous stem cells in the hemopoietic tissue under the effect of hyaluronidase were reported [5,13]. At the same time, the role of HA in the regulation of stem cells (SC) and the possibility of modulating functional activity of endogenous SC with HA remain little studied.

Here we analyzed the effect of various doses of hyaluronidase on the state of bone marrow and circulating pools of mesenchymal precursor cells of different degree of maturity and studied its capacity to affect mobilization of these elements induced by granulocyte CSF (G-CSF).

MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice weighing 18-20 g (n=268, conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences). Hyaluronidase (Lidase) dissolved in 0.5 ml physiological saline was intraperitoneally injected to intact mice once a day for 2 days in a dose of 20 or 100 U/mouse. Some animals in parallel with hyaluronidase received subcutaneous injections of granulocyte CSF (G-SCF, Neupogen, Hoffman-la Roche, once a day for 5 days) in 0.2 ml RPMI-1640 (Sigma). Control mice

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received equivalent volumes of physiological saline or G-CSF. The number of committed mesenchymal precursor cells (fibroblast CFU, CFU-F) in the bone marrow and peripheral blood was determined on days 3, 5, and 8 after the start of treatment using the method of cell cloning [2]. The content of mesenchymal stem cells (MSC) in the bone marrow and circulating blood was evaluated on day 3 using the method of limiting dilutions with long-term cell culturing [6,9]. The data were processed statistically using Student's *t* test, and nonparametric Wilcoxon and Mann—Whitney tests. The incidence of MSC in the bone marrow and peripheral blood was evaluated using generalized linear model for Poisson distribution. The correspondence of limiting dilutions to unidimensional Poisson model was evaluated by linear log-log regression. The distribution of theoretic fraction of negative wells μ_i was described by an equation: $\mu_i=\exp(-fx_i)$, where f is the incidence of MSC and x_i is the number of cells seeded to the well [6.9]. Statistica 6.0 software was used.

RESULTS

In intact animals, hyaluronidase in a dose of 20 U/mouse considerably increased the number of both committed stromal precursors on days 3 and 5 (maximum 234.7% on day 5) and MSC (to 333.3% from the control on day 3) in the hemopoietic tissue. These changes were probably a result of generation of considerable amounts of intermediate-molecular-weight forms of HA stimulating cell proliferation and differentiation [11,12]. High doses of hyaluronidase (100 U/mouse) produced different effects on the studied parameters. Enhanced hydrolytic degradation of HA was associated with a significant decrease in the content of CFU-F in the bone marrow on days 5 and 8 (to 28.3% on day 8). At the same time, the content of MSC in the hemopoietic tissue corresponded to that after administration of 20 U hyaluronidase (333.3% from the background value). The described differences in the state of pools of mesenchymal precursors of different degree of maturity probably resulted from

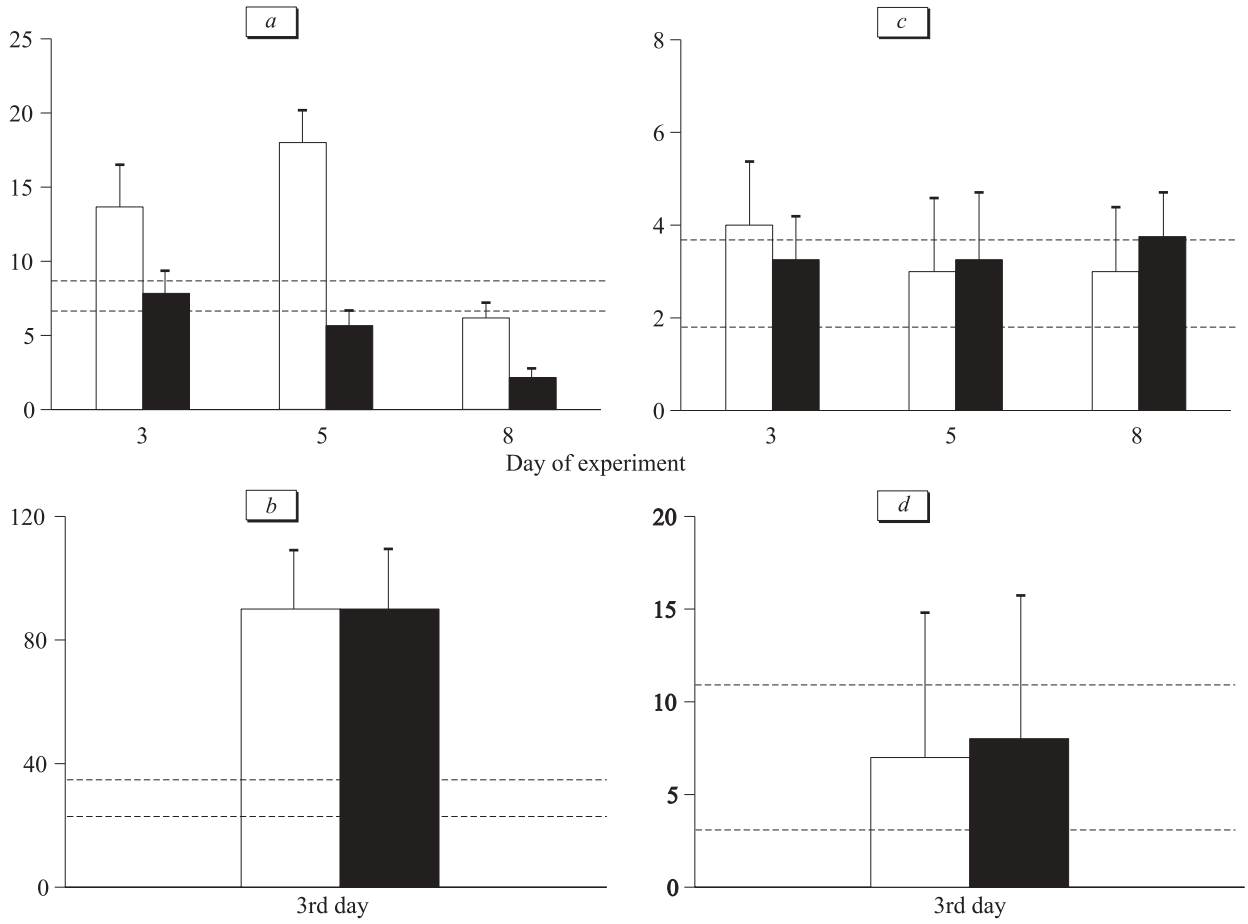

Fig. 1. Content of CFU-F (*a*) and MSC (*b*) in the bone marrow and CFU-F (*c*) and MSC (*d*) in peripheral blood of CBA/CaLac mice receiving hyaluronidase in doses of 20 (open bars) and 100 U/mouse (dark bars). Here and on Fig. 2: Ordinate: parameter per 2.5×10^5 myelokaryocytes (*a*); per 10^6 myelokaryocytes (*b*); per 2.5×10^5 mononuclears (*c*); per 10^6 mononuclears (*d*); area between dotted lines shows confidence interval for the test parameter in intact mice at $p<0.05$.

uncoupling of proliferation and differentiation of early progenitor elements in animals receiving 100 U hyaluronidase. This assumption is also confirmed by the fact that administration of hyaluronidase in both doses had no effect on the content of precursor cells in the peripheral blood (Fig. 1). Hence, the observed decrease in the content of CFU-F in the group receiving 100 U hyaluronidase cannot be explained by their mobilization. In general, the observed changes suggest that degradation of HA, the main component of extracellular matrix and glycocalyx [8], disturbs the functions of mesenchymal precursor cells (and, probably, other clonogenic elements of SC).

At the next stage we studied the possibility of regulating the function of mesenchymal precursor cells by combined treatment with G-CSF inducing SC mobilization [1,3,4] and hyaluronidase. Our experiments showed that administration of G-CSF reduced the content of CFU-F in the bone marrow on day 3 of the experiment, but then their number increased (days 5 and 8) against the background of unchanged content of MSC in the hemopoietic tissue. We observed an increase of both early progenitor cells (to 311.1% from the background level on day 3) and their committed precursors (CFU-F, peaked on day 3: 152.4% from the background level) in the peripheral blood (Fig. 2). G-CSF produced a stimulating effect on proliferation and differentiation status of committed stromal precursors and considerable mobilizing effect on mesenchymal precursor cells of different degree of maturity.

Additional administration of hyaluronidase in a dose of 20 U/mouse against the background of G-CSF treatment changed the pattern and magnitude of the forming shifts. In particular, we observed accumulation of MSC and CFU-F in the bone marrow on day 3 of the experiment (in contrast to animals receiving G-CSF alone), while the content of CFU-F in the hemopoietic tissue was below the control level on days 5 and 8. At the same time, combined treatment with G-CSF and hyaluronidase (20 U/mouse) more markedly increased the number of MSC (to 1000% from the background level on day 3) and CFU-F (peaked on day 3: 211.9% from the background level) in the peripheral blood (Fig.

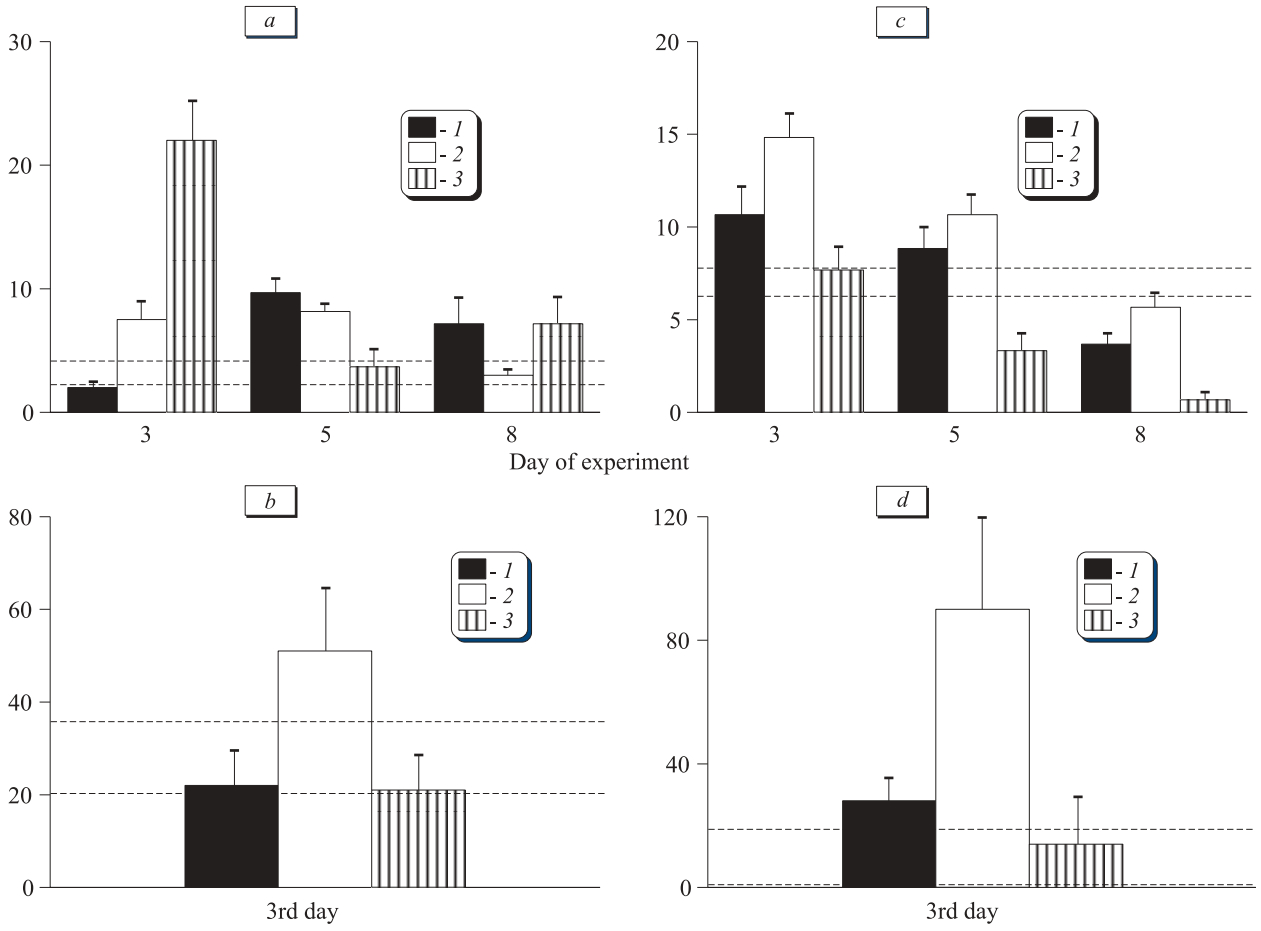

Fig. 2. Content of CFU-F (*a*) and MSC (*b*) in the bone marrow and CFU-F (*c*) and MSC (*d*) in peripheral blood of CBA/CaLac mice receiving G-CSF (*1*) and G-CSF in combination with hyaluronidase in doses of 20 (*2*) and 100 U/mouse (*3*).

2). Against the background of combined treatment with these preparations, the content of committed mesenchymal precursor cells in the peripheral blood decreased from day 3 though 8 with the same rate as in the control: the content of CFU-F in the groups receiving G-CSF alone and in combination with hyaluronidase decreased to 75.6 and 74.3%, respectively. These findings indirectly suggest that engrafting capacity of CFU-F is preserved after exposure to low dose of hyaluronidase, which is important for possible application for transplantation of mononuclear fraction of the peripheral blood (enriched with progenitor elements) obtained after combined treatment with these preparations.

Treatment with G-CSF and 100 U/mouse hyaluronidase led to more pronounced increase in the count of CFU-F in the bone marrow on day 3 of the experiment and more pronounced decrease in this parameter on day 5 (compared to the group receiving G-CSF alone). The content of CFU-F in the peripheral blood decreased (minimum 9.6% on day 8). Different picture was observed in the MSC pool. On day 3, the number of MSC in the hemopoietic tissue decreased (compared to the group receiving 20 U hyaluronidase). The changes developed against the background of blocked MSC release into the circulation (Fig. 2). Taking these findings into account, we can hypothesize that the pronounced accumulation of CFU-F in the bone marrow (which cannot result from the absence of their mobilization) was related to enhanced formation of these elements due to stimulation under the effect of G-CSF of MSC differentiation into more mature stromal elements forming the microenvironment [1]. At the same time, we cannot exclude increased sensitivity of CFU-F to growth factors (in particular, to G-CSF) at the early terms after exposure to the high dose of the enzyme. The results suggest that increasing the dose of hyaluronidase against the background of G-CSF treatment impairs mobilization capacity of precursor cells and probably changes the proliferation—differentiation balance of MSC and CFU-F.

Thus, HA and the ratio between its various molecular forms *in situ* play an important role in the regulation of functions of progenitor cells [11,12], including bone marrow cells. The state of different pools of mesenchymal precursors can be pharmacologically corrected via modulation of the properties of the extracellular matrix of the hemopoietic tissue with hyaluronidase. Our experiments demonstrated the efficiency of low doses of this enzyme for the stimulation of proliferation and differentiation potencies of mesenchymal precursor cells and potentiation of their G-CSF-induced mobilization.

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