Effect of Hyaluronidase Immobilized Using Electron-Beam Synthesis Nanotechnology on Sensitivity of Progenitor Cells to Regulatory Factors

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In vitro experiments demonstrated increased colony-forming capacity of erythroid, granulomonocytic, and mesenchymal progenitors of the bone marrow and parenchymal progenitor elements of the liver after treatment with immobilized hyaluronidase. Increased sensitivity of these progenitor cells to erythropoietin, granulocyte colony-stimulating factor, fibroblast growth factor, and stem cell factor, respectively, was demonstrated. Immobilized hyaluronidase enhanced the formation of tissue-specific hepatic CFU against the background of reduced yield of stromal precursors in liver tissue culture containing insulin.

Key Words: *immobilized hyaluronidase; cytokines; progenitor cells; drugs; nanotechnology*

Creation of new drugs based on endogenous regulators of functions synthesized by using genomic and post-genomic technologies (cytokines, hormones, and other bioactive substances) is a promising trend in pharmacology. Of particular interest is the use of stem cell (SC) growth factors for the treatment of diseases [8,9]. At the same time, most bioengineering products may cause serious complications when used as pharmacological agents. This fact severely limits or excludes the use of these drugs in clinical practice in optimal doses and modes [1,13]. This circumstance is not only due to protein nature of these substances, but in many ways to their pleiotropy and multifunctionality determining high probability of specific adverse effects [8,11]. In this context, studies aimed at minimizing the risk of growth factor application for therapeutic purposes, including those reducing the dose of administered potential drugs, are now in progress. Experiments performed at the Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences, demonstrated the possibility of *in vivo* potentiating the hemostimulating and SC-mobilizing effects of granulocytic CSF by using hyaluronidase (HD), an enzyme cleaving hyaluronic acid (HA) to polymers activating proliferation and differentiation of stem cells and weakening their adhesion to the stroma of tissue depots [2-4]. However, these effects of native HD are observed only when the enzyme is used in high toxic doses. At the same time, HD immobilized using nanotechnology of electron-beam synthesis produces an independent specific effect on stem cells in relatively low doses [6].

Here we studied the effect of immobilized HD on the realization of the growth potential of progenitor

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cells belonging to different classes exposed to specific growth factors.

MATERIALS AND METHODS

Bone marrow and liver of 2-month old male CBA/CaLac mice (n=20) weighing 18-20 g were examined *in vitro*. The animals were obtained from the nursery of Experimental Biological Clinic of Laboratory Animals (Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences). To modulate the effects of bioactive substances, preparation of immobilized HD was used. The preparation was developed at the Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk in cooperation with Scientific Future Management Company Group (Novosibirsk). Enzyme immobilization was carried out on polyethylene glycol with a molecular weight of 1500 Da by exposing to directed flow of accelerated electrons with initial electron energy of 2.5 MeV (absorbed dose 10.2 kGy, dose rate 1.65 kGy/h).

Erythropoietin (Recormon), granulocyte colony-stimulating factor (granulocytic CSF, Neupogen), basic fibroblast growth factor-1 (FGF; Sigma), stem cell factor (SCF, Sigma), and insulin (Sigma) were used as growth factors affecting progenitor cells. The sensitivity of erythroid, granulomonocytic (GM), fibroblast, and liver colony-forming units (CFU) preincubated for 30 min with immobilized HD (0.1 U/ml) in DMEM (Sigma) to the test substances was determined.

After preincubation, we analyzed myelokaryocytes capacity to form erythroid CFU, CFU-GM, and fibroblast CFU in semisolid culture medium [5] upon adding erythropoietin, granulocytic CSF and FGF, respectively, the capacity of liver cells to form liver and fibroblast CFU in a liquid medium [12] containing insulin and to form only hepatic CFU in a medium containing SCF. In all cases, colony forming capacity of cells not treated with immobilized HD and cells cultured without growth factors served as the control.

The results were processed using Student's t test and nonparametric Mann–Whitney U test.

RESULTS

Myelokaryocyte pretreatment with the enzyme considerably increased the formation of erythroid CFU, GM-CFU, and fibroblast CFU in methylcellulose medium without additional growth factors (Fig. 1). In this case, colony-stimulating activity of the culture

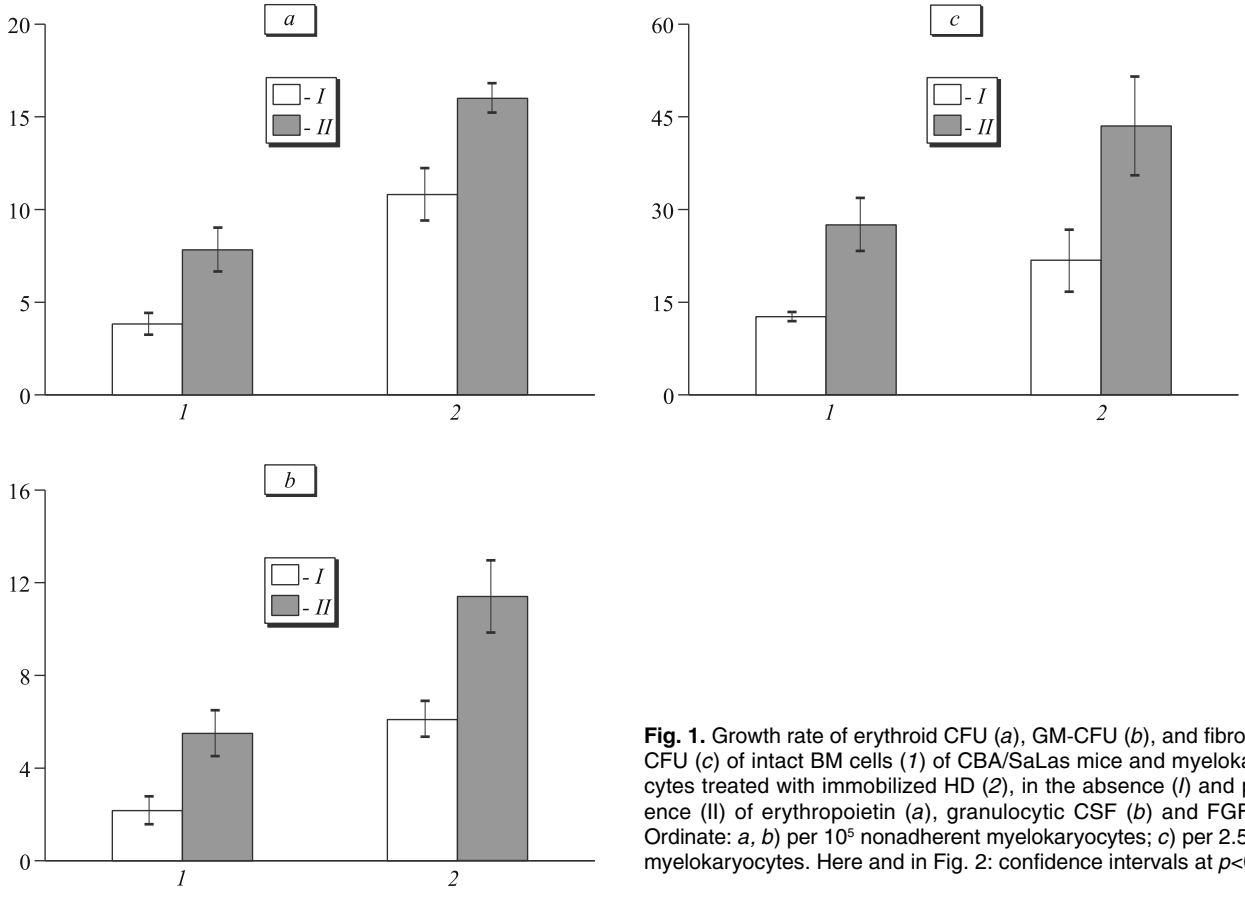

Fig. 1. Growth rate of erythroid CFU (*a*), GM-CFU (*b*), and fibroblast CFU (*c*) of intact BM cells (*1*) of CBA/SaLas mice and myelokaryocytes treated with immobilized HD (*2*), in the absence (*I*) and presence (II) of erythropoietin (*a*), granulocytic CSF (*b*) and FGF (*c*). Ordinate: *a, b*) per 10^5 nonadherent myelokaryocytes; *c*) per 2.5×10^5 myelokaryocytes. Here and in Fig. 2: confidence intervals at $p<0.05$.

medium was determined by the complex of bioactive substances of fetal calf serum [5].

Preincubation of liver cells in enzyme solution also increased the formation of parenchymal liver CFU (Fig. 2), but inhibited the growth of fibroblast CFU from liver tissue. This phenomenon, apparently, was determined by the influence of the enzyme on differentiation potential of parental cells [3,10] and by stimulation of their growth in tissue-specific, parenchymatous direction. Moreover, the cells incubated with immobilized HD were significantly more receptive to specific growth factors than intact blood nuclears. The capacity of erythroid CFU, CFU-GM and CFU fibroblast CM to respond to erythropoietin, granulocytic CSF, and FGF by stimulation of colony forming increased by 48.1, 86.9 and 99.5%, respectively (Fig. 1), and the sensitivity of parenchymal liver CFU to SCF increased by 95.4% (Fig. 2, *a*).

Thus, immobilized HD promoted realization of growth potential of progenitor cells of different classes. The maximum changes were observed in the earliest progenitor cells from BM, primarily fibroblast CFU containing not only stromal precursors, but also multipotent SC [7,12] and in hepatic progenitor cells.

At the same time, analysis of colony-forming capacity of hepatic progenitor cells in the presence of insulin in tissue culture yielded ambiguous results. We observed increased yield of parenchymal CFU, on the one hand, and reduced formation of stromal colonies (fibroblast CFU), on the other. Taking into account incomparable changes in the yield of tissue-specific and fibroblast CFU (number of hepatic CFU increased by 39.0%, while the number of fibroblast CFU decreased by 92.7%), these data suggest that immobilized HD not only affects differentiation status of progenitor cells, but also potentiates the inhibitory effects of insulin on the growth of fibroblast CFU (Fig. 2, *b, c*).

The results indicate a significant increase in the sensitivity of progenitor elements exposed to immobilized HD to various bioactive substances, including early and linearly restricted growth factors [11]. The mechanism of these phenomena is probably a modification of the susceptibility of receptors to regulatory molecules due to modulation of their state caused by degradation of hyaluronic acid in the glycocalyx [14,15] of effector cells.

Our findings and published data on specific activity of immobilized HD [6,9] and, obviously, its low

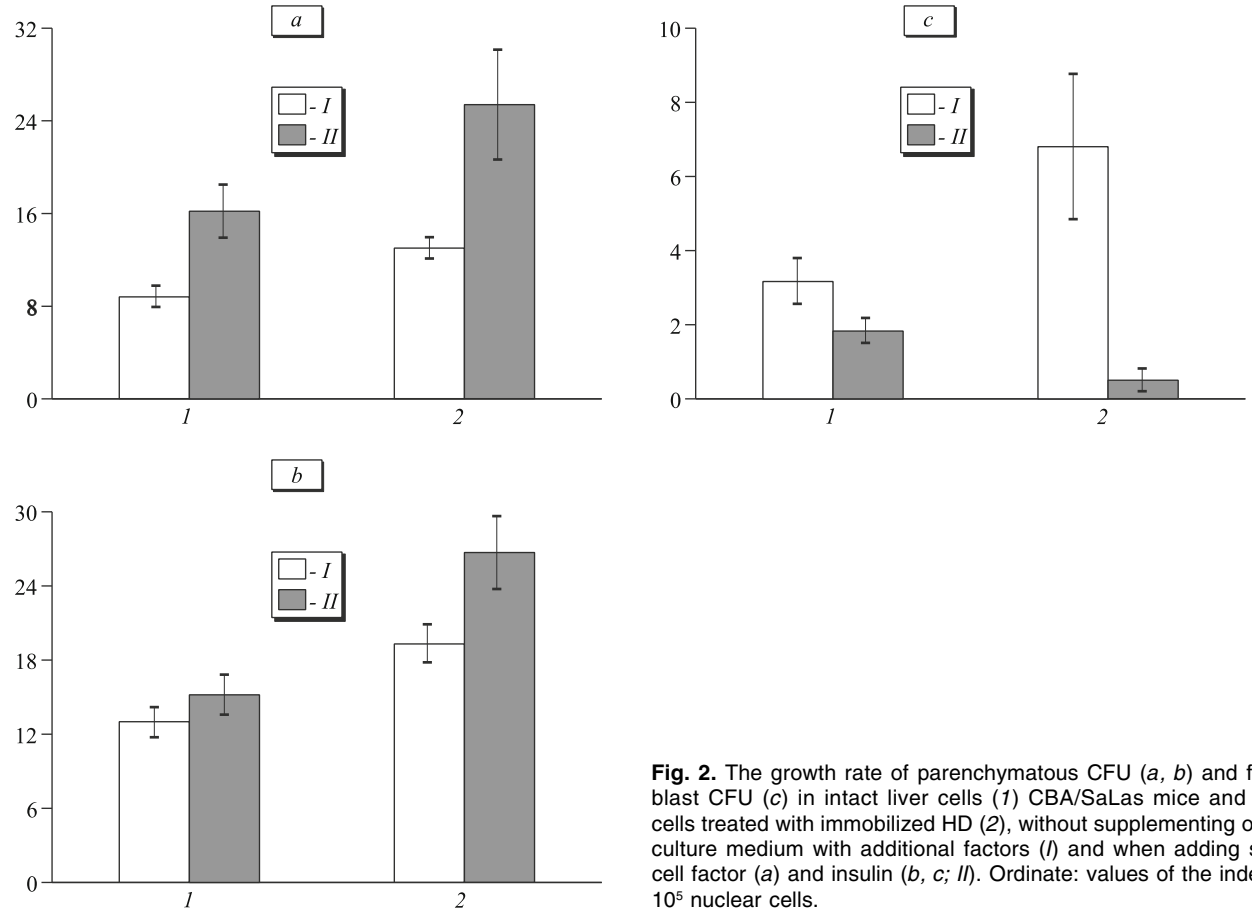

Fig. 2. The growth rate of parenchymatous CFU (*a, b*) and fibroblast CFU (*c*) in intact liver cells (*1*) CBA/SaLas mice and liver cells treated with immobilized HD (*2*), without supplementing of the culture medium with additional factors (*I*) and when adding stem cell factor (*a*) and insulin (*b, c; II*). Ordinate: values of the index at 10^5 nuclear cells.

toxicity [7] suggest that the development of drugs for regenerative medicine on the basis of HD [8,9] and approaches for improving the efficiency and safety of therapy of various diseases with recombinant and other bioengineered protein drugs as analogs of endogenous regulators of physiological functions are promising trends.

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REFERENCES

1. M. A. Volkova, *Ter. Arkhiv*, No. 4, 80-84 (1998).
2. E. D. Gol'dberg, A. M. Dygai, G. N. Zyuz'kov, *et al.*, *Byull. Eksp. Biol. Med.*, **144**, No. 12, 652-656 (2007).
3. E. D. Gol'dberg, A. M. Dygai, G. N. Zyuz'kov, *et al.*, *Klet. Tekhn. Biol. Med.,* No. 2, 115-119 (2007).
4. E. D. Gol'dberg, A. M. Dygai, G. N. Zyuz'kov, *et al.*, *Byull. Exp. Biol. Med.*, **145**, No. 6, 628-633 (2008).
5. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
6. A. M. Dygai, A. V. Artamonov, A. A. Bekarev, *et al., Byull. Eksp. Biol. Med.*, **149**, No. 5, 528-531 (2010).
7. A. M. Dygai, E. I. Vereshchagin, G. N. Zyuz'kov, *et al*., *Klet. Tekhn. Biol. Med.,* No. 2, 63-66 (2009).
8. A. M. Dygai and G. N. Zyuz'kov, *Science in Russia*, **169**, No. 1, 4-8 (2009).
9. A. M. Dygai, G. N. Zyuz'kov, V. V. Zdanov, *et al., Vestn. Ross Akad. Med. Nauk*, No. 11, 6-9 (2009).
10. G. N. Zyuz'kov, V. V. Zdanov, A. M. Dygai, *et al.*, *Byull. Exp. Biol.Med.*, 144, No. 12, 690-695 (2007).
11. *The System of Cytokines: Theoretical and Clinical Aspects* [in Russian], Eds. V. A. Kozlov and S. V. Sennikov, Novosibirsk (2004).
12. O. I. Epstein., G. N. Zyuz'kov, N. V. Sotnikova, *et al*., *Klet. Tekhn. Biol. Med.,* No. 4, 194-198 (2005).
13. J. A. Anderson and N. F. Jr. Adkinson, *JAMA*, **268**, No. 20, 2845-2857 (1992).
14. C. B. Henry and B. R. Duling, *Am. J. Physiol*., **277**, No. 2, Pt. 2, 508-514 (1999).
15. R. Stern, *Glycobiology,* **13**, No. 12, 105-115 (2003).