Hemopoiesis-Stimulating Activity of Immobilized Oligonucleotides and Hyaluronidase during Cytostatic-Induced Myelosuppression

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 11, pp. 545-549, November, 2010
Original article submitted August 30, 2009

The hemopoiesis-stimulating effect of combined treatment with immobilized oligonucleotides and hyaluronidase preparations was studied during cytostatic-induced myelosuppression caused by cyclophosphamide administration. Immobilized hyaluronidase was shown to increase the efficiency of correction of changes in the erythroid and granulocytic hemopoietic stems with immobilized oligonucleotides. This potentiation of the effect of immobilized oligonucleotides by immobilized hyaluronidase was related to an increase in functional activity of committed hemopoietic precursors.

Key Words: *immobilized oligonucleotides; immobilized hyaluronidase; hemopoiesis; hemopoietic precursor cells; cytostatic*

The toxic effect of antineoplastic drugs on hemopoiesis is the most common complication of chemotherapy. It is manifested in a variety of blood disturbances, including leukopenia, agranulocytosis, pancytopenia, anemia, and thrombocytopenia [1]. Nucleic acids and their derivatives hold much promise for the correction of cytostatic-induced myelosuppressions. Nucleic acid products stimulate granulocytopoiesis, normalize cellularity of the lymphoid and thrombocytic hemopoietic stems, increase functional activity of T helper cells and T killer cells, and activate B cell proliferation and antibody synthesis under conditions of suppressed hemopoiesis [7,8]. These properties are probably related to the ability of nucleic acids to eliminate free radicals from the body. The inflow of nucleotides into the cytostatic-damaged hemopoietic tissue has a stimulatory effect on nucleic acid synthesis, which probably results in an increase in the division rate of hemopoietic precursors and rapid recovery of bone marrow cells [5].

The use of nucleic acids (oligonucleotides) in clinical practice is limited by low level of intracellular transport and rapid degradation with lysosomal nucleases [6]. Stability of these substances and the duration of therapeutic action can be increased via modification of bioactive compounds by their attachment to polymeric molecules. The attachment of polyethylene glycol molecules (pegylation) is a widely distributed method of drug modification. Pegylation increases the solubility due to an increase in hydrophilicity, size, and weight of the particle, and thereby reduces the intensity of renal excretion. The immunogenicity and availability of particles for proteolytic enzymes were shown to decrease after this treatment [4,9,12,13].

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0007-4888/11/1505-0614

Moreover, a modulating effect of hyaluronic acid fragments on progenitor cells was demonstrated [3].

The targeted transport of medicinal products is an urgent problem of pharmacotherapy. The supply of pharmacological agents to target cells can be improved by treatment with compounds reducing viscosity of the intercellular space. For example, hyaluronidase increases the permeation of medicinal products (G-CSF and insulin) due to degradation of hyaluronic acid into glucosamine and glucuronic acid [3,10].

Here we studied hemopoiesis-stimulating activity of immobilized oligonucleotides (imON) and hyaluronidase (imHD) and evaluated the effect of combined treatment with these agents under conditions of cyclophosphamide administration.

MATERIALS AND METHODS

Experiments were performed on 610 CBA/CaLac mice (class I conventional strain) at the age of 2-2.5 months obtained from the nursery of the Institute of Pharmacology (certified animals).

Cytostatic myelosuppression was induced by an intraperitoneal injection of the alkylating agent cyclophosphamide in a single dose 200 mg/kg. The mice were divided into 4 groups. Group 1 animals received physiological saline (0.2 ml orally) for 7 days after cytostatic treatment (cytostatic control). Group 2 animals received imON (Scientific Features Management Company) in a dose of 200 mg/kg (beginning from the next day after cytostatic treatment). The preparation represents highly purified short fragments from sturgeon milt DNA (molecular weight 500-700 kDa) immobilized on polyethylene glycol by the nanotechnology method of electron-beam synthesis. imHD was given to group 3 mice once a day (50 U orally) on days 1 and 2 after cytostatic treatment (Scientific Features Management Company). Group 4 animals received the immobilized substances as follows: imHD, days 1 and 2 after cytostatic treatment; and imON, 1 h after administration of imHD (for 7 days). Baseline parameters were measured in intact animals (intact control).

Standard blood tests were performed on days 2-6, 8, 10, and 12 after cytostatic treatment [2]. The mice were euthanized by CO_2 overdose. The total karyocyte count (TKC) and count of morphologically distinguishable cells of the bone marrow were determined. The content of committed granulocyte-macrophage (CFU-GM) and erythroid precursors (CFU-E) in the bone marrow was estimated routinely [2]. The degree of differentiation of hemopoietic precursor cells was evaluated from the index of maturation (cluster/colony ratio in a well). The colony-stimulating and erythropoietic activity in supernatants from adherent and nonadherent cells of the hemopoiesis-inducing microenvironment was determined from the effect of conditioned media on the formation of CFU-GM and CFU-E in nonadherent myelokaryocyte cultures of intact mice [2].

The results were analyzed by standard methods of variation statistics. The significance of differences was evaluated by parametric Student's t test or nonparametric Mann–Whitney U test. Exact Fisher test was used to analyze the rated data.

RESULTS

Experiments showed that the dynamics of TKC in cyclophosphamide-treated mice after treatment with imON or imHD did not differ from that in animals of the cytostatic control group (physiological saline). Analysis of the bone marrow showed that imON significantly increased the count of immature (day 8) and mature neutrophilic granulocytes (day 4, Fig. 1). The content of erythroid cells was elevated on day 4 after cytostatic treatment. This drug increased the number of neutrophilic granulocytes (up to neutrophilia) in the peripheral blood on days 6, 8, 10, and 12 after treatment (Fig. 2). We revealed a more significant increase in the count of immature neutrophils (294% of the baseline *vs*. 220% in the cytostatic control group; day 5) and erythrokaryocytes (50-87% of the baseline *vs*. 11-49% in the cytostatic control group; days 5 and 6) in imHD-treated animals. The test preparations had little effect on lymphopoiesis.

Combined administration of these preparations increased TKC compared to not only the cytostatic control group, but also imON and imHD groups on day 3 after treatment (Fig. 1). Morphological study of the bone marrow showed that this effect was related to an increase in the count of immature neutrophils, erythrokaryocytes, and lymphocytes. The mice were characterized by an increase in the number of neutrophilic granulocytes (days 4 and 6) and erythroid cells (days 4 and 5). These changes were not observed on the 3rd day. It should be emphasized that the content of myelokaryocytes in these animals was much higher than in mice receiving imHD or imON. The number of peripheral blood neutrophilic granulocytes after combined treatment with imON and imHD was higher than after administration of imON alone (days 6 and 10; Fig. 2).

Our results indicate that imHD and imON potentiate the effects of each other. It is manifested in accelerated regeneration of the hemopoietic tissue and recovery of cell number in the peripheral blood during cytostatic-induced myelosuppression.

We studied the mechanisms for action of immobilized substances on hemopoiesis. imON administra-

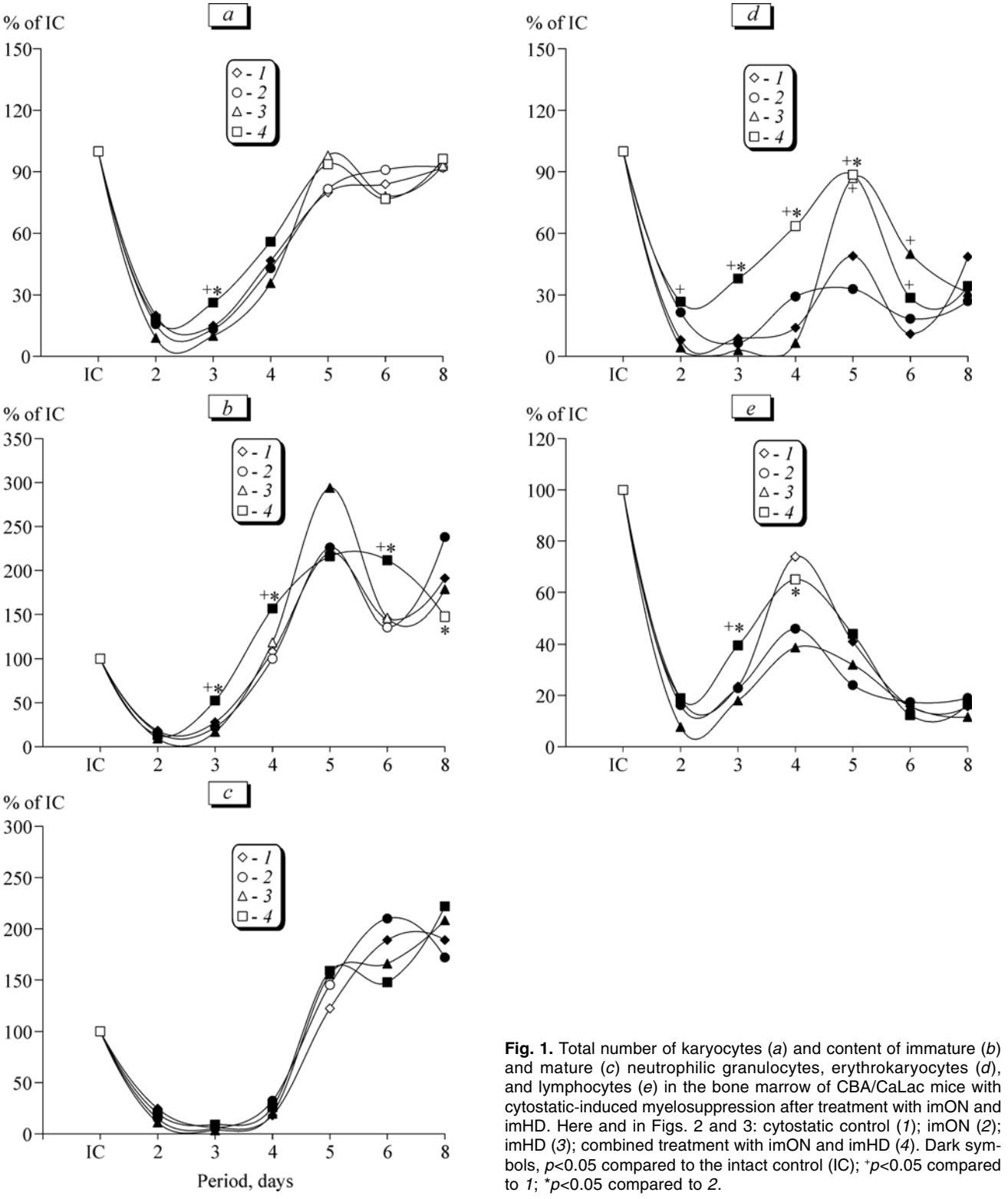

Fig. 1. Total number of karyocytes (*a*) and content of immature (*b*) and mature (*c*) neutrophilic granulocytes, erythrokaryocytes (*d*), and lymphocytes (*e*) in the bone marrow of CBA/CaLac mice with cytostatic-induced myelosuppression after treatment with imON and imHD. Here and in Figs. 2 and 3: cytostatic control (*1*); imON (*2*); imHD (*3*); combined treatment with imON and imHD (*4*). Dark symbols, $p<0.05$ compared to the intact control (IC); $^{+}p<0.05$ compared to *1*; $^{*}p<0.05$ compared to *2*.

tion during cytostatic-induced myelosuppression was followed by an increase in the content of CFU-GM (day 2) and CFU-E (days 3 and 4) in the bone marrow and stimulation of differentiation of committed hemopoietic precursors (days 2 and 3; Fig. 3). This preparation abolished the increase in the rate of CFU-GM maturation, which was observed in animals of the cytostatic control group (day 6). imHD had no effect on erythroid precursors and decreased the content of CFU-GM (day 6).

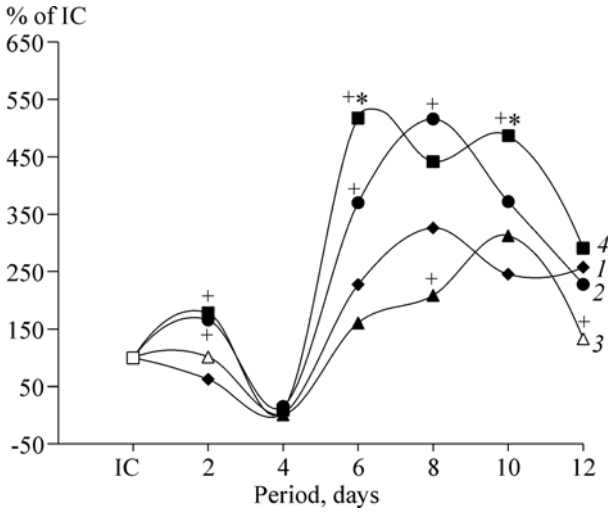

Fig. 2. Number of segmented neutrophils in the peripheral blood from CBA/CaLac mice with cytostatic-induced myelosuppression after treatment with imON and imHD.

Combined treatment with imON and imHD significantly increased the content of bone marrow CFU-GM (days 3 and 4 after administration of the alkylating agent; Fig. 3). Study parameter was 95% higher compared to that in imON-receiving mice ($p<0.05$). An imON-induced increase in the content of CFU-E was less pronounced under the influence of imHD (reduction from 282 to 185%, day 4). The maturation index of erythroid precursor cells in animals of the imON+imHD group did not differ from that in imHD-treated mice. This index was much lower than in animals of the intact control group.

Individual or combined treatment with imON and imHD abolished the increase in the colony-stimulating (day 2) and erythropoietic activity of blood serum (day 4). These preparations impaired the production of growth factors CFU-GM (day 5) and CFU-E (day 3) by cells of the hemopoietic microenvironment in cyclophosphamide-treated animals.

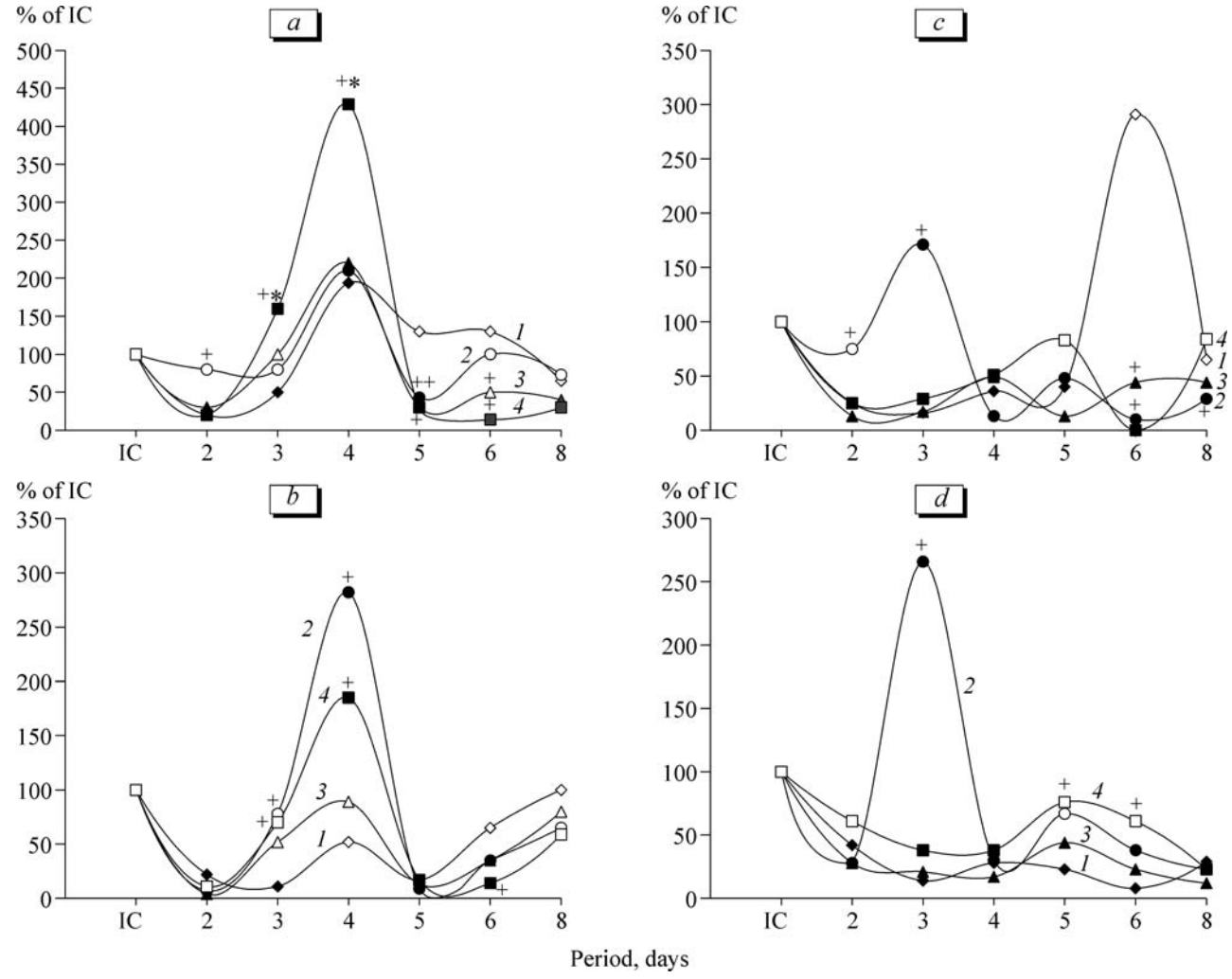

Fig. 3. Content of CFU-GM (*a*) and CFU-E (*b*) and differentiation of CFU-GM (*c*) and CFU-E (*d*) in the bone marrow of CBA/CaLac mice with cytostatic-induced myelosuppression after treatment with imON and imHD.

Therefore, imHD potentiated the stimulatory effect of imON on CFU-GM and decreased the rate of CFU-E recovery during cytostatic-induced myelosuppression. Additional treatment with imHD had no effect on functional activity of the hemopoietic microenvironment.

It should be emphasized that imON not only modulated the function of committed precursor cells. Previous experiments showed that oligonucleotides *in vitro* stimulated the formation of colonies from granulocyte-erythroid-macrophage-megakaryocyte CFU (CFU-GEMM) [12]. Published data and results of our studies showed that hyaluronidase-induced changes in the properties of glicocalix and intercellular matrix had a modulatory effect on cell function [3,11,14]. These data suggest that accelerated regeneration of the hemopoietic tissue and recovery of cell number in the peripheral blood depend on an imHD-induced change in the response of hemopoietic precursor cells to regulatory signals of imON. It cannot be excluded that degradation products of hyaluronic acid also have a stimulatory effect on hemopoiesis. The increase in granulomonocytopoiesis is probably associated with stimulatory effect of glucuronic acid on the proliferation of CFU-GM and formation of granulocytic hemopoietic islets [1].

This work was supported by the Federal Target Program "Scientific and Scientific-Pedagogical Personnel of Innovative Russia for 2009-2013" (State contract No. 02.740.11.0781).

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