Effect of Dimethylsulfoxide on the Functions of Mesenchymal and Hemopoietic Precursors

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The effects of dimethylsulfoxide on the state of mesenchymal precursors *in vivo* were demonstrated. Treatment with dimethylsulfoxide reduced the content of stromal clonogenic elements in the bone marrow and inhibited mobilization of mesenchymal precursors induced by granulocyte colony-stimulating factor. In *in vitro* system, dimethylsulfoxide inhibited proliferation of fibroblast, erythroid, and granulomonocytic colony-forming units and stimulates maturation of hemopoietic precursors.

Key Words: *stem cells; mesenchymal precursor cells; hemopoietic precursors; dimethylsulfoxide; granulocyte colony-stimulating factor*

High quality of the transplant during its storage is an important problem determining the efficiency and results of the used methods and procedures in modern transplantology and cell therapy. The quality of the transplant depends on the choice of cryoprotectors and freezing regimen [1,5] preserving functional properties of the transplanted tissues and elements, including various types of stem cells, after their defrosting. Dimethylsulfoxide (DMSO) is the most widely used compound protecting cell material from damaging factors during cryopreservation [1,5]. Taking into account the wide spectrum of pharmacological effects of DMSO in experimental and clinical practice [4] and recent data on the effects of DMSO on the cell genome [11], the study of the effect of this compound on the functions of various types of cell precursors seems to be actual.

Here we studied the effects of DMSO on the state of bone marrow pools of mesenchymal precursor cells of different maturity, their mobilization capacity, and possible effects of DMSO on hemopoietic precursors.

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MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=286, weight 18-20 g) conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences). DMSO (Dimeksid, 20% solution, Red'kin Experimental Plant) was administered to experimental animals in a dose of 2 g/kg daily for 5 days through a gastric tube. Some animals in parallel with DMSO received subcutaneous injections of granulocyte CSF (G-SCF, Neupogen, Hoffman-la Roche, once a day for 5 days) dissolved in 0.2 ml RPMI-1640 (Sigma). Control mice received physiological solution or G-CSF according to the same schemes. 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 [3]. The content of mesenchymal stem cells (MSC) in the bone marrow and circulating blood was evaluated on day 3 using the method of limiting dilution [10]. In addition, direct effects of DMSO added *in vitro* to a final concentration of 0.2% on the growth of

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fibroblast, erythroid, and granulomonocyte colonies and on proliferative and differentiation status of hemopoietic precursors [3] and CFU-F were evaluated. Proliferative activity of CFU-F was studied by the method of cell suicide. To this end, myelokaryocytes were preincubated with hydroxyurea (Sigma) and then divided into adherent and nonadherent fractions. Nonadherent and adherent cells were washed by centrifugation and by 10-fold washout with DMEM (Sigma), respectively. Nonadherent myelokaryocytes were then placed onto the washed adherent cells. Bone marrow cells not treated with hydroxyurea were processed similarly and their colony-forming capacity was taken as the control. The pool of S-phase CFU-F was determined by the formula for hemopoietic precursors [3]. The intensity of CFU-F differentiation (index of differentiation) was evaluated by the ratio of fibroblast cluster-forming units (focus of mesynchymopoiesis, 5-50 cells) to CFU-F (>50 cells). The data were processed by methods of variation statistics using Student's *t* test and nonparametric Mann—Whitney *U* test. The incidence of MSC in the bone marrow and peripheral blood was evaluated using generalized lineary 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 [10]. Statistica 6.0 software was used.

RESULTS

During the initial terms, DMSO administered to intact animals had practically no effect on the growth of CFU-F from the bone marrow, but later we observed a sharp decrease in their content in the hemopoietic tissue (to 28.3% from the background value on day 8). The number of CFUF in the peripheral blood tended to decrease by day 8. These elements do not enter the circulation, therefore we can hypothesize that changes in the bone marrow pool of CFU-F are a result of inhibition of proliferation or stimulation of maturation of stromal precursors under the effect of DMSO [9,15]. At the same time, DMSO had practically no effect on the state of bone marrow and circulating pools of MSC (Fig. 1).

On the other hand, DMSO considerably modulated the effect of granulocyte CSF, a factor inducing mobilization of stem cells, on the function of mesenchymal precursors [2]. Experiments showed that G-CSF reduced the number of CFU-F in the bone marrow on day 3 of the experiment. Then, the number of CFU-F in the hemopoietic tissue

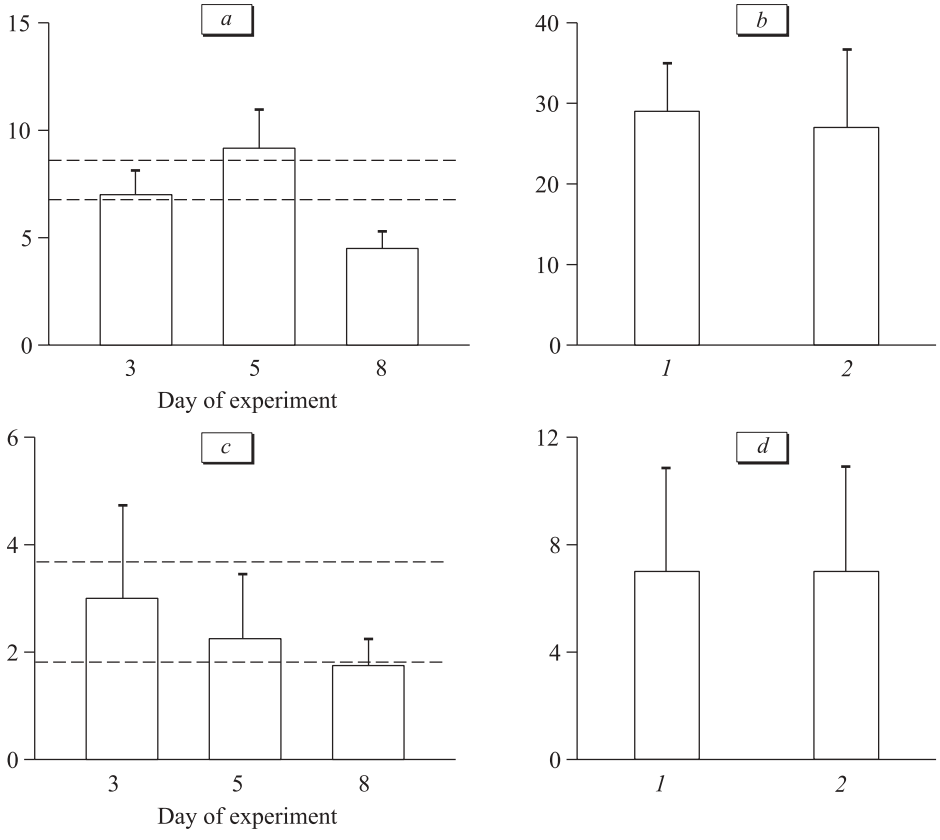

Fig. 1. Content of CFU-F (*a, c*) and MSC (*b, d*) in the bone marrow (*a, b*) and peripheral blood (*c, d*) in CBA/CaLac mice receiving DMSO. *1*) baseline; *2*) day 3. Here and on Fig. 2: ordinate: *a*) per 2.5×10^5 myelokaryocytes; *b*) per 10^6 myelokaryocytes; *c*) per 2.5×10^5 mononuclears; *d*) per 10^6 mononuclears. Here and on Fig. 2, 3: area between dotted lines shows confidence interval for the test parameter in intact mice at $p<0.05$.

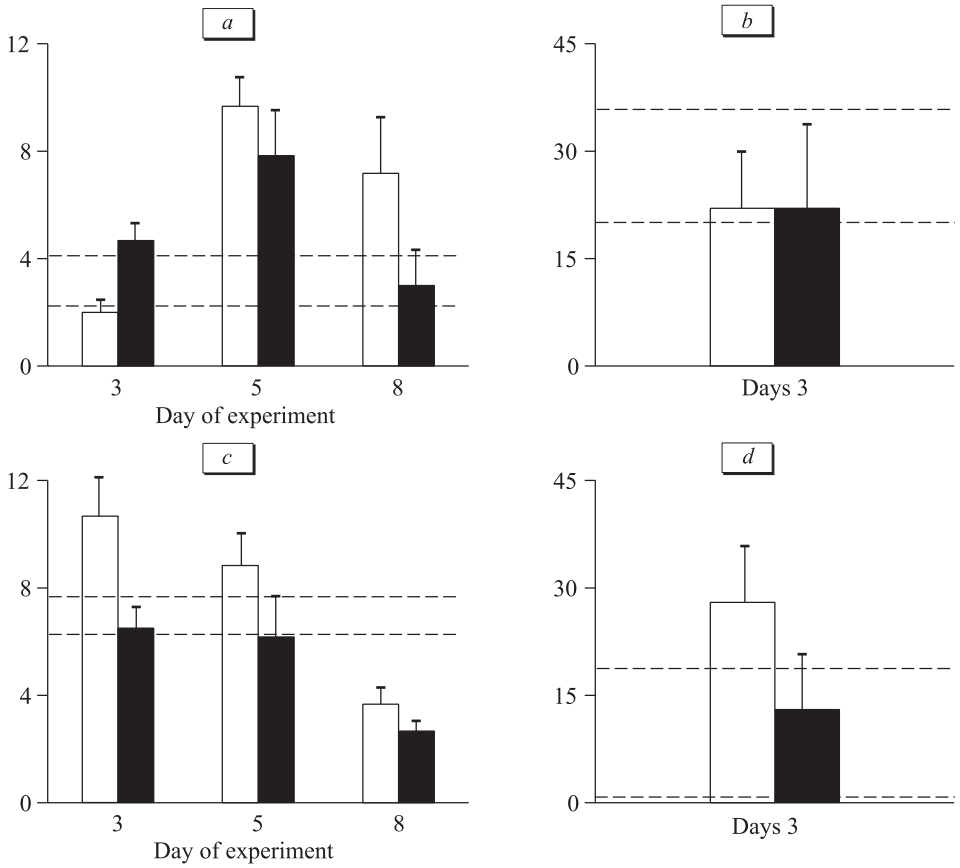

Fig. 2. Content of CFU-F (*a, c*) and MSC (*b, d*) in the bone marrow (*a, b*) and peripheral blood (*c, d*) in CBA/CaLac mice receiving G-CSF (open bars) and G-CSF+DMSO (shaded bars).

increased (days 5 and 8) against the background of unchanged content of MSC. We observed an increase of both early MSC precursors (to 311.1% from the background level on day 3) and committed precursors (CFU-F, peaked on day 3: 152.4% from the background level, Fig. 2). DMSO considerably modulated these changes. The content of CFU-F in the bone marrow increased on day 3 and sharply decreased to the end of the experiment (to 41.8% of the control on day 8). The content of MSC in the hemopoietic tissue on day 3 remained virtually unchanged. DMSO considerably decreased the number of mobilized cell elements. In the peripheral blood we observed a decrease in the content of both MSC (by 53.6% from the control on day 3) and CFU-F (throughout the experiment). These changes (impaired release of mesenchymal precursors into circulation) can led to accumulation of CFU-F in the hemopoietic tissue at the initial terms of the experiment in animals receiving G-CSF and DMSO (Fig. 2).

Thus, DMSO administered *in vivo* considerably affected the state of the bone marrow pool of stromal precursors, which was probably related to the effect of this substance on proliferation and differentiation status of these cells [7,9,12,15] and was associated with disturbances in G-CSF-induced mobilization of mesenchymal precursor cells of different maturity.

At the next stage of the experiment, we evaluated direct *in vitro* effects of DMSO on clonogenic cell elements (mesenchymal and hemopoietic precursors). DMSO added to complete nutrient medium in a concentration of 0.2% considerably decreased the number of fibroblast, erythroid, and granulomonocyte colonies (by 91.1, 50.0, and 70.7%, respectively) compared to the corresponding values in the bone marrow cultures without DMSO (Fig. 3). Proliferative activity of precursor cells decreased in all cases. The number of S-phase CFU-F, erythroid and granulomonocyte CFU decreased by 60.2, 55.0 and 43.0% from the control, respectively. Moreover, DMSO considerably accelerated maturation of bone marrow erythroid and granulomonocyte precursor cells (to 393.4 and 424.2% from the control, respectively, Fig. 3).

Thus, our findings and published reports [7,9, 11,15] on the modulating effect of DMSO even in low doses (0.02-0.25%) on proliferation and differentiation status of precursor cells *in vitro* and reports on the development of complications after transplantation of DMSO-treated materials [6,8] suggest that new methods of cryopreservation without DMSO should be developed. The use of DMSO considerably modulating the epigenetic profile of cells [11] undoubtedly changes biological proper-

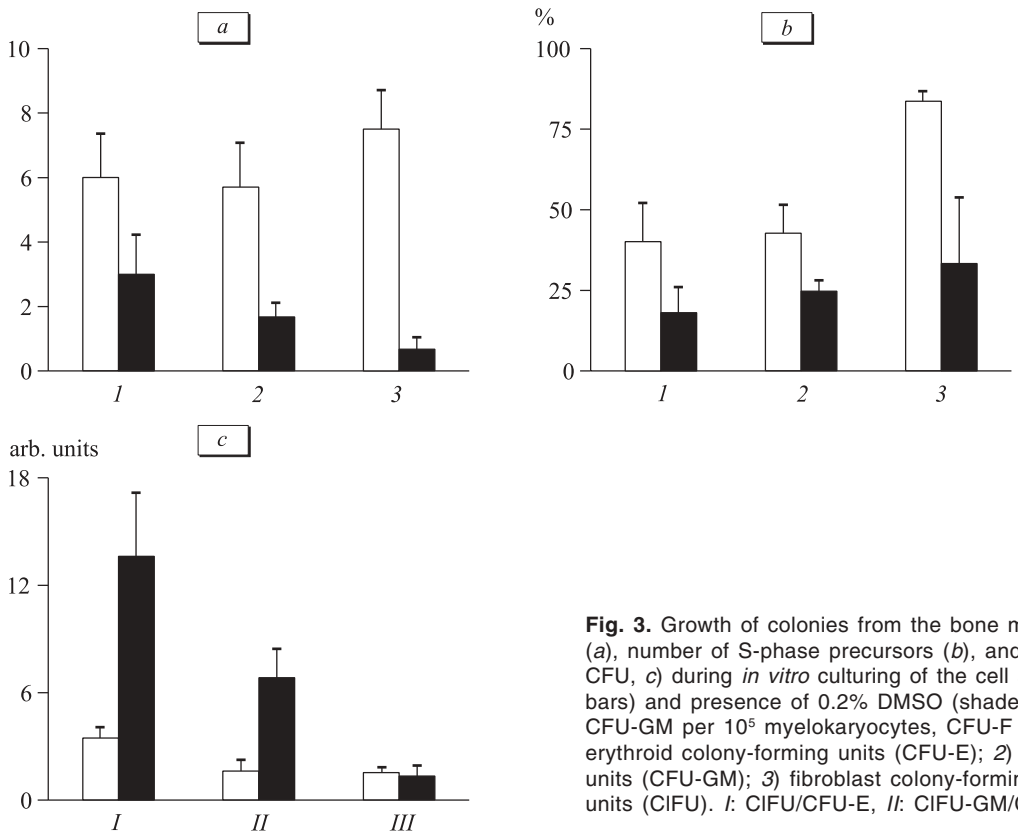

Fig. 3. Growth of colonies from the bone marrow of intact CBA/CaLac mice (*a*), number of S-phase precursors (*b*), and index of their maturation (CIFU/CFU, *c*) during *in vitro* culturing of the cell suspension in the absence (open bars) and presence of 0.2% DMSO (shaded bars). Ordinate: *a*) CFU-E and CFU-GM per 10^5 myelokaryocytes, CFU-F per 2.5×10^5 myelokaryocytes; *1*) erythroid colony-forming units (CFU-E); *2*) granulonomocyte colony-forming units (CFU-GM); *3*) fibroblast colony-forming units (CFU-F); cluster-forming units (CIFU). *I*: CIFU/CFU-E, *II*: CIFU-GM/CFU-GM, CIFU-F/CFU-F.

ties of the transplanted materials, first of all materials containing stem cells.

At the same time, DMSO modulating the functions of stem cells (*e.g.* inducing differentiation of early stem cells to various directions [7,9,12,15]) can be used at final stages of cell therapy. In this case, stimulation of maturation of exogenous stem cells populating the targeted niches or mobilized endogenous stem cells (in the direction determined by tissue-specific environment) can accelerate clinical effects of treatment and/or reduce the risk of complications associated with tumor transformation of the transplanted material [12,13].

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