Effect of Imbalance in Plasma Oxidant Homeostasis on the Choice of Stromal Differentiation of Polypotent Bone Marrow Stem Cells in Mice Receiving Estrone Injection

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The choice of stromal differentiation by polypotent stem cells was studied by morphological and chemiluminescent methods. The differentiation pathway is selected on day 7 after heterotopic bone marrow transplantation and injection of estrone. The imbalance in plasma oxidant homeostasis in mice on day 7 after estrone injection is associated with drastic intensification of free-radical oxidation and blockade of antioxidant activity.

Key Words: *imbalance of oxidant homeostasis; plasma; polypotent bone marrow stem cells; differentiation; stroma; estrone; mice*

Regulation of differentiation pathways of bone marrow polypotent stem cells (PSC) remains an unsolved problem of hematology for many decades. The possibility of choosing differentiation pathways by these stem cells was demonstrated by French scientists in 1998, when they modified the technology of *in vitro* culturing of porcine embryonal stem cells [10].

Until recently the choice of differentiation pathway of bone marrow PSC (transplantanble stem elements forming a heterotopic hemopoietic focus — HHF) after heterotopic bone marrow transplantation (HBMT) was evaluated morphologically by their final phenotype in regenerating bone marrow [3,8]. HBMT followed by injection of curantyl (dipyridamole) and estrone led to the formation of HHF with predominance of hemopoietic or stromal component, respectively [3], from the same substrate, while in control mice both the hemopoietic and stromal components were present. However, morphological methods did not help to answer the question why this or that differentiation pathway was selected.

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Free oxygen radicals are involved in the basic biochemical processes [1,9]. Hyperproduction of free radicals or weakening of the antioxidant defense systems creates conditions for cell damage [1,9,11]. Free oxygen radicals are highly active and attack lipids, nucleic acids, and proteins in cells of various tissues of the organism. An intricate multichannel system of antioxidant defense, whose main component is total antioxidant activity (TAA), protects from destructive effects of free-radical oxidation (FRO) [1,9]. TAA regulates the intensity of prooxidant processes in the organism under physiological conditions and maintains the oxidant homeostasis in the organism. The imbalance in oxidant homeostasis caused by changes in the pro- and antioxidant processes, serves as one of the main mechanisms of cell and tissue damage in various diseases [6,11].

We hypothesized that the choice of the differentiation pathway by transplantable stem elements of regenerating bone marrow is determined by the imbalance of oxidant homeostasis, caused by impaired function of the antioxidant system and dysregulation of FRO intensity in biological fluids [5].

This concept was confirmed experimentally with the choice of hemopoietic differentiation of bone marrow PSC during long-term platelet disaggregation with curantyl on a model of HBMT [5].

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The cause of choice of stromal differentiation of bone marrow PSC after HBMT and injection of estrone was unclear up to the recent time. As active antioxidants, estrogens are involved in the maintenance of antioxidant homeostasis of the organism [6,7].

The aim of our experiment was to evaluate the effect of plasma oxidant imbalance on the choice of stromal differentiation pathway by bone marrow PSC after HBMT in mice receiving estrone.

MATERIALS AND METHODS

The comparison of our present results with the findings of previous studies with curantyl was correct, because we used the same protocol as in experiments with curantyl [5].

Experiments were carried out on 120 male $(C57Bl\times CBA)F_1$ mice weighing 18-20 g. Bone marrow from the femur was transplanted (under hexenal narcosis) under the renal capsule to 60 syngeneic recipient mice [8]. Recipient mice of experimental group (n=30) were subcutaneously injected with estrone (Sigma) in 0.2 ml olive oil (0.5 mg/kg, once a week for 30 days).

Control mice (n=30) received olive oil according to the same protocol [3].

Mice of both groups were decapitated on days 2, 7, and 30 of estrone treatment, and HHF, blood, and plasma were examined.

Morphological picture of the bone marrow in HHF was examined on deparaffinized sections routinely stained with hematoxylin and eosin.

Blood smears were fixed, stained by the method of Romanowskii, and platelets were counted routinely. Plasma was obtained after 15-min centrifugation at 3000 rpm with 4% sodium citrate [1].

The intensity of plasma FRO was evaluated by luminol-dependent chemiluminescence. TAA was evaluated on days 2, 7, and 30 of the experiment (10 animals per point).

Chemiluminescence in the reaction mixture containing 700 µl phosphate buffer (pH 7.4), 50 µl 0.1 mM luminol, and 50 µl plasma was recorded on an Emilite-1105 luminometer. Chemiluminescence was initiated by adding 200 µl 20 mM H_2O_2. Photosum was measured at 37°C during 2 min, the maximum flash was expressed in mV/sec [1]. The intensity of FRO chemiluminescence was expressed in arbitrary units/mg protein measured after Lowry [12].

TAA was evaluated by the reaction of riboflavin with H_2O_2 in the presence of Fe^{2+} and expressed in arbitrary units/mg protein [1,13] and then converted into platelets/liter and expressed in arbitrary units [6,13].

The correlation between the intensity FRO and TAA and between platelet count and TAA in the experimental and control groups was analyzed using Statistica 5.0 software.

RESULTS

Morphological analysis of the bone marrow in HHF after estrone treatment confirmed the choice of stromal differentiation of bone marrow PSC on day 30 of the experiment [3].

On day 2 after HBMT and estrone injection HHF in the experimental group were characterized by intensive vascularization compared to the control. On day 7 of the experiment the transplant morphologically did not differ from the bone marrow in HHF of control mice and was presented by poorly differentiated bone marrow cells. On day 30, bone marrow in HHF of experimental mice was presented mainly by the stromal component. The bone marrow contained numerous megakaryocytes without apparent morphological disorders. Similar intensification of megakaryocytopoiesis in HHF was observed in previous experiments with curantyl. However curantyl treatment induced pathological changes in megakaryocytes and their destruction because of accumulation of giant lipid drops [3]. Estrone did not cause cell destruction.

At the early stages of estrone treatment the morphological picture of the bone marrow in HHF virtually did not differ from that during curantyl treatment. However, after 30 days of estrone treatment bone marrow PSC formed HHF consisting primarily of stromal component, while after curantyl treatment they formed HHF consisting of hemopoietic cells. On day 2 of estrone treatment the intensity of FRO in the plasma was virtually the same as in the control. Together with the results observed by this term in the curantyl group (Fig. 1, *a*), these data confirm the capacity of the antioxidant system to maintain oxidant homeostasis in the plasma at early stages irrespective of the treatment.

Starting from day 7 of the experiment, pro- and antioxidant processes were different by the absolute values and were oppositely directed (Fig. 1, *a*, *b*). The intensity of plasma FRO in mice receiving estrone 10-fold surpassed the control (Fig. 1, *a*). By contrast, in mice receiving curantyl (hemopoietic differentiation of PSC) the intensity of FRO processes considerably decreased at this term (Fig. 1, *a*). Hence, day 7 as can be considered as the term when stromal or hemopoietic differentiation is chosen.

Opposite direction of FRO processes during the choice of differentiation pathway by transplantable elements after HBMT confirms different mechanisms of action of curantyl and estrone on cells: mediated effect of curantyl and direct effect of estrone [3].

Sharp stimulation of FRO in mouse plasma on day 7 of estrone treatment does not allow us to refer

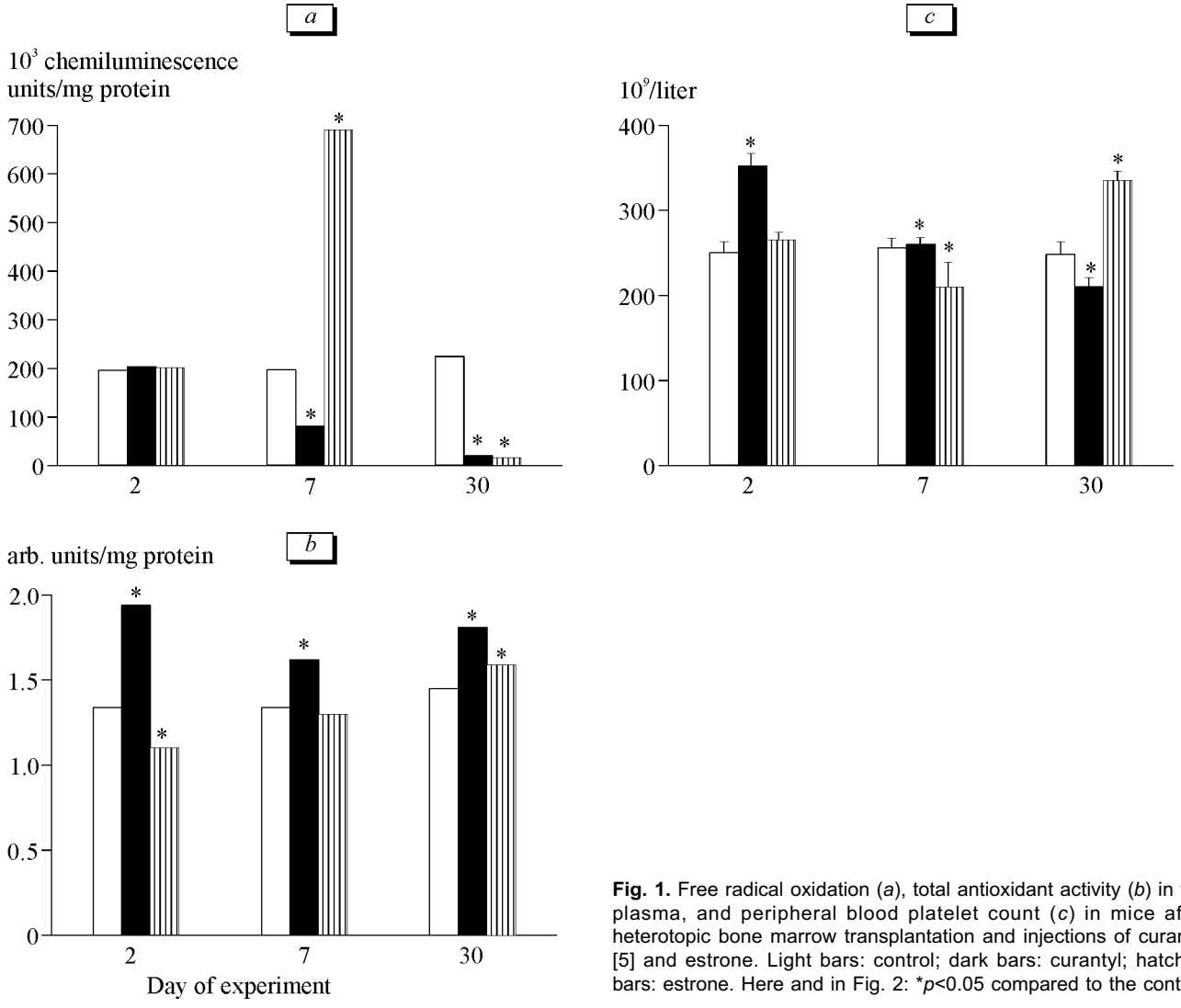

Fig. 1. Free radical oxidation (*a*), total antioxidant activity (*b*) in the plasma, and peripheral blood platelet count (*c*) in mice after heterotopic bone marrow transplantation and injections of curantyl [5] and estrone. Light bars: control; dark bars: curantyl; hatched bars: estrone. Here and in Fig. 2: *$p<0.05$ compared to the control.

this estrogen hormone to antioxidants, as was previously done [6,7]. On day 30 of estrone treatment the intensity of FRO processes was blocked and dropped below the control level (Fig. 1, *a*). Hence, the results indicate that stimulation or blocksde of FRO processes in mouse plasma depend on the hormone dose. Similar data on the dose-dependent effects of hydrocortisone and thyroxine on the intensity of pro- and antioxidant processes were obtained in study of the role of these hormones in LPO regulation in rat brain [2].

The pattern of changes in the total antioxidant activity of the plasma of experimental mice differed from that of FRO processes (Fig. 1, *b*). Prooxidant processes in mouse plasma were maintained at the control level on day 2 of estrone therapy due to more pronounced blockade of total antioxidant activity and its maintenance at a level significantly below the control. When bone marrow PSC selected (under the effect of curantyl) the hemopoietic differentiation, the maintenance of FRO at the control level was, by contrast, paralleled by intensification of total antioxidant activity, the count of disaggregated platelets being increased (Fig. 1, *b*).

The maintenance of oxidant homeostasis in mouse plasma on day 7 after estrogen stimulation was also due to blockade of total antioxidant activity in the plasma, as a result of which its values did not surpass the control level. Total antioxidant activity slightly increased compared to the control only by the end of the experiment (Fig. 1, *b*). Drastic dose-dependent activation of FRO on day 7 of estrone therapy was paralleled by a dose-dependent blockade of plasma antioxidant defense system.

It is known that curantyl-disaggregated platelets increase plasma TAA [4]. Moreover, estrone treatment stimulates thrombocytopoiesis in the blood (Fig. 1, *c*). However, the maximum platelet count during estrone treatment was observed only by the end of the experiment, while in mice receiving curantyl the same increase in platelet count was observed on day 2 (Fig.

1, *c*). A drastic increase in platelet count after 4 injections of estrone was paralleled by a slight increase in plasma TAA. On day 30 of treatment the ratio of plasma TAA to platelet count decreased compared to the corresponding parameter in control mice (Fig. 2). Hence, estrogen stimulation, in contrast to curantyl disaggregation, blocked the capacity of platelets to increase plasma TAA.

Therefore, judging from opposite changes in FRO and various pathways of oxidant homeostasis maintenance, day 7 is the most likely term when stromal or hemopoietic differentiation of bone marrow PSC is selected. Morphological study detects this choice much later, only on day 30 of the experiment. The choice of stromal differentiation of regenerating bone marrow PSC after estrone treatment is paralleled by a sharp intensification of FRO in the plasma and inhibition of plasma TAA. Presumably, different changes in FRO and pathways maintaining oxidant homeostasis in the plasma of mice injected with estrone and curantyl are explained by different mechanisms of their action on cells [3].

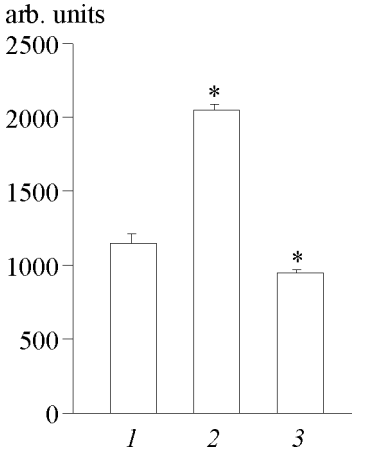

Fig. 2. The ratio of total antioxidant activity to platelet count (per liter of blood) in the plasma of mice on day 30 after heterotopic bone marrow transplantation and treatment with curantyl [5] and estrone. *1*) control; *2*) curantyl; *3*) estrone.

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