Effect of Disturbed Plasma Oxidant Homeostasis on Hemopoietic Differentiation of Polypotent Bone Marrow Stem Cells in Mice Treated with Dipyridamole

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The choice of hemopoietic differentiation by polypotent bone marrow stem cells was studied by morphological and chemiluminescent methods. This choice occurred on day 7 after heterotopic bone marrow transplantation and dipyridamole administration, under the effect of oxidative homeostasis imbalance in the plasma. Dipyridamole increased total antioxidant activity and 10-fold reduced the intensity of free radical oxidation. Disaggregation therapy with dipyridamole reduced platelet count in the peripheral blood and decreased total plasma antioxidant activity.

Key Words: *oxidative homeostasis; dipyridamole; polypotent stem cells; heterotopic bone marrow transplantation; hemopoietic differentiation*

Regulation of differentiation of polypotent stem cells (PSC) in the bone marrow remains an actual problem of hematology for many decades.

Recently, the possibility of changing PSC differentiation by modulating the conditions of cell isolation and culturing was demonstrated in experiments aimed at elaboration of *in vitro* technology of obtaining embryonic porcine PSC with preserved polypotent properties [6]. These changes are possible only if PSC are sensitive to the given influence, which is characteristic of PSC ready to exit from the stem cell compartment. These stem cells are used for heterotopic transplantation of the bone marrow (HTBM). The cells called polypotent transplanted elements participate in the formation of the heterotopic hemopoiesis focus [2,5].

The possibility of changing PSC differentiation towards the formation of heterotopic hemopoietic focus with prevalence of bone marrow hemopoiesis was shown on the model of HTBM combined with long-term dipyridamole-induced platelet disaggregation [3]. Dipyridamole-induced reduction of the osteogenic component of the bone marrow was associated with massive degeneration of pathologically changed megakaryocytes. However, changes in thrombocytopoiesis under these conditions were not considered. Further studies showed that megakaryocyte degeneration during long-term dipyridamole treatment was associated with enhanced synthesis of long-chain fatty acids and accumulation of giant lipid drops in the cytoplasm of these bone marrow cells. The growth of these lipid granules caused membrane rupture and release of cytoplasm content from damaged megakaryocytes to the intercellular space [3].

Enhanced production of free oxygen radicals is the main mechanism of cell and tissue damage during various pathological conditions [1,7]. Free radical oxidation (FRO) impairs the integrity and permeability of the plasma membrane, induced structural changes in DNA molecules, modifies mitotic cycle, and, finally, leads to cell death [8,9].

Destructive changes in cells and tissues result from imbalance of the oxidant homeostasis, which is determined by the ratio between FRO and total antioxidant activity (TAA) in the body.

Despite the importance of modulation of PSC differentiation, this fundamental problem was not studied

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from the viewpoint of the regulatory role of the FRO-antioxidant defense imbalance in the body.

The purpose of the present study was to examine the effect of impaired oxidative homeostasis in the plasma after HTBM and long-term platelet deaggregation treatment with dipyridamole on the choice of differentiation pathway in mouse bone marrow PSC.

MATERIALS AND METHODS

The study was carried out on 120 (60 donors and 60 recipients) male (C57Bl×CBA)F_1 mice weighing 18-20 g. HTBM was performed under hexenal narcosis [5]. After HTBM experimental mice (n=30) received intraperitoneal injections of dipyridamole (30 mg/kg in 0.2 ml isotonic NaCl, daily, for 30 days), while control mice (n=30) received an equivalent volume of physiological saline according to the same scheme [3].

The animals were decapitated on days 2, 7, and 30 after HTBM (10 mice per point from each group). Morphological changes in the bone marrow in the thorax and femur were evaluated using histological methods [3]. Morphological changes in the thoracic bone marrow were compared to changes in FRO and TAA in the plasma. Platelets count was evaluated routinely in blood smears obtained during decapitation. The plasma was isolated by centrifugation at 1500 rpm for 15 min. FRO intensity and TAA level were evaluated in the plasma by the method of H_2O_2-induced chemiluminescence (CL). Luminescence was measured on a Emilite-1105 luminometer.

CL was recorded in a reaction mixture containing 700 μl phosphate buffer (pH 7.4), 50 μl 0.1 mM luminol, and 50 μl plasma.

CL was initiated by 200 μl 2% H_2O_2 and measured at 37°C for 2 min. The maximum flash of CL was expressed in mV/sec. CL intensity was expressed in arb. units/mg protein. Protein content was measured by the method of Lowry.

TAA was evaluated by chemiluminescent reaction between riboflavin and H_2O_2 in the presence of Fe^{2+} [1,10].

Correlation analysis between plasma FRO intensity and TAA level and statistical processing of the results were using Statistica 5.0 software.

RESULTS

As in our previous studies, the formation of ecotopic hemopoietic organ presented mainly by the hemopoietic bone marrow component was completed by day 30 of the experiment [3].

At this term, numerous pathologically changed or degenerating megakaryocytes were found in the red bone marrow. Destruction of these cells serving as a source of platelets was associated with significant changes in FRO and TAA activity in mouse plasma.

However, as in our previous experiments (by day 7 after HTBM), morphological characteristics of the thoracic bone marrow were similar in the control and experimental animals and the bone marrow was presented by low differentiated cells [2].

On day 2 of the experiment, FRO processes in the experimental group did not differ from those in the control group, while on day 7 after HTBM the intensity of free oxygen radical production in the experimental group decreased dramatically and attained a minimum by the end of the experiment (Fig. 1, *a*).

Oxidative homeostasis by day 2 of dipyridamole treatment was maintained at the control level due to increased plasma TAA (Fig. 1, *b*). This increase in TAA observed during subsequent dipyridamole injections sharply reduced FRO intensity in mouse plasma starting from day 7 of the experiment. In the experimental group, TAA surpassed the control level throughout the experiment (Fig. 1, *b*).

These findings suggest that day 7 corresponds to the choice of hemopoietic differentiation pathway by bone marrow PSC after HTBM. This also coincided with pronounced imbalance in plasma oxidative homeostasis.

This choice of bone marrow PSC differentiation was morphologically confirmed only on day 30 of the experiment.

Platelet count in the peripheral blood of experimental mice surpassed the control level throughout the entire observation period (Fig. 1, *c*) attaining a maximum on day 2 of the experiment.

Previous experiments with long-term platelet disaggregation treatment with dipyridamole showed that the increase in TAA on day 30 of treatment was due to dipyridamole-disaggregated cells. Thus, plasma TAA calculated per 10^9 platelets/liter comprised 1200 and 2300 arb. units in control and experimental groups, respectively [4].

Correlation analysis revealed neither direct nor inverse correlation between plasma FRO intensity and TAA level.

Thus, long-term platelet disaggregation treatment with dipyridamole ensures high antioxidant activity of plasma due to increased platelet count in the blood, which results in a dramatic decrease of FRO intensity. These extreme conditions promote hemopoietic differentiation of PSC in regenerating bone marrow after HTBM. The imbalance in oxidative homeostasis blocks stromal (osteogenic) pathway of differentiation of transplanted bone marrow stem elements. This is confirmed by the fact that in dipyridamole-treated mice the ectopic hemopoietic organ on day 30 after HTBM was presented by hemopoietic component without osteal capsule,

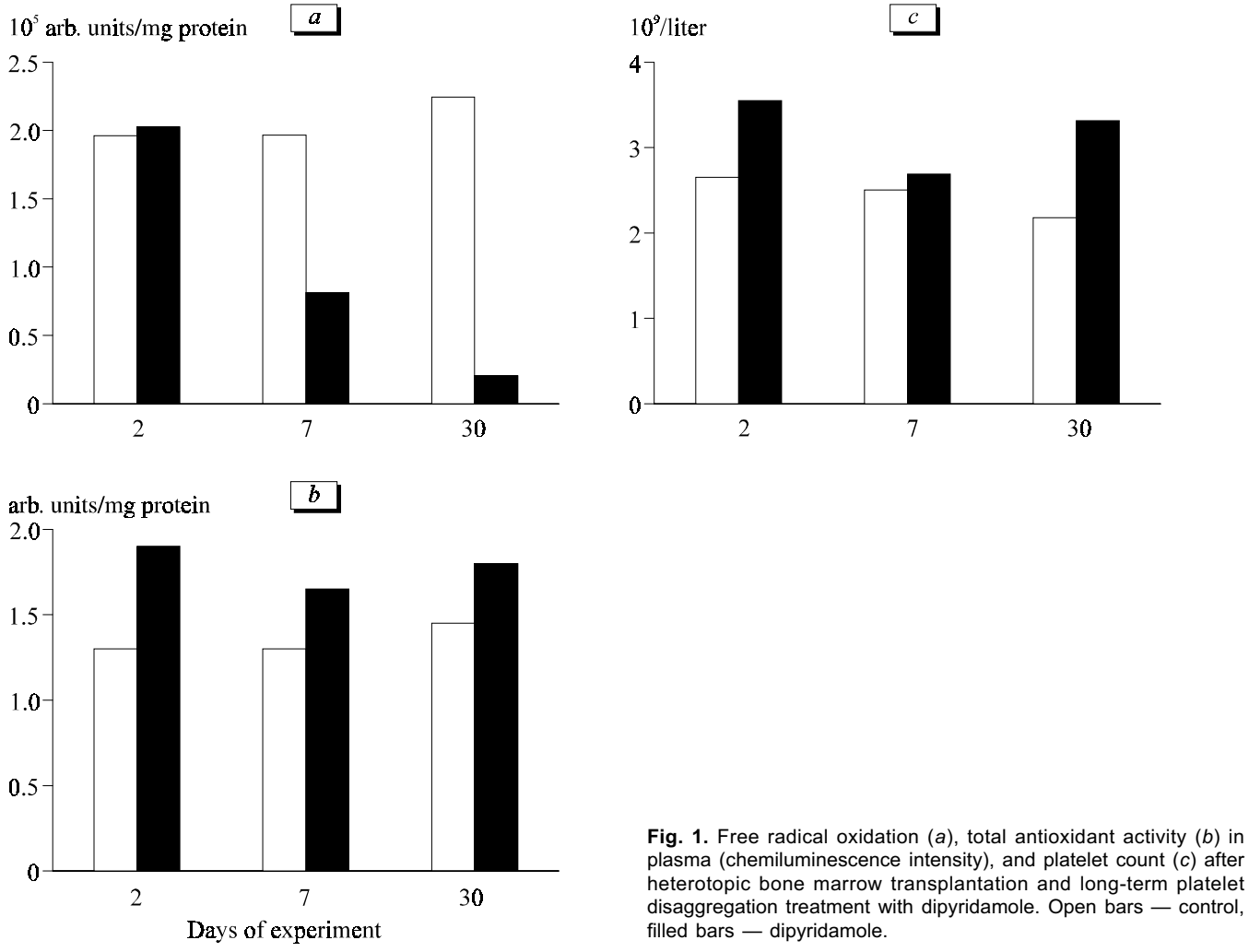

Fig. 1. Free radical oxidation (*a*), total antioxidant activity (*b*) in plasma (chemiluminescence intensity), and platelet count (*c*) after heterotopic bone marrow transplantation and long-term platelet disaggregation treatment with dipyridamole. Open bars — control, filled bars — dipyridamole.

whereas in the thoracic bone marrow of control mice both the osteal and hemopoietic components were present.

Thus, the imbalance of oxidative homeostasis in the plasma produces a regulatory effect on the choice of hemopoietic differentiation of transplanted bone marrow stem elements on day 7 after heterotopic transplantation and platelet disaggregation treatment with dipyridamole. However, additional data on the effect of estron on osteogenic differentiation of transplanted bone marrow stem elements [2] are required to confirm that day 7 of the experiment corresponds to the choice of hemopoietic differentiation of bone marrow PSC.

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