Role of PI3K, MAPK/ERK1/2, and p38 in Implementation of the Proliferative and Differentiation Potential of Erythroid Progenitors after Blood Loss


The involvement of PI3K, ERK and p38-dependent signaling system in the regulation of functional activity of erythroid precursors after blood loss (30% of circulating volume) was studied. We demonstrated the important role of PI3K and p38 in the suppression of differentiation of erythroid precursors the contribution of p38 to stimulation of mitotic activity of erythroid CFU, which maintains the growth potential of the precursors at the optimal physiological level. The classical MAPK/ERK-kinase pathway does not determine the proliferative and differentiation status of erythroid CFU.

Key Words: PI3K; MAPK/ERK1/2; p38, erythroid precursors; blood loss

According to previous results, stimulation of erythropoiesis during the posthemorrhagic period after loss of 30% circulating blood volume is determined by functional activation of erythroid precursors, in particular, acceleration of their division and maturation [1,9]. However, the molecular mechanisms increasing the proliferative and differentiation status of cells under these conditions have not yet been studied. PI3K is an essential regulatory protein that controls the key cell function (proliferation, differentiation, and apoptosis) and is involved in various signaling pathways. Moreover, its serine-threonine protein kinase activity plays an important role in the regulation of cellular MAP kinases (ERK, p38) that also control the growth potential of progenitor cells [3,5,8].

Here we studied the role of PI3K, ERK, and p38 in the regulation of functional activity of erythroid precursors after loss of 30% of circulating blood volume.

MATERIALS AND METHODS

The study was carried out on 2-month-old (n=32) male C57Bl/6J mice weighing 20-22 g. The animals were obtained from the Laboratory of Experimental Biological Models, E. D. Goldberg Research Institute of Pharmacology, Siberian Division of Russian Academy of Medical Science (certificate available).

The experimental group included mice with posthemorrhagic anemia modeled by retro-orbital sinus puncture followed by single-step 30% bloodletting through heparinized graduated capillary tube. The volume of sampled blood was calculated assuming that the amount of circulating blood in rodents is 1/13 of body weight [9]. Intact group (baseline) included 8 mice.

The number of erythrocytes, reticulocytes, hemoglobin content, and hematocrit were measured on day 1, 3, and 6 after blood loss using Abacus automatic hematolohy analyzer (Diatron) in the veterinary mode. Total myelokaryocyte count in the bone marrow and their qualitative composition were studied [2,9].
The direct effect of inhibitors of PI3K (LY294002), MAPK/ERK1/2 (PD98059), and p38 (SB203580) (all inhibitors from Calbiochem) on colony formation from bone marrow erythroid precursors (CFU- and cluster-forming units, CFU-E) of experimental animals was studied by cultural methods. The intensity of maturation of committed erythroid precursors was evaluated by index of maturation (ratio of clusters to colonies grown in the same well) [2,3,8]. Precursors were cultured under standard conditions (MethoCult M3334 methylcellulose medium with erythropoietin; STEMCELL Technologies) or under above conditions in the presence of inhibitors in working concentration 50, 100, and 10 μM, respectively (the most effective concentration determined in preliminary experiments). Proliferative activity of CFU-E was assessed by the hydroxyurea suicide technique (Sigma) after preincubation with inhibitors for 24 h [2,3,8].

The data were processed by methods of variation statistics using Student’s t test and nonparametric Mann–Whitney U test.

RESULTS

Blood loss resulted in anemia on experimental day 1. Reduced erythrocytes, hemoglobin, and hematocrit (by 1.2 times in comparison with intact animals) was found in parallel with the development of severe reticulocytosis on days 1-6 that attained 656.88% from baseline on experimental day 3 (Table 1). The increase in total bone marrow cellularity in mice with posthemorrhagic anemia observed at all stages of the experiment was due to an increase in erythrokaryocyte and monocyte contents that peaked on day 6: 268.46 and 236.84% from baseline, respectively (Table 2).

These changes in the erythron system after blood loss were linked with enhanced colony-forming capacity of the bone marrow on experimental days 1, 3, and 6 (by ~2 times) along with increased intensity of DNA synthesis processes in CFU-E in comparison with similar parameters in intact animals in the same period (Fig. 1, a, b). At the same time, significant inhibition of maturation of erythroid precursors (by 2.02 and 3.88 times, respectively) was observed on days 1 and 6 with an increase in this parameter by 73% from the baseline on day 3 (Fig. 1, c). Thus, accumulation of erythroid precursors in the bone marrow (underlying stimulation of erythropoiesis in the posthemorrhagic period) is associated with a significant increase in their proliferative activity and delayed differentiation.

Bearing in mind that kinases PI3K, ERK, and p38 are the major regulatory proteins that control the key functions of the cell (proliferation, differentiation, and apoptosis) [4-6], we studied the involvement of the relevant kinase-dependent pathways in the implementation of growth potential of erythroid precursors in the posthemorrhagic period.

Addition of all examined inhibitors of signaling molecules to the myelokaryocyte culture from intact animals enhanced colony-forming capacity of bone marrow cells. However, significant changes in CFU-E yield were found only during culturing with specific MAPK/ERK1/2 blocker (Fig. 1, a). In this case, the intensity of differentiation and the rate of erythroid precursor division were changed only after blockade of PI3K as follows: the number of S-phase erythroid precursors increased by 2.96 times and maturation index decreased to 2.79 vs. 6.06 in the absence of the inhibitor (Fig. 1, b, c). Thus, under conditions of balanced hemopoiesis, kinetic characteristics of hemopoietic progenitors are determined by signaling pathways regulated by PI3K, which is consistent with published reports [5].

The study of the role of these signaling cascades in the formation of proliferative and differentiation status of erythroid precursors revealed some regularities. Thus, blockade of PI3K in vitro stimulated maturation of erythroid precursors at all terms without changes in their proliferative activity in comparison with the group without inhibitor (Fig. 1, b, c). In this case, the observed significant decrease in colony formation on days 1 and 3 of the posthemorrhagic period (to 6.95

<table>
<thead>
<tr>
<th>Group</th>
<th>Erythrocytes</th>
<th>Reticulocytes</th>
<th>Hematocrit</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact mice (baseline)</td>
<td>10.84±0.14</td>
<td>22.50±1.55</td>
<td>34.60±0.48</td>
<td>16.25±0.22</td>
</tr>
<tr>
<td>Mice after blood loss</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>day 1</td>
<td>8.88±0.56*</td>
<td>55.14±2.90*</td>
<td>28.40±1.68*</td>
<td>13.08±0.80*</td>
</tr>
<tr>
<td>day 3</td>
<td>10.08±0.22</td>
<td>147.80±3.79*</td>
<td>33.60±1.17</td>
<td>15.14±0.36</td>
</tr>
<tr>
<td>day 6</td>
<td>10.82±0.17</td>
<td>119.75±5.89*</td>
<td>34.80±0.49</td>
<td>16.04±0.21</td>
</tr>
</tbody>
</table>

Note. Here and Table 2: *p<0.05 in comparison with baseline.