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Erythropoietin and platelet production

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Abstract

Background and Objective. **Erythropoietin (Epo) is the primary growth factor for the red cell lineage but treatment with recombinant human Epo (rHuEpo) has been shown to increase platelet counts. In several animal species treatment with rHuEpo stimulated platelet production, but platelet counts tended to normalize after 1-2 weeks and large, chronic doses even caused thrombocytopenia. This paper aims at reviewing the evidence about the effects of Epo on megakaryopoiesis.**

Information sources. **We examined the literature published in journals covered by Medline®, concerning the effect of Epo, hypoxia and iron deficiency on megakaryopoiesis and platelets. The reference list of each article was reviewed to try to identify further contributions.**

State of the Art. In vivo **data have shown that moderate Epo stimulation, i.e. that produced by standard doses of rHuEpo, short-term hypoxia or moderate iron deficiency, causes a moderate elevation of platelet counts, whereas intense Epo stimulation, as produced by high doses of rHuEpo, prolonged hypoxia or severe iron deficiency, causes some degree of thrombocytopenia. In the latter case, there appears to be a diphasic response to Epo, the initial positive response (a stimulation of platelet production) being followed by thrombocytopenia. Contrarily to the thrombocytopenia due to increased platelet destruction induced by other growth factors, Epo-induced thrombocytopenia is the result of an inhibition of platelet production.**

Conclusions and Perspectives. **Stem-cell competition between erythroid and platelet precursors appears to be the cause of these phenomena in situations of prolonged, intense stimulation by Epo. *In vitro* data support the existence of a common erythrocytic and megakaryocytic precursor. It remains to be determined whether these effects of rHuEpo are a result of the dose itself or of the magnitude of the erythropoietic effect of that dose. It is not known whether a lower dose given in a patient with decreased marrow function would bring about the same biological effects as those induced by high doses of rHuEpo in the presence of a normal marrow function. Caution should be exercised before using high doses of hematopoietic growth factors.**

Key words: Erythropoietin, hypoxia, iron deficiency, erythropoiesis, megakaryopoiesis, platelets

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Erythropoietin (Epo) is the primary growth factor for the red cell lineage, controlling the survival, proliferation and differentiation of erythroid precursors.[1] Inadequate erythropoietin production, as observed in patients with end-stage renal disease, results in an anemia that can be best treated with recombinant human erythropoietin (rHuEpo).[1,2] Treatment with rHuEpo has been shown to improve platelet function in hemodialysis patients, as assessed by platelet adhesion, platelet aggregation, and serum levels of various factors involved in platelet function.[3-7] However, large clinical trials have shown that rHuEpo therapy also resulted in increased platelet counts.[8,9] In several animal species treatment with rHuEpo has been shown to stimulate platelet production, but platelet counts tend to normalize after 7 to 15 days.[10-13] In fact, large, chronic doses of rHuEpo even caused thrombocytopenia in rats, and *stem-cell competition* between erythroid and platelet precursors has been suggested as the cause of this phenomenon.[14]

We review here the published evidence about the effects of erythropoietin on megakaryopoiesis. We first present data obtained during *in vivo* models of hypoxia, rHuEpo therapy as well as other experimental or clinical conditions. The second part of the article reviews the potential mechanisms involved in these phenomena, including changes in plasma volume or platelet survival, the response of megakaryocytes to erythropoietin, the global response of the bone marrow as an organ, and functional iron deficiency. We conclude that a large body of data supports the existence of a common erythrocytic and megakaryocytic precursor, as hypothesized earlier.[15]

In vivo observations of concomitant changes in erythropoiesis and megakaryopoiesis

The model of hypoxia

Experiments in guinea pigs[16] and rats[17] have shown that acute blood loss in normal animals is followed by increased platelet production and platelet counts. Short-term hypoxia in normal mice induced both erythrocytosis and thrombocythemia,[18-21] without an increase in ^{35}S incorporation into platelets, suggesting that platelet production may be increased without true stimulation of megakaryopoiesis.[20] However, chronic hypoxia decreased platelet production[18-25] and this was shown to result from decreased differentiation of

hematopoietic precursors into the megakaryocytic lineage.[19,25-27] Despite a small increase in megakaryocyte size,[28] total circulating platelet counts[20] as well as total megakaryocyte mass[28] were reduced. There was no change either in platelet survival[18] or in megakaryocyte DNA content,[19] activity of differentiated megakaryocytes[29] or TPO production.[29] Relative numbers of spleen megakaryocytes are reduced by hypoxia to a degree similar to that of marrow megakaryopoiesis.[30] However, cycling of splenic erythropoiesis may explain why absolute numbers of megakaryocytes are normal in the spleen.[25,30] Splenectomy causes a further degree of hypoxia-induced thrombocytopenia in hypertransfused mice.[23] On the other hand, marrow ablation causes the spleen to compensate by increasing platelet production.[31] However, this inverse relationship between erythropoiesis and megakaryopoiesis during hypoxia in mice also depends upon the strain studied. Responses in Balb/C mice are not so great as in C3H mice.[22,28,32] This is due to the former's relatively defective Epo response to hypoxia[33] linked to lower hemoglobin O_2 affinity.[32] Increased erythropoiesis, and not elevated RBC (as produced by transfusion), is required for the thrombocytopenia to occur.[21,34] Accordingly, polycythemia induced by transfusion or previous exposure to low barometric pressure is accompanied by increased platelet counts and ^{35}S incorporation into platelets.[24] On the other hand, the post-hypoxic recovery phase is not associated with the expected rebound thrombocytosis.[35] These data indicate that short-term hypoxia is associated with thrombocytosis but more prolonged exposure results in thrombocytopenia.

Treatment with rHuEpo

In vitro experiments have demonstrated that hypoxia also exerts direct effects, which are independent of Epo, on erythroid progenitors.[36,37] For instance, although the oxygen tension had no effect on BFU-E formation when Epo was present, BFU-E production under hypoxia without Epo was equivalent to that under normoxia in the presence of Epo.[37] Very severe hypoxia was associated with enhanced formation and maintenance of BFU-E, but also with inhibition of their terminal expansion and maturation.[36] Furthermore, hypoxia reduced megakaryocyte number and size while increasing the number of CFU-Mk.[37] Therefore, the model of hypoxia may not be ideal for studying the effect of Epo on hematopoiesis and direct application of recombinant human erythropoietin (rHuEpo) may be more appropriate.

In large clinical trials of rHuEpo treatment in renal failure patients, platelet counts increased significantly, then gradually returned to pretreatment levels after months of maintenance therapy.[8] In another study, platelet increments over baseline occurred in parallel with expansion of erythropoietic activity.[9] In chronic liver disease, rHuEpo therapy improved platelet counts.[38] Mean platelet volumes dropped but platelet counts remained unchanged in patients receiving rHuEpo and oral iron before scheduled surgery.[39] In adult, but not in infant, monkeys, rHuEpo therapy resulted in elevated platelet counts throughout the 6-week treatment followed by rapid normalization thereafter.[40] Short-term treatment of normal dogs, mice and rats with high doses of rHuEpo stimulated platelet production, as evaluated by platelet counts, platelet sizes, proportion of reticulated platelets, seleno-methionine incorporation into platelets and thymidine incorporation into megakaryocytes, but the effect on megakaryocyte and CFU-Meg numbers has been less reproducible.[10-13,41] The effect on platelet counts may be partially masked by splenic pooling and the effect on megakaryocyte and CFU-Meg numbers may be limited to the spleen in intact mice but become significant in the bone marrow in splenectomized animals.[12,42,43] The initial elevation of platelet counts was, however, followed by a return to control levels after 7 or 15 days.[11,12] In fact, large, chronic doses of rHuEpo caused thrombocytopenia, decreased seleno-methionine incorporation into platelets, and reduced number of megakaryocytes in normal rats.[14] Transgenic mice expressing the human Epo gene develop both polycythemia and a moderate degree of thrombocytopenia.[44] Together, these findings demonstrate that conventional doses of rHuEpo produce some elevation of platelet counts, whereas higher doses cause thrombocytopenia.

Other experimental or clinical conditions

Similarly to hypoxia or rHuEpo, thyroxine[45] and actinomycin D[17] were both shown to induce reciprocal changes in erythropoiesis and megakaryopoiesis. In contrast, testosterone stimulated platelet production in mice, perhaps acting on a more primitive bipotential precursor.[46] Conversely, acute thrombocytopenia caused decreased erythropoiesis in some[14] but not other[47] studies. Short-term thrombopoietin (TPO) treatment in normal mice[48] as well as chronic exposure to TPO after gene transfer[49] induced erythroid hypoplasia in the bone marrow with declining peripheral red cell numbers and hemoglobin. However, this was at least partially explained by the development of myelofibrosis. Moreover, in myelosuppressed animals[50] or after stem cell transplantation,[51] TPO generally also accelerated erythroid recovery, with prominent erythropoietic stimulation even to the point of inducing functional iron deficiency.[50] Although experimental conditions are often of larger magnitude than physiologic conditions and of more acute onset (thus not allowing for progressive adaptation), there are also a few clinical conditions in which this reciprocal evolution was observed. Thrombocytopenia was frequently observed,[52] and tended to increase with phlebotomy, in cyanotic congenital heart disease.[53] Persistent thrombocytosis after splenectomy was associated with continuing anemia.[54] Therefore conditions other than hypoxia or rHuEpo therapy point to an inverse relationship between erythropoiesis and megakaryopoiesis.

Mechanisms accounting for these observations

Several mechanisms could account for the platelet response to erythropoietin, i.e. changes of plasma volume, alterations of platelet survival, a direct effect on megakaryocytes, a global response of bone marrow as an organ, and functional iron deficiency. Reduction of plasma volume occurs only after prolonged treatment with rHuEpo has produced changes in the red cell mass[55] and thus cannot explain the early increase in platelet counts. Furthermore, the effect should not be limited to platelets alone but also cause a similar early elevation of hematocrit and white blood cells, which was not, however, observed.[9] Although rHuEpo therapy may produce hyperreactive new platelets[41] and correct part of the hemostatic defect of uremia,[3-7] it has never been proved that rHuEpo has an effect on platelet survival[18] and there is no reason why this would be limited to patients in whom the red cell production is adequately stimulated.[9]

Megakaryocyte response to erythropoietin

Rat and mouse megakaryocytes have been shown to express high-affinity binding sites for erythropoietin,[56] resulting in enhanced growth in the presence of Epo.[57] Low-affinity binding sites for Epo have also been demonstrated on undifferentiated cells from a mouse megakaryoblastic cell line, and Epo-induced differentiation was associated with the additional expression of high-affinity binding sites.[58,59] Introduction of Epo receptor DNA into pluripotent progenitors induced these cells to proliferate in response to Epo without preferential erythroid differentiation.[60] Mice infected with a retrovirus expressing an oncogenic Epo receptor showed stimulation of both erythropoiesis and megakaryopoiesis, with a substantial early increase of platelet counts.[61] *In vitro* studies in man as well as in mice have demonstrated that Epo promoted megakaryocytic colony formation and increased the size, ploidy and number of megakaryocytes, as well as their cytoplasmic process formation, even if the presence of serum or cell conditioned-medium was often required.[57,62-69] In serum-free conditions, only those megakaryocyte progenitors previously stimulated by SCF and IL-3 responded to Epo.[70] Stimulated platelet production was not reported in these experiments but Epo stimulated DNA and protein synthesis in megakaryocytes.[71] It is therefore possible that rHuEpo exerts a direct positive effect on megakaryopoiesis.

Global marrow response to erythropoietin

Treatment of patients with the anemia of end-stage renal failure with rHuEpo produced, after 2 weeks, a 4-fold increase of erythroid progenitors, as well as a 2-fold increase of CFU-Meg and CFU-GM, suggesting that in the short-term human marrow responded to rHuEpo as an organ.[72] This hypothesis was also supported by the fact that platelet increments over baseline correlated with the degree of expansion of erythropoietic activity as measured by soluble transferrin receptor (sTfR) and hematocrit changes. It was shown that patients with functional iron deficiency did not increase sTfR levels in response to rHuEpo.[73] Also, platelet counts did not change in non-responders until the Epo dose was increased and erythropoiesis began to expand.[9] In chronic liver disease, changes in platelet counts with rHuEpo therapy occurred only in patients who also showed a response of the erythroid lineage.[38] In mice, elevated platelet counts correlated with increased Hct after 5 days of rHuEpo therapy.[12] All these observations appear to support the concept that rHuEpo exerts a positive effect on platelet production, which is proportional to its effect on the red cell lineage.

Iron deficiency and platelets

Iron deficiency was shown to be associated with reactive thrombocytosis.[74-78] However, when iron deficiency became very severe, platelet counts tended to normalize,[79-81] megakaryocyte numbers decreased[79] and even thrombocytopenia occurred,[82-84] possibly as a result of altered activity in iron-dependent enzymes. This could, however, also be consistent with the previously described diphasic pattern of increased stimulation by endogenous Epo. Iron supplementation was rapidly followed by a return of platelet counts to normal levels in rats with moderate iron deficiency anemia, whereas it had little effect in rats with severe iron deficiency anemia and normal platelet counts.[79,80] Iron-deficient infants treated with oral iron developed decreased platelet counts but this was followed by reactive thrombocytosis when the reticulocyte peak receded.[84] Surprisingly, parenteral iron produced thrombocytosis without the preceding decrease in platelet counts.[84] In patients with very severe iron deficiency, iron therapy may even be associated with thrombocytopenia.[85] In iron-deficiency anemia, erythroblast and CFU-E – but not BFU-E or CFU-GM – numbers were elevated and iron therapy further increased erythroblast numbers while decreasing CFU-E frequency in a manner reciprocal to changes in hematocrit.[86] Therefore iron therapy rapidly enhanced erythropoiesis and caused a drop in Epo levels even before any change in hematocrit was observed.[87] This normalization of endogenous Epo levels with intense stimulation of erythropoiesis may explain why thrombocytopenia can occur during treatment of severe iron deficiency anemia. All these data confirm that the effects of iron status and iron therapy on platelet production depend on the severity of iron deficiency.

Increased erythropoiesis and functional iron deficiency

The observed alterations of platelet counts in iron deficiency anemia could however represent in part an effect of increased endogenous Epo stimulation in response to the anemia rather than an effect of iron deficiency *per se*. Moderate iron deficiency would cause a moderate elevation of serum Epo with subsequent

increase in platelet production, whereas more severe anemia would induce a major Epo response with ensuing thrombocytopenia. However, data from models characterized by intense erythropoietic activity and no functional iron deficiency or iron deficiency without elevated Epo levels are lacking. Transfusion of red cells into iron deficient rats decreased platelet production, whereas transfusion into normal animals had no effect.[80] This is consistent with the modifications of Epo levels induced by these manipulations. The absence of an elevation of platelet counts after acute hemolysis could be explained by splenic pooling in an enlarged spleen.[12,80] Platelet counts are usually normal in patients with pure red cell aplasia, but absolute changes in platelet counts in response to increased Epo stimulation are too small to be detected in these patients unless platelet counts before the onset of red cell aplasia are available for comparison.

However, enhanced erythropoiesis is also associated with functional iron deficiency, i.e. an imbalance between iron needs in the bone marrow and iron supply from stores, which may develop even in the presence of adequate storage iron when these stores cannot be mobilized rapidly enough.[88] The distinction of functional iron deficiency from expanded erythropoietic activity is thus very difficult in patients treated with rHuEpo. In patients with renal failure receiving rHuEpo, relative platelet increments over baseline correlated inversely with relative changes of serum iron or transferrin saturation (an indication of erythroid marrow activity) rather than with absolute serum iron and transferrin saturation values (an indication of functional iron deficiency) or with ferritin levels (an indication of iron stores), emphasizing the role of marrow response to rHuEpo rather than that of iron deficiency alone.[9] However, in a recent study it was shown that whereas iron overloaded rats developed the classical diphasic platelet response to rHuEpo, normal animals appeared to be protected from secondary thrombocytopenia by the development of (functional) iron deficiency.[89] Therefore, the effect of rHuEpo on platelet counts appears to be strongly modulated by the iron status.

A common erythrocytic and megakaryocytic progenitor

Thus a large body of data support the concept that megakaryocytic and erythrocytic cell lineages share a common progenitor.[15] Megakaryocytes have been shown to express erythroid-specific transcription factors, such as GATA factors,[90] a specific DNA-binding protein[91] or a nuclear factor involved in the regulation of globin transcription.[92] On the other hand, cord blood $CD34^+$ cells induced into the megakaryocytic lineage by TPO also had erythroid potential,[93] although the erythroid-enhancing effect of TPO was mainly directed toward pure erythroid rather than bipotent progenitors.[94] An erythroleukemic cell line has been shown to express numerous megakaryocyte markers.[95] Multipotential[59] or bipotential[96-98] cell lines derived from patients with megakaryoblastic leukemia have been obtained, in which erythroid differentiation can be induced through the action of Epo[59,96] and megakaryocytic differentiation by TPO.[96,98] These cells were shown to express Epo receptors.[59,97] A population of probable erythrocytic and megakaryocytic cell lineage precursors co-expressed glycophorin A and glycoprotein IIIa.[99] Such a bipotent erythromegakaryocytic progenitor has been characterized in human bone marrow.[100] It was found mostly in the $CD34^+/CD38^{low}$ cell fraction and required the combination of stem cell factor (SCF), interleukin (IL)-3 and Epo for its growth in serum-free conditions. The biological and clinical importance of this common erythrocytic and megakaryocytic progenitor remains to be clarified.

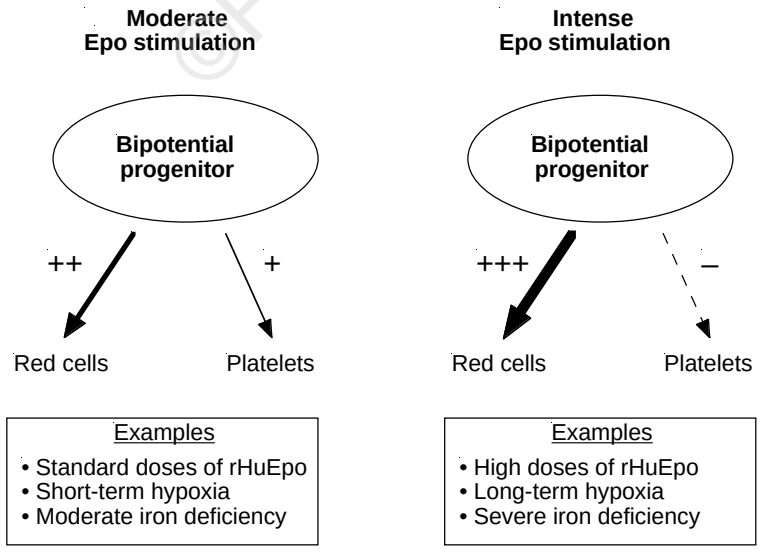

Figure 1. A model for the effect of Epo on platelet production. Moderate Epo stimulation, i.e. that obtained by standard doses of rHuEpo, short-term hypoxia or moderate iron deficiency, causes a moderate elevation of platelet counts. Intense Epo stimulation, as produced by high doses of rHuEpo, prolonged hypoxia or severe iron deficiency, causes some degree of thrombocytopenia.

Conclusions

This review has indicated that Epo could induce both thrombocytopenia and thrombocytosis. We show that these apparently contradictory data are in fact the result of a diphasic response to erythropoietin. Both in models of hypoxia and of rHuEpo therapy the initial positive response (a stimulation of platelet production) was followed by a negative impact on megakaryopoiesis (an inhibition of platelet production). Figure 1 provides a model for the effect of Epo on platelet production. Moderate Epo stimulation, i.e. that obtained by standard doses of rHuEpo, short-term hypoxia or moderate iron deficiency, causes a moderate elevation of platelet counts. Intense Epo stimulation, as produced by high doses of rHuEpo, prolonged hypoxia or severe iron deficiency, causes some degree of thrombocytopenia. Conversely intense stimulation of thrombopoiesis could induce anemia. *Stem-cell competition* between erythroid and platelet precursors has been suggested as the cause of these phenomena in these situations of prolonged, intense stimulation.[14] However, this concept of *stem-cell competition* is still speculative because it has not been proved in appropriate *in vitro* experiments[101] and its physiologic basis, for instance modulation of TPO receptors, has not been established.

A question remaining to be examined is whether the differing effects of rHuEpo on platelet production are a result of the dose itself or of the magnitude of the erythropoietic effect of that dose. For example, could a lower dose given in a patient with decreased marrow function (because his number of hematopoietic progenitors is decreased by post-chemotherapy stem cell damage or in the context of transplantation) bring about the same biological effects as those induced by higher doses of rHuEpo in the presence of a normal marrow function? This implies that careful consideration should be given before using high doses of hematopoietic growth factors in cancer patients.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers

Manuscript processing

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