Effect of Recombinant Human Erythropoietin on Platelet Production in Dialysis Patients

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ABSTRACT
Two hundred forty-four anemic hemodialysis patients were randomized into recombinant erythropoietin and placebo-treated groups during a 12-wk double-blind phase, followed by a 24-wk open-label period. Mean platelet count rose from the baseline value of 242 x 10^9/L to 264 x 10^9/L on day 5 of epoetin therapy (P < 0.001, paired t test). Mean platelet count peaked at 290 x 10^9/L on day 40 and remained at a significantly elevated level below the peak thereafter. The peak platelet count did not exceed the normal range in a majority of cases. Platelet count was unaffected by placebo. Patients without an erythropoietic response during the first few weeks of therapy exhibited a rise in platelet count comparable to that in patients with a satisfactory erythropoiesis. Patients with low initial serum ferritin concentrations had baseline platelet counts comparable to those with normal or high ferritin values and showed a similar rise in platelet count during therapy. As a group, patients with baseline platelet counts above 400 x 10^9/L showed no rise in platelet count, whereas those with normal or reduced platelet counts showed a marked thrombopoietic response to epoetin. Erythropoietin therapy did not significantly alter the incidence of blood access thrombosis when compared with placebo treatment.

Key Words: Dialysis, recombinant erythropoietin, platelets

Chronic renal failure is associated with impaired production of erythropoietin, the major erythropoietic growth factor. In most cases, the resulting erythropoietin deficiency leads to hypoproliferative anemia, roughly correlating in severity with the degree of renal insufficiency. Before the advent of recombinant human erythropoietin (epoetin), a great majority of patients with ESRD exhibited moderate to severe anemia, with blood transfusion required in approximately 25% of cases. However, widespread use of epoetin has greatly improved anemia-associated morbidity and markedly lowered the need for blood transfusion in the ESRD population (1-3).

Although the primary effect of epoetin is stimulation of erythropoiesis, in vitro studies have suggested an effect on platelet production as well (4-7). In addition, in vivo animal experiments and human trials of epoetin have raised the possibility of enhanced platelet production and function (3,8-10).

The use of epoetin has been suspected to increase the risk of thrombosis, particularly of the vascular access and hemodialysis circuit (11,12). Accordingly, a possible effect of epoetin on platelet production and function is not only of physiologic interest but of clinical significance as well. This article reports the effect of epoetin therapy on platelet production and the rate of vascular access thrombosis in chronic hemodialysis patients studied during two identical, multicenter, phase III clinical trials.

METHODS

Patients
Two hundred forty-four chronic hemodialysis patients (138 men and 106 women; mean age, 50.9 yr) were studied at 20 different sites. Patients underwent in-center hemodialysis treatment three times weekly.

To be included in the study, patients met the following criteria: baseline hemoglobin concentration less than 85 g/L, clinical stability on hemodialysis for at least 3 months, and age of older than 18 yr. Women were postmenopausal or treated with oral contraceptives. Exclusion criteria included: poorly controlled hypertension, a history of myocardial infarction within 1 yr, a history of cerebral infarction or seizure, significant hepatic or neoplastic disease, sickle cell anemia, presence of antibodies to HIV, evidence of hemolysis or blood loss other than through the dialyzer, evidence of aluminum toxicity or otherwise unexplained microcytosis, substance abuse within 5 yr, and recent therapy with androgens
Recombinant Human Erythropoietin

Recombinant human erythropoietin (epoetin beta; Marogen®; Chugai-Upjohn, Inc., Rosemont, IL) was provided by Chugai Pharmaceutical Co., Ltd. Epoetin beta has 165 amino acid residues and a molecular size of approximately 30 kd, with 40% carbohydrate. Endogenous human erythropoietin, epoetin beta, and epoetin alfa have identical biologic properties, identical amino acid sequences, and similar carbohydrate structures. Epoetin beta is a lyophilized powder that was reconstituted with saline on the day of use and was injected iv at the end of each dialysis session.

Study Design

The study protocol was approved by the Institutional Review Board of each of the participating centers, and all patients gave informed consent before participating in the study.

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The study period consisted of two parts. The first part was a 12-wk placebo-controlled, double-blind period. Patients were randomized at a 2:1 ratio to receive either epoetin, 100 IU/kg, or vehicle alone three times per week. After 6 wk, the dose of epoetin was increased to 150 IU/kg if the hemoglobin concentration was below 95 g/L and had not increased by at least 20 g/L over baseline. The dose was reduced to 50 IU/kg for hemoglobin values greater than 125 g/L and temporarily withheld when hemoglobin concentration was greater than 135 g/L.

The second part was a 24-wk open-label period. Patients who completed the double-blind portion of the study could elect to enter the 24-wk open-label period. Epoetin was administered to all patients at an initial dose of 50 IU/kg for 6 wk, regardless of their hemoglobin levels at the end of the double-blind period.

Thus, patients who had been randomized to receive placebo during the initial 12 wk constituted a group for whom this open-label period was the first exposure to epoetin, at a dose of 50 IU/kg. In contrast, patients randomized to receive epoetin at the beginning of the double-blind study had received an initial dose of 100 IU/kg, allowing a comparison of the effects of two different dosages and placebo.

After the first 6 wk of the open-label period, dosages were adjusted at 4-wk intervals as follows: the drug was continued at the same dose if hemoglobin concentration was between 95 and 125 g/L or if it had increased by at least 20 g/L since the beginning of the open-label period; the dose was reduced by 50% for hemoglobin concentrations above 125 g/L and temporarily withheld for values exceeding 135 g/L. If the hemoglobin concentration remained below 95 g/L and had not increased by at least 20 g/L compared with the concentration at the beginning of the open-label period, the dose was increased by 50 IU/kg.

Ferrous sulfate, 325 mg thrice daily, was administered to all patients whose baseline serum ferritin was below 300 ng/mL. Intravenous iron dextran and blood transfusion were administered as needed. Antihypertensive and other medications were instituted or continued as appropriate.

Hemoglobin, hematocrit, and platelet counts were determined in predialysis arterial blood samples before the first dose of epoetin and were repeated at days 5, 12, 26, 40, 54, 82, 110, 138, 166, and 250 of the study.

Patients were carefully monitored for dialysis arterial access thrombosis during the study period. In patients experiencing more than one episode, the thromboses were considered to be separate and distinct events only if they were separated by at least 4 days.

**STATISTICAL ANALYSIS**

Data for hematologic parameters were analyzed by t test, paired t test, and logistic or linear regression analysis as appropriate. The rate of hemodialysis access thrombosis, comparing the placebo and treatment groups and the multiple study sites, was compared by logistic regression, the Mantel-Haenszel test, and the Wilcoxon rank sum test. Survival analysis was used to compare differences between treatment and placebo groups and between the study sites, with the occurrence of access thrombosis defined as the survival endpoint.

Results are presented as mean ± SD unless otherwise noted. A P value less than 0.05 was considered significant.

**RESULTS**

**Effect on Platelets**

Platelet count immediately before epoetin administration was 242 × 10^9/L ± 103 × 10^9/L (range 67 × 10^9 to 837 × 10^9/L; N = 244). Thrombocytopenia (platelet count <150 × 10^9/L) was found in 33 patients (13%), and thrombocytosis (platelet count >400 × 10^9/L) was found in 8 patients (3%). No significant difference was present in baseline platelet counts between the group treated with 100 IU/kg and those treated with 50 IU/kg as the initial dose.

Mean platelet count in patients receiving epoetin rose from the baseline value to 264 × 10^9/L at day 5 of therapy (P < 0.001; paired t test), reaching a peak value of 290 × 10^9/L (a 19.8% rise above baseline) at day 40 (Figure 1). Subsequently, it remained signifi-
Platelet counts in the group receiving epoetin compared with the group receiving placebo. Data are mean ± SE. * P < 0.05 between groups; † P < 0.05 versus baseline value.

Figure 1. Platelet counts in the group receiving epoetin compared with the group receiving placebo. Data are mean ± SE. * P < 0.05 between groups; † P < 0.05 versus baseline value.

Significantly elevated at a level below the peak. For each individual patient, the peak platelet count occurred within the first 54 days of therapy in the majority of cases. The mean of the individual peak values achieved at any point during the course of epoetin therapy was $332 \times 10^9/L$, representing an average increase of 43% compared with the baseline value. Mean platelet count remained unchanged in patients receiving placebo (Figure 1).

Baseline platelet counts in patients with initial serum ferritin concentrations below 100 µg/L ($N = 55$) were not different from those of the remainder of the study group. Baseline platelet counts did not correlate with serum ferritin concentrations for the study group as a whole. Moreover, changes in platelet count did not correlate with changes in serum ferritin concentration during epoetin therapy.

Serum ferritin concentration decreased significantly during epoetin therapy in patients with initial values above 500 µg/L ($1,883 \pm 1,768 \mu g/L$ at baseline to $998 \pm 1,412 \mu g/L$ at day 251; $P < 0.05$) but remained largely unchanged in those with low baseline values receiving iron supplementation.

To examine the relationship between changes in erythrocyte and platelet production during the first few weeks of therapy, patients with complete reticulocyte and platelet data during the first 26 days were divided into two subgroups. The first subgroup ($N = 47$) consisted of patients who showed no initial erythropoietic response to epoetin (i.e., an unchanged or decreased reticulocyte count at day 5 compared with the baseline value). The second subgroup ($N = 166$) consisted of those exhibiting a rise in reticulocyte count. A significant increase in platelet count was noted in both subgroups at day 5. A similar comparison was made between 126 patients exhibiting a hematocrit rise of at least 0.01 (mean hematocrit increased from 0.21 to 0.25; $P < 0.05$) and 90 patients who showed no rise in hematocrit during the first 12 days of epoetin therapy (mean hematocrit, 0.24 versus 0.23; $P = \text{not significant (NS)}$). These two subgroups showed comparable baseline platelet counts ($239 \times 10^9/L$ versus $241 \times 10^9/L$; $P = \text{NS}$) and nearly identical rises in platelet count with epoetin administration (Figure 2). No correlation was found between changes in hematocrit and platelet count during the initial 6 wk of therapy.

Patients with initial platelet count below 150 $\times 10^9/L$ ($N = 33$) showed a significant rise in mean platelet count from $125 \times 10^9/L$ at baseline to $191 \times 10^9/L$ by day 26 of epoetin therapy ($P < 0.01$). Platelet count remained significantly above the baseline value throughout the study in this subgroup. In contrast, patients with initial platelet counts greater than $350 \times 10^9/L$ (mean $434 \times 10^9/L$; $N = 26$) showed virtually no change in platelet count, whereas those with baseline values between the two extremes showed an intermediate rise. The relative change in platelet count during epoetin therapy correlated negatively with the baseline platelet count ($r = -0.26$; $P = .001$ comparing baseline to day 27 values) (Figure 3).
Initial dosage of epoetin was 50 or 100 IU/kg, thrice weekly. Periodic dosage adjustments were made according to the protocol. Of the 244 patients initially enrolled in the double-blind study, 185 completed both the 12-wk double-blind and 24-wk open-label periods, at which time the mean dose was 91 IU/kg thrice weekly (range, 6 to 300 IU/kg) (Figure 4). At the conclusion of the study, 11 (6%) of 185 patients were receiving doses greater than 250 IU/kg, whereas 42 (23%) of 185 were receiving less than 50 IU/kg. Eighteen patients (8%) failed either to achieve a hemoglobin concentration above 95 g/L or to experience a hemoglobin rise exceeding 20 g/L by day 82 of epoetin therapy.

At the time of the first dosage adjustment (day 40), 18 (29%) of the 62 patients who first received epoetin at a dose of 50 IU/kg thrice weekly in the open-label phase of the trial required an upward dosage adjustment, whereas 44 (71%) of 62 required no change in epoetin dose; none required a dose reduction. In the double-blind phase, 31 (20%) of 156 patients receiving epoetin, 100 IU/kg thrice weekly, required upward adjustment, 123 (79%) remained on the same dose, and 2 (1%) required downward adjustment. As expected, the group receiving placebo failed to exhibit a discernible erythropoietic response to either the initial dose or subsequently increased doses of the vehicle.

**Effect on Erythrocytes**

A significant increase in mean reticulocyte count was observed by day 5 of epoetin therapy at both initial dosages ($P < 0.001$). Reticulocyte counts in the 100-IU/kg group increased steadily from $1.7 \pm 1.3\%$ to a peak of $4.2 \pm 4.7\%$ at day 26 and remained significantly above the baseline thereafter. The group receiving 50 IU/kg as initial dosage followed a similar course, exhibiting a rise from the baseline
value of 1.8 ± 1.7% to a peak reticulocyte count of 3.3 ± 4.1%.

With epoetin administration, hemoglobin and hematocrit rose to values that were significantly greater than those found at baseline and in comparison to those of the placebo-treated group at day 12 (Figure 5). The observed rise was greater in patients given 100 IU/kg than that found in patients treated with 50 IU/kg. Consequently, hemoglobin and hematocrit values in the 100-IU/kg group were significantly greater than those in the 50-IU/kg group at days 26 to 82.

Figure 4. Distribution of epoetin dosages at the conclusion of the study. The drug was administered iv, three times weekly, with dosages adjusted to maintain hemoglobin concentration between 95 and 125 g/L.

Figure 5. Mean hematocrit values during the first 82 days of epoetin administration. Three groups are shown: patients receiving initial dosages of 50 IU/kg and 100 IU/kg of recombinant erythropoietin and the group receiving placebo. * P < 0.05, for each of the epoetin-treated groups compared with placebo. # P < 0.05, comparing the 50 and 100 IU/kg groups with each other. Data shown are mean ± SE.
Reticulocyte count, hemoglobin, and hematocrit values did not change in patients receiving placebo.

Effect on the Rate of Venous Access Thrombosis

During the 83-day double-blind period, 21 epoetin-treated patients (12%) experienced 31 episodes of graft thrombosis (0.58 events/patient year). In the placebo-treated group, six patients (8%) exhibited single episodes of access thrombosis (0.34 events/patient year) during the observation period (P = NS comparing epoetin-treated and placebo groups). The rate of access thrombosis observed during the open-label period (0.57 events/patient year) was comparable to that observed in the epoetin-treated patients during the double-blind period. Access survival, defined as time to first thrombotic event, did not differ significantly between the epoetin-treated and placebo-treated groups. Stratification of the data by treatment site demonstrated no significant inter-site differences in the rate of thrombosis or access survival time. Moreover, access thrombosis was not correlated with baseline or subsequent platelet count or hematocrit during the study period.

DISCUSSION

Erythropoietin, a 30-kd glycoprotein, is the primary hormonal regulator of erythropoiesis. Erythropoietin promotes proliferation and differentiation of erythrocyte progenitor cells, leading to production and release of mature reticulocytes. Concurrently, erythropoietin stimulates transferrin receptor expression and hemoglobin synthesis (13).

A number of observations suggest that, in addition to its erythrocytopoietic property, erythropoietin may also influence platelet production. This study examined the effect of epoetin on platelet count, erythropoiesis, and incidence of vascular access thrombosis in a large group of anemic patients maintained on hemodialysis. As expected, a significant increase in reticulocyte count was observed within the first week of therapy in a great majority of patients, preceding a detectable rise in hemoglobin and hematocrit values. In addition, our patients exhibited a rise in platelet count that reached statistical significance in 1 wk. On the average, platelet count rose by nearly 20% and remained significantly elevated at a level below the peak for the duration of the study. The observed rise was confined to the normal limits in the large majority of cases. These results are suggestive of a persistent stimulatory effect of epoetin on platelet production.

A significant rise in platelet count was found whether or not reticulocytosis had occurred during the first week of epoetin administration. Likewise, platelet count remained significantly above the baseline with epoetin administration during the ensuing 3 wk whether a rise in hematocrit or hemoglobin concentration had occurred or not. Moreover, changes in platelet count did not correlate with the resultant changes in hematocrit and hemoglobin concentration. It thus appears that the effect of epoetin on platelet production is largely independent of the associated erythropoietic response. Although hematocrit rose somewhat more rapidly with the higher initial dose of epoetin (100 IU/kg) than with the lower initial dose (50 IU/kg), the rate of rise in platelet count was similar with both dosages. This observation suggests that the maximum thromboipoietic effect may be achieved at an iv dose of 50 IU/kg or less.

Patients with initial platelet counts below the normal range showed a greater rise, reaching normal values within a few weeks of therapy in most cases, as compared with those with initial thrombocytosis who, as a group, showed no significant increase in platelet count.

Abnormal platelet counts have been described in patients with iron storage or availability disorders (14,15). However, in our patients, the size of tissue iron stores (as indicated by serum ferritin concentration) at the beginning of the study did not correlate with the subsequent changes in platelet count during epoetin administration. Moreover, platelet count in a subgroup of 55 patients with iron depletion was not significantly different from that seen in their iron-replete counterparts. Hence, the associated rise in platelet production is not mediated by epoetin-induced iron depletion and appears to represent a direct effect on megakaryocytopoiesis instead.

The regulation of megakaryocytopoiesis is complex and incompletely understood, apparently involving a variety of humoral factors. Among these are interleukins-1α,-3,-4, and -5, granulocyte macrophage-colony-stimulating factor, transforming growth factor beta, and thrombopoietin (4,5,16). Thrombopoietin, whose structure has not yet been elucidated, is the major growth factor for platelet progenitors. Whereas thrombopoietin activity has been shown to increase in thrombocytopenia (16), the nature of the feedback mechanism remains unknown.

Several clinical and experimental observations have suggested a role for erythropoietin in the regulation of platelet production. In vitro experiments with various preparations of erythropoietin have demonstrated increased megakaryocyte colony formation in several assay systems with plasma clot, agar, and serum-free culture media (4-7,17-20). Recent studies with recombinant erythropoietin have shown stimulation of megakaryocyte colony growth and promotion of the differentiation of murine megakaryocytes in serum-free liquid cultures (6,21). In several studies, recombinant erythropoietin alone stimulated megakaryocytopoiesis (6,9), whereas in
others, an effect was seen only in combination with other cytokines such as interleukin-1α, interleukin-3, or granulocyte macrophage–colony-stimulating factor (5,22). In contrast, a few studies found erythropoietin to have no apparent effect (23,24).

In vivo studies have yielded similar results. The administration of epoetin to rats resulted in a dose-dependent increase in both reticulocyte and platelet counts, with an effect on megakaryocytes observed as early as 24 h after the initial dose. Higher doses were required to significantly raise platelet count as compared with those required to produce reticulocytosis. Peak values for both reticulocytes and platelets were observed after 4 to 6 days of epoetin administration, with platelet count rising by 20% and subsequently returning to baseline levels despite continued epoetin administration (8).

Clinical trials in anemic ESRD patients have demonstrated the efficacy of epoetin in raising hemoglobin and hematocrit, but the reported effects on platelets have been inconsistent. Although some studies have reported no change in platelet count (1), others have demonstrated rises in the order of 20% or more (3,25,26).

The mechanism by which erythropoietin alters platelet production is unknown. However, a direct effect on platelet precursors is suggested by the demonstration that megakaryocytes express erythropoietin receptors and that epoetin binds to the high-affinity receptors on murine megakaryocytes. The apparent receptor density is similar to that of pronormoblasts (6,500 receptors/cell) and increases with advancing megakaryocyte differentiation. Mature murine platelets, however, are devoid of erythropoietin receptors (27). Thrombopoietin, the major regulator of terminal megakaryocyte differentiation, is believed to act through as-yet-unidentified cell membrane receptors. Whether erythropoietin and thrombopoietin share a common receptor is uncertain. Likewise, the interaction, if any, between erythropoietin and thrombopoietin remains unclear. However, the observation that epoetin promoted a marked rise in platelet count in patients with low platelet count and presumably high thrombopoietin, but had no effect in those with thrombocytosis, is of interest. These observations may suggest that the effect of epoetin on thrombopoiesis may be influenced by the presence of thrombopoietin. Although the molecular structure of thrombopoietin has not yet been fully elucidated, some investigators have postulated structural similarities between erythropoietin and thrombopoietin (9,16).

In conclusion, this study demonstrates that, in addition to promoting erythropoiesis, epoetin enhances platelet production. The associated rise in platelet count is evident within the first week of epoetin therapy. Platelet count generally increases by as much as 20% and remains elevated throughout the course of therapy. The epoetin-induced thrombopoiesis is independent of the associated erythropoietic response and ferritin values but is inversely related to the baseline platelet concentration. Finally, epoetin administration does not appear to significantly increase the rate of hemodialysis access thrombosis.

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