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# HIGH DOSES OF RECOMBINANT HUMAN ERYTHROPOIETIN FAIL TO ACCELERATE PLATELET RECONSTITUTION IN ALLOGENEIC BONE MARROW TRANSPLANTATION. RESULTS OF A PILOT STUDY

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**ABSTRACT**

**Background and Objective.** The effectiveness of recombinant human erythropoietin (rhEpo) in accelerating erythroid engraftment in patients undergoing allogeneic bone marrow transplantation (BMT) has been demonstrated in previous studies. On the other hand, there are experimental data suggesting that high doses of rhEpo might also exert a stimulatory effect on thrombopoiesis.

**Methods.** We carried out a pilot study on the use of high doses of rhEpo (500 U/kg/day for 30 days after transplant) in ten patients (HD-Epo group) receiving BMT to evaluate the effects on both erythroid and platelet (Plt) engrafment. This group was compared to ten BMT patients who had not received the hormone (Placebo group).

**Results.** The HD-Epo group patients showed signs of accelerated erythropoietic recovery; in fact, the time required to reach a reticulocyte count higher than $30\times10^9$/L was significantly shorter than in the Placebo group, while the number of high RNA content reticulocytes (HFR) was about three times greater. Circulating transferrin receptor (TfR) levels 30 days after BMT were also significantly higher in the HD-Epo group than in the other. Finally, the number of red blood cell (RBC) transfusions in the first 30 days following BMT was about twofold lower in the HD-Epo group; moreover, 4/10 patients who were treated with HD-Epo did not require any RBC units. No significant effects on the engraftment of platelets or on the number of Plt transfusions were observed in the HD-Epo as compared to the Placebo group. No adverse effect was noted on granulocytopoiesis, nor were any adverse clinical experiences found in patients who had been treated with erythropoietin at high dosages.

**Interpretation and Conclusions.** These data confirm that rhEpo may stimulate erythroid reconstitution after BMT, while its effects on Plt engraftment and on Plt transfusion requirements are minimal.


*Key words: bone marrow transplantation, erythropoietin, erythropoiesis, thrombopoiesis, platelet transfusion*

Erythropoietin (Epo) is the main regulatory factor of erythroid proliferation and maturation.[1-5] However, functional Epo receptors are also expressed on megakaryocytes,[6] and several studies have demonstrated an effect of Epo on the megakaryocytic lineage. In fact, *in vitro* Epo stimulates both megakaryocyte colony growth[7,8] and the maturation of megakaryocytes,[9] while *in vivo* increased Plt production and Plt counts have been observed in animals injected with relatively high amounts of the hormone, in both acute and chronic administration models.[10,11] On the other hand, the effects of rhEpo on thrombocytopoiesis in humans appear to be inconsistent, with only a minority of renal failure patients showing an increase in Plt count.[12-14] The effects of rhEpo in patients undergoing allogeneic BMT have been evaluated in several studies which demonstrated accelerated erythroid reconstitution and a reduction in red blood cell (RBC) transfusion needs.[15-19] Since relatively high doses of erythropoietin were necessary to show any thrombopoietic effect in *in vivo* models,[10,11] we wondered whether the administration of higher doses of rhEpo than usually employed in BMT could have any beneficial action on Plt reconstitution. In this paper, we report the results of a pilot study in which twenty BMT patients were randomly assigned to received rhEpo, at a total daily dose of 500 U/kg, or placebo.

## *Patients and Methods*

Twenty adult patients undergoing allogeneic BMT from an HLA-matched, ABO compatible sibling donor in our center were enrolled in the study, after informed consent, to randomly receive either rhEpo at high dosage (500 U/kg/day; HD-Epo group) or placebo (Placebo group). Patient data, including diagnosis and conditioning regimen, are reported in Table 1.

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Fourteen out of 20 patients were conditioned with the BU/CY protocol (busulphan 4 mg/kg/day for four days, followed by cyclophosphamide 50 mg/kg/day for four days); in one patient etoposide (25 mg/kg/day on days -5 and -4) and in another melphalan (140 mg/m$^2$ on day -1) were added to the BU/CY regimen. The remaining four patients were conditioned with TBI-containing regimens; TBI was performed by delivering eleven 120 cGy fractions in four days at a dose rate of 18 cGy/min for a total of 1320 cGy, followed by the administration of cyclophosphamide (60 mg/kg/day for two days) in two patients, of etoposide (25 mg/kg/twice) in one and of both drugs in the fourth patient. All patients had a central venous catheter implanted and were treated until discharge in single, positive-pressure rooms with Hepa-filtered air. They all received oral antibiotics and fluconazole for selective decontamination of the gut, and prophylactic i.v. acyclovir. Patients were routinely screened for cytomegalovirus (CMV) infection by conventional serological assays, virus cultures, and viral DNA probes. Cyclosporine and methotrexate were given as a prophylaxis against graft-versus-host disease (GVHD) according to Storb *et al*.[20]

Red blood cell transfusions were administered in order to keep hemoglobin levels above 8.0 g/dL. Single-donor platelet apheresis were routinely carried out to keep platelet count above 30×10$^9$/L. All blood products were leukocyte-free and irradiated. Arterial pressure and heart rate were determined at least every eight hours during hospital stay.

Recombinant human erythropoietin (rhEpo) was delivered at a total daily dose of 500 U/kg in continuous infusion, from day +1 to day +30.

Peripheral blood counts were determined daily with an electronic particle counter; biochemistry data were also obtained daily using standard laboratory techniques. The absolute number of circulating reticulocytes was determined weekly with a Sysmex R-1000 automated reticulocyte flow cytometry analyzer (TOA Medical Electronics GMBH, Hamburg, Germany). Three populations of reticulocytes are recognizable on the basis of fluorescence intensity: low, middle and high fluorescence ratio reticulocytes (LFR, MFR, HFR, respectively). High RNA content reticulocytes (HFR) are an early and predictive index of erythropoietic engraftment.[21,22] Quantitative determination of circulating transferrin receptor was performed using an enzyme linked immunosorbent assay (ELISA) (Quantikine, R&D System, Inc., Minneapolis, USA).

White blood cell engraftment was defined as the day when granulocytes were >0.5×10$^9$/L, platelet engraftment as the day when the unsupported platelet count was >50×10$^9$/L, and erythroid engraftment as the day when the hematocrit was stabilized at >30% in the absence of RBC transfusions.

All data are presented as means and standard deviations (mean±SD). Data were analyzed according to the CSS (Statsoft, Tulsa, OK, USA) program. The Friedman ANOVA test was used for multiple comparisons among groups, while the Wilcoxon's rank sum test and the Mann-Whitney U-test were utilized for data analysis. A probability of 5% or less was considered to be statistically significant.

## Results

The administration of high doses of rhEpo did not cause any significant adverse events. There was no increment in mean arterial pressure or in heart rate, as compared with pre-study levels. The incidence of epistaxis, hematuria and hemorrhagic cystitis was similar between the two groups; no patient suffered from veno-occlusive disease (VOD). No virologically documented CMV infection occurred during the study period (from day +1 to day +30). The number of BM cells infused was comparable in the two groups, as was the day of WBC engraftment (Table 1). Two patients in the Placebo group died of grade III-IV acute GVHD beyond the study period (day +54 and +56, respectively), while one patient presented with grade II skin acute GVHD on day +27 which later resolved upon prednisone therapy. In the HD-Epo group, three patients developed grade I-II acute skin GVHD on day +25, +29, and +37, respectively, and were successfully treated with prednisone.

Patients receiving HD-Epo showed earlier erythroid reconstitution: in fact the time required to reach a reticulocyte count higher than 30×10$^9$/L was significantly shorter (11.6±1.6 days) in comparison with the Placebo group (15.5±2.5 days ) ($p < 0.01$) (Table 2). Moreover, HD-Epo increased the number of HFR in the circulation, and on day +30 following BMT there were three times more of them in this group than in the controls ($p < 0.01$; Table 2). Finally, HD-Epo patients showed a significantly higher TfR concentration on day +30 after BMT (8791±2198 µg/dL) than the controls (2696±933 µg/dL) ($p < 0.01$). The stimulation of erythropoiesis by rhEpo also resulted in a reduction of RBC requirements. The number of RBC units

Table 1. Patient characteristics.

| *Characteristics* | *HD-Epo (n=10)* | *Placebo (n=10)* |
|---|---|---|
| Age (median)/(range) | 37/(17-55) | 39/(19-44) |
| Male/female | 5/5 | 7/3 |
| *Diagnosis* | | |
| AML | 6 | 5 |
| ALL | 2 | 1 |
| CML | 1 | 3 |
| MM | 1 | 1 |
| *Conditioning regimen* | | |
| Including TBI | 2 | 2 |
| Not including TBI | 8 | 8 |
| *No. of BM cells infused* x10$^8$/kg | 4.2±1.4 | 3.6±1.4 |
| *WBC engraftment (day)* mean±SD | 15.4±2.1 | 16.7±3.6 |

*AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myelogenous leukemia; MM: multiple myeloma.*

Table 2. Effects of rhEpo on erythroid engraftment and transfusion requirements.

| | *HD-Epo (n=10)* | *Placebo (n=10)* |
|---|---|---|
| RBC units transfused within day +30 | 2.2±2.5* | 4.2±2.7 |
| Day Hct >30% | 25.6±11.1 | 36.6±18.1 |
| Day of last RBC transfusion | 15.3±7.0 | 30.0±15.0 |
| Day reticulocyte engraftment | 11.6±1.6° | 15.5±2.5 |
| Max number of HFR (x10$^9$/L) within day +30 | 32.7±15.0° | 9.8±6.4 |
| TfR levels on day +30 (µg/dL) | 8791±2198° | 2696±933 |

**p<0.05; °p<0.01.*

Table 3. Effects of rhEpo on platelet engraftment and transfusion requirements.

| | *HD-Epo (n=10)* | *Placebo (n=10)* |
|---|---|---|
| Plt transfusions within day +30 | 4.5±1.8 | 5.7±2.8 |
| Plt engraftment (day) | 26.9±7.0 | 32.2±9.6 |
| Day of last Plt transfusion | 15.3±7.0 | 25.3±15.6 |

required in the period from day +1 to day +30 was significantly lower in the HD-Epo group (2.2±2.5) than in the Placebo group (4.2±2.7) ($p < 0.05$). Four patients treated with HD-Epo did not require any RBC units during the study period; moreover, in the HD-Epo group the day of last RBC transfusion occurred two weeks earlier (15.3±7.0) than in the control group (30.0±15.0), although this difference was not statistically significant due to a relatively broad distribution of individual values.

On the other hand, there was no statistically significant difference between the two groups in the time to Plt engraftment (27 versus 32 days), or in the number of Plt transfusions delivered from day +1 to day +30 (Table 3). Patients treated with HD-Epo showed a shorter transfusion dependence time, and received the last platelet transfusion ten days earlier than the placebo group (15±7 and 25±15, respectively); however, the difference did not reach the statistical level.

## *Discussion*

Previous studies on allogeneic BMT patients have shown that serum Epo levels are inappropriately low for the severity of anemia in the period following transplantation.[24-27] Several factors may play a role in hindering the Epo response after allogeneic BMT: renal toxicity from cyclosporin A (CsA) used for GVHD prevention, interactions between host and donor marrow, active CMV infection.[28] CsA has been demonstrated to impair Epo production both *in vivo* and *in vitro*; in fact, therapeutical doses of the drug down-modulated Epo production *in vivo* in anemic mice,[29] while *in vitro* CsA was able to inhibit the release of Epo in the medium by a human hepatoma cell line (Hep3B),[30] which produces Epo in a regulated fashion following a hypoxic stimulus.[31] It has also been reported that cytokines such as TNF-α, IL-1 and IFN-γ released by alloreactive donor lymphocytes and/or monocytes can both decrease Epo production by the kidney[32] and exert a suppressor effect on erythropoiesis.[33,34]

According to the results of several trials, treatment with rhEpo accelerates erythropoietic engraftment and reduces RBC transfusion requirements after allogeneic BMT.[15-19] Our present study on the administration of rhEpo at high dosage in patients undergoing allogeneic BMT confirms that rhEpo is able to determine earlier erythroid reconstitution and reduced transfusional needs; whether higher amounts of rhEpo can increase the beneficial effect of the conventional dosage on erythroid engraftment after BMT remains to be assessed.

The effects of rhEpo on thrombocytopoiesis in BMT patients have been reported to be minimal or null in most studies.[15-19] However, Steegman *et al.*[35] described earlier platelet engraftment and a lower number of platelet transfusions in twenty-four patients treated with 100 U/kg/day rhEpo from day +1 to +7, followed by 150 U/kg/day from day +8 to +30. Notably, however, the incidence of hepatic VOD was lower in the Epo group than in the controls, and since VOD is associated with platelet consumption, the significance of this observation was questionable. In addition, Locatelli *et al.*[36] observed fewer Plt transfusions in children receiving allogeneic BMT who were treated with 75 U/kg/day from day +1 to day +30 after transplant.

Experimental data showing that high doses of rhEpo possess a stimulatory effect on thrombopoiesis induced us to evaluate whether the administration of greater doses of rhEpo than usually employed in allogeneic BMT patients could exert a beneficial effect on thrombopoiesis, but we failed to observe any significant effect on Plt engraftment or on the number of Plt transfusions required in the HD-Epo group as compared to the Placebo group.

Although it is conceivable that the small size of the trial could have hampered the detection of subtle differences between the two groups, our data do not support the idea of using high doses of rhEpo with the aim of hastening Plt engraftment after BMT in adults. It has been shown that stem cell factor participates in the regulation of megakaryocytopoiesis and that its administration might have a role in the treatment of disorders of platelet production.[37,38] However, the recent cloning of thrombopoietin,[39] a relatively specific stimulator of thrombopoiesis, and the preliminary results of phase I trial[40] suggest that the scenario of platelet reconstitution after BMT will soon be changed by the introduction of this novel cytokine into clinical use.

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