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Change in Erythropoietin Pharmacokinetics Following Hematopoietic Transplantation

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Abstract

Pre-clinical studies have demonstrated that bone marrow ablation has a profound effect in decreasing erythropoietin (EPO) elimination. The study's objective was to determine in humans if EPO pharmacokinetics (PKs) are perturbed following bone marrow ablation. EPO PK studies were performed in eight subjects, aged 4 to 61 years, undergoing fully myeloablative hematopoietic stem cell transplantation. Serial PK studies using intravenous injection of recombinant human EPO (92 ± 2.0 U/kg) (mean $\pm$ SEM) were carried out during four periods of altered marrow integrity: baseline pre-ablation, post-ablation pre-transplant, early post-transplant pre-engraftment, and late post-transplant full engraftment. Compared with baseline, post-ablation pre-transplant and early post-transplant EPO PKs demonstrated declines in clearance increases in terminal elimination half-life of 36 and 95%, respectively. Clearance and half-life returned to baseline following full engraftment. The association of EPO elimination with decreased bone marrow activity in patients undergoing transplantation conclusively establishes the bone marrow as a key determinant of EPO elimination in humans.

Erythropoietin (EPO), a 34-kDa glycoprotein hormone, has a dominant action in the regulation of erythrocyte production.¹ EPO exerts its biological effect in stimulating the proliferation and differentiation of erythroid progenitors by binding to specific cell-surface receptors (EPO-Rs) that are in greatest abundance on erythroid progenitors located primarily in the bone marrow.² EPO-Rs are also located on virtually all non-hematopoietic tissues and have diverse additional biologic effects.³

There is a paucity of information regarding which organ(s) and tissue(s) are important in EPO metabolism and elimination. Previous *in vivo* studies demonstrated that the kidney and liver exert no measurable effect on EPO *in vivo* elimination.^{4–6} Erythropoietic tissues in rats, *e.g.*, bone marrow and spleen, have also been shown to rapidly metabolize EPO with tissue uptake and clearance directly correlated with the number of erythroid colony-forming units.⁷ These pre-clinical *in vivo* studies are supported by *in vitro* studies demonstrating that EPO is rapidly degraded by ligand-specific EPO-R erythroid progenitors,^{8,9} and by clinical studies in anemic patients with hypoplastic marrows manifesting high serum EPO levels relative to their hemoglobin (Hb) levels.^{10–12}

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

In adults, EPO has demonstrated nonlinear pharmacokinetic (PK) behavior, with EPO clearance decreasing as the administered EPO dose increases.^{12–14} The half-life of EPO ranges from 4 to 8 h in healthy adults given therapeutic doses of EPO.¹³ In very-low-birth-weight premature infants, EPO also manifests nonlinear PK behavior, with clearances approximately threefold greater than adults.¹⁵ Notably premature and term infants also manifest proportionally greater red marrow space than children and adults.¹⁶ Together, these observations indicate that *in vivo* EPO's PK behavior is nonlinear—perhaps related to the limited number of EPO-Rs located on the finite, but expandable, number of bone marrow erythroid progenitors.

In this study, we hypothesized that EPO clearance decreases following myeloablative therapy in hematopoietic stem cell transplant patients and subsequently increases following marrow reconstitution. To test our hypothesis, sequential EPO PK studies were performed under four distinct bone marrow conditions anticipated to differ in their erythroid activity as follows: (1) pre-bone marrow myeloablation baseline; (2) post-ablation/pre-transplant when erythroid progenitors would be absent; (3) early post-transplant pre-engraftment when erythroid progenitors would be starting to become reestablished; and (4) late post-transplant full engraftment recovery when erythroid progenitors are fully reestablished. This study design provides an opportunity to demonstrate the extent that the bone marrow may exert an important effect on EPO elimination in humans, recognizing that final proof will rely on additional mechanistically based studies yet to be done.

RESULTS

The eight study subjects included children and adults from 4 to 61 years of age (Table 1). There were five males and three females. All patients were in remission from their primary disease and had evidence of normal hematopoiesis as indicated by normal blood and reticulocyte counts. All patients received myeloablative-conditioning regimens that lasted 4–7 days, with a 1–3 day rest period before infusion of hematopoietic stem cells.

Four subjects received allogeneic transplants from a matched sibling or unrelated donor and four received autologous transplants (Table 1). The former received allogeneic bone marrow or peripheral blood stem cells that were T cell depleted with anti-T cell antibody therapy using OKT3 plus complement or with positive CD34 selection using CliniMACS® (Miltenyi Biotec, Auburn, CA). The four autologous transplant donors received granulocyte colony stimulation factor before marrow harvest to mobilize peripheral blood stem cells.¹⁷ Patients who received autologous transplants received higher doses of nucleated cells per kg administered, relative to those receiving allogeneic transplants ($P = 0.003$). CD34+ cell counts were obtained primarily for autologous transplants with the dose of cells administered varying from 5.42 to 32×10^6 CD34 cells/kg. All patients demonstrated prompt erythroid recovery, *i.e.*, between 8 and 17 days post-transplant as defined by the number of days since the last post-hematopoietic stem cell transplantation (HSCT) red blood cell transfusion. Erythroid recovery, defined by the time of the last red blood cell transfusion following stem cell infusion, occurred at a median of 18 days (range 5 to 88 days) post-transplant. Two of the study subjects experienced late deaths at 230 and 941 days post-HSCT and were not available for late post-transplant PK study.

Laboratory testing on whole blood before each PK study demonstrated no change in Hb level, but significant change in white blood cell (WBC) and reticulocyte counts were observed (Figure 1). Compared with pre-ablation baseline values, WBC and reticulocyte counts both decreased significantly during the post-ablation pre-transplant and the early post-transplant periods. Although the number of subjects available for analysis for the late post-transplant PK analysis was small, both WBC and reticulocyte counts approached baseline values at this time.

The myeloablative-conditioning regimen leading to bone marrow ablation had a marked effect in decreasing EPO elimination as indicated by more gradual decline in plasma EPO levels following recombinant human EPO (r-HuEPO) bolus administration during the post-ablation pre-transplant and early post-transplant periods (Figure 2). On the basis of our previous experience with adult sheep subjected to busulfan-induced bone marrow ablation,¹⁸ this period coincided with the period of minimal marrow cellularity and no hematopathologic evidence of erythroid precursors.

The slower declines in plasma EPO during the post-ablation and early post-transplant period were manifested in the PK analyses by significant reductions in EPO clearance and prolongation of terminal elimination half-life ($t_{1/2}$) (Figure 3a). Pre-ablation EPO clearance and the late post-ablation clearance (done at 183–500 days post-bone marrow transplant) were similar. The baseline EPO clearance we observed was 5.61 ± 0.41 mL/(kg-h), which is similar to what has been previously reported and decreased to 3.65 ± 0.34 mL/(kg-h) in the post-ablation pre-transplant period. The ablated clearance was $36 \pm 4\%$ of the total initial (normal) clearance. When compared with the six adults, the two pediatric study subjects demonstrated similar qualitative and quantitative changes in EPO PK, *i.e.*, from baseline to post-ablation pre-transplant EPO clearance in the two pediatric subjects decreased from 5.69 and 6.01 to 4.92 and 4.97 mL/(kg-h).

Terminal elimination $t_{1/2}$ increased significantly from its pre-ablation value of 6.4 to 12.5 h (Figure 3b) immediately post-ablation, a 95% increase. Following HSCT, the mean elimination half-life remained elevated during the early post-transplant period before returning to near pre-ablation baseline values by the late post-transplant period. The apparent volume of distribution at baseline remained unchanged throughout the study (data not shown) with mean values approximating the plasma volume (range 40–43 mL/kg) based on an estimated blood volume or 80 mL/kg.

DISCUSSION

The mechanisms of EPO's *in vivo* elimination in humans are incompletely understood and the site(s) of its elimination remain controversial.¹³ In sheep, we have previously demonstrated that the bone marrow contributes significantly to the elimination of EPO,¹⁸ whereas the liver and kidney exert no measurable effect.¹⁹ In these ovine studies where the clearance of ¹²⁵I-r-HuEPO was determined pre- and post-busulfan-induced bone marrow ablation, we demonstrated a profound 80% reduction of EPO clearance (from 40.1 ± 7.4 to 9.4 ± 2.4 mL/kg-h) in association with markedly decreased bone marrow cellularity, *i.e.*, to $<10\%$ of baseline. This study indirectly demonstrates in humans the important role of the bone marrow in EPO elimination.

The kinetic mechanism(s) responsible for EPO's *in vivo* metabolic fate remains a major unanswered question.^{4,20} Because plasma levels of drugs, including EPO,²¹ are related to efficacy, understanding EPO's disposition is important in optimizing its efficacy. Stohlman²² was among the first to call attention to the bone marrow as the primary site of EPO metabolism. Stohlman²² observed that following the cessation of hypoxia-plasma, EPO levels remained abnormally high in dogs that had been sub-lethally irradiated. His conclusion was that EPO levels were significantly "influenced by the functional state of the erythroid tissue of the marrow". Subsequently, other investigators documented that following bone marrow ablation,^{12,23,24} or in patients with aplastic anemia,²⁵ plasma EPO levels were disproportionately increased relative to slightly decreased Hb levels. Conversely, individuals with hyperactive marrow owing to hemolytic anemia had disproportionately low plasma EPO levels^{26–28} and rapid EPO plasma disappearance.^{29,30}

More direct preclinical evidence implicating the primary role of the bone marrow in EPO metabolism has been provided by Kinoshita *et al.*³¹ and by Spivak and Hogans.³² Both intravenously administered ¹²⁵I-r-HuEPO to rats and the highest organ-specific radioactivity uptake in bone marrow and spleen. ¹²⁵I-r-HuEPO uptake by these organs was transient and observed only at low doses. These data, which suggest a saturable process for EPO elimination, are consistent with clinical reports of EPO's nonlinear behavior.^{15,33}

In contrast, some studies have demonstrated, that the kidney and liver contribute to EPO elimination.^{34–36} Urinary excretion has been shown to contribute in a minor way to EPO disposition with only approximately 5% of the administered dose excreted unchanged into the urine.^{4,20} Similarly, non-excretory renal catabolism of EPO contributes in only a minor way.^{37,38} Because of the liver's importance as a metabolic organ, it has received attention as a candidate for EPO elimination. Although several studies demonstrated clear evidence that asialo-EPO is removed by the liver in a matter of minutes,^{39,40} fully intact r-HuEPO is cleared only after several hours.^{19,32,41}

In this study, baseline pre-ablation EPO clearance was somewhat less than in our previous report in normal human volunteers, *i.e.*, 5.6 vs 7.9 mL/kg-h.¹⁵ Although this study was not substantiated by bone marrow examination, the slightly lower clearance may have been the result of somewhat depressed marrow function in study subjects with malignancies. A key finding of this study was that during the post-ablation period, EPO clearance had decreased significantly to 3.7 mL/kg-h. This finding is qualitatively similar to our previous report in busulfan-treated sheep.¹⁸ As maturation of the erythroid series in the bone marrow spans approximately 5 days and as our sheep study demonstrated complete ablation by 7–8 days, the ablative regimens employed were anticipated to have completely eliminated erythropoiesis at the time of the post-ablation pre-transplant testing. The marked decrease in reticulocyte counts post-myeloablation supports this speculation. By 3–9 days post-transplant (*i.e.*, during the early post-transplant-phase PK study), we speculated that the return of EPO PK results towards baseline had begun, with marrow recovery antedating improvement of peripheral WBC and reticulocyte counts because of a lag time required for myeloid/erythroid differentiation and emigration from the marrow space to the periphery. We further speculate that rapid expansion of erythroid precursors in the bone marrow at the time of the third EPO PK study contributed to the increase in EPO clearance and the shortening of EPO half-life. Clearly, future pre- and post-marrow ablative human studies are needed to confirm and extend these speculative statements extrapolated from pre-clinical studies in sheep.

It should be noted that present findings, our previous PK report in busulfan-treated sheep,¹⁸ and the findings of previous clinical studies noted above differ from those reported by Piroso *et al.*⁴² in rats. The latter authors observed that EPO clearance and half-life were not significantly altered by hypo or hyperplastic marrow states.⁴² These authors used cyclophosphamide to induce marrow hypoplasia and phenylhydrazine or bleeding to induce marrow hyperplasia. It is possible that the extremely high EPO levels observed among the phenylhydrazine and phlebotomized groups of study animals (540 and 186 mU/mL respectively) had saturated EPO's nonlinear clearance mechanism,^{6,43,44} thereby accounting for why Piroso *et al.*⁴² were not able to detect pre- and post-ablation differences in EPO PK. Finally, it should be noted that in the few animals studied with the hypoplastic marrows, a trend towards longer EPO $t_{1/2}$ values was observed.

The present observations in humans that EPO clearance is dependent on the bone marrow is consistent with what has been observed with some other EPO products and with other hematopoietic growth factors.^{45–47} The novel erythropoiesis-stimulating protein that has recently been introduced into clinical practice, darbepoetin alpha, has PK behavior that is distinct from r-HuEPO in that it has a markedly reduced clearance and a prolonged $t_{1/2}$.⁴⁸ This

behavior may be because of this protein's reduced affinity for EPO-Rs relative to r-HuEPO. This further supports our studies implicating EPO-Rs in the bone marrow having primary responsibility for EPO clearance. Nonlinear PK behavior has also been observed for other hematopoietic growth factors. For example, pegfilgrastim is eliminated entirely by a saturable neutrophil receptor-mediated process.⁴⁶ Consistent with this, pegfilgrastim's clearance is markedly diminished in neutropenic states, whereas it is increased under neutrophilic conditions.⁴⁶ In a similar manner, thrombopoietin has also demonstrated nonlinear behavior that is associated with the pool of receptors on platelets and megakaryocytes.⁴⁷

In summary, this study's identification of a significant direct association of EPO elimination with decreased bone marrow compartment activity in patients undergoing HSCT conclusively establishes the bone marrow as a key determinant of EPO elimination in humans. Although there were too few subjects completing the late post-transplant full engraftment recovery EPO PK study for performing definitive statistical testing, the PK available data at this period adds further support of our study's primary findings. Although the greatest decline in baseline EPO clearance was noted during the post-ablation pre-transplant period, the subsequent significant increase in clearance observed during early post-transplant period suggests that significant engraftment of erythroid progenitors—with their relatively abundant cell surface EPO-Rs—had occurred within approximately 1 week. An implication of this is that the administration of r-HuEPO relatively early after marrow ablation to patients undergoing HSCT might stimulate erythropoiesis before a significant peripheral reticulocytosis is detectable. Ongoing studies in our laboratory continue to address this and other important issues related to optimal stimulation of erythropoiesis using erythropoietic agents.

METHODS

Study subjects

The study was approved by the University of Iowa Human Subject IRB Committee; all patients and/or their parents signed informed consent. A convenience sample of eight pediatric and adult subjects undergoing full myeloablative chemotherapy preparative regimens before HSCT therapy were enrolled between May 1998 and November 2002. At entry, subjects were scheduled to undergo either autologous or allogeneic HSCT for malignant disorders. Subjects were required to be red blood cell transfusion independent, to have Hb levels >12 g/dl and peripheral absolute neutrophil counts >1,500/ μ l, and to be not receiving r-HuEPO therapy. The transplantation protocol required that renal and hepatic function be not more than minimally impaired as defined by baseline plasma creatinine less than $1.5 \times$ normal, hepatic transaminases less than $1.5 \times$ normal, and total bilirubin within the normal range.

Study protocol

Study subjects were scheduled to undergo sequential EPO PK studies at times representing four different erythropoietic marrow conditions as a consequence of their myeloablation hematopoietic stem cell therapy. The four marrow conditions included (1) pre-ablation baseline; (2) post-ablation pre-transplant; (3) early post-transplant post-engraftment; and (4) late post-transplant full engraftment. The timing of the post-ablation pre-transplant EPO PK study paralleled that which resulted in complete marrow aplasia in adult sheep.¹⁸ The first of the post-transplant EPO PK studies was chosen to examine early, but subclinical, recovery of hematopoiesis. The rationale for including the post-ablation pre-transplant and early post-transplant studies was to account for potential independent effects of HSCT therapy on EPO PK. It was predicted that the baseline and late post-transplant full engraftment EPO PK studies would reflect similar levels of marrow function, as both were performed at times when marrow function was anticipated to be comparable and normal.

WBC counts and Hb were monitored daily and reticulocyte counts intermittently following initiation of myeloablative therapy until discharge. Throughout the study, subjects were prohibited from receiving EPO treatment. Instead, all received red blood cell transfusions that were administered to maintain Hb levels >12 g/dl for at least 24 h before a PK experiment, thereby avoiding the confounding effects of increased endogenous plasma EPO levels and perturbing EPO PK.^{15,33}

Assays

Plasma r-HuEPO levels were measured in triplicate using a double-antibody radioimmunoassay.⁴⁹ Linear assay values for r-HuEPO concentrations are obtained between 4 and 62.5 mU/mL. The third International Reference Preparation of EPO served as the human standard. The linear portions of the standard curve were 4–2.5 mU/mL. Intra-assay coefficients of variation for three plasma pools spanning the assay's useful range were 1.4–5%. To reduce variability, samples for each subject were measured in the same assay.

EPO PK analysis

EPO PK parameters were determined based on frequent blood sampling following intravenous bolus administration of r-HuEPO (92±2.0 U/kg (mean±SEM). Blood samples (~3 mL/sample) were collected at 0.0, 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h after dose administration. In determining EPO PK parameters, post-dose serum EPO concentrations were corrected for the pre-dose baseline EPO concentration by subtracting the baseline EPO value from all post-dose samples. PK parameters were determined by non-compartmental analysis using WinNonlin Professional version 4.1 (Pharsight, Mountain View, CA). To quantify the influence of bone marrow ablation on EPO elimination, the clearance following marrow ablation was expressed as the percent reduction from the baseline clearance value.

Hb, WBC count, and reticulocyte count were measured by flow cytometry (Advia 120, Bayer, Tarrytown, NJ).⁵⁰

Statistical analysis

Data are presented as mean±SEM. The α significance level applied was 0.05. Statistical testing included repeated measure analysis of variance using SAS/STAT (SAS Institute, Cary, NC). The linear mixed model analysis was used to compare mean values for the PK and clinical laboratory variables. Because of the small number of study subjects included in the final late post-ablation PK study, those PK studies were not included in the comparisons. If a significant overall difference among the remaining three study periods was shown based on the analysis of variance F value, a pair-wise comparison of means was performed using *post hoc* Tukey's testing procedure. Bonferroni adjustments were made for multiple comparisons when the overall F value indicated significance.

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Figure 1. Hematopoietic changes at the time of the four transplant conditions for (a) hemoglobin, (b) reticulocyte count, and (c) WBC count. Error bars indicate mean $\pm$ SEM.

Figure 2. Plasma EPO concentration–time profiles for each of the four EPO PK studies, *i.e.*, pre-ablation baseline, post-ablation pre-transplant, early post-transplant pre-engraftment, and late post-transplant full engraftment. Error bars indicate mean $\pm$ SEM.

Figure 3. EPO PK changes at each of the four transplant conditions for: (a) EPO clearance, and (b) terminal half-life. Error bars indicate mean $\pm$ SEM.

Table 1

Subject characteristics, marrow ablative regimens, stem cell/bone marrow replacement, and myeloid recovery

Subject number	Gender	Age (year)	Diagnosis	Conditioning regimen	Type of transplant	Nucleated cells administered (×10 ⁶ /kg)	CD34 ⁺ cells administered (×10 ⁶ /kg)	Myeloid recovery ^a (days)	Erythroid Recovery ^b (days)	Outcome
1	M	23	Anaplastic large cell lymphoma	BVAC	Auto PBSC	536	6.2	17	5	Survived
2	M	51	Acute myelogenous leukemia	ACT	Allo BM, MSD, TCD	124	ND	12	88	Died
3	M	19	Hodgkin's lymphoma	BU/CY/VP	Allo BM, MUD, TCD	223	ND	14	31	Survived
4	M	19	Acute lymphocytic leukemia	CY/TBI/VP	Allo PBSC, MSD, CD34+ select	9	7.55	12	80	Survived
5	M	57	Diffuse large B-cell lymphoma	BEAM	Allo BM, MSD	166	ND	13	51	Died
6	F	9	Recurrent Wilm's tumor	VPI6/THIO/CY	Auto PBSC	1,028	5.44	12	12	Survived
7	F	4	Recurrent medulloblastoma	BCNU/THIO	Auto PBSC	649	32	8	43	Survived
8	F	61	Mantle cell lymphoma	BCNU/VP/Ara-C/MEL	Auto PBSC	1,128	5.42	12	8	Survived

Allo; allogeneic; Auto; autologous; BEAM; BCNU; VP, Ara-C, MEL; BU/CY, busulfan, cyclophosphamide; CYT; cytarabine; MEL, melphalan; THIO, thiotepa; VP, etoposide; BM; bone marrow; BVAC; ACT, Ara-C, cyclophosphamide, THIO; BCNU (carmustine), VP-16, Ara-C (CYT), cyclophosphamide; MSD; matched sibling donor; MUD; matched unrelated donor; PBSC; peripheral blood stem cells; TCD; T cell-depleted marrow; ND; not done as CD34+ counts were not routinely measured on allogeneic donor marrows.

^aMyeloid recovery defined as the first of three consecutive days when absolute neutrophil count exceeded 500 per μ l.

^bErythroid recovery defined as the days since the last post-HSCT RBC transfusion.