# ERYTHROPOIETIN: A REVIEW

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**The fact that a plasma factor was responsible for the stimulation of red cell production has been known for more than 35 years. However, it is only recently that the gene responsible for its production and its molecular structure has been identified. Furthermore, recombinant human erythropoietin is now available for clinical use. This article details the molecular biology and clinical pharmacology of this remarkable growth factor. (*J Natl Med Assoc.* 1994;86:129-135.)**

**Key words** • erythropoietin • red blood cell production

The humoral regulation of erythropoiesis was identified more than 35 years ago.[1] Since then, the rate of accumulation of knowledge about erythropoietin (EPO) has accelerated. Erythropoietin, produced primarily in the kidney by the endothelial, peritubular, and epithelial tubular cells[2] with a small contribution from the liver, is an obligatory growth factor for the proliferation and differentiation of committed erythroid progenitor cells. It is produced in response to hypoxia, and there is an inverse relationship between EPO levels in serum and urine and the level of tissue oxygenation.[3]

There is a derangement of EPO homeostasis in a range of diseases characterized by anemia. Moreover, EPO has been studied extensively as replacement and pharmacological therapy in these disorders. This article discusses the biochemistry and physiology of EPO, as well as some of the diseases in which EPO metabolism is abnormal. Also, the use of EPO in the treatment of various types of anemia is addressed.

## STRUCTURE OF ERYTHROPOIETIN

Erythropoietin, a member of a family of growth factors, is a 166-amino acid glycoprotein, MW 39 000 d. It is heavily glycosylated with approximately 40% of the molecule composed of sialic acid. These carbohydrate moieties are important in the biologic activity and stability of EPO, as desialation reduces the half-life of EPO in the circulation. It is a hydrophobic molecule and requires intact disulfide bonds for its activity. Nonetheless, the structure of the active site remains to be fully characterized.

## PHYSIOLOGY AND BIOCHEMISTRY OF ERYTHROPOIETIN

Hypoxia stimulates production of EPO; moreover, hyperoxia and polycythemia suppresses it. Nevertheless, elevation of serum EPO levels in response to hypoxia is a transient process; such increased EPO concentration eventually will return to normal even if hypoxia persists, and even primary or secondary polycythemia will not entirely suppress EPO production. Thus, it is worth emphasizing that measuring serum EPO in a single sample may be misleading.[4]

Erythropoietin increases the number of developing erythroid precursors and accelerates release of reticulocytes from the bone marrow, but does not alter cell-cycle length or the number of meioses involved in erythroid differentiation in stimulating such activity. There is additional evidence that EPO also increases the pool of cells capable of erythroid differentiation.[5]

Hematopoiesis begins with primitive pluripotent stem cells capable of self-renewal and differentiation into multipotent progenitor cells. While EPO is the important hormone influencing erythroid differentiation, it does not seem to interact with pluripotent or multipotent hematopoietic progenitor cells, that is, EPO-responsive cells do not seem to arise directly from pluripotent stem cells[6] and are not capable of self-renewal.[7] However, it is known that EPO does interact with early (burst-forming unit-erythroid [BFU-e's]), as well as late erythroid progenitor cells (colony-forming unit-erythroid [CFU-e's]), the first cells recognizable as committed to erythroid differentiation.

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Young BFU-e's are relatively insensitive to EPO in vivo as well as in vitro[8] and can proliferate in the absence of exogenous EPO if burst-promoting activity is present.[9] As BFU-e's mature, however, their sensitivity to EPO (as seen by proliferation) increases,[10] and CFU-e's, which derive from but lack the proliferation capacity of BFU-e's, are exquisitely sensitive to EPO. Colony-forming unit-erythroid has an essential requirement for EPO for colony formation and terminal differentiation.[11] Moreover, in the absence of EPO, CFU-e's are irrevocably lost after one cell cycle.[12] However, despite the role of EPO as an obligate growth factor for CFU-e's, EPO independent differentiation of CFU-e's has been demonstrated[13]; the physiologic significance of this observation remains to be clarified.

Erythropoietin also seems to stimulate proliferation of proerythroblasts and basophilic erythroblasts,[14] the first morphologically recognized erythroid elements; however, EPO is not an obligate growth factor here as it is for CFU-e's, as erythroid differentiation at this point can continue in the absence of EPO.[15]

That BFU-e's and CFU-e's are responsive to EPO (although to differing degrees) invokes the presence of an EPO receptor. Krantz and Goldwasser[16] have found two specific high-affinity sites for EPO on erythroid progenitor cells, and eight molecules of EPO are bound to progenitor cells for their terminal differentiation. They, along with Sawyer et al,[17] also have found that EPO is internalized and metabolized by these target cells. More recent work by D'Andrea and Lodish[18] has shown that the EPO receptor is a double-stranded 507 amino acid polypeptide with a single membrane-spanning domain; the extracytoplasmic domain is the EPO-binding domain, and the cytoplasmic tail contains the signal-transduction domain. D'Andrea and Zon[19] have shown that there are approximately 200 EPO receptors present on the surface of purified erythroid progenitors and that certain cell lines may increase this number to as much as 1000 on stimulation. Furthermore, they have shown that these EPO receptors have low or high affinity for EPO, probably accounting for the different cellular responses to EPO.

Ertythropoietin-EPO receptor interaction initiates a series of events that are associated with terminal differentiation and enucleation. First, there is an increase in synthesis of a variety of RNA species; protein synthesis is not required for this activation of transcription of the EPO gene.[1] How such interaction initiates this series of biochemical events remains to be elucidated; the signal may involve activation of adenyl or guanyl cyclase, activation of an intrinsic receptor kinase, activation of protein kinase C, alteration of intracellular cation concentration or pH, or proteolytic liberation of an intracellular protein. Further studies on EPO receptor signalling are necessary to clarify these events. Nonetheless, work by Misiti and Spivak[20] has shown that there is an important role for $Ca^{+2}$ ions in both the intra- and extracellular events associated with the interaction of EPO with CFU-e's. $Ca^{+2}$ appears to be required early in such interaction and seems to be involved in the binding of EPO to its target cells as well as in the initiation of erythroid differentiation as an induction agent to induce a subpopulation of erythroid progenitor cells to respond to EPO.

Some studies have evaluated the effect of EPO on megakaryocytes. Jackson et al[21] have shown that there may be a connection between the production of platelets and EPO. Dessypris et al[22] have demonstrated that megakaryocyte colony-stimulating factors increased with EPO. However, there was no increase in platelet production. At high doses of recombinant human (rHuEPO), McDonald et al[23] have shown that megakaryocyte production in vitro increased without a change in platelet production. In clinical trials,[24,25] the platelets of patients with end-stage renal disease who received rHuEPO increased. These suggest that EPO may be beneficial in hypoproductive thrombocytopenic states. However, the data are not strong enough to recommend its use for this purpose as yet.

## REGULATION OF ERYTHROPOIETIN GENE EXPRESSION

As noted above, EPO is produced in the kidney in response to hypoxia, yet little is known about the exact nature of this oxygen sensor. Preliminary investigations have revealed that detection of oxygen availability involves a membrane-bound heme protein[26] that somehow communicates with the cell nucleus to activate EPO gene expression.

Goldwasser and Beru[27] have proposed that the EPO gene is under dual control by transacting factors: one protein, 47 KDal that is produced constitutively, acts positively to activate EPO gene expression by binding to a 17-base region at the 5′ end of the EPO gene. However, this 47 KDal protein cannot do so until one or more of the ribonucleoproteins that bind to the same sequence has been dissociated from the chromosome or until the synthesis of this ribonucleoprotein(s) has been arrested. Thus, EPO gene regulation involves loss of binding of a negative factor, which allows binding of a positive factor.

Goldberg et al[28] concur that EPO gene regulation

occurs at the level of EPO mRNA; nevertheless, posttranscriptional events are involved as well. Also, while EPO mRNA concentration increases 50-fold in response to hypoxia, the EPO gene transcription rate increases only 10-fold; they describe a mechanism whereby EPO mRNA degradation requires both transcription and translation of rapidly turning over mRNA(s) whose protein product(s) participates in the degradation of EPO mRNA. Hypoxia somehow decreases the activity of this protein, thus increasing EPO mRNA stability. Erythropoietin gene regulation may well involve increased gene transcription coupled with increased mRNA stability, thus significantly amplifying EPO mRNA levels in response to hypoxia.

## ERYTHROPOIETIN IN HEALTH AND DISEASE

Under normal steady-state conditions, the concentration of circulating EPO is that amount necessary to maintain the red cell mass and to replace senescent and dying cells.[29] Sensitive radioactive assays of EPO have revealed this level normally to be approximately 10 to 25 mU/mL.[30] However, serum titers can vary considerably. For example, at a hematocrit of 30%, EPO levels can range from 50 to 500 mU/mL. It was once believed that the overall inverse relationship between serum EPO and hematocrit in anemic patients was lost once hematocrit fell below 33%,[31] and it now seems that the inverse correlation between serum EPO level and hematocrit holds in anemic patients even if hematocrit is greater than 33%.[32]

Despite the difficulty in assigning a strict value to normal EPO levels, it is generally recognized that patients with diseases associated with varying degrees of anemia manifest EPO levels outside the generally accepted range. For example, endocrine renal function decreases in parallel with excretory renal function resulting in EPO deficiency and anemia once creatinine clearance falls below 40 mL/min/1.73 $m^2$.[33] However, even severely diseased kidneys are capable of producing some EPO; despite the deficiency, the inverse relationship between EPO level and hematocrit remains intact, although it functions at a lower level.

Ninety percent of patients with the myelodysplastic syndrome present with anemia.[34] However, their anemia is not secondary to EPO deficiency as their renal function usually is intact. The myelodysplastic syndrome comprises a group of disorders in which a clonal abnormality of hematopoietic stem cells exists that may progress to an acute leukemia state. Ineffective hematopoiesis is an early feature of myelodysplastic syndrome[35] and causes the associated anemia, but may occur in nonanemic patients.[36] In contrast to the situation in chronic renal failure, there is only a weak inverse correlation between hematocrit and EPO level despite the presence in many myelodysplastic syndrome patients of active erythropoiesis and even erythroid hyperplasia.[37] While EPO levels vary greatly in myelodysplastic syndrome patients for the same or similar hemoglobin concentrations,[38] often serum EPO levels are elevated, but there is not the expected close relationship of EPO level with the degree of anemia. Jacobs et al[39] suggested that these observed EPO levels may be secondary to reduced utilization by EPO-responsive cells. Interestingly, the highest EPO levels are seen in those patients with erythroid hypoplasia.

Furthermore, patients with sickle cell anemia have low EPO levels for their degree of anemia. Sherwood et al[40] propose a mechanism of ongoing renal damage interfering with the synthesis of EPO or with the function of the putative oxygen sensor.

Human immunodeficiency virus (HIV) infection is associated with defects in hematopoiesis, including decreased proliferation of hematopoietic progenitor cells and increased destruction of mature cells. These events may be secondary to the influence of HIV infection of progenitor cells on bone marrow stromal elements.[41] Regulatory cytokines also are disturbed; thus, hematopoietic cytopenias are common. In patients with acquired immunodeficiency syndrome (AIDS) treated with zidovudine, which causes additional significant bone marrow suppression, two distinct types of anemia can be observed[42]: one associated with macrocytosis and low serum EPO and the other with normocytic red blood cells and high serum EPO. This distinction becomes important when considering treatment of such patients with exogenous EPO.

Polycythemia rubra vera is a myeloproliferative disorder marked by autonomous overproduction of erythrocytes and variable overproduction of granulocytes and platelets. Evidence suggests there is an acquired sensitivity of erythroid precursors to EPO, and EPO levels in polycythemia rubra vera are usually low.[8] However, Cotes et al[4] have questioned the value of a single EPO determination in the diagnosis of polycythemia rubra vera versus secondary erythrocytosis. They found 61% of polycythemia rubra vera patients had EPO levels in the "normal" range, suggesting that while the erythrocytosis may suppress EPO secretion, this suppression is somehow incomplete. While they did find elevated EPO titers in 25% of patients with secondary erythrocytosis and levels elevated above

67% of those with polycythemia rubra vera, they believe that the most important use of serum EPO levels in patients with erythrocytosis is in those with erythrocytosis of unknown cause, ie, those with erythrocytosis in whom polycythemia rubra vera and secondary erythrocytosis have been ruled out. In such patients, mechanisms of erythrocytosis may include an abnormality of feedback control of EPO secretion, ectopic EPO secretion, or a possible "pre-polycythemia rubra vera" condition yet to be characterized in which erythrocytosis occurs with low EPO levels.

Total red blood cell mass decreases in the first trimester of pregnancy but gradually increases to nonpregnant values by week 30 and further increases later in pregnancy.[43] Because the life span of red blood cells remains unchanged during pregnancy,[44] this change in red blood cell mass is most likely secondary to decreased erythropoiesis. Although absolute EPO levels increase over nonpregnant values throughout pregnancy and correlate with hematocrit in the third trimester, delivery, and postpartum, no such correlation is evident earlier in pregnancy. Erythropoietin levels remain relatively low for the degree of anemia at this stage, thus likely accounting for the observed decrease in erythropoiesis and total red blood cell mass seen early in pregnancy. Nevertheless, Beguia et al[43] caution that these observed alterations in the rate of erythropoiesis are adaptive and require no therapeutic intervention, as may be the case in the aforementioned conditions.

## ERYTHROPOIETIN THERAPY

Recombinant human EPO has been approved by the US Food and Drug Administration for the treatment of the anemia of chronic renal failure since June 1989. Approval for such use also has been obtained in western Europe and Japan.[45] In an important study, Eschbach et al[24] found that chronic renal failure patients on hemodialysis responded to rHuEPO IV three times per week with elevation in hematocrit in a dose-dependent fashion; furthermore, his group found this response to be uniform and predictable above 15 U/kg. In another study, Eschbach et al[46] reported similar results in predialysis CRF patients. Additionally, Steinhauer et al[47] found not only that subcutaneous rHuEPO effectively corrected anemia in continuous ambulatory peritoneal dialysis patients, but also that rHuEPO subcutaneously increased peritoneal ultrafiltration. Although rHuEPO is approved at this time only for chronic renal failure, rHuEPO has been studied extensively for its therapeutic value in a host of other conditions manifested by varying degrees of anemia despite the sometimes already increased serum EPO titers observed in many of these conditions.

To assess the effectiveness of rHuEPO therapy in nonchronic renal failure patients, it is instructive to categorize patients as to their level of endogenously produced EPO. Patients with rheumatoid arthritis, chronic inflammatory and infectious disorders, solid tumors, and multiple myeloma, as well as chemotherapy patients, often have endogenous EPO levels of 20 to 200 mU/mL and experience relatively mild anemia similar to that seen in chronic renal failure patients.[28] As such, these patients usually respond favorably to rHuEPO. For example, patients with multiple myeloma or other bone marrow-infiltrative diseases, such as low-grade non-Hodgkin's lymphoma, despite their manifestation of endogenous EPO overexpression, respond to rHuEPO with increased erythropoiesis and elevation of hemoglobin levels.[48] Nevertheless, in patients with normal renal function, doses slightly higher than those used in chronic renal failure patients generally are used.

Patients with endogenous EPO levels of 200 to 2000 mU/mL generally have more severe anemia with hematocrits 20% to 30%. This group of anemic patients includes those with myelodysplasia, sickle cell anemia, and patients with AIDS, particularly those receiving zidovudine. Unfortunately, such AIDS patients seem to respond variably to rHuEPO. In a randomized, placebo-controlled clinical trial of 63 AIDS patients treated with zidovudine,[42] Fischl et al[41] found that patients responded to 100 U/kg rHuEPO ($\times 3$ months) only if endogenous EPO levels were below 500 U/L. However, Fischl et al did concede that the optimal doses of rHuEPO in this population of patients remains to be established. Notwithstanding, subcutaneous rHuEPO has been used together with granulocyte-colony stimulating factor, correcting anemia and leukopenia sufficiently so that AIDS patients may resume zidovudine therapy.

Transfusion-dependent patients include those with aplastic anemia, hemolytic anemia, or severe myelodysplastic syndrome; serum EPO levels may be on the order of 2000 to 20 000 mU/mL. Unfortunately, rHuEPO given at doses appropriate for the anemic conditions described above probably are not useful in severely anemic patients. Several investigators have confirmed that none to perhaps only 10% to 20% of myelodysplastic syndrome patients (all with varying degrees of anemia) respond to various doses of rHuEPO with increasing hemoglobin. While stimulation of

erythropoiesis by rHuEPO remains intact, rHuEPO seems to be affecting only ineffective erythropoiesis. The few myelodysplastic syndrome patients who have responded to rHuEPO have, as in AIDS patients on zidovudine, lower EPO levels.[38]

There has even been an anecdotal report of healing of chronic leg ulcers in a sickle cell patient using rHuEPO,[49] but it is in the near-elimination of transfusion requirements in patients with Hodgkin's disease and others who respond to rHuEPO that the drug has truly proven its value.

Repeated transfusions may result in iron overload, infections including HIV and, more commonly, hepatitis B and C, reactions to leukocyte antigens, or the development of cytotoxic antibodies that may produce eventual renal transplantation; moreover, blood transfusion has been shown to exert an immunosuppressive effect in a variety of conditions, including organ transplantation. Thus, rHuEPO has proved invaluable in avoidance of the dangers inherent in homologous blood transfusion.

Furthermore, rHuEPO has been used successfully in nonanemic patients to enhance autologous blood donations before elective surgery and to help normalize hemoglobin after such donations; rHuEPO two to three times several days before surgery can allow the collection of an additional one to two predeposit units. However, evidence supporting use of rHuEPO in avoidance of postoperative homologous blood transfusion is currently inconclusive.

Recombinant human EPO also has been used in Jehovah's Witnesses who, in deference to their religious beliefs, refuse transfusion of blood products. However, it is worth noting that human albumin, a blood product, is used as a diluent in the two currently available rHuEPO preparations (Epogen, Amgen Inc, Thousand Oaks, California and Procrit, Ortho Biotech, Raritan, New Jersey).[50] Thus, devout Jehovah's Witnesses may not permit treatment with rHuEPO unless the diluent is changed.

An important controversy concerning rHuEPO use in CRF and other conditions that arose with the initial clinical trials was the optimal dosage and route of administration of the drug. For example, Eschbach et al[24] found optimal results in patients with Hodgkin's disease using >15 U/kg IV three times per week, while Steinhauer et al[47] found success in continuous ambulatory peritoneal dialysis patients using >50 U/kg subcutaneously twice a week. Cazzola et al[38] used a median dose of 75 U/kg subcutaneously five times per week, and Zappacosta et al[51] used 75 to 150 U/kg subcutaneously once a week. Clearly, the treating physician must handle each case individually according to the symptoms attributable to the anemia; nevertheless, Erslev[52] does recommend an initial dose of 35 U/kg three times per week in chronic renal failure to raise and maintain hematocrit at 30% to 34%. He adds that it is rarely necessary to exceed 80 U/kg three times a week in these patients. For patients with nonrenal anemia who have endogenous EPO levels <500 U/L, Erslev recommends 80 U/kg twice weekly; it is unclear whether patients with endogenous EPO >500 U/L would respond to even higher doses.

Intravenous, subcutaneous, and intraperitoneal routes for administration of rHuEPO have all been used, but subcutaneous administration has won favor with investigators for a variety of reasons. Egrie et al[53] found that after subcutaneous injection, serum EPO levels peak at 8 to 12 hours and are maintained for another 12 to 16 hours, while for IV injection, EPO's $t_{1/2}$ is less than 10 hours. Zappacosta et al[51] reports continued absorption of a subcutaneous dose of rHuEPO for up to at least 70 hours, while the $t_{1/2}$ of rHuEPO IV is approximately 6 to 9 hours. Subcutaneous injection thus results in more sustained plasma levels of EPO, permitting the use of lower effective doses, resulting in lower treatment costs. Subcutaneous administration of rHuEPO is also more convenient than the intravenous route, particularly for continuous ambulatory peritoneal dialysis and predialysis patients.[54] Even Hodgkin's patients benefit from the lower doses used. The subcutaneous route is also superior to the intraperitoneal route in continuous ambulatory peritoneal dialysis patients because of the greater bioavailability of rHuEPO afforded by subcutaneous administration.[55]

Because of the lower effective doses subcutaneous use of rHuEPO can achieve, patients on such therapy are at lower risk for adverse effects of rHuEPO. While some investigators have reported no serious side effects attributable to rHuEPO in their studies, others have seen an increased incidence of hypertension, seizures (often secondary to increased diastolic blood pressure), and fistula occlusion.[56,57] These investigators have attributed their findings to the increased red blood cell mass and whole blood viscosity, as well as increased peripheral vascular resistance caused by rHuEPO as opposed to any direct effects of the drug. But, Samtleben et al[57] have concluded that these complications are found most often in those already predisposed. Finally, aggravation of splenomegaly has been observed in myelodysplastic syndrome patients on rHuEPO, probably secondary to extramedullary hematopoiesis.

Other less serious reported effects of rHuEPO have included transient myalgia and flulike symptoms (occurring shortly after the first few doses of rHuEPO), headaches and conjunctival injection, nausea, vomiting and diarrhea,[54] and slight but significant increases in serum potassium requiring dietary counseling.[58] There have been no reports of antibody formation to rHuEPO.[59]

One final effect occurring most commonly in patients using rHuEPO is the development of functional iron deficiency, a major cause of nonresponsiveness to rHuEPO. Because of the magnitude of this problem, Erslev[52] and MacDougall et al[54] recommend routine assessment of iron stores (iron, TIBC, and ferritin) before initiation of rHuEPO therapy. Despite its occurrence, iron deficiency is easily treated by oral or IV (if necessary) iron supplementation, to provide additional iron to meet the increased demands afforded by stimulation of erythropoiesis by rHuEPO. Furthermore, iron supplementation may be necessary even in patients replete with iron or even iron-overloaded, as they may eventually become iron deficient on rHuEPO.

## CONCLUSION

Although much remains to be learned about the biochemistry and mechanism of action of EPO, the efficacy of treatment of the anemia of chronic renal failure, as well as nonrenal anemias, with rHuEPO seems to be improving exponentially. Recombinant human EPO can restore normal hematocrit, eliminate the need for transfusions (with their inherent risks and costs), and improve the quality of life of its recipients. Thus, rHuEPO is a safe drug with a high benefit-risk ratio.

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