

Review

Therapeutic implications of recombinant human erythropoietin in anaemic related clinical manifestations

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The introduction of recombinant human erythropoietin (RHUEPO) has revolutionised the treatment strategies for patients suffering with anaemia of chronic renal disease and chronic heart failure. Clinical studies and several observational evidences have demonstrated that RHUEPO is also useful in various non-uraemic conditions including haematological and oncological disorders, prematurity, HIV infection and preoperative therapies. The successful treatment of all the anaemic related malfunctions with recombinant human erythropoietin (RHUEPO) has become a standard treatment tool for dialysis patients and as an interesting therapeutic option for several forms of non-renal anaemia. As a consequence of both, RHUEPO has achieved the highest annual sales worldwide and the potential of it increases its scope in the future prospective also.

Key words: Recombinant human erythropoietin, anaemia, recombinant human erythropoietin therapy, erythropoietin-mediated malfunctions.

INTRODUCTION

Erythropoietin is a glycoprotein which stimulates the red blood cells in the bone marrow. In 1985, the human erythropoietin gene was cloned (Tabbara et al., 1993). The introduction of Recombinant Human Erythropoietin (RHUEPO) has revolutionized the therapeutic approaches in treating the patients with anaemia of chronic renal disease. Clinical studies have demonstrated that recombinant human erythropoietin is also useful in various-uraemic conditions including hematological and oncological disorders, prematurity, HIV infection and other therapies (Ng et al., 2003). Several studies showed that anaemia observed in patients with chronic heart failure (CHF) is associated with worsened symptoms and survival, a significant improvement is seen when treated with erythropoietin (EPO) (Wiek Van Gilst et al., 2003,

Glaspy et al., 2001, Locatelli et al., 2001)

Despite impressive advances in safety of blood supply (Levin et al., 1999), the search for therapeutic alternatives to blood continues (Floey et al., 1996; Parfrey et al., 1996). Several studies in dialysis patients, including an analysis of the US Medicare database (Ma et al., 1999) demonstrated an inverse relationship between haematocrit and patient survival. Prospective Cohort studies identified anaemia as an independent risk factor for growth in the dialysis and pre-dialysis population (Kaiuwe Eckardt, 2001). An uncontrolled pilot study and a small controlled trial by the same investigators have suggested recently that in patient with severe heart failure, an increase in haemoglobin levels following combined therapy with recombinant human erythropoietin and iron leads to an impressive improvement of symptoms and retards disease progression (Silverberg et al., 2003). Knowledge of scientific and physiologic bases for the pharmacological use of erythropoietin has led to clinical

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trials in patients undergoing elective surgery especially in the United States (Krantz, 1991; Adamson et al., 1994). This review reveals the possible applications of erythropoietin, its potential and an over-view on overcoming some of the anaemic related malfunctions in humans with RHUEPO.

LOCATION AND SPECIFICATION OF ERYTHROPOIETIN

Erythropoietin is a glycoprotein with a molecular weight of 30.4 kD. The human erythropoietin gene is situated at chromosome 7q11-22, consisting of five exons and four introns, which produce a post transcriptional single polypeptide containing 193 aminoacids (Jelkmann, 1992). During the post-translational modification, glycosylation occurs with the addition of three N-linked (at Asn-24, Asn-38 and Asn-83) and O-linked (at Ser-126) acidic oligosaccharides, the formation of two disulphide bonds at Cys-7 to Cys-161 and at Cys-29 to Cys-33, concomitant with the removal of the 27 aminoacid hydrophobic secretory sequence (Law et al., 1986). Circular dichroism spectral analysis has proposed that its secondary structure contains 50% of helix moiety, with spatial arrangement of two helical pairs running antiparallel similar to that of growth hormone (Inoue et al., 1995). The N-glycosylated moiety of recombinant human erythropoietin has three main functional units; the main core, the branched portion and the terminal component with each unit having a specific role. The function of the O-glycosylated unit, a component constituting about 3% of the total mass of recombinant human erythropoietin, remains to be defined (Ng' et al., 2003).

ROLE OF ERYTHROPOIETIN IN HUMANS

The Erythropoietin (EPO), a glycoprotein hormone produced primarily by cells of the peritubular capillary endothelium of the kidney, is responsible for the regulation of red blood cell production. Secondary amounts of the hormone are synthesized in liver hepatocytes of healthy adults. In premature as well as full term infants, liver is the primary site of EPO production (Hawazin Faruki et al., 1995). The Erythropoietin has the ability to reduce the diseases related with heart, kidney, lungs, bone marrow, brain and other exercise parameters such as muscle oxidative metabolism and fore arm vasodilation.

Anaemia is commonly observed in patients with chronic heart failure (CHF) and is related to the severity of disease (Wiek Van Gilst et al., 2003). Evidence for a relation between anaemia and cardiac morbidity and mortality was provided by retrospectively examined studies (Al – Ahmad et al., 2003). The role of recombinant human erythropoietin therapy in CHF patients showed many beneficial effects (Silverberg et al., 2001). Clinical studies have shown that recombinant human erythropoietin therapy corrects the anaemia of chronic renal failure, avoids

blood transfusions and improves quality of life (Eschbach, 1994). Furthermore, it optimizes patient's haemodynamic status thus minimizing the risk of progression of left ventricular hypertrophy and its associated mortality; it leads to the improvement of physical performance and cognitive function (Mocks, 2000; Silverberg et al., 2001). Some clinical conditions give rise to tissue hypoxia including anaemia, lung disease, or cyanotic heart disease which further leads to the increase in the levels of serum erythropoietin (Hawazin Faruki et al., 1995.) Certain bone marrow disorders, such as myelody-splastic syndrome and aplastic anaemia, may also be associated with high serum levels of erythropoietin. A primary elevation of erythropoietin levels can also occur in certain tumors, resulting in erythropoiesis (Tabbara, 1993) and recombinant human erythropoietin is also used in the treatment of tumors. The multifunctional role of erythropoietin has further been confirmed by the discovery of a specific erythropoietin receptor (EPOR) system in the central nervous system (Buemi et al., 2000). The hypothesis suggested that the brain of a patient with depression may be in state of hypoxia, which may induce erythropoietin production (Nakamura et al., 2000). Recently it was discovered that erythropoietin also protects neurons. Recombinant human erythropoietin is given after chemotherapy and used in the treatment of solid tumors. (Case et al., 1993; Casinu et al., 1994). RHUEPO prevents chemotherapy-induced anaemia in children (Csaki et al., 2005). EPO was also applied as performance enhancing drug in athletes (Adamson et al., 1991; Ekblom et al., 1991). Therefore the RHUEPO has significant implications concerning its use in clinical practice. Thus RHUEPO therapy is strongly recommended in humans.

Deficiency of EPO leads to diverse abnormalities

The lack of erythropoietin in humans leads to several anaemic related malfunctions as well as other disorder. Anaemia is frequently observed in patients with chronic renal failure (CRF). Renal anaemia is an important risk factor for the development of cardiovascular disease (Levin et al., 1999). Besarab et al. (1998) studied haemodialysis patients with clinically evident ischaemic heart disease (or) CHF (Fellner et al., 1993; Metivier et al., 2000). Patients with higher haemoglobin levels showed a significantly reduced left ventricular end-diastolic diameter haemodynamic status (Mocks, 2000). It also increases hypertension and results in some adverse events (Cody et al., 2001). The decreased dosage of RHUEPO provides an additive and rapid effect in the correction of renal anaemia during the pre-dialysis period (Eknoyan, 2001). The reduction in EPO causes delayed graft function (or) graft thrombosis and also reduces sensitisation as a result of random blood transfusion (Muirhead, 1999). A recent study in Sweden has shown that pre-transplant correction of haemoglobin reduced the necessity of post

– operative blood transfusion with no evidence of worsening the transplant outcome (Linde et al., 2001).

There exists an evidence for a relation between anaemia and cardiac morbidity and mortality (Horwich et al., 2002). The random studies revealed that CHF is diagnosed in many people and became the risk of death (Clellan et al., 2002). Mild anaemia in diabetics and non-diabetics resulted in an improved cardiac function (Silverberg et al., 2003). Heart failure is one of the results due to the decrease in the haemoglobin levels (Besarab et al., 1998). The severity of anaemia increases with the worsening of congestive cardiac failure (Silverberg et al., 2002). The potential mechanisms by which anaemia would worsen CHF include exacerbation of myocardial and peripheral hypoxia, increased venous return and cardiac work and consequent left ventricular hypertrophy (Al-Ahmad et al., 2001). Hypoxia could also potentially lead to activation of neurohormones and cytokines. In turn, cytokines can exacerbate the anaemia, leading to a vicious cycle, the recently coined cardiorenal anaemia syndrome (Means et al., 1999). The increased production of EPO in CHF patients with anaemia may reflect the presence of renal hypoxia and a compensatory attempt to augment O₂ delivery to peripheral tissues through erythrocytosis (Volpe et al., 1994; Kumagai et al., 1999). Large doses of EPO results increase in blood pressure (Silverberg et al., 2001), and there is no effect on resting or exercise blood pressure (Maschio, 1995; Fishbane et al., 1995).

Apoptosis has been implicated as a mechanism that contributes to the loss of cardiomyocytes in CHF and ischaemic injury. (Olivetti et al., 1997; Siren et al., 2001). The implication of recombinant human erythropoietin also has beneficial effects on these anaemic diseases as well. (Saraste et al., 1997). Many experiments with mice which included the knocking out of the gene expressing EPO resulted in ventricular hypoplasia (Vander Meer et al., 2003). The defect in the expression of EPO gene results in the effect of EPO on angiogenesis (Ashley et al., 2002). Recent studies have been conducted to evaluate the possible effect in cardiac ischaemia and myocardial infection assessed by the role of EPO *in vitro* and *in vivo* with adult rat models (Calvillo et al., 2003).

Low level expression of EPO and its receptors has since long been reported in the brain (Tan et al., 1991; Marti et al., 1996). High doses of RHUEPO was found to be neuro protective in different models of brain injury, even when the application was started several hours after the injury (Brines et al., 2000). In general, administration of EPO protects retinal neurons from acute ischemia-reperfusion injury (Junk et al., 2005). EPO may in addition, become an emergency medication, in patients with stroke or traumatic brain injury.

Other than the several anaemias (Table 1), there exists some other disorders such as haemolysis i.e., the fall of haemoglobin as a result of RBC disorders like hereditary spherocytosis and haemoglobinopathies (Tchernia et al.,

2000; Shapira et al., 2001) and neuroprotection i.e., the use of RHUEPO has been shown to limit the degree of ischaemic cerebral damage and spinalcord injury, together with expending neurological recovery (Cerami, 2001; Gorio et al., 2002).

Lack of EPO also results in the decrease in the functioning of skeletal muscle and O₂ use as well as endothelial function (Mohan et al., 1999). The decreasing levels of EPO have a drastic effect on the exercise capacity, especially in athletes (Ma et al., 1999).

Clinical treatments for malfunctions

There are several clinical treatments other than the introduction of recombinant human erythropoietin therapy. These clinical therapies are designed as medical therapies, surgical therapies and downshifting of risk for new surgical therapy. The medical therapies include the administration of nitrated, mercurial diuretics and other chemicals from early part of the twentieth century. The usage of thiazide diuretics was on the way even before twentieth century (Calduch et al., 1995; Cattaneo et al., 1996). Other chemicals such as nitropruside and hydratazine isosorbide dinitrate combination came into existence as medical therapies. The surgical therapies were done mainly for the treatment of chronic heart failure and severe heart attacks. The artery bypass surgery clarified its role in overcoming the coronary heart disease (Digicaylioglu et al., 1995; Dobbing et al., 1999).

The downshifting of risk for new surgical therapy recognizes the success or new surgical process followed in some cases by a cycle of improving definition for these evolutionized therapies (Eckardt et al., 1990). The recommended specific medication of iron, vitamin – B₁₂ and folic acid promotes erythropoiesis (Lawrence Goodnough et al., 1997). Rather than all these therapies, the recombinant human erythropoietin therapy is the forward step-in process leading to recuperate all the anaemic related malfunctions.

CLONING AND PRODUCTION OF RECOMBINANT HUMAN ERYTHROPOIETIN

The production of recombinant human erythropoietin began with a search for the gene coding for erythropoietin (EPO). Two radiolabeled pools of oligonucleotide probes were designed based on amino acid sequence information obtained from human urinary EPO. Each probe, consisting of complex mixtures of 128 short synthetic sequences of DNA, was used to search a human genomic library for clones containing the human EPO gene sequence. To verify that the isolated clones contained the complete functional gene encoding human EPO, these sequences were expressed in Chinese hamster ovary cells, and the secreted recombinant human erythropoietin was purified, characterized, and compared with the human urinary hormone using a variety of differ-

Table 1. Anaemias resulting from lack of erythropoietin.

Disorder	Author
Anaemia associated with bone marrow and stem cell transplantation	Klaesson, 1999
Anaemia of prematurity	Reiter et al., 2000; Ledbetter et al, 2000
Anaemia associated with HIV infection	Volberding, 2000
Anaemia associated with malignancy	Van Bommel et al., 2001; Fernandes Jr. et al., 2001
Anaemia associated with surgery	Yazicioglu et al., 2001; Stovall, 2001

ent techniques. Results of these studies indicate that recombinant human erythropoietin is virtually indistinguishable from human urinary EPO in its biochemical and immunological properties. It is a 165-amino acid protein whose primary sequence is identical to that of urine-derived EPO (Inoue et al., 1995). The gene coding for human erythropoietin was obtained from human genomic DNA. The gene used does not include sequences from regions at 1 (5' of first translated ATG and 2) 3' of the stop codon of the erythropoietin gene. The gene was cloned into an expression plasmid for eukaryotic cells that have a sole expression control elements, the early promoter of SV40 virus and its polyadenylation signal. Recombinant cells resulting from transfection with genetic constructs provided an unexpectedly high level of protein expression of 50 mg of recombinant human erythropoietin per litre of culture medium per day (Kurnet et al., 2003). The subsequent development of large-scale cell culture and production techniques has made available sufficient amounts of recombinant human erythropoietin for clinical use in the treatment of the debilitating anemia that almost invariably accompanies chronic renal failure (Egrie, 1990). The erythropoietin gene, when introduced into chinese hamster ovary cells produces erythropoietin that is biologically active *in vitro* and *in vivo* (Lin et al., 1985).

Stimulation of erythropoiesis by *in vivo* gene therapy

Erythropoietin is the principal regulator of erythropoiesis. To evaluate the concept that *in vivo* gene transfer might be used as an alternative to recombinant human EPO in applications requiring a 1- to 3-week stimulation of erythropoiesis, the replication-deficient recombinant adenovirus AdMLP.EPO was constructed by deleting the majority of E1 from adenovirus type 5, and replacing E1 with an expression cassette containing the adenovirus type 5 major late promoter (MLP) and the human EPO gene, including the 3' cis-acting hypoxia response element. *In vitro* studies showed that infection of the human hepatocyte cell line Hep3B with AdMLP.EPO resulted in a 15-fold increase in EPO production in 24 h that was enhanced to 116-fold in the presence of a hypoxic stimulus. One-time *in vivo* administration of AdMLP.EPO (7×10^9 plaque-forming units/kg) to the peritoneum of cotton rats caused a marked increase in

red blood cell production, with a 2.6-fold increase in bone marrow erythroid precursors by day 4, and sevenfold increase in reticulocyte count by day 7. The hematocrit increased gradually, with a maximum of $64 \pm 4\%$ at day 14 (compared with an untreated baseline of $46 \pm 2\%$), and a level of $55 \pm 1\%$ at day 24. Furthermore, one-time subcutaneous administration of AdMLP.EPO caused an increase in hematocrit that peaked at 14 days ($57 \pm 2\%$) and was still elevated at day 42. Hematocrit level in animals receiving subcutaneous administration of AdMLP. EPO sustained a long-term increase compared with animals receiving intraperitoneal administration. (Setaguchi et al., 1994). However considering the complexity and sensitivity of the physiological oxygen dependent control of erythropoietin production in kidneys, it will be extremely difficult to achieve a similar regulation through autoregulated gene therapy (Rinsch et al., 1997). Alternatively, systems of ligand dependent transgene regulation could be used, which involve specific activation of a transgene through different drugs. (Ye et al., 1999). The most important of the currently developed drug systems are those driven by tetracycline, a synthetic antiprogesterin or chemical dimerizers such as rapamycin. With all three systems, iterative regulation of erythropoietin secretion and haematocrit has been demonstrated in experimental animals depending on the systemic application of the respective ligand (Abbruzzese et al., 2000). In the context of these observations, gene therapy with a single administration of an adenovirus vector containing the human EPO gene may provide a means of significantly augmenting the circulating red blood cell mass over the 1- to 3-week period necessary for many clinical applications (Eckardt et al., 2001).

Potential of RHUEPO therapy

There are currently four different RHUEPOs: α , β , δ and ω . However, only EPO- α and EPO- β , are commercially available in the UK. Although these RHUEPOs act on the same erythropoietin receptor, there are some variations on the degree of glycosylation which lead to the differences in the pharmacokinetics and pharmacodynamics among the RHUEPOs (Macdougall, 2001). It requires a less frequent dosing schedule and produces a similar clinical outcome and safety profile as RHUEPOs in treating anaemia of chronic renal disease and of malignancy.

nancy (Locatelli et al., 2001; Glaspy et al., 2001). Another strategy to enhance the biological activity of RHUEPO is to provide a "protective vehicle" so as to decrease the rate of elimination, thus prolonging the half-life of RHUEPO (Smith et al., 2001).

The mechanism of action of erythropoietin is essential for the proliferation, differentiation and maturation of RBCs in bone marrow. Moreover, erythropoietin is critical for the survival of RBC progenitors in bone marrow and may also have immunomodulatory activity (Silva et al., 1996). Erythropoietin functions by binding to the erythropoietin receptor, a member of the superfamily of cytokine receptors (Huraib et al., 1997). The number of erythropoietin receptors varies during RBC differentiation. The number of erythropoietin to its receptor results in homodimerisation of the receptor followed by activation of several signal transduction pathways: JAK 2/STAT5 system, G-protein (RAS), calcium channel and kinases (Tilbrook et al., 1999). RHUEPO can also be administered to marrow donors. Engraftment was also obtained in all patients who received the marrow from the donors treated with rhEPO (Akiyama et al., 2005).

Clinical studies have demonstrated that the subcutaneous route offers a few advantages over intravenous administration for instance; subcutaneous administration is more convenient as it does not require any venous access (Macdougall, 1998). A large dose of RHUEPO may be required to maintain the same haemoglobin level if RHUEPO has to be applied intraperitoneally (Johnson et al., 1999). It remains uncertain whether similar measure will be applied to the other recombinant erythropoietins (Ng' et al., 2003).

The frequency of administration by both intravenous and subcutaneous RHUEPO can be given from once daily to thrice, twice and once weekly in renal patients, depending on the clinical status of the patients. Recombinant human erythropoietin is very much used as a vital and novel treatment for several anaemic related malfunctions and disorders.

Increased scope of recombinant human erythropoietin therapy

The increased scope of RHUEPO therapy impairs the factors such as renal dysfunction, hyperviscosity, inflammation, infection, neoplasia, cancer chemotherapy, bone marrow transplantation, surgery, prematurity, pregnancy (Jerry and Spivak, 2002). Several findings suggest that treatment with EPO could be beneficial in anaemic patients with CHF, CRF and CNS. These benefits effects on exercise and quality of life are seen whether the anaemia is true or dilutional in nature (Silverberg et al., 2000; Silverberg et al., 2001).

The novel treatment approaches are irrespective of how different potential future indications for RHUEPO will be, they will altogether increase the total need of the hormone, in particular, since several indications may req-

uire doses much higher than those currently used in the dialysis population. The future of EPO will therefore, very much depend on its price. Expiration of current patents is foreseeable and will probably have a significant impact. Other developments are underway, which are likely to increase the number of providers, may result in alternative and less expensive methods of administration or will even lead to new therapeutic agents capable of mimicking and amplifying the action of EPO (Eckardt, 2001).

CONCLUSION

Based on the effect of recombinant human erythropoietin on its receptor, investigations have been focused on searching for alternatives to enhance and stimulate erythropoiesis. In the future, it is likely to envisage new development which optimizes and maximises erythropoiesis, thus shifting the paradigm of anaemia management. However, it will be necessary to define precisely the cost/benefit relationship for different indications as well as the optimal ways to use EPO and related therapies. The RHUEPO has become a challenge for surgeons, anesthesiologists, and transfusion as well as in combining the use of his product of biotechnology with other conservation strategies. Given the enormous interest in this therapeutic approach, it seems to be promising in the future. Finally, the randomized clinical trails have to prove, if and at which speed, the theoretical advantages will be translated into clinical practice promoting strong calls for continued research.

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