Novel aspects of erythropoietin response in renal failure patients

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Abstract

The invention of recombinant human erythropoietin (rHuEpo) for the treatment of renal anaemia was a hallmark in the care of patients with renal insufficiency. Recently published guidelines (European Best Practice Guidelines, NKF-DQOIQ) have set the target haemoglobin to be reached by treatment with rHuEpo to >11 g/dl. Normalizing haemoglobin levels may reduce morbidity and mortality and improve quality of life in haemodialysis patients. During long-term treatment, most patients will not respond adequately to therapy with rHuEpo alone. The most important confounding factor, limiting the effectiveness of rHuEpo, is absolute or functional iron deficiency, which is now recognized and treated in many dialysis units. However, there are several other adjuvant treatment options which may help to optimize the response to treatment with rHuEpo. A weekly dose of 2–3 mg of folic acid and 100–150 mg of vitamin B6 is recommended for haemodialysis patients on rHuEpo therapy. The addition of 0.25 mg/month of vitamin B12 may be necessary in selected patients. Vitamin C (1–1.5 g/week) was shown to overcome functional iron deficiency in patients with high ferritin levels. The potential increase of oxidative stress induced by intravenous iron therapy may be blunted by concomitant administration of vitamin E (1200 IU). There is clear evidence from the literature that treatment of secondary hyperparathyroidism by vitamin D improves erythropoiesis. The most recently discovered biological effects of rHuEpo include the induction of several genes in endothelial cells as well as a role for erythropoietin in the outcome of plasmad infection. A new erythropoietin-like molecule is novel erythropoiesis stimulating protein (NESP), which is as effective and safe as rHuEpo, with the potential advantage of less frequent dosing.

Keywords: adjuvant therapy; anaemia; chronic renal failure; rHuEpo

Introduction

Erythropoietin (Epo) is a 34 kDa glycoprotein, which is indispensable for the proliferation, differentiation and survival of erythroid progenitors and precursor cells [1]. In 1985 the human Epo gene was cloned [2], providing the basis for correction of renal anaemia in patients with end-stage renal disease [3,4].

In this article we will focus on four important issues related to therapy with recombinant human Epo (rHuEpo):

1) potential benefits of increasing the target haemoglobin into the normal range;
2) adjuvant therapies which improve the response to rHuEpo;
3) novel biological effects of rHuEpo; and
4) new treatment options for renal anaemia.

Target haemoglobin

Two major recommendations for treatment of renal anaemia were published: in 1997 the NKF-DQOI guidelines [5], and in 1999 the European Best Practice Guidelines [6]. Both guidelines recommend maintenance of the target haemoglobin at >11 g/dl, with or without an upper limit. The question therefore arises as to why we should keep the haemoglobin level >11 g/dl?

During the last few years a considerable body of evidence has accumulated in the literature, showing that haemodialysis patients benefit from increasing and normalizing haemoglobin with regard to quality of life, a reduction in hospitalization and a reduction in mortality.

The Spanish quality of life study clearly showed that increasing the haematocrit has a beneficial effect on quality of life and is safe [7]. In this study, 156 patients from 34 dialysis units were followed for...
6 months, during which time the haemoglobin was raised from 10.2 ± 0.7 to 12.5 ± 0.9 g/dl. The authors examined quality of life, potential adverse events of rHuEpo, and frequency and length of hospitalizations. There was an improvement in quality of life as determined by the Sickness Impact Profile (SIP) score (8.9 ± 1.4 to 7.3 ± 1.3; P < 0.001) and by the Karnovsky scale (75.6 ± 2.7 to 78.4 ± 2.8; P < 0.01). There was no worsening of hypertension and a significant reduction in frequency (19 to eight hospitalizations; P < 0.05) and length (152 to 47 days; P < 0.05) of hospitalization. In summary, this study shows that the increase of a mean haemoglobin of > 2 g/dl may be associated with improved patient outcomes. However, one has to consider that this multicentre trial was an uncontrolled observation.

The association of haematocrit and risk for hospitalization was also examined in a large retrospective analysis including 71 717 US patients on haemodialysis [8]. In this study the risk for hospitalization was set at 1.0 for patients with a haematocrit of 30–33%. A haematocrit of < 27% increased this unadjusted risk by 1.3 (95% confidence interval (CI): 1.26–1.34). A decrease of the risk for hospitalization by 0.89 (95% CI: 0.86–0.91) was observed in patients with a haematocrit between 33 and 36%. In a comparable study by the same authors, the mortality of diabetic and non-diabetic patients also decreased with increasing haematocrit [9]. The strength of both studies is the large patient number. However, the power of these studies is compromised due to their retrospective nature.

Because the benefits and risks of normalizing haemoglobin levels in haemodialysis patients without symptomatic cardiac disease were unknown, 146 haemodialysis patients from Canada with either concentric left ventricular hypertrophy or left ventricular dilation were randomly assigned to a target haemoglobin level of 10 or 13.5 g/dl [10]. After 48 weeks of epoetin treatment, normalization of haemoglobin did not result in regression of established concentric left ventricular hypertrophy or left ventricular dilation. It might, however, have prevented the development of left ventricular dilation, and it resulted in an improved quality of life [10]. These data suggest that early correction of renal anaemia is probably needed in order to reduce development of left ventricular hypertrophy or left ventricular dilation.

**Adjuvant therapies to rHuEpo treatment**

In the European Best Practice Guidelines, iron and other potential adjuvant therapies to rHuEpo therapy have been summarized [6]. The most important treatment options are shown in Table 1.

**Iron**

Iron deficiency is the most important reason for a poor or suboptimal response to therapy with rHuEpo [11–14]. Iron deficiency, functional or absolute, can be treated and prevented by adequate monitoring of iron status and iron therapy [11,12].

The diagnosis of absolute or functional iron deficiency relies on the measurement of serum ferritin levels, the transferrin saturation and the percentage of hypochromic red blood cells [15]. However, the diagnosis in the individual patient is sometimes not without problems [16]. Another parameter of iron metabolism, the soluble transferrin receptor (sTFR), is a valuable tool for diagnosis of iron deficiency in patients with anaemia of chronic disease [17]. However, in the setting of concomitant therapy with rHuEpo and iron, the diagnostic value of the measurement of sTFR is limited due to the increase in serum levels during enhanced erythropoiesis and during development of iron deficiency [18].

According to the European Best Practice Guidelines, ferritin levels should be kept between 200 and 500 ng/ml. The saturation of transferrin is recommended to be 20–40%, and the percentage of hypochromic red blood cells should be < 10% [6].

The European Best Practice Guidelines also suggest that 1000 mg of elemental iron should be given over 6–10 weeks in case of iron deficiency. In the maintenance phase, the weekly dose is 25–100 mg, resembling 10–20 mg per haemodialysis treatment or 50–100 mg every 2 weeks [6].

An important issue is the cost effectiveness of combined rHuEpo and iron therapy, because intensive iron therapy can reduce rHuEpo outlays by 30–70% [13]. In a recent study conducted by Besarab et al. [19], rHuEpo requirements decreased by ~40% during aggressive iron substitution in 42 haemodialysis patients. The haemoglobin was kept constant and the iron use increased dramatically. Overall, a cost reduction of US$1308 per patient per year could be achieved. However, the cut-off point for ferritin levels (1500 ng/ml) in this study would not be accepted by many nephrologists.

**Safety of iron therapy**

Iron overload may be associated with an increased risk for infectious complications [20]. In this context, several pivotal functions of polymorphonuclear leukocytes were shown to be impaired in iron-treated chronic haemodialysis patients with ferritin levels > 650 ng/ml [21]. Another important issue is the potential association of iron overload with cardiac disease [22,23].

**Table 1. Most important issues related to the improvement of the response to rHuEpo in renal failure**

<table>
<thead>
<tr>
<th>Treatment of iron deficiency</th>
<th>Adequacy of dialysis</th>
<th>Therapy with vitamin D metabolites</th>
<th>Subcutaneous administration of rHuEpo</th>
<th>Correction of folate and vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency</th>
<th>Administration of vitamin C in case of functional iron deficiency</th>
<th>Reduction of oxidative stress by vitamin E</th>
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**Iron deficiency, functional or absolute, can be treated and prevented by adequate monitoring of iron status and iron therapy [11,12].**
There may be also acute, life-threatening reactions to injections of iron compounds [24,25]. However, Eschbach et al. [26] recently showed that the use of non-iron dextran preparations is safe. They performed a prospective cross-over, blinded, placebo- and historically controlled study to characterize adverse events and to identify the rate of life-threatening reactions using the sodium ferric gluconate complex. A large number (1117) of haemodialysis patients have been treated with both placebo and a single intravenous bolus of 125 mg iron as sodium ferric gluconate complex (undiluted, over 10 min and without a test dose). Seventy-three of these patients (6.5%) had a history of iron dextran sensitivity. The rate of blinded ‘life-threatening’ reactions was 0.09% (1/1117) and consisted of immediate flushing, diaphoresis, nausea, vomiting, back pain and wheezing. Subcutaneous epinephrin and diphenhydramin were required. Another patient developed hypotension (80/40 mmHg) and required hypertonic saline. It was concluded that the low incidence of reactions to sodium ferric gluconate complex represents an important advance as compared with iron dextran [26].

At present it appears to be wise to avoid overtreatment with iron in renal failure patients, not allowing elevation of ferritin levels to > 800–1000 ng/ml.

Vitamins and rHuEpo therapy

B vitamins and folic acid. The importance of folic acid and vitamin B therapy for optimizing response to rHuEpo is unclear. Most authorities claim that folic acid and vitamin B12 deficiencies should be treated. Unfortunately, it is unclear how to diagnose folate or vitamin B12 deficiency in the renal failure setting. A recently published consensus report recommends supplementation for haemodialysis patients, particularly those on high-flux dialysis, with some of these vitamins. For rHuEpo treated patients, the following recommendations were made: 100–150 mg of vitamin B6 per week, 0.25 mg vitamin B12 per month, and 2–3 mg of folic acid per week [27].

A sensitive marker for vitamin B12 or folate deficiency is the plasma concentration of total homocysteine (tHcy), which will rapidly decrease following vitamin therapy in many patients [28]. However, a short course of high-dose folic acid therapy lowered tHcy plasma levels but failed to improve erythropoietic response in haemodialysis patients with stable haemoglobin levels [29].

Vitamin C. It has been shown that intravenous administration of vitamin C can overcome resistance to erythropoietin therapy in haemodialysis patients with functional iron deficiency [30–32]. The workshop recommendation included the advice to give 1–1.5 g of vitamin C orally or to administer 300 mg of vitamin C intravenously three times a week to haemodialysis patients [27]. At present, there are no long-term data available, since the studies on intravenous vitamin C lasted only 8 weeks. Nevertheless, these studies demonstrated the effectiveness of vitamin C therapy in increasing haemoglobin levels by the mobilization of iron. Plasma oxalate levels did not increase during these short-term studies [31].

Vitamin D. Parathyroidectomy improves erythropoiesis in renal failure patients and several studies have shown that treatment with vitamin D improves anaemia and the response to rHuEpo [27]. Calcitriol was given to 28 haemodialysis patients by Goicoechea et al. [33]. Response to treatment, in terms of a decrease of elevated serum PTH levels, was paralleled by an increase of haemoglobin from 10.6 to 12.5 g/dl. Similarly, Albitar et al. observed an increase of haemoglobin from 8.7 to 10.3 in 12 haemodialysis patients treated with alfacalcidol [34].

Thus, treatment of secondary hyperparathyroidism improves anaemia, suggesting an indirect effect of calcitriol via suppression of PTH. It has, however, been shown that vitamin D metabolites enhance maturation and proliferation of stem cells, suggesting also a direct effect of these compounds. Overtreatment with vitamin D should be avoided to reduce the risk of development of adynamic bone disease [27].

Vitamin E. Haemodialysis therapy can induce oxidative stress. In a study from Japan, the authors have shown that vitamin E-coated dialysers reduce haemodialysis-induced oxidative stress [35]. Twelve patients were haemodialysed with or without a vitamin E-coated membrane. There was a significant increase in oxidized low density lipoprotein (LDL) during haemodialysis, which was attenuated using vitamin E-coated dialysers. In the same manner, flow-mediated vasodilatation was impaired during conventional dialysis and improved during dialysis with a vitamin E-coated membrane [35]. Reduced DNA damage and improved immune function of leukocytes were also attributed to vitamin E-coated membranes by Tarng et al. [36]. It was speculated that this kind of membrane may reduce the risks of cancer and infection in chronic haemodialysis patients [36].

Following intravenous injections of high molecular weight iron complexes, low molecular weight potential toxic iron complexes can be formed and detected in serum by the bleomycin method [37]. In a study from Austria, a single oral dose of vitamin E reduced the formation of malondialdehyde and of bleomycin-detectable iron following intravenous injection of iron sucrose in 22 haemodialysis patients. The authors concluded that vitamin E may attenuate oxidative stress induced by intravenous iron in haemodialysis patients [38]. Whether vitamin E therapy enhances the response to rHuEpo in uraemic patients needs to be demonstrated.

New aspects of biological effects of rHuEpo

Erythropoietin and the endothelium

Although rHuEpo exerts many beneficial effects in reversion of anaemia, pharmacological doses of
rHuEpo may show side-effects arising from the vascular system. A complication of rHuEpo therapy is the development or aggravation of arterial hypertension, which has been observed in about one-third of end-stage renal disease patients maintained on haemodialysis and rHuEpo treatment [3–8]. Other side-effects that are possibly attributable to treatment with rHuEpo include thrombotic events [9] and seizures [5,6]. The effect of intense rHuEpo therapy in patients with cardiovascular disease is not clear at present. The underlying mechanisms, however, are largely unknown.

To elucidate downstream events following erythropoietin receptor triggering in endothelial cells, a differential display mRNA analysis was performed [39]. Eight genes were identified that were upregulated by rHuEpo. The genes coded for proteins that may be assigned to four different groups: (i) proteins implicated in the regulation of vascular functions (thrombospondin-1, 20 kDa myosin regulatory light chain); (ii) gene products involved in gene transcription and/or translation (c-myc purine-binding transcription factor PuF, tryptophanyl-tRNA synthetase, S19 ribosomal protein); (iii) subunits of mitochondrial enzymes related to energy transfer (NADH dehydrogenase subunit 6, cytochrome C oxidase subunit 1); and (iv) a regulator of signal transduction, protein tyrosine phosphatase G1. This study provides new insights into the molecular changes induced by rHuEpo in human vascular endothelial cells. We could show that several genes may be activated in human endothelial cells, which may contribute to potential side-effects of rHuEpo therapy.

Erythropoietin and malaria

During plasmodium infections there is a high female-to-male ratio of parasites, which is paralleled by Epo suppression. Furthermore, immune response and recovery are better in animals with a higher male ratio of parasites, which coincides with intense host erythropoietic activity.

Paul et al. [40] showed that hypoxia, phlebotomy and mouse Epo increase the number of male parasites, which was associated with a more favourable course of malaria. Thus, natural and artificial induction of erythropoiesis in vertebrate hosts provoked a shift towards male parasite production. This change led to a reduced reproductive success in the parasite, which suggests that sex determination is adaptive and is regulated by the haematological state of the host. Whether or not this observation has implications for the clinical situation remains to be clarified.

New treatment options for renal anaemia

A new erythropoietin-like molecule that will enter the US American and European market in the near future is novel erythropoiesis stimulating protein (NESP) [41]. NESP is a hyperglycosylated analogue of rHuEpo and has a slightly different amino acid sequence. The molecular weight is 38 kDa and it stimulates erythropoiesis by binding to the erythropoietin receptor in a similar fashion as rHuEpo. In vitro studies showed that the affinity of NESP for the receptor is only marginally less than for rHuEpo. The pharmacology of NESP was authoritatively summarized by Macdougall [42]. The main difference to rHuEpo is the terminal half-life of NESP, which is prolonged by factor 3 and allows for less frequent dosing (once every week or fortnight) of NESP [43]. Clinical data, until now only presented in abstract form, have shown the efficacy and safety of NESP [42,44,45]. Dose finding studies in haemodialysis and peritoneal dialysis patients with an open label dose escalating design examined the efficacy of NESP. The main conclusions of these two studies were: (i) NESP is safe and effective in the treatment of renal anaemia; (ii) the optimal weekly dose is 0.45 µg/kg body weight per week; and (iii) once-weekly dosing with NESP is possible for both intravenous and subcutaneous administration [42,44,45]. In subsequent studies, NESP was as effective as rHuEpo in maintaining haemoglobin levels in chronic haemodialysis patients. There was no difference between NESP and rHuEpo for adverse events. Furthermore, no antibodies to NESP or rHuEpo were detected [46]. The first clinical studies also confirmed that the conversion algorithm for NESP from rHuEpo, exchanging 200 IU of rHuEpo with 1 µg of NESP, works satisfactorily [46]. In summary, NESP appears to be a novel, interesting therapeutic option with advantages for renal failure patients due to less frequent dosing.

References