ERYTHROPOIETIN – AN OUTSTANDING SUCCESS FOR GENETIC MODIFICATION

*R.M. Temple**

HISTORICAL BACKGROUND

Just over a century ago, Viault, a French physician, established the link between low atmospheric oxygen at high altitude and rapid physiological acclimatisation as a result of increased red cell production. Viault venesected himself, several mining employees, a llama and a 'lively little bitch' during a 15-day train journey from Lima at sea level to Morococha, a mining town at an altitude of almost 4,400 metres in the Peruvian Andes, and demonstrated profound increases in red cell counts.[1] Early this century, Carnot and Deflandre reported animal experiments supporting the presence of a circulating factor with the potential to influence erythropoiesis.[2]

Four decades later, an elegant parabiotic animal model confirmed humoral control of erythropoiesis.[3] In two rats joined by a vascular union it could be demonstrated that when one rat was rendered hypoxic by breathing air at low oxygen pressure an erythropoietic signal (erythropoietin) was transmitted to the normal parabiotic partner via the blood stream. Nephrectomised rats failed to produce erythropoietin in response to anaemia implicating the kidney as the major site for production of this factor.[4]

Over the next two decades tantalising glimpses of the therapeutic potential of erythropoietin were demonstrated in animals,[5] but investigation was hampered by the restricted amount of the hormone available. The initial breakthrough, which ultimately led to erythropoietin becoming available in abundance, was made by Miyake and co-workers.[6] In a heroic study this group purified sufficient human erythropoietin from 2,550 litres of urine from patients with aplastic anaemia to determine partial amino-acid sequences of this protein, leading subsequently to isolation and cloning of the erythropoietin gene.[7,8] Using a plasmid vector the human gene was transfected into a mammalian cell line, the Chinese hamster ovary cell, to provide recombinant human erythropoietin (rHuEPO) in unlimited quantities.

THE ANAEMIA OF CHRONIC RENAL FAILURE

The development of dialysis therapy for end-stage renal failure from an experimental technique in the 1960s to standard therapy in the 1980s is one of the great medical advances of this century. However, the success of chronic haemodialysis exposed the limitations imposed by the anaemia that accompany such therapy and restricted rehabilitation. Anaemia is almost invariable in patients with chronic renal failure and is characteristically normocytic, normochromic and hypoproliferative. The principal cause for this anaemia is deficient erythropoietin production by the failing kidney. In the healthy adult man, 90% of erythropoietin is produced by the kidneys and 10% by the liver.[9]

**Consultant Nephrologist, Birmingham Heartlands Hospital*

Prior to rHuEPO becoming available, nephrologists relied on repeated blood transfusion to ameliorate chronic anaemia. In addition folate, parenteral iron, androgens and even cobalt chloride were used to improve haemoglobin concentration. However, many patients became transfusion-dependent. Repeated blood transfusion was associated with an increased risk of HLA sensitisation (reducing the potential for transplantation), iron overload, transmission of viral infection and suppression of endogenous erythropoiesis.[10] Although androgens partially corrected anaemia, their use was limited by potential adverse effects of virilisation, disordered liver function and the possibility of hepatocellular carcinoma.[11]

THE EARLY CLINICAL TRIALS OF RECOMBINANT ERYTHROPOIETIN

The first clinical trials of recombinant human erythropoietin (rHuEPO) were commenced in 1985 in Seattle by Eschbach,[12] and in 1986 in London and Oxford by Winearls.[13] Treatment was highly effective and benefits included improved well-being, increased exercise tolerance and the abolition of the need for regular transfusion. A pronounced fall in serum ferritin was noted and Eschbach's group also reported 'functional' iron deficiency, attributed to the demand for iron for haemoglobin synthesis exceeding the ability of the reticuloendothelial system to supply it.[12] The reduced erythropoietic response in patients with functional iron deficiency could be restored by the administration of parenteral iron.

Given the long anticipated therapeutic role of erythropoietin perhaps the most striking finding of these pioneering studies was not the haematological effect of treatment but the range of adverse effects reported. In these early clinical trials, hypertension occurred in one third of patients with, more disturbingly, the occurrence in some patients of uncontrolled hypertension, encephalopathy and seizures. There were also concerns about dialysis clearance in patients receiving rHuEPO. Severe hyperkalaemia was reported in two patients studied by Eschbach's group[12] other minor adverse effects included flu-like symptoms with transient aches and pelvic and limb pain, chills and sweating.

rHuEPO IN CHRONIC RENAL FAILURE PATIENTS

rHuEPO is now established treatment for anaemia of chronic renal failure in dialysis and pre-dialysis patients. Its clinical use has proved remarkably effective, abolishing symptoms of anaemia and improving well-being and exercise tolerance. In pre-dialysis patients there is no evidence that rHuEPO accelerates the progression to end-stage renal failure.

Dialysis patients are generally more anaemic than patients whose renal failure is less severe due to the more profound deficiency of endogenous erythropoietin, uraemia, blood loss (occult and haemodialysis-related) and reduced red cell survival. The proportion of pre-dialysis patients receiving regular treatment with rHuEPO in the UK is small and is

influenced and controlled by regional prescribing protocols. However, rHuEPO has become recommended standard therapy for dialysis patients particularly those maintained on haemodialysis, and it is estimated that up to 75% of these patients would benefit from treatment.[14] As experience with rHuEPO has grown within renal units the decision to initiate treatment has become based more on symptoms related to anaemia, rather than a specific threshold haemoglobin concentration. In general, rHuEPO is recommended when the haemoglobin falls below 9 g/dl or when patients are intolerant of more modest anaemia, particularly those with angina and cardiac failure.

Recommended starting doses are 50 international units (i.u.)/kg bodyweight three times per week when given by the intravenous route or 50 i.u./kg bodyweight twice weekly subcutaneously (s/c). In practice the contents of a complete vial or pre-filled syringe are given, with 3,000 units twice weekly s/c being a common initial dose. Subsequently, the dose is titrated as necessary, to achieve an ideal rate of haemoglobin response of 1 g/dl/month. Most patients in the UK receive rHuEPO by the subcutaneous route and overall the evidence suggests that this is the more economical route.[15] The annual cost of rHuEPO for a patient maintained on 4,000 i.u. twice-weekly in the UK is approximately £4,000. This is a recurring cost, as treatment is indefinite unless the patient receives a renal transplant, which adds considerably to the financial burden of end-stage renal disease.

The optimum target haemoglobin concentration for patients receiving rHuEPO is uncertain. In 1989 the National Kidney Foundation recommended a target haematocrit of 30% (haemoglobin 10 g/dl)[16] as sufficient to ameliorate many of the symptoms of anaemia and perhaps being associated with a lower risk of side-effects from rHuEPO than higher target levels. Current target haemoglobin concentrations are commonly in the range 10-13 g/dl. There is an increasing trend to individualise target haemoglobin with higher levels being chosen for younger dialysis patients seeking unrestricted effort tolerance and patients with cardiac disease.

rHuEPO hyporesponsiveness and iron deficiency

Many factors other than erythropoietin deficiency may cause anaemia in uraemic patients; the most common of these is unrecognised iron deficiency. Iron deficiency must be sought in all patients before commencing rHuEPO. Absolute iron deficiency (serum ferritin < 30 µg/l) should be corrected with intravenous iron before rHuEPO is commenced. However, the storage iron required to expand the red cell mass is considerable and the assessment of iron stores is not straightforward. Serum ferritin may not accurately estimate the amount of iron available for erythropoiesis during rHuEPO treatment. In part this is due to ferritin being an acute phase reactant which will therefore rise non-specifically in the presence of acute or chronic inflammation. Haemoglobin correction of 5 g/dl requires at least 750 mg of storage iron,[17] which roughly equates to a ferritin of 100 µg/l. This serum ferritin value is considered to represent satisfactory storage iron prior to initiation of rHuEPO. Nevertheless, during rHuEPO treatment some patients with baseline ferritin values above this threshold fail to mobilise storage iron fast enough and develop a 'functional' iron deficiency. The transferrin saturation (serum iron divided by the total iron binding capacity expressed as a percentage) reflects iron that is readily available for erythropoiesis. A fall in transferrin saturation to below 20% is an important indicator of functional iron deficiency. Values for the percentage of hypochromic red cells greater than 10% are also a useful indicator of iron deficiency. Although there is no single ferritin or transferrin saturation value which conclusively indicates functional iron deficiency the lower the values of each, the greater is the likelihood of iron deficiency.

Many nephrologists have lost faith in chronic oral iron supplementation in patients receiving rHuEPO due to poor compliance and the small amount of iron absorbed. Intravenous iron supplementation has become standard with protocols for regular or intermittent infusions governed by the response to rHuEPO and serial assessment of iron status. Generally parenteral iron is given as a series of 6-10 intravenous infusions providing up to 1,000 mg of elemental iron over ten weeks. Total dose infusion protocols have not been utilised, possibly due to concerns about iron overload and dose-related side-effects associated with older parenteral iron formulations including arthralgia and hypotension.

Other potential causes of hyporesponsiveness to rHuEPO include chronic occult inflammation, malignancy, haemorrhage and bone disease secondary to severe secondary hyperparathyroidism and aluminium intoxication. Any condition causing an acute phase response also has the potential to inhibit erythropoiesis and C-reactive protein (CRP) is commonly used as a screening test for such conditions.

Adverse effectsof rHuEPO

The early concerns about a wide variety of adverse effects associated with the use of rHuEPO have not been substantiated, and treatment has proved remarkably safe. The common major adverse effect is hypertension reported in approximately 35% of haemodialysis patients participating in large trials.[18] The hypertension has been attributed to increases in total peripheral resistance following amelioration of hypoxic vasodilatation.

Despite adoption of higher target haemoglobin levels, reports of hypertensive encephalopathy and seizures are now much less frequent. rHuEPO treatment protocols place considerable emphasis on slow correction of anaemia with an ideal increment of 1 g/dl/month, and this approach together with greater awareness and prompt treatment of hypertension may have influenced the frequency of reported adverse effects.

Flu-like symptoms occasionally occur following initial injections but are not troublesome enough to require a change in dosage. Although early reports suggested that higher haematocrit values may lead to underdialysis, no clinically significant deterioration in biochemical control has been confirmed. Early trials also suggested an increased risk of thrombosis of arteriovenous dialysis fistulae. There does appear to be a slightly increased incidence of fistula thrombosis, but this is almost invariably in access sites which are already compromised.[18]

THE USE OF rHuEPO IN NON-RENAL ANAEMIA

The advent of reliable correction of renal anaemia with rHuEPO has led to investigation of its potential use in non-uraemic patients. Assays for serum erythropoietin concentrations, now widely available, indicate that some

non-renal anaemias are associated with an inadequate erythropoietin response to anaemic hypoxia. Anaemic patients with serum erythropoietin concentrations of < 500 i.u./ml seem to have a greater likelihood of a response to therapy.[19]

The normocytic, normochromic, hypoproliferative anaemia, which characterises many chronic diseases including malignancy, inflammatory bowel disease, AIDS during treatment with AZT, and rheumatoid arthritis has been shown to respond to rHuEPO.[20-23] Patients with a variety of malignancies also responded to rHuEPO in a large randomised placebo-controlled trial.[24] The response rate was greater in those patients not receiving chemotherapy and the dose used, 100-150 i.u./kg s/c three times per week, was considerably higher than standard doses used to correct renal anaemia.

rHuEPO has also been used successfully to augment the amount of blood that can be removed in the weeks prior to surgery to make blood available for peri-operative autologous transfusion.[25] The administration of rHuEPO for several days prior to elective orthopaedic surgery has also been shown to reduce the risk of severe anaemia and the requirements for blood transfusion.[26]

rHuEPO – THE FUTURE

Reliable sustained correction of the chronic anaemia in patients with end-stage renal failure has transformed their rehabilitation prospects. A new paradigm for haemoglobin concentration in dialysis patients has revealed a previously unrecognised contribution of anaemia to impaired rehabilitation, particularly in relation to intellectual function.[27]

Two outstanding questions remain unanswered:

- What is the optimal target haemoglobin (Hb) for patients receiving rHuEPO?
- Can rHuEPO reduce the cardiovascular mortality and morbidity that blights the lives of patients with end-stage renal failure?

Since a target haematocrit of 30% was recommended in 1989,[16] revision upwards has been modest. An opinion poll conducted amongst nephrologists at a meeting to review the use of erythropoietin in 1994 revealed a consensus favouring a target haemoglobin of 10-12 g/dl.[28] The target haemoglobin at which the patient derives optimal benefit and the least risk of adverse effects remains uncertain. Inevitably there are economic considerations. Increasing the target haemoglobin from 9.5-11 g/dl to 11.5-13 g/dl in the Canadian erythropoietin study required a mean of 44 i.u./kg/week more rHuEPO per patient.[29] If adverse effects are not a major concern, the question becomes: what is the target Hb above which additional benefit is marginal and increased dosage not justified?

Ironically a large controlled prospective study was recently stopped prematurely because of concern about adverse effects. One thousand two hundred and thirty-three haemodialysis patients with clinical evidence of cardiac disease were recruited to the Amgen trial in the US and randomised to a target haematocrit of either 42 or 30 (haemoglobin of 14 or 10 g/dl). The trial was halted after 29 months because an excess number of deaths and non-fatal myocardial infarctions in the normal haematocrit group.[30] However the difference between primary end-points occurring in each group (time to death or first non-fatal myocardial infarction) did not reach statistical significance. Higher haematocrit values in both groups were associated with lower mortality and the trial outcome could not explained by differences in haematocrit or rHuEPO dosage. More patients in the normal haematocrit group received intravenous iron dextran and in greater quantities, and this treatment was associated with an increased risk of death in this group. Higher iron stores may predispose to an increased risk of myocardial infarction[31] and infection,[32] and an excess number of deaths due to infection were noted in the normal haematocrit group. Hopefully these issues will be clarified by two large prospective trials assessing the effect of normalisation of haemoglobin, currently being conducted in Canada and Scandinavia.

Support for the concept that more haemoglobin is not necessarily better, has come from one of the very few placebo-controlled trials of rHuEPO. In an elaborate multi-centre study the Canadian Erythropoietin Study Group failed to show a significant difference in quality of life or exercise capacity in patients randomised to a target haemoglobin of 11.5-13 g/dl compared to 9.5-11g/dl.[29]

Cardiovascular disease is the major cause of premature death in patients with end-stage renal disease (ESRD) with a mortality of 9% per year - approximately 30 times the risk of the general population. Even after stratification for age, gender, race, and presence or absence of diabetes, cardiovascular disease remains 10-20 times higher than in the general population.[33]

Three decades of extraordinary advances in the treatment of ESRD have had little impact on the toll of cardiovascular disease. The potential for rHuEPO to have an impact on cardiovascular pathology has roused considerable interest. Left ventricular hypertrophy is a risk factor for coronary artery disease, and has a high prevalence and adverse prognostic effect in ESRD,[34] and although many factors may influence left ventricular hypertrophy, there is a clear relationship with anaemia.[34] Reduction in left ventricular mass, left ventricular wall thickness and end-diastolic diameter have been reported with rHuEPO,[35,36] despite the potential for this treatment to increase blood pressure. Reduction in left ventricular mass is a key recommendation of the National Kidney Foundation Task Force on cardiovascular disease,[37] and a reduction in overall cardiovascular events and angina have been reported in a few small studies. However, as in the debate on optimal target haemoglobin, the results of large prospective trials are awaited.

POSTSCRIPT

Recombinant human erythropoietin is the prodigal son that failed to turn up at the baptism of renal replacement therapy. Decades later renal replacement is post-adolescence and the arrival of the long-lost son is greeted with a great fanfare and not a little trepidation. A spectacular family reunion follows. Initial concerns are largely cast aside and years of family schism forgotten. However the wily family elders agree 'it's good to have him with us, but now the lad needs to earn his keep'.

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