Recombinant human erythropoietin: 10 years of clinical experience

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Abstract  The need for a renewable source of erythropoietin to treat the anaemia of chronic renal failure was first recognized in the 1960s, but cloning and expression of the human gene was not achieved until 1983. Clinical testing of recombinant human erythropoietin (r-HuEPO) began in 1985, leading to the first licence as a therapeutic agent in 1988. The first clinical trials showed that an intravenous dose requirement of about 200 IU/kg/week would increase haemoglobin concentrations to 10–12 g/dl in >90% of haemodialysis patients. Subcutaneous administration has subsequently been found to be effective, and may allow lower maintenance doses. It is now the route of choice in Europe, but not the USA. The best marker of benefit of the introduction of r-HuEPO is the reduction in need for regular blood transfusions. A marked improvement in anaemia-related symptoms has been clearly demonstrated. The most important factor in optimizing the response to r-HuEPO is iron supply. The marrow should be stimulated slowly, to allow mobilization of iron stores. Functional or absolute iron deficiency should be pre-empted by regular iron supplementation. It is also important to recognize resistant states induced by inflammation and bleeding, and to exclude severe hyperparathyroidism, aluminium overload and other haematological diseases. The most important adverse events associated with r-HuEPO are increased blood pressure and a possible increased risk of access failure. These are, however, challenges to improve practice, not reasons to avoid the use of r-HuEPO.

Key words: anaemia, chronic renal failure, erythropoietin, iron

History of the development of r-HuEPO

The availability of recombinant human erythropoietin (r-HuEPO) is now taken for granted. Yet it is worth remembering how long we waited for r-HuEPO in relation to the evolution of renal replacement treatment. Haemodialysis for end-stage renal failure (ESRF) was first described in 1960 but r-HuEPO only became available 28 years later (Figure 1). The need was predicted by Hegstrom et al. in their paper entitled ‘Haemodialysis in the treatment of chronic uraemia’, published in the transactions of American Society for Artificial Internal Organs in 1961, in which they wrote ‘Supplementary erythropoietin, were it available, might reduce significantly the transfusion requirements’ [1].

The management of anaemia before the availability of r-HuEPO was a struggle. Faith was put in ‘good dialysis’, minimizing blood loss, avoiding nephrectomy, administering parenteral iron, pharmacological doses of folic acid, and androgens. Restrictions were placed on sampling for laboratory tests, exercise was advocated and blood transfusions were given as infrequently as possible to avoid suppressing endogenous erythropoietin (EPO) production. All these measures could at best achieve an average haemoglobin of about 8 g/dl but still left about 10% of patients dependent on regular blood transfusion.

The industrial production of r-HuEPO would not, of course, have been possible without the basic research into gene structure, function and manipulation. The efforts of the pioneers in EPO research such as Ersliev, Goldwasser, Cotes, Eschbach and Adamson ran in parallel, allowing the cloning and expression of the human gene in 1983 and the physiological justification for clinical trials in man [2]. It is remarkable that r-HuEPO was administered to man within 2 years of cloning the gene, and licensed as a therapeutic agent within 5 years.

- 1960 → Haemodialysis for ESRF
- 1966 → Brescia-Cimino fistula
- 1978 → CAPD
- 1979 → Vitamin D analogues
- 1985 → Cyclosporin
- 1988 → r-HuEPO

Fig. 1. The advent of r-HuEPO in the evolution of renal replacement treatment for end-stage renal failure.
Review of initial clinical studies

Once the pilot studies were complete, a number of multicentre trials was performed. Reassuringly, the doses of r-HuEPO required to reverse anaemia and to maintain the haemoglobin concentrations were found to be similar in the USA and Europe. It was shown in several hundred patients [3–5] that the intravenous (i.v.) administration of different preparations of r-HuEPO at doses of approximately 200 IU/kg/week would increase the haemoglobin concentration to between 10 and 12 g/dl in >90% of subjects.

Haematological effects of r-HuEPO

In normal subjects the relationship between erythropoietin secretion and erythropoiesis is beautifully balanced, with red cell production sustained at the correct rate to replace red cell loss (Figure 2). The effect of EPO on the erythroid marrow is mediated following binding to specific receptors on erythroid precursors (intermediate-stage erythroid burst-forming units or BFU-E and the erythroid colony-forming unit or CFU-E) that have already differentiated from pluripotential stem cells (see Figure 3). Differentiation to this stage is not dependent on EPO. This has been shown by Wu et al. (1995), who produced ‘knockout’ mice that lacked either the EPO or the EPO receptor genes [6]. Mice with either mutation were incapable of erythropoiesis but committed CFU-E and BFU-E were present in the foetal livers. Thus, the expression of the receptor is itself an essential part of the mechanism by which erythropoiesis is controlled. That the presence of EPO is an absolute requirement for erythropoiesis is obvious; however the mechanism by which this route of differentiation and maturation is favoured is not. It may act as no more than a permissive survival factor, preventing apoptosis and thereby allowing red cell precursors to reach a stage at which they will inevitably mature into red cells [7].

As expected, the erythrogenic studies performed on the first dialysis patients treated with r-HuEPO showed that the total red cell volume was below normal before treatment and increased after treatment. These increases in red cell volume were accompanied by reciprocal changes in plasma volume, so that blood volume was barely changed. Red cell survival in non-transfused patients was only modestly shortened before treatment and was not altered by treatment. In transfusion-dependent patients, red cell survival was much shorter than normal, but this could be explained by the shorter survival of the transfused cells. The erythron transferrin uptakes (ETUs), an accurate measure of erythroid marrow activity, were subnormal before treatment, and an overall twofold increase was observed after treatment was stabilized [8].

Studies of the response of bone marrow erythroid progenitors to r-HuEPO treatment showed that after treatment, the number of bone marrow BFU-E had fallen to a mean of 24% of pre-treatment values, but there was no significant change in the number of CFU-E. The surprising decrease in BFU-E could be explained by depletion of this compartment by EPO-driven maturation to CFU-E without a commensurate replenishment from the pluripotential stem cell pool. The failure to change the numbers of CFU-E would have to be explained by self-renewal and replenishment from the BFU-E, keeping the size of this compartment constant [9].
**Route of administration**

The i.v. route was chosen as a matter of convenience and in ignorance of the pharmacodynamics of r-HuEPO. What is the best route? Continuous ambulatory peritoneal dialysis (CAPD) and pre-dialysis patients have little choice—subcutaneous (s.c.) administration is the only practicable route—but haemodialysis patients do have the i.v. option. Pharmacokinetic studies showed that administration of r-HuEPO by thrice-weekly i.v. pulses would saturate the EPO receptors but, because the half-life of r-HuEPO in plasma is approximately 8 hours, the concentration would fall to baseline levels before the next scheduled administration. Subcutaneous administration of r-HuEPO results in a prolonged elevation of the plasma EPO concentration, which should achieve a greater effect on erythropoiesis. By 1991, 11 of 15 reports comparing both routes of administration had concluded that the latter allowed lower maintenance doses. Since then three prospective, randomized, comparative trials have been performed, yet the matter remains unresolved. Muirhead et al. found that the target haemoglobin was reached more rapidly in patients receiving r-HuEPO s.c. but, although the doses at the time of stabilization and at 24-week follow-up were lower, the difference from the i.v. group at this latter time point was not statistically significant [10]. Hörfl found that switching patients maintained on i.v. r-HuEPO to thrice-weekly s.c. administration allowed a 32% reduction in mean dose but daily s.c. administration allowed no greater dose reduction. Weekly s.c. administration did not allow a reduction in total dose [11]. Taylor et al. found that route of administration made no difference to the rate of response, absolute reticulocyte counts and maintenance doses in 16 haemodialysis patients who completed a randomized crossover study [12].

The most recently reported trial comparing the two routes has given the most striking result [13]. After 6 months of i.v. treatment, the experimental subjects were switched to receive weekly s.c. r-HuEPO at one-third the weekly dose for 10 months and compared with control subjects who continued to receive r-HuEPO i.v. After this second phase, the s.c. group reverted to i.v. administration. There was no significant change in haematocrit in the experimental group during the 10 months of s.c. administration, although one patient had to revert to the i.v. route because of a drop in haematocrit. This study can, however, be criticised on the grounds that it was not randomized and 17 of the subjects failed to complete the study.

Some patients found that s.c. administration caused local pain or discomfort. Blinded comparisons from three centres showed that this was more likely with epoetin-alfa than epoetin-beta [14–16]. A change in the vehicle buffer (substituting phosphate for citrate) has solved this problem. Despite the balance of evidence favouring the s.c. route, most US haemodialysis patients continue to receive r-HuEPO i.v. This is apparently explained by regulations governing reimbursement.

**Secondary benefits**

The best marker of benefit of the introduction of r-HuEPO to clinical practice is the reduction in need for regular blood transfusion [17]. The improvement in symptoms attributable to anaemia is unequivocal and was confirmed in a Canadian randomized, placebo-controlled study [18], among others [19].

There are a number of secondary benefits to removing or ameliorating anaemia in dialysis patients. The cardiovascular system is the chief beneficiary, providing the blood pressure is controlled. Improved oxygen delivery reduces the incidence and severity of angina and improves exercise capacity. There are reductions in left ventricular mass and dimensions, which may have implications for long-term survival.

The haemostatic defect of anaemia is improved by raising the haematocrit. This is usually a benefit but may contribute to thrombosis of faltering vascular access. There are also reports of improvement in sexual function, the immune system and pruritus [reviewed in 20].

**Audit of current usage**

Estimates of the likely numbers of patients who would benefit from r-HuEPO were made for the Oxford Renal Unit in 1988, based on the distribution of haemoglobin concentrations in 225 haemodialysis patients, 69 CAPD patients and 88 pre-dialysis patients with plasma creatinines > 350 μmol/l. What has happened in practice is very close to these predictions (Figure 4).

**Factors affecting the response**

In the early clinical trials of r-HuEPO there was a gratifyingly high and almost uniform response [4,5,21].

Fig. 4. Assuming that a haemoglobin of <10 g/dl would be an indication for r-HuEPO, 67% of CAPD patients (dark bar) and 81% of haemodialysis patients (dark bar) would have been candidates in 1988. The light bars show the actual numbers receiving treatment in 1996.
The situation in clinical practice after licensing has proved rather different; 40–60% of US haemodialysis patients fail to achieve haematocrits of >30% [22]. Audit of our own unit showed that in 30% of patients, the haemoglobin concentrations were <10 g/dl (Figure 5). Why should this be? The first reason is inadequate doses. There is a genuine variation in individual requirement, which may depend on red cell survival and endogenous EPO production. In clinical trials there is no constraint on increasing the dose, whereas in clinical practice each dose increment of 1000 IU/week will cost approximately an extra £500 per annum. The tendency, therefore, is to titrate the dose towards the minimum and clinicians have been very successful in this respect. Average doses are half of the 200 IU/kg/week that were originally predicted (Figure 6). Figure 7 shows the large variation in the relationship between dose and achieved haemoglobin in our unit. One could argue that the patients in panel A who have below average haemoglobins received below average doses of r-HuEPO (100 IU/kg/week) which, if increased, could put them into panel C or D. It is the patients in panel B who need attention for they have below average haemoglobins and they receive above average doses. The reasons that must be considered are shown in Table 1.

Iron deficiency, both absolute and relative, was recognized very early as the most important limiting factor to achieving target haemoglobin levels of 10–12 g/dl at average doses. In the US Amgen multicentre trial Eschbach found that iron deficiency was recognized in as many as 50% of cases.

Infection and inflammation, often not clinically obvious, are the next most important causes. Routine uncomplicated surgery, rejecting previously failed transplants and minor dental sepsis are examples that are commonly encountered in clinical practice. These produce a suppression of erythropoiesis which, when observed in patients with inflammatory diseases, is referred to as the ‘anaemia of chronic disease’. It is explained in part by the effect of interleukin-1 (IL-1) and tumour necrosis factor (TNF), which act on T-cells and marrow stromal cells to release interferon (IFN)-γ and IFN-β both of which inhibit the development of CFU-E to erythroblasts [23] (Figure 8).

Nephrologists sometimes fail to recognize that their patients are suffering from other blood disorders such as thalassaemia, myelofibrosis or even myeloma. Ifudu et al. have shown that under-dialysis is an independent predictor of a requirement for a higher dose of r-HuEPO [22]. The importance of this is that one can take remedial action. Aluminium intoxication is now rare but an increased load below the overtly toxic

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range may shift the dose-response curve adversely [24]. The effect of hyperparathyroidism is more controversial. Rao et al. believe it has a major influence but this is not supported by others [25,26]. There is only one reported case of r-HuEPO resistance explained by an antibody to the hormone [27].

**Adverse events**

At first it was difficult to distinguish which of the adverse events experienced by patients receiving r-HuEPO were coincidental, which are directly related to administration and which a consequence of changes in haematocrit. A small number of patients (approximately 4%) reported 'flu-like symptoms after receiving r-HuEPO [4,5,28] but the Canadian placebo-controlled study found no difference in the numbers reporting this symptom [18]. This may be accounted for by the fact that the initial reports were in patients receiving high doses as a rapid bolus, which was not the case in the Canadian study. This side effect has not been a problem in patients receiving r-HuEPO s.c.

In about one-third of treated patients there was a clinically significant rise in blood pressure, which has been attributed to an increase in peripheral resistance following the relief of hypoxic vasodilatation, and an increase in whole blood viscosity [4,5,29]. In some of the early trials there was a disturbing incidence of hypertensive encephalopathy and seizures [30]. Reports of this are now much less frequent, perhaps because reversal of anaemia is being effected more slowly and clinicians are reacting immediately to any changes in blood pressure.

There does seem to be a slightly higher incidence of vascular access failure after increasing the haemoglobin but this is often in access sites with pre-existing problems, and grafts, rather than A-V fistulae [4,28]. This subject is reviewed by Muirhead et al. [31]. In this context the results of the US Amgen Normal Haematocrit study will provide data on the relationship between target and achieved haematocrit and the risk of vascular access failure.

Small increases in pre-dialysis concentrations of creatinine, phosphate and potassium were observed. Apart from potassium [32] these were not usually clinically significant and probably reflected changes in diet and dialysis efficiency [4,5,33]. A more precise assessment of the effect of haematocrit on dialysis was conducted by Movilli et al. [34], who showed a decrease in Kt/V in seven patients whose haematocrits rose following r-HuEPO treatment.

**Conclusion**

It has been said that r-HuEPO is the single most important advance in the management of ESRF in the last 20 years. Although the ‘downsides’ of r-HuEPO use continue to exist for nephrologists, these are challenges to improve practice not reasons to avoid its use.

**References**


