Erythropoiesis-stimulating Agents – Present and Future

a report by
Huub Schellekens

Director, Central Laboratory Animal Institute, Utrecht University
DOI:10.17925/EE.2006.00.02.1k

Huub Schellekens is Director of the Central Laboratory Animal Institute of Utrecht University. He teaches medical biotechnology in the Department of Innovation Studies and has a position at the Faculty of Pharmaceutical Sciences at the same university. Previously, he was Deputy Director of the Dutch Primate Center, Director of Medscand Ingeny and medical microbiologist at the Reinier de Graaf Hospital in Delft. Dr Schellekens is a member of the Dutch Medicine Evaluation Board and a national expert of the European Medicine Evaluation Agency (EMEA). He is a medical microbiologist by training and works on the preclinical development of biopharmaceuticals. Dr Schellekens received his PhD at Erasmus University in Rotterdam in 1980, having carried out his training in medical microbiology and medicine studies at the same university.

Recombinant erythropoietin (EPO) is a 165-amino-acid glycoprotein with a molecular weight of 30.4 kilo Dalton (kDa) and approximately 40% carbohydrates. It binds to a single receptor that is widely distributed within the body, but bone marrow is the main target of EPO, where it stimulates both the production and the survival of erythrocytes. Recombinant EPOs are the most successful biotechnology-derived therapeutic proteins, and the market worldwide is currently at approximately US$8 billion.

The initial introduction of EPOs around 1989 concerned the treatment of renal anaemia, which is caused by a deficiency in the production of endogenous EPO. In 90% to 95% of renal patients, EPO dramatically improves the quality of life. Within weeks after the start of treatment, haemoglobin and red blood cell count start to rise. This reduction in anaemia has a number of secondary effects. For example, the cardiovascular function improves, which not only enhances the feeling of wellbeing, but also reduces mortality.

The list of other beficial effects is impressive and include enhanced immune and endocrine functions, improvements in memory, concentration and other cerebral functions and decreased bleeding tendency through improved platelet function.

EPOs are also succesfully being used in the treatment of cancer-associated anaemia. In cancer, the endogenous EPO production is suppressed and EPO substitution leads to a reduction in transfusion requirements. Furthermore, in cancer patients, EPO increases the quality of life by reducing the fatigue level. This improvement is particularly notable in patients with mild anaemia, where transfusion dependency is not an issue.

The two first generations of biotechnology-derived EPOs available in Europe are epoetin alpha (Eprex) and epoetin beta (Neorecormon). These two products have an identical amino acid sequence, but they differ in glycosylation and, therefore, in isoform composition. Both are produced in Chinese hamster ovary (CHO) cells and their difference in glycosylation probably reflects a difference in the purification process. The formulation of the products is different and this can influence safety. Their pharmacokinetic characteristics are comparable and their biological and clinical efficacy are similar.

Millions of patients have been treated with recombinant EPOs in the last 20 years, and these products have been shown to be among the safest biotechnological products. The side effects seen are caused by the increase in haematocrit and not by direct toxicity of EPOs. The main side effect is hypertension, which occurs almost exclusively in renal patients during the acute reduction in anaemia and is less during the maintenance phase.

The other, more rare side effects include influenza-like symptoms and increased blood viscosity, leading to clotting of lines and vascular access thrombosis. Pure red cell aplasia (PRCA), associated with the use of subcutaneous epoetin alpha, will be discussed later. Based on animal studies, suggestions have been made as to the increase of tumour progression through EPOs. However, a careful evaluation of all available clinical data from the different manufacturers have failed to confirm this effect in cancer patients.

New Epoetins

Second Generation – Darbepoetin

Darbepoetin alpha is the most recent entrant into the erythropoiesis-stimulating agents (ESAs) field. Its molecular structure was modified from its predecessors, with the substitution of two of the 154 amino acids in the protein and the attachment of two additional high molecular weight carbohydrate chains. The impact of these changes on pharmacokinetic properties include a relative extension of elimination half-life *in vivo*. Darbepoetin alpha demonstrates a two- to three-fold longer elimination half-life compared with epoetin alpha.

The clinical programme for darbepoetin has focused on less frequent dosing, with once-weekly administration being the focus of the initial trials programme, regardless of disease state, treatment phase or route of administration.

As once-weekly maintenance dosing is also possible with epoetin beta for renal and haem/oncology patients, the use of darbepoetin beyond the periodicity predicted by its half-life has been explored. Darbepoetin is now licensed, in some countries, for use at two, three and four weekly intervals, depending on disease type and within limited patient populations. The data published to date on these less frequent dosing regimes are mixed, and whether long-term safety and efficacy can be sustained at equivalent dose and cost to the established regimens across a broad population remains to be proven.

Third Generation – Continuous EPO Receptor Activator

The chemically synthesised continuous EPO receptor (EPO-R) activator (CERA) differs from EPO through its integration of amide bonds between amino groups and methoxy polyethylene glycol-succinimidyl butanoic acid. This results in a molecular weight of approximately 60kDa.

In contrast to EPO, CERA shows a different activity at the receptor level, characterised by a reduced specific activity *in vitro*, an increased activity *in vivo* and an increased half-life. These different pharmacological properties are relevant in order to achieve the desired characteristics of the molecule. Clinical development activities are under way to fully explore the potential advantages offered by CERA in the management of anaemia in patients with kidney disease and anaemia in patients with cancer.

New Indications

The new areas currently undergoing the most intensive research are anaemia post renal transplantation, anaemia in congestive cardiac failure (CCF) and series of investigations that can be grouped under the heading of the non-erythropoietic effects of EPOs (i.e. non-blood-forming). This latter group can be sub-divided into two entities sharing a common underlying mechanism of effect:

- the prevention and treatment of ischaemic organ damage; and
- the reduction of reperfusion injury (e.g. following coronary thrombolysis or percutaneous coronary artery stenting).

Other areas, closer to existing usage, have already been the subject of more extensive study, but remain of interest to the clinical community – e.g. anaemia in myelodyplastic syndrome, surgical anaemia and anaemia in ribavirin-treated hepatitis C patients.

Although incidences vary between countries, up to half of the patients receiving renal replacement therapy are renal transplant recipients. Despite their prevalence, anaemia is an under-studied phenomenon in this group. Up to one-third of patients are believed to suffer from anaemia as a consequence of both on-going, or progression of, renal impairment and the myelosuppressive effects of anti-rejection therapies. A number of large studies are currently under way to improve post-transplant anaemia and the role of EPO therapy.

Work is intensifying in the field of anaemia in CCF, where early non-randomised human studies of EPOs have shown highly encouraging results, with improvements in cardiac and physical functioning, reduction in hospitalisation and reduction in death, compared with historical controls. The first large-scale randomised studies are now under way.

EPO has been shown to prevent loss of neurons, endothelial cells and cardiomyocytes. It is now clear that EPO modulates an array of vital cellular functions that involve progenitor stem cell development, cellular protection, angiogenesis, DNA repair and the promotion of cellular longevity. Recombinant human EPO (rHuEPO) may therefore have potentially beneficial effects in a variety of conditions, including cerebral ischaemia, myocardial infarction (post-reperfusion) and acute renal injury. Early exploratory human studies have been launched in these areas.

Biosimilars

The basic patent of recombinant DNA-derived epoetin has expired in most European countries. In the case of classical drugs, expiration of patents opens the possibility of the introduction of generic products. Limited documentation showing chemical similarity and bioequivalence in a small study of volunteers is in general sufficient to obtain marketing authorisation.

However, the concept of generics developed for small therapeutic molecules cannot be extrapolated to the majority of biopharmaceuticals that are large and complex proteins. There is no technology with which to establish whether the structure of two biopharmaceuticals is completely identical. Moreover, the properties of biotechnology products are highly dependent on the type of host

cells used for the production, the downstream processing and the purification process. In addition, formulation and storage can influence their biological and clinical behaviour.

The most important difference between therapeutic proteins, such as epoetin, and classical drugs is the potential of therapeutic proteins to induce antibodies. This immunogenicity of bio-pharmaceuticals is a good illustration of the difficulties in comparing biotechnology products. It also shows that the clinical consequences of insufficient biological characterisation may be severe. Antibodies may lead to general immune effects, such as allergic reaction and serum sickness-like symptoms. In the majority of cases, the clinical effect of immunogenicity is loss of efficacy of the protein.

In some cases, these antibodies may cross-react with the native protein. If this native protein has a unique and important biological function, dramatic side effects may occur. This has recently been observed in patients treated with Eprex. The antibodies induced by Eprex cross-neutralised endogenous EPO, resulting in pure red cell aplasia (PRCA) in approximately 225 patients. This side effect was only seen in patients with chronic renal failure after subcutaneous treatment. At the end of 2002, most European countries have contra-indicated this use of Eprex and since then, the number of cases has fallen dramatically.

This upsurge of PRCA is associated with a formulation change in 1998, when human serum albumin as a stabiliser was removed and replaced with sorbitol 80, a detergent that is used widely in protein formulations. Additional potential factors have been suggested, such as inappropriate handling of the product for self-administration by patients or the use of the subcutaneous route of administration, which may have further enhanced the inherent immunogenicity of the epoetin alpha molecule in the new formulation.

In a number of recent papers, the manufacturer claims that an adjuvant effect by compounds leaked from uncoated rubber stoppers of sorbitol 80 enhanced the immunogenicity of Eprex. However, the poor quality of the research and the large deficits in the data and description of the material and methods do not allow any conclusions to be drawn.