SEMINARS IN
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Recent Advances in Erythropoietic Agents in Renal Anemia

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Red cell production in chronic kidney disease is usually too low to maintain a normal haemoglobin, and thus anaemia develops in a large proportion of patients. The ability to stimulate erythropoiesis in the bone marrow by the use of therapeutic agents has only been possible in the last 20 years, initially with recombinant human erythropoietin (epoetin), and later darbepoetin alfa. Many new agents are, however, in clinical development, and these include CERA, Hematide, and HIF stabilisers, in addition to the imminent launch of biosimilar epoetins. The main issue with biosimilars is the unknown risk of immunogenicity. CERA is a large molecule, approximately twice the size of epoetin, which was created by integrating a single polymer chain into the erythropoietin molecule. CERA has a much prolonged half-life, and Phase II and III clinical trials have investigated administration of CERA every 3 or 4 weeks. Hematide is derived from original research on the erythropoietin-mimetic peptides, and is in Phase II of its clinical trial programme. Again, this compound is being investigated as a once-monthly administration. The HIF stabilizers are orally-active inhibitors of the enzyme that degrades hypoxia-inducible factor (prolyl hydroxylase), and this leads to upregulation of erythropoietin gene expression. Other strategies for stimulating erythropoiesis, briefly described in this review, are at an earlier stage of development. This is an exciting and rapidly developing area of scientific and translational research.

KEYWORDS novel erythropoietic agents, anaemia, epoetin, darbepoetin alfa, biosimilars, CERA, Hematide, HIF stabilisers

It has been recognized for more than 30 years that the vast majority of patients suffering from the anemia associated with chronic kidney disease have inappropriately low levels of circulating erythropoietin for their hemoglobin level, in contrast to other causes of anemia such as iron deficiency and aplastic anemia.[1] This is not unduly surprising, given that the kidney is the major source of erythropoietin in adults. Thus, even though many patients had plasma levels of erythropoietin in the same range as normal healthy individuals, red cell production was insufficient to maintain a normal hemoglobin level.[2] The rationale for administering exogenous synthetic erythropoietin therefore was very strong, and the expectation was that this would increase erythropoietic activity and thereby restore the decreasing hemoglobin level to more normal levels.

Before the mid-1980s, however, this was no more than a dream, and there was really no effective therapeutic means of stimulating erythropoiesis. Patients with the severe anemia that accompanies chronic kidney disease therefore were managed by regular blood transfusions, which were required to be given as often as every 2 to 3 weeks. Androgen therapy was attempted with the knowledge that this may potentiate erythropoiesis both in vitro and in vivo,[3] but the effect was too weak and unpredictable to be adopted as a routine therapeutic strategy, even ignoring the fact that the side-effect profile (hirsutism, virilization, and liver toxicity) was unacceptable. As a consequence, many patients developed transfusional iron overload, with acquired hemosiderosis in several key organs such as the heart, liver, and pancreas. Regular red cell transfusions also exposed the patient to the risk of transmissible infections (particularly viral), along with sensitization to major histocompatibility antigens that reduced the chances of successful kidney transplantation.

This devastating situation prompted researchers to pursue the development of recombinant human erythropoietin by means of gene technology. The major breakthrough that made this possible came in 1977 with the successful purifi-

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Table 1 Products Currently in Clinical Development for Stimulating Erythropoiesis

Biosimilar epoetins
CERA
Hematide
HIF stabilizers

cation of small amounts of human erythropoietin from the urine of patients with aplastic anemia.[4] Based on limited peptide sequence information of this purified material, the gene for human erythropoietin was isolated and cloned in 1983,[5] and the use of genetic engineering techniques then allowed the large-scale production of recombinant human erythropoietin from a Chinese hamster ovary cell line. Every nephrologist is aware of the clinical success of this product, which became available commercially in the United States in 1989, and in Europe in 1990.

Recombinant human erythropoietin has an excellent benefit: risk ratio, with high efficacy and low safety concerns, and therefore attempts to improve on the innovator product focused mainly on strategies to increase the ease of administration rather than enhancing its biological action. In the early 1990s, the erythropoietin molecule was modified by substituting 5 amino acids using site-directed mutagenesis, which allowed the introduction of an additional 2 N-linked glycosylation chains.[6] This biochemical modification resulted in a molecule with a significantly longer plasma half-life,[7] and this second-generation erythropoietic agent, now called *darbepoetin alfa*, was able to be administered less frequently to patients.[8,9] Thus, in contrast to recombinant human erythropoietin, darbepoetin alfa was developed as a once-weekly or even once-every-alternate week injection, whereas epoetin initially was dosed 2 or 3 times per week. At the present time, therefore, our therapeutic armamentarium for stimulating erythropoiesis consists of several epoetins (which differ with regard to their manufacturing technique, resulting in subtle changes in the molecular structure while retaining the basic 165 amino acid-protein backbone and the carbohydrate chains), and darbepoetin alfa (with its 2 additional glycosylation chains).

Further scientific developments, however, have resulted in new molecules that have the potential to promote erythropoiesis. The majority of these are still in the laboratory stage of development, and are undergoing preclinical studies, but a small number have now entered the clinical phase of development and are being tested in phase II and III clinical trials in chronic kidney disease patients. This review focuses mainly on the latter group of drugs because these are the ones that potentially could be commercially available within the next 5 years or so (Table 1). These include the biosimilar epoetins, Continuous Erythropoietin Receptor Activator (CERA), Hematide, and the hypoxia-inducible factor (HIF) stabilizers.

Biosimilar Epoetins

Before the reporting of cases of antibody-mediated pure red cell aplasia in 2002,[10] many generic pharmaceutical companies were preparing for the expiration of the patent for recombinant human erythropoietin. Indeed, at least 20 companies were pursuing this potentially lucrative avenue of product development, and several had already begun phase I and II clinical trials. When Casadevall et al[10] reported the initial series of 13 cases of pure red cell aplasia associated with the development of antibodies against both recombinant erythropoietin and the endogenous hormone, increased scrutiny for this potential complication of epoetin therapy ensued. With the discovery of further cases appearing on an escalating basis, the drug regulatory authorities on both sides of the Atlantic began to discuss this problem. Tighter regulations were imposed on the production of biotherapeutics,[11] including recombinant proteins, and the clinical development of further recombinant erythropoietin products became much more costly and labor intensive. As a result, many companies since have abandoned their programs of clinical development, and only a few remain.

The efficacy of these biosimilar epoetins in stimulating erythropoiesis and maintaining correction of anemia is, to a large extent, assumed, but virtually no data have been published. The largest hurdle faced by both the pharmaceutical companies and the regulatory authorities lies, however, in ascertaining the level of potential immunogenicity of these products. Although epoetin-associated pure red cell aplasia has been reported with all the existing products on the market, the absolute incidence of this condition is extremely low, of the order of 1:10,000 patient-years. Thus, proving that a biosimilar epoetin does not have (for example) a 5-fold increase in the risk of immunogenicity would be impossible, given the current scale of clinical development programs. Companies manufacturing biosimilar epoetins may be granted a product license if a rigorous clinical trial program has been undertaken, but even then, postmarketing surveillance may be mandated. Before the advent of epoetin-associated pure red cell aplasia, nephrologists looked forward to the introduction of biosimilar epoetins, with the hope and expectation that such products would be cheaper and more widely available. Given the escalating costs of bringing these products to market, however, these hopes have been ever-diminishing. The critical question here is at what price would a nephrologist be happy to prescribe a biosimilar epoetin for their patients, given the unknown risk of pure red cell aplasia? It is difficult to contemplate a positive benefit:risk ratio with the biosimilar epoetins at present, and therefore cost and/or marketing is likely to be the crucial factor influencing their potential use.

Outside the tight regulatory influences of the Food and Drug Administration and the European Medicines Agency (EMEA) many other biosimilar epoetins already are marketed in countries such as India, China, Korea, Argentina, and Cuba. The quality of these products has been shown to be highly variable, and the biological activity also can vary enormously from one product to another.[12] In China, for example, switching from one brand of epoetin alfa to another at the same dose may result in a very different biological response, and this too has important implications for their use outside the United States and Europe. In certain countries, however,

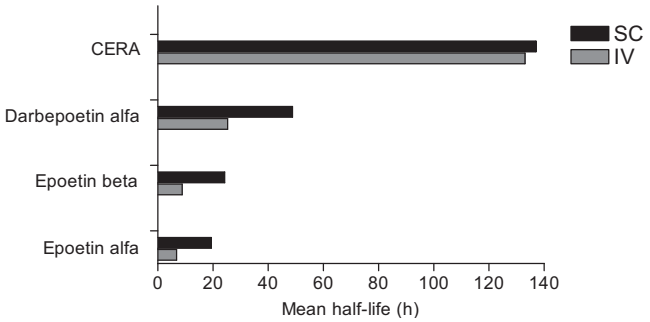

Figure 1 Mean half-lives of erythropoiesis-stimulating agents: CERA, darbepoetin alfa, epoetin alfa, and epoetin beta. ■, subcutaneous; ▨, intravenous. Reprinted with permission from Macdougall.[13]

economic factors may override quality and safety, and it could be argued that some of these products that never could be accepted onto the market in the United States, Canada, Europe, and Australia still might be better than no erythropoietin at all in some instances.

CERA

CERA is the next erythropoietic agent that is likely to be licensed for the treatment of renal anemia, having recently completed phase III of its clinical development program. CERA was created by the integration of a single 30-kd polymer chain into the erythropoietin molecule, thus increasing the molecular weight to twice that of epoetin at approximately 60 kd, and its elimination half-life in human beings is considerably greater (≈130 h) (Fig 1). This methoxy-polyethylene glycol polymer chain is integrated via amide bonds between the N-terminal amino group or the ε-amino group of lysine (predominantly lysine-52 or lysine-45), using a single succinimidyl butanoic acid linker. The hypothesis that was tested in the phase II and III clinical studies was that CERA can be administered safely and effectively every 3 to 4 weeks, and the preliminary data suggest that this is the case.[13] Most of these studies, however, have been reported only in abstract form to date, and therefore have not been subjected to rigorous peer-review. Once-monthly administration may be more suitable for patients with chronic kidney disease who do not yet require dialysis, and who therefore are not attending the renal center on a regular basis.

The preclinical studies of CERA compared the erythropoietic activities of this new molecule with epoetin beta in vitro by measuring their effect on the proliferation of a human acute myeloid leukemia cell line (UT-7) that expresses the erythropoietin receptor. Across the dose range of 0.003 to 3 U/mL, epoetin beta stimulated greater proliferation of UT-7 cells than did CERA.[14] However, in vivo studies in normocythemic mice comparing identical amounts of protein across the dose range of 60 to 1000 ng of protein per animal have shown that CERA was more effective than epoetin at stimulating bone marrow precursor cells and increasing reticulocyte count. At a dose of 1000 ng, CERA increased the mean reticulocyte count by 14%, compared with 9% with epoetin.[14]

Further preclinical studies in various other animal models have investigated the pharmacodynamic and pharmacokinetic properties of CERA, administered both intravenously and subcutaneously. Data from these studies showed that CERA had a lower systemic clearance and a longer half-life compared with epoetin, and this also was confirmed subsequently in healthy volunteers[15] and CKD patients.[16]

Phase II studies of CERA have been published in abstract form and again have investigated both the intravenous and subcutaneous routes of administration.[13,17-20] Dosage frequencies of once every 2 weeks, once every 3 weeks, and once every 4 weeks have been examined, and the phase III studies were designed to show correction of anemia (2 studies) and maintenance of hemoglobin correction (4 studies) in both dialysis and nondialysis CKD patients. Again, only abstracts from a few of these phase III studies are available, but they confirm the efficacy of CERA at a reduced dosage frequency compared with epoetin. A phase II dose-escalation study of CERA also has been conducted in 64 patients with multiple myeloma and anemia, and a dose-dependent erythropoietic response at doses of up to 4.2 μg/kg was observed.[21] In all the studies reported to date, CERA generally has been well tolerated with no unexpected safety concerns. There has been no evidence of antibody production in any patient treated with CERA to date.

Hematide

Hematide is likely to be the first erythropoietin-mimetic peptide to become available commercially. About a decade ago, a family of peptides was identified that were found to have erythropoietin-mimetic activity both in vitro and in vivo.[22] These peptides bind to the erythropoietin receptor and activate the same JAK-2/STAT-5 intracellular signaling system that mediates the action of erythropoietin. Interestingly, there is no structural homology between these peptides and erythropoietin protein. The first erythropoietin-mimetic peptides (EMP) were identified after screening of a peptide library in search of agonist peptides that bound to the erythropoietin receptor, as shown by phage display technology. The first such peptide to be identified (EMP1) was a cyclic oligopeptide with an amino-acid sequence completely unrelated to native or recombinant erythropoietin.[22] Nevertheless, this peptide shared many of the functional and biological properties of erythropoietin: it competed with radiolabeled erythropoietin for the erythropoietin receptor, it induced proliferation of an erythropoietin-responsive cell line, it caused colony forming unit-erythroid growth in human bone marrow, and it was active in 2 different in vivo models of erythropoiesis. Furthermore, it was shown to induce the same intracellular tyrosine phosphorylation pattern as erythropoietin.[22] The development of EMPs has continued, with the aim of increasing their biological potency, and a further product has been synthesized. This compound has a new sequence of amino acids, again completely unrelated to the sequence of EPO and with a modified peptide architecture that includes dimerization and intramolecular cyclization. The new dimer peptide was found to be equipotent to

recombinant erythropoietin in various receptor binding and in vitro proliferation assays. The dimer peptide was pegylated, and the resultant compound (Hematide Affymax, Palo Alto, CA) showed a long circulating half-life in rats and monkeys, along with an extended duration of erythropoietic action.[23] Interestingly, antierythropoietin antibodies do not cross-react with Hematide nor do they neutralize its biological activity in vitro. Indeed, Hematide has been shown to stimulate erythropoiesis and increase the hemoglobin level in animals with circulating antierythropoietin antibodies, suggesting that this compound potentially could be used as rescue therapy for patients with antierythropoietin antibody–mediated pure red cell aplasia.[24] Phase I studies of Hematide in healthy volunteers[25] and CKD patients with renal anemia have been conducted, and phase II studies now are underway.

HIF Stabilizers

Hypoxia-inducible factor is a heterodimeric transcription factor that occurs in 2 forms: alfa and beta. Although HIF beta is expressed constitutively, HIF alfa levels are regulated by oxygen. Under hypoxic conditions, HIF alfa is able to upregulate endogenous erythropoietin gene expression, with the production of increased amounts of erythropoietin from the kidney (and to a lesser extent, the liver).[26] In short, HIF alfa is the oxygen-sensing protein that controls erythropoietin production. Recently, specific enzyme (prolyl hydroxylase) inhibitors have been synthesized that stabilize HIF and mimic the effect of hypoxia. One such orally active prolyl hydroxylase inhibitor recently was reported to induce erythropoietin production and stimulate erythropoiesis in healthy human beings[27] and nondialysis CKD patients.[28] Phase II studies of this prolyl hydroxylase inhibitor currently are being conducted, but it is fascinating scientifically that enhanced erythropoietin production can occur in anephric animals, suggesting an extrarenal mechanism. This is an orally active therapy, but there is some concern about the ubiquitous nature of this gene upregulation in that this transcription factor may upregulate many other HIF target genes in addition to erythropoietin.[29] Some of these genes may be involved in neoangiogenesis, and there also are concerns about increased malignant potential. If the safety and efficacy of the HIF stabilizers are confirmed in larger numbers of patients, then this could be the first orally active therapy for stimulating erythropoiesis. It might also be the first agent to do so without being a direct agonist of the erythropoietin receptor.

Other Strategies for Stimulating Erythropoiesis

The products described thus far in this article already have been subject to clinical trials in CKD patients (Table 1). There are other molecules that have been tested in the laboratory setting that potentially could yield therapeutic agents in the future. Some of these are listed in Table 2.

Table 2 Other Strategies That May Be Used to Stimulate Erythropoiesis

Synthetic erythropoiesis protein
Erythropoietin fusion protein
Non-peptide erythropoietin-mimetics
Hemopoietic cell phosphatase (SHP-1) inhibitors
GATA inhibitors
Erythropoietin gene therapy

Synthetic erythropoiesis protein is another erythropoietin polymer that exploits recent advances in the chemical synthesis of proteins and polymers. By using solid-phase peptide synthesis and branch precision polymer constructs, a 51-kd protein-polymer construct has been made that contains 2 covalently attached polymer moieties. Synthetic erythropoiesis protein stimulates erythropoiesis via activation of the erythropoietin receptor.[30]

Erythropoietin fusion protein is derived from a complementary DNA encoding a fusion protein of 2 complete human erythropoietin domains linked by a 17 amino acid flexible peptide. The molecular weight of this product is 76 kd, and a single subcutaneous administration of this compound to mice increased red cell production within 7 days at a dose at which recombinant human erythropoietin was ineffective.[31]

In addition to erythropoietin-mimetic peptides, small molecule libraries also have been screened to identify a nonpeptide molecule that binds to the erythropoietin receptor. One such compound was found to bind to a single chain of the erythropoietin receptor, but this was not active biologically.[32] The compound was ligated to allow it to interact with both domains of the receptor, and this second molecule was shown to stimulate erythropoiesis. The further development of nonpeptide erythropoietin-mimetics potentially could lead to the production of an orally active erythropoiesis-stimulating agent in the future.

Inhibitors of an intracellular enzyme called *hemopoietic cell phosphatase* (otherwise known as SHP-1) potentiate erythropoiesis by preventing dephosphorylation of the intracellular signaling molecule JAK-2 and STAT-5 in erythroid progenitor cells.[33] Hemopoietic cell phosphatase inhibitors are active orally and potentially could be used alone or in conjunction with another erythropoiesis-stimulating agent.

GATA inhibitors act via a similar mechanism of action to the prolyl hydroxylase inhibitors by selectively inhibiting HIF degradation and thereby prolonging endogenous erythropoietin activity. GATA-binding transcription factors are essential for the proliferation and survival of hemopoietic cells.

Erythropoietin gene therapy is still very much at an experimental stage, but various strategies have been devised to deliver this therapy to the intact animal, such as adenovirus transfection[34] and transplantation of autologous or allogeneic cells manipulated ex vivo.[35] The main issue with respect to erythropoietin gene therapy is the ability to achieve some measure of feedback control, but in animals it has been

shown that it is possible to link the erythropoietin transgene to a hypoxia-responsive DNA element (ie, the HIF binding site) to establish an oxygen-dependent feedback regulation of the transgene, similar to that of the endogenous gene.[36]

Conclusions

We have come a long way in the past 20 years with regard to pharmacologic stimulation of erythropoiesis. With the exception of the very rare complication of antibody-mediated pure red cell aplasia, the erythropoiesis-stimulating agents that are available to date have been proven to be highly effective with a very acceptable safety profile. Adverse events are few, and are related mainly to overstimulation of erythropoiesis with hemoglobin levels that are too high. Only time will tell whether the same safety profile will exist with the newer agents, but within the next 5 to 10 years nephrologists may have other products available to them for stimulating erythropoiesis and treating the anemia of chronic kidney disease. This is an exciting area of scientific and translational research.

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