New erythropoiesis-stimulating agents – an update

Introduction of recombinant human erythropoietin (epoetin) was a milestone in the treatment of anaemia in patients with chronic kidney disease. The further development of a new group of drugs – erythropoiesis stimulating agents was initially based on the modification of the aminoacide sequence of the EPO molecule and their hyperglycosylation. These modifications improved the pharmacokinetics of the new drugs (darbepoetin and CERA) by prolongation of the serum elimination half-life. The search for small molecules with EPO mimetic properties focused on two different approaches: moieties enable to cause the dimerisation and activation of EPO receptors or substances targeting the intracellular signalling pathway of the EPO receptor. Recently, quite a new approach has appeared – the innovation of inhibitors of prolyl hydroxylase and GATA-2 transcription factor orally active compounds enhancing the endogenous EPO synthesis. This paper summarise the recent advances in the development of the new erythropoiesis stimulating agents.

(NEPHROL. DIAL. POL. 2006, 10, 173-176)

Zastosowanie ludzkiej erytropoetyny otrzymanej metodą rekombinacji genetycznej miało przełomowe znaczenie w leczeniu niedokrwistości towarzyszącej przewlekłym chorobom nerek. Obecnie, poprzez modyfikację sekwencji aminokwasowej i hiperglikozylację cząsteczki erytropoetyny zsyntetyzowano nowe leki stymulujące erytropoezę (darbepoetyna, CERA), które charakteryzują się lepszym profilom farmakokinetycznym. Poszukiując małych cząsteczek o właściwościach nasadzających działanie erytropoetyny odkryto substancje, które powodują dimeryzację i aktywację receptora erytropoetyny oraz cząsteczki, które nasilają wewnątrzkomórkowe przewodnictwo sygnału tego receptora. Ostatnio prowadzone są badania nad zastosowaniem inhibitorów hydroksylazy prolinowej HIF oraz czynników transkrypcyjnych GATA-2. Obydwa te czynniki podawane doustnie zwiększają stężenie hemoglobiny poprzez nasilenie syntezy endogennnej erytropoetyny. Artykuł jest podsumowaniem aktualnego stanu wiedzy o nowych czynnikach stymulujących erytropoezę.

(NEFROL. DIAL. POL. 2006, 10, 173-176)

Introduction
The management of anaemia in patients with chronic kidney disease was revolutionised in the late eighties by introduction of the recombinant human erythropoietin (rHu-EPO) [9]. In the following 15 years a considerable number of clinical trials proved their efficacy and safety. Low haemoglobin level has been identified as a risk factor for cardiovascular complications in patients with chronic kidney diseases (CKD) [8]. In the late eighties and nineties of the XX century, when epoetins were introduced to clinical practice, almost all patients started treatment simultaneously with dialysis initiation. They already had cardiovascular complications enhanced by long lasting anaemia at that time. Early correction of anaemia in CKD patients (before renal replacement therapy), when cardiovascular complications are less advanced is crucial for the risk reduction of cardiac disease and mortality [39]. The recommended target haemoglobin (Hb) concentration in CKD patients is lower than in general population and in the same range for men and for women, regardless of different physiological levels in both sexes. The target Hb in anaemia management guidelines have not changed very much since 1997 in Europe, USA and Australia. According to European Best Practice Guidelines the lower Hb limit is >11 g/dl [21]. The new National Kidney Foundation guidelines of anaemia management in CKD recommended similar lower Hb limit and pay attention on upper Hb concentration, which should not be higher as 13 g/dl [3]. The results of CREATE trial (Cardiovascular risk Reduction by Early Anaemia Treatment with Epoetin) performed in non-dialysed CKD patients showed significant improvement of quality of life, without significant impact on mortality in patients with Hb concentration 13-15 g/dl [6]. In the other trial – CHOIR, the non-
dialysed CKD patients treated with epoetin α with target Hb of 13.5 g/dL showed increased risk of composite endpoint events (mortality, stroke, heart attack, hospitalisation) comparing to patients with target Hb 11.3 g/dL [32]. The last results are similar to the results in dialysis patients, in whom Hb normalisation increased mortality risk [2]. Currently there are inconclusive evidence for a specific upper Hb limit in CKD patients. 

Endogenous EPO is predominantly produced in renal peritubular, fibroblast-like cells in response to oxygen demand [9]. Erythropoietin binding to the extracellular domain of the EPO receptor on the erythroid progenitor cells results in dimerisation of two molecules [35]. Stimulation of these receptors prevent apoptosis of burst-forming unit erythroid (BFU-E), colony-forming unit erythroid (CFU-E) and normoblasts, thus enhancing the erythropoiesis [35].

Commercially available three isoforms of rHuEPO – epoetin-α (Eprex®), Procrit®, epoetin-β (Neupogen®), epoetin-τ (Epomax®) are glycoproteins produced by Chinese hamster ovary cells (epoetin-α and epoetin-β) or baby hamster kidney cells (epoetin-α containing EPO gene. Although these glycoproteins differ in their carbohydrate structures and pharmacokinetic activity, this difference does not result in longer elimination half-life, the treatment efficacy and the route of administration (only parenteral) are almost identical.

Further modification of the erythropoietic peptide structure by hyperglycosylation and changing of the aminocides strain (darbepoetin alfa modifications) (Novel Erythropoietin Receptor Activator – CERA and Synthetic Erythropoiesis Protein – SEP) brought additional improvement in it’s pharmacokinetic.

The attempt to design proteins with greater than rHuEPO hemopoietic activity, resulted in the creation of dimeric fusion proteins (including synthetic polypeptide – NESP (Darbepoetin alfa)) or baby hamster kidney cells (epoetin-α containing EPO gene. Although these glycoproteins differ in their carbohydrate structures and pharmacokinetic activity, this difference does not result in longer elimination half-life, the treatment efficacy and the route of administration (only parenteral) are almost identical.

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The "hunting" for non-peptide erythropoietic agents – EPO-mimetics, did not bring any spectacular discovery.

Recently, quite a new approach has appeared – enhancement of endogenous EPO synthesis by overexpression of the prolactin, xylase and GATA-2 inhibitors. The efficiency of such management, even in case of cirrhotic kidneys, brought a new hope for the procurement of an orally active drug in the future.

On the other hand, patents for a number of approved biopharmaceutical agents including epoetins has expired or will run out soon. The patents for epoetin-α expired in Europe in 2004 and biosimilar versions of this therapeutic agent (i.e. biosimilars) are likely to emerge into the market soon [30]. The development of biosimilar proteins is more complicated and requires a new approach than for conventional generic drugs. The biopharmaceuticals are heterogeneous proteins produced by different manufacturing processes. The pharmacodynamics and clinical effectiveness of such biopharmaceuticals is unclear. The change in the formulation of epoetins highlights the potential for increased risk of immunogenicity and higher occurrence of antibody mediated pure cell aplasia (PRCA). Therefore the approval of these agents require more than the confirmation of pharmacokinetic bioequivalence. The regulatory requirements for the approval of biosimilars have not been yet fully established. Companies manufacturing biosimilar epoetins may get a product licence if rigorous clinical trial program will be undertaken and the postmarketing surveillance may be mandatorily frequent. In the mentioned problems some companies have abandoned their programs of development of biosimilar epoetins. Biosimilar epoetins are already marketed in countries such as China, India, Korea and Cuba. The quality and biological activity of these products has been shown to be very variable [24].

Novel erythropoiesis stimulating peptide – NESP (Darbepoetin alfa) Based on the knowledge that sialic acid residues in vivo are responsible for maintaining the biological activity of EPO, a new molecule has been created (as native moiety of the human EPO), but five N-linked oligosaccharides chains (NESP), was synthesised [23,29]. The original amino acid sequence of EPO was modified at 5 positions (Ala30Asn, His32Thr, Pro87Val, Tyr88Asn and Pro90Thr) in order to attach additional oligosaccharides (including an asparagine residues in 30, 31, 40, 41, 48 [23,29]). Darbepoetin alfa binds to the EPO receptor in an indistinguishable from native EPO manner, to stimulate intracellular pathway of signalling. However, the pharmacokinetics of darbepoetin alfa is different. The glycoprotein clearance is slower and serum elimination half-life is longer (3-4 times longer) vs 8.5 hr in haemodialysis patients after intravenous injection) [23,29]. The serum elimination half-life of darbepoetin alfa can be further elongated to 48.4 hr after subcutaneous injection [23] (table I). Longer serum elimination half-life enables less frequent administration of darbepoetin alfa than epoetin. Darbepoetin alfa may be administered once a week or every second week in the initial treatment of anaemia and once every 3-4 weeks for the maintaining of haemoglobin concentration [14].

The treatment of renal anaemia with darbepoetin alfa is equally effective as for epoetin [27]. The drug was introduced into the clinical practice in 2002. There is still not enough information concerning the cost ratio over the currently used epoetins [27].

It is worth to stress, that until now, no case of antibody-mediated PRCA associated with darbepoetin alfa elation has been reported.

Synthetic Erythropoiesis Protein (SEP) Kochendoerfer et al. described 166-aminoacid polypeptide of similar sequence to EPO, which differs strikingly in the number and type of polymers attached to the main chain [16]. Two precisely constructed branched peptides were designed to bear negative charges and give optimal potency and prolonged duration of action in vivo [16]. The biological activity of SEP is superior to epoetin alfa. The serum half-lives were 2-times higher, however not as long or even as for darbepoetin alfa [16]. This fact limits at the least above mentioned SEP applicability in the future.

Continuous Erythropoietin Receptor Activator (CERA)

CERA is an innovative erythropoietic agent (synthetic polypeptide conjugated with methoxy-polyethylene glycoluscinimidylbutanoic acid), which currently entered preclinical (phase III) studies in renal anaemia. Four placebo-controlled phases I studies demonstrated dose-dependent erythropoietic response, which was higher than for darbepoetin half-life [12]. Both, after intravenous and subcutaneous injections, serum elimination half-lives exceed 130 hrs [25] (table I). This unique feature suggests that CERA may be administered in longer than darbepoetin intervals. It has been already proven, that the mean increase of haemoglobin level is similar in patients receiving CERA once a week and every third week [22].

The results of larger studies with CERA in dialysis patients should be available this year.

Dimeric fusion peptides The binding of native EPO to its receptor results in dimerisation, which is required to trigger the biological response [10]. This finding initiated search for dimeric fusion peptides providing two polypeptide fragments with high affinity domains for EPO receptor and a linker peptide [33].

Dimeric EPO-EPO protein with 6-aminoacid linker obtained by DNA recombinant technique provided a new protein with a similar to EPO monomer pharmacokinetics (weaker binding affinity and 3-4 times higher biological activity) [5]. It has been demonstrated that the length or even more importantly – the structure of the linker may influence the activity of dimeric fusion protein. When the linker was too long, the activity of such a protein was similar to the monomer [33].

Another dimeric fusion protein was composed of GM-CSF and EPO [4]. The idea of that invention was based on the fact, that burst forming unit-erythrocyte (BFU-E) re-

Table 1

| Table 1 Serum half-life (hours) of epoetins, darbepoetin alfa and CERA administered intravenously and subcutaneously [6,11,13]. |
|-----------------|-----------------|-----------------|
|                 | Intravenous     | subcutaneous    |
| Epoetin alfa    | 6.8 ± 0.6       | 19.4 ± 2.5      |
| Epoetin beta    | 8.8 ± 0.5       | 24.2 ± 2.6      |
| Epoetin omega   | not determined  | not determined  |
| Darbepoetin alfa| 25.3 ± 2.2      | 48.8 ± 5.2      |
| CERA            | 133 ± 10        | 137 ± 22        |

mean values ± SEM
EMP1 (EPO mimetic peptide 1) is a small (20-aminoacides) cyclic peptide, containing some conservative amino acid residues of native EPO structure [33]. EMP1 shows similar binding affininity to EPO receptor and activates intracellular pathway as native EPO [15,37]. However, even after EMP1 dimerization, its activity – both in vitro and in vivo conditions – remains much lower [37]. This diminished EMP1 dimer activity is not related to its lower binding affinity, which suggests that natural ligand activates EPO receptor in multiple ways. This complex interaction between EPO and EPO receptor damp our zeal for rapid invention of small non-peptide orally delivered EPO-mimetics. The second approach targets the intracellular signal transduction of EPO receptor [1]. After the EPO receptor dimerisation, JAK2 protein undergoes autophosphorylation and subsequently phosphorylates the EPO receptor and STAT protein, which translocate to the nucleus and activate the transcription of the target genes [10]. De- phosphorylation of JAK2 protein by the haematopoietic cell phosphatase (HCP) is a negative regulator of the EPO receptor signalling (Figure 1) [10]. Thus, the inhibition of the HCP could augment the receptor stimulation [1]. A number of HCP inhibitors were tested [24]. No data concerning its efficacy are available.

Pegylated Peptide-Based Erythropoiesis-Stimulating Agent

Hematide (AF37702) developed by Affymax is a synthetic dimeric polypeptide linked to polyethylene glycol [38]. In contrast to dimeric fusion peptides, its aminoacidic sequence is unrelated to that of EPO. Thus, antibodies do not cross-react with rHuEPO and AF37702. Regardless of this modification, the binding affinity to erythropoietin receptor is maintained. The erythropoietic activity and efficacy of AF37702 were confirmed in vitro and in vivo studies [38]. Pharmacokinetic data showed extended, in comparison with epoetin alfa, serum elimination half-life (58.4 hrs) [38].

AF37702 may be especially useful in patients with previous PRCA, as neutralisation antibodies against rHuEPO do not cross-react with its moiety [38].

Inhibition of Hypoxia-Inducible Factor (HIF) prolyl 4-hydroxylase

The endogenous EPO production is stimulated by hypoxia. It is currently believed, that a key element of the oxygen sensing mechanism is Hypoxia Inducible Factor (HIF) [19]. HIF is a cytosolic transcriptional factor constitutively produced in the majority of cells [19]. In normoxic conditions, HIFα is rapidly degraded [11]. As shown on figure 2 this process is mediated by the von Hippel-Lindau tumor suppressor protein, which enables ubiquitination and degradation of HIFα by targeting it to the proteosome [40]. After cell exposure to hypoxia, a stabilisation of HIFα is observed with subsequent dimerization with HIFβ [31]. HIF complex mediates the transcriptional response to hypoxia including enhancement of EPO synthesis [19,31]. The mechanism of oxygen dependent HIF degradation is regulated by hydroxylation...
tion of HIF proline residues [20]. This modification is carried out by HIF-specific prolyl hydroxylase [26]. Recently it has been found that quite similarly to hypoxia, FG-2216 (manufactured by FibroGen) inhibits prolyl hydroxylase leading to HIF stabilization and induction of EPO synthesis not only by the kidney, but also in the liver [34]. It is worth to stress, that FG-2216 shows high bioavailability after oral administration (over 75%) [34]. It is also characterised by high efficacy. In animals, up to 300-fold elevation of plasma EPO concentration after oral FG-2216 administration was observed [18]. Also, after the bilateral nephrectomy, an increase of plasma EPO concentration was observed [18].

In healthy human subjects, FG-2216 given orally in dose 10mg/kg or higher twice a week, effectively inducted endogenous EPO synthesis [34]. Also in predialysis patients with chronic kidney disease the dose of 6mg/kg of FG-2216 weekly resulted in 19-fold increase of haemoglobin concentration after 3 weeks therapy [36].

GATA-2 inhibitors

GATA-2 transcription factor suppresses erythropoietin gene transcription by binding to the highly conservative GATA motif in the promotor region, that also include hypoxic response element – HIF-1 binding sequence [17]. Inhibitors of transcriptional factor GATA-2 stimulates erythropoietin gene transcription, quite similarly to the prolyl hydroxylase inhibitors. Both, intraperitoneal injection of K-7174 and oral administration of K-11706 (GATA-2 specific inhibitors), in animal models improved erythropoietin secretion and erythropoiesis suppressed by IL-1b or TNF-α [13,28]. These studies raised the possibility, that GATA-2 inhibitors in future might be suitable for treating patients also with refractory renal anaemia in inflammatory states.

Conclusion

There are several new erythropoiesis stimulating agents that may potentially improve the management of anaemia in patients with chronic kidney disease in the near future. The new agents will be administered less frequent, with better stabilisation of blood haemoglobin concentration. There are also promising attempts to overcome the parterreal way of drug administration. Additionally it is a real chance that the new compounds will be much more cost-effective than currently used epoetins. This fact will certainly improve the management of anaemia, quality of life and hopefully also survival of patients with chronic kidney diseases.

References