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NEPHROLOGY FORUM

Novel erythropoiesis-stimulating protein in the management of the anemia of chronic renal failure

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CASE PRESENTATION

Patient 1. A 41-year-old woman with end-stage renal disease secondary to chronic pyelonephritis and hypertension commenced hemodialysis three years ago. A forearm arteriovenous fistula had been created eight months prior to start of dialysis. A congenital absence of the sacrum had resulted in a neurogenic bladder associated with vesico-ureteric reflux and recurrent urinary tract infections. A colonic conduit had been constructed in childhood with no evidence during follow-up of carcinoma in the urinary diversion. The patient had a normochromic normocytic anemia (hemoglobin, 9.3 g/dL) and was iron replete at the start of dialysis (serum ferritin >100 μg/L, transferrin saturation >20%).

She was treated with recombinant human erythropoietin (rHuEpo) with the aim of achieving a target hemoglobin of > 11 g/dL. Intravenous iron saccharate was given once weekly to optimize iron stores. Her hemoglobin was maintained in the range of 11 to 12 g/dL by once-weekly subcutaneous rHuEpo injections. She later enrolled in a trial of subcutaneous novel erythropoiesis-stimulating protein (NESP) for the management of renal anemia. She was converted from rHuEpo therapy to NESP with a subcutaneous injection given every two weeks. Apart from a transient stinging sensation at the injection site following the first dose of NESP, no other side effects have been observed over one year of treatment. She continues to have well-controlled blood pressure. Her hemoglobin concentration has remained stable over one year in the range of 11.0 to 12.5 g/dL despite NESP being injected less frequently than was rHuEpo.

Patient 2. A 69-year-old man was referred to the Belfast City Hospital four years ago for management of advanced chronic renal failure secondary to atherosclerotic renal vascular disease and hypertension. His medical history also included ischemic heart disease, coronary artery bypass surgery eight years earlier, and elective abdominal aortic aneurysm repair six months prior to nephrology referral. Physical examination revealed: heart rate, 70 beats/min; blood pressure, 160/80 mm Hg; a grade III/VI mitral systolic murmur; and clear lung fields. No jugular venous distension was present. Epigastric and femoral artery bruits were present and the foot pulses were weak. His medications included aspirin, dipyridamole, simvastatin, amlodipine, doxazosin, isosorbide mononitrate, and frusemide. Laboratory data included: hemoglobin, 11.7 g/dL; serum creatinine, 474 μmol/L (5.4 mg/dL); creatinine clearance, 15 mL/min; and serum total cholesterol, 5 mmol/L. Two months later he had a cerebrovascular accident resulting in a left-sided hemiparesis from which he made a good functional recovery.

Ten months after his original referral, he had reached end-stage renal failure and was admitted for placement of a tunneled internal jugular vein dialysis catheter. An echocardiograph at that time revealed diffuse impairment of left-ventricular function, ejection fraction of 35%, and moderate calcification of the mitral valve.

Three months after starting dialysis, he complained of fatigue and increased frequency of angina. He had a normochromic normocytic anemia (hemoglobin 9.6 g/dL) and was iron replete (ferritin >100 μg/L; transferrin saturation >20%). He was enrolled in a clinical trial of once-weekly intravenous NESP for treatment of renal anemia. The dose was escalated until the hemoglobin level was within the target range of 11 to 13 g/dL. His iron stores have been maintained with intravenous iron saccharate. He has now been treated with NESP for 33 months and has not required blood transfusion over this period. He has remained clinically stable on hemodialysis with only one admission to hospital for elective arteriovenous fistula construction. He has had no apparent side effects related to NESP.

DISCUSSION

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Renal Medicine, Queen's University of Belfast, Northern Ireland): These patients illustrate that therapy with novel erythropoiesis-stimulating protein (NESP) (darbepoetin alfa, ARANESP™, Amgen Inc, Thousand Oaks, CA, USA) can successfully manage the anemia secondary to chronic renal failure. In the first case, the patient's therapy was converted from recombinant erythropoietin (rHuEpo) to less frequent dosing with NESP while effective control of hemoglobin concentration was maintained. In the second case, the patient had never received rHuEpo and was anemic when hemodialysis was initiated. The NESP therapy successfully corrected his anemia and during prolonged follow-up has maintained the patient's hemoglobin above the European Best Practice Guidelines (EBPG) target of 11 g/dL [1] and within the target hematocrit range of 33% to 36% detailed in the NKF-DOQI guidelines for renal anemia management [2].

The development of rHuEpo and its subsequent widespread use for treating renal anemia is arguably the most significant advance in clinical nephrology in the last 20 years. An effective alternative to rHuEpo, NESP has now been licensed for treatment of the anemia of chronic renal failure. In this Forum I will describe how knowledge of the structural biology of erythropoietin was crucial in the development of NESP and discuss the initial clinical data supporting its use for the management of renal anemia. I also will briefly review the search for alternative molecular therapies to stimulate erythropoiesis in renal failure.

Erythropoietin is the principal regulator of the process of erythropoiesis, which maintains an optimal circulating red cell mass for oxygen delivery to the tissues [3]. Under basal conditions, erythropoietin is present in serum at picomolar concentrations (0.8 to 4.0 picomoles/L), and in adults its primary site of synthesis is the kidney. A homeostatic feedback mechanism links renal oxygen sensing with erythropoietin production. Erythropoietin binds to and activates specific receptors on red cell progenitors in the bone marrow. Signal transduction via the erythropoietin receptor prevents apoptosis of these erythroid cells, permitting their further proliferation and differentiation into mature erythrocytes [4]. The subsequent increase in red cell mass improves tissue oxygenation and thereby reduces the stimulus for erythropoietin production.

Opinion is divided regarding both the primary source of erythropoietin and its eventual metabolic fate. In situ hybridization studies have demonstrated erythropoietin gene expression in renal peritubular and tubular cells [5–8]. Subsequently transgenic mice models identified erythropoietin synthesis in renal peritubular, interstitial, and proximal tubular cells [9–11]. The secreted hormone occupies a volume of distribution equivalent to the plasma volume, and its circulating half-life and clearance are similar in normal and uremic individuals [12, 13]. Some studies indicate that the kidney has a role in the elimination of erythropoietin [14, 15] but the liver has been regarded as the primary site of erythropoietin metabolism [16]. The major site for elimination of both endogenous erythropoietin and rHuEpo remains unclear, given reports that in animals removal of the liver or kidneys does not affect the half-life of erythropoietin [17]. In addition, the pharmacokinetics of rHuEpo in patients with liver cirrhosis are similar to those of normal individuals [18]. Recent evidence suggests that the bone marrow is the major erythropoietin elimination pathway [19].

Human erythropoietin is a glycoprotein with a molecular weight of 30,400 daltons [20]. The molecule consists of a 165 amino acid polypeptide chain linked by two disulfide bonds. Following translation the protein is heavily glycosylated, with the additional sugar chains accounting for 40% of the mass of the secreted hormone. The carbohydrate moieties are added to three specific asparagine or N-glycosylation sites (Asn 24, 38 and 83) and one serine or O-linked site (Ser 126) in both the natural and recombinant human erythropoietins [21]. Human erythropoietin is an elongated molecule with structural features of a left-handed 4-helix bundle similar to other hemopoietic growth factors [22]. The amino acids engaged in receptor binding are at the opposite end of the molecule to the cluster of four carbohydrate chains. Each of the N-linked carbohydrate chains can contain two, three, or four branches tipped by a negatively charged sugar molecule, sialic acid. The single O-linked carbohydrate can have as many as two sialic acid residues. All the other sugars within the carbohydrate chains are neutral. Endogenous erythropoietin molecules therefore have a variable number of sialic acid residues, to a maximum of 14. Recombinant human erythropoietin is purified to contain 9 to 14 sialic residues per molecule [23]. A fuller understanding of the heterogeneity of these carbohydrate structures was crucial to the subsequent development of novel erythropoiesis-stimulating protein [24–26].

The carbohydrate portions of erythropoietin are essential for its biologic activity in vivo [27]. Removal of sialic acid residues increases activity in vitro but severely limits activity in vivo presumably because of increased clearance of the molecule by hepatic asialoglycoprotein receptors [16]. The addition of carbohydrate to erythropoietin appears to be necessary for both its cellular secretion and solubility [28, 29]. The in vivo efficacy of isoforms of recombinant erythropoietin, with variable numbers of sialic acid residues, was tested in normal mice by assessing the effect of repetitive dosing on hematocrit [23]. A marked difference in biologic response was noted: the isoforms with the highest sialic acid content induced the greatest increase in hematocrit.

Two potential mechanisms could account for the greater activity of the sialic acid-rich isoforms, namely, increased erythropoietin receptor binding or a longer serum half-life. Utilizing a radioreceptor assay, Egrie and Brown examined the relative affinity of different isoforms for the erythropoietin receptor [23]. Isoforms with the lowest sialic acid content had the highest affinity for the receptor. For instance, the relative affinity of the isoform with six sialic residues was sevenfold greater than the isoform with the maximum 14 sialic acids. Conversely, in experiments designed to determine the circulating half-life of various erythropoietin isoforms, the molecules with the highest sialic acid content had much slower clearance from the circulation. For comparison, the half-life of the isoform with 14 sialic acid residues was more than threefold longer than the molecule with 6 sialic acids. Thus, the sialic acid content was directly related to biologic activity through its influence on serum half-life and inversely related to receptor affinity. Furthermore, an increase in serum half-life had a greater effect on activity in vivo than did decreased receptor affinity. Exploiting this relationship between serum clearance and the extent of glycosylation of erythropoietin led to the design and development of novel recombinant erythropoiesis-stimulating molecules.

Development of novel erythropoiesis-stimulating protein

A hyperglycosylated analog of rHuEpo in theory should have a much longer circulating half-life than native human erythropoietin. The N-linked carbohydrate chains are attached at consensus amino acid sequences (Asn-XXX-Ser/Thr) to the polypeptide portion of erythropoietin [30]. Several novel consensus sequences were engineered by site-directed mutagenesis of the cloned human erythropoietin gene [23]. It was essential that these sequence changes permit the additional glycosylation without significantly disrupting erythropoietin receptor binding or biologic activity. An additional concern, which was addressed in the clinical trials of NESP, was that altering amino acid sequence might introduce epitopes into the tertiary structure of an analog, rendering it immunogenic. A 5 N-linked chain analog of erythropoietin, NESP differs in its amino acid sequence from human erythropoietin at five positions (Ala30Asn, His32Thr, Pro87Val, Trp88Asn, and Pro90Thr). The asparagine residues at positions 30 and 88 provide the anchoring points for the two additional N-linked carbohydrate chains. Therefore, NESP is biochemically distinct from recombinant erythropoietin by virtue of its higher molecular weight, sialic acid content, and net negative charge (Table 1).

Table 1. Biochemical properties of rHuEpo compared to NESP

rHuEpo	NESP
Up to 14 sialic acids/molecule	Up to 22 sialic acids/molecule
3 N-linked carbohydrate chains	5 N-linked carbohydrate chains
1 O-linked carbohydrate chain	1 O-linked carbohydrate chain
165 amino acids	165 amino acids
40% carbohydrate	51% carbohydrate
30,400 daltons	37,100 daltons
pI 4.0	pI 3.3

Initially the efficacy of NESP compared with rHuEpo was assessed in vivo by measuring the increase in hematocrit in mice injected with equimolar doses of peptide over a 6-week period [23]. The NESP increased the hematocrit by 33.9% compared to an increase of 22.8% induced by rHuEpo. The increased activity of NESP was associated with a prolonged half-life but reduced erythropoietin receptor binding affinity. This finding was consistent with the data obtained in studies of erythropoietin isoforms that differed in their sialic acid content. Intravenous NESP was approximately 3.6-fold more potent than intravenous rHuEpo when administered three times weekly. When NESP and rHuEpo were administered once weekly in mice, NESP was approximately 13-fold more potent. In these animal studies, achieving the same increase in hematocrit required a higher weekly dosage of NESP when administered once weekly compared to thrice weekly. In contrast, the clinical trials of NESP revealed no apparent difference between once weekly and thrice weekly administration in terms of the total weekly NESP dosage (abstract; Macdougall et al, *J Am Soc Nephrol* 9:A1317, 1998).

Intravenous rHuEpo treatment given once weekly is less efficient compared to the same total weekly dose divided thrice weekly [31]. The pharmacokinetics of less frequently administered rHuEpo predict that the plasma level will fall below a threshold for effective erythropoiesis. Selective hemolysis of young red blood cells (neocytolysis), because of their relative erythropoietin deficiency, also can be a factor in poor response to once-weekly intravenous rHuEpo therapy [32].

In most published studies, subcutaneous administration of rHuEpo is more cost effective than intravenous administration at the same dosing schedule [33–36]. The optimal frequency of dosing with subcutaneous rHuEpo is unclear. In one study, the efficacy of once weekly subcutaneous rHuEpo was equivalent to that seen with a twice or three times weekly regimen [37].

Pharmacokinetics of NESP in dialysis patients

Single-dose pharmacokinetic studies of equimolar doses of NESP versus rHuEpo were performed in patients on peritoneal dialysis [38]. Serum levels of NESP and rHuEpo were determined at regular intervals after injection by

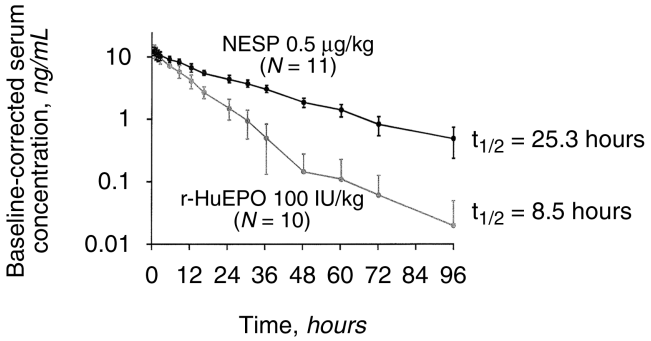

Fig. 1. Comparison of single-dose intravenous pharmacokinetics of novel erythropoiesis-stimulating protein (NESP) compared with recombinant human erythropoietin (rHuEPO; Epoetin alfa). Reproduced by the kind permission of Lippincott-Williams & Wilkins, from Macdougall et al, *J Am Soc Nephrol* 10:2392–2395, 1999.

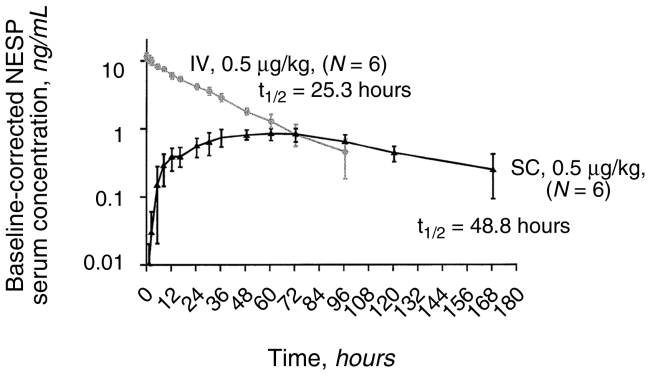

Fig. 2. Comparison of subcutaneous (SC) and intravenous (IV) pharmacokinetic profiles of NESP. Reproduced by the kind permission of Lippincott-Williams & Wilkins from Macdougall et al, *J Am Soc Nephrol* 10:2392–2395, 1999.

immunoassay (Fig. 1). The mean half-life of NESP following intravenous injection was three times that of rHuEpo (25.3 vs. 8.5 hours). In addition, the area under the curve (AUC) for intravenous NESP was twice that of intravenous rHuEpo (291 ng/h/mL vs. 131.9 ng/h/mL). The volume of distribution was similar for NESP (52.4 mL/kg) and rHuEpo (48.7 mL/kg) and is equivalent to the plasma volume. As expected, the mean half-life of NESP administered by subcutaneous injection was longer at 48.8 hours compared to 25.3 hours for the intravenous route (Fig. 2). The serum concentration peaked at 54.1 hours and the bioavailability of NESP was estimated to be 36.9%. The pharmacokinetic data from this first clinical study extended the observations in animals, which predicted that treatment of renal anemia would require less frequent dosing with NESP compared to rHuEpo.

The longer half-life of subcutaneous NESP reflects the balance between continued absorption from the subcutaneous site and its circulating metabolism. The subcutaneous half-life of NESP is approximately twice that reported for subcutaneous rHuEpo [31, 39–41]. The bioavailability of NESP is comparable to that of subcutaneous erythropoietin [42, 43]. Both the site of administration and skin-fold thickness influence the bioavailability of rHuEpo [44, 45], although these factors have not been assessed yet for NESP.

Studies of the pharmacokinetic profile of NESP following chronic intravenous and subcutaneous administration disclosed that NESP does not accumulate over time (abstract; Allon et al, *J Am Soc Nephrol* 11:A1308, 2000; abstract; Lerner et al, *J Am Soc Nephrol* 11:A1479, 2000). The elimination half-life of intravenous NESP remained three times that of intravenous rHuEpo.

Therapy

Initial therapeutic trials have addressed three areas of interest to nephrologists: the correction of anemia, long-term maintenance of hemoglobin concentration, and conversion from rHuEpo to NESP therapy. Correction of anemia with NESP was studied in patients who had been stabilized on dialysis but who had not been previously treated with rHuEpo. Utilizing a dose-escalation protocol, two multicenter trials were conducted involving hemodialysis and peritoneal dialysis patients, respectively (abstract; Macdougall et al, *J Am Soc Nephrol*, 9:A1317, 1998). The same total weekly dose of NESP was administered either once weekly or three times weekly to hemodialysis patients by intravenous injection or to peritoneal dialysis patients by subcutaneous injection. A weekly NESP dose of 0.45 μg/kg produced mean hemoglobin increases of 1.01 g/dL following subcutaneous administration and 1.08 g/dL with intravenous injections after four weeks. Neither the dosing schedule nor the route of administration appeared to influence the erythropoietic response to NESP. This finding differs from the clinical experience with rHuEpo [36, 46]. In a further study, patients with chronic renal failure (creatinine clearance <30 mL/min) and anemia (hemoglobin <11 g/dL) who were not undergoing dialysis were randomized to receive NESP (0.45 μg/kg once weekly) or rHuEpo (50 IU/kg twice weekly) (abstract; Locatelli et al, *J Am Soc Nephrol* 11:A1486, 2000). The primary outcome was a composite defined as a rise in hemoglobin of >1 g/dL and absolute hemoglobin level of >11 g/dL. A similar erythropoietic response was seen in both treatment groups (93% NESP, 92% rHuEpo), with a mean hemoglobin rise after 4 weeks of 1.38 g/dL (NESP) and 1.4 g/dL (rHuEpo).

Two studies have evaluated the efficacy of NESP in maintaining hemoglobin levels. The objective was to confirm that the hemoglobin concentration would remain stable after conversion to NESP even when it was administered less frequently compared to rHuEpo. In the North American hemodialysis trial, 507 patients were

enrolled into a double-blind study (abstract; Nissenson et al, *J Am Soc Nephrol* 11:A1326, 2000). Patients were randomized in a 1:2 ratio to receive intravenous NESP once weekly plus placebo twice weekly or to continue receiving thrice-weekly intravenous rHuEpo. The NESP dosage was calculated by a conversion formula (1 μg NESP = 200 IU rHuEpo) that equates the protein mass of the two compounds. No statistically significant difference in hemoglobin concentrations between baseline and the evaluation period was detected during weeks 21 through 28.

In the European/Australian multicenter study, 522 dialysis patients (either hemodialysis or peritoneal dialysis) were randomized in a 2:1 ratio to NESP or to continue receiving rHuEpo with no change in the route of administration (abstract; Vanrenterghem et al, *J Am Soc Nephrol* 10:A1365, 1999). Patients previously receiving twice- or thrice-weekly rHuEpo were converted to once-weekly NESP. Patients on once-weekly rHuEpo were converted to NESP once every 2 weeks. No significant changes in hemoglobin concentration between the groups occurred from baseline to the evaluation period (24 to 32 weeks). The NESP dosing was similar regardless of the route of administration over the course of the study. However, the weekly dosage of subcutaneous rHuEpo dosage was on average 22% lower compared to intravenous rHuEpo administration.

The longer term safety and efficacy of NESP in maintaining hemoglobin concentrations has been evaluated in a further European/Australian multicenter study that enrolled 703 dialysis patients who were treated with NESP for as long as one year (abstract; Graf et al, *J Am Soc Nephrol* 11:A1317, 2000). Again, patients receiving rHuEpo twice or three times weekly were converted to once-weekly NESP, and those receiving rHuEpo once weekly were converted to NESP every 2 weeks. No significant change in hemoglobin concentration was found from baseline to 36 weeks, and 96% of patients were managed at a reduced dosage frequency.

Data have been presented on more than 1500 patients who have received NESP and more than 500 who have been treated with rHuEpo in comparative controlled trials. The overall proportion discontinuing treatment due to adverse events was 4% for rHuEpo and 2% for NESP. Side effects attributed to NESP included hypertension and vascular access thrombosis; however, these sequelae did not correlate with the rate of rise of hemoglobin or the hemoglobin concentration.

One safety concern regarding NESP is the potential immunogenicity of the molecule, as its sequence differs at five amino acid positions compared to endogenous erythropoietin. If antibodies to NESP were to develop, they might be non-neutralizing or neutralizing (rendering NESP ineffective). Both types of antibodies could also in theory cross-react with erythropoietin. Several design features of NESP suggest that antibodies are unlikely to develop. Because NESP is a heavily glycosylated molecule, the carbohydrate chains might act as a barrier to immune surveillance of the underlying polypeptide structure. The amino acid changes are at sites distal to the receptor-binding domain. Therefore, any antibodies induced by these changes should be non-neutralizing. Carbohydrate chains themselves are rarely immunogenic, and all the additional residues on NESP are found on the N-linked chains of erythropoietin. Patients have been regularly tested for the development of NESP antibodies in all the clinical trials; none of these antibodies has been detected to date [47].

Several conclusions can be drawn from the clinical trials involving NESP. Once-weekly NESP administration by either subcutaneous or intravenous injection can effectively correct renal anemia in both pre-dialysis and dialysis patients who have not previously been treated with rHuEpo. When NESP is administered at a dosage of 0.45 μg/kg, the mean rate of rise of hemoglobin has been within the European Best Practice Guidelines for anemia management of 1 to 2 g/dL/month [1]. Conversion of dialysis patients from treatment with rHuEpo to less-frequently administered NESP by the same route has not been associated with any significant change in mean hemoglobin concentrations. A dosage conversion formula of 200 IU rHuEpo = 1 μg NESP for equivalent efficacy is supported by the trial data. In contrast to the clinical experience with rHuEpo, there is no difference in the mean weekly NESP dosage requirement when administered either via the intravenous or subcutaneous routes.

Future developments

The development of NESP from a design concept to a therapeutic reality has provided nephrologists with a new molecule for treating renal anemia. The increasing focus on earlier correction of anemia in pre-dialysis patients [48–50] and the upward trend in desired and achieved target hemoglobin concentrations in dialysis patients [1, 2, 51, 52] will place additional pressures on nephrology budgets. The cost and inconvenience of chronic parenteral administration of either NESP or rHuEpo will remain impediments to their wider use.

Alternative novel strategies for augmenting erythropoiesis are being explored, including the synthesis of erythropoietin mimetic compounds and gene therapy [53–57]. The erythropoietin receptor is a member of a super-family of cytokine receptors and possesses an extracellular ligand-binding domain, transmembrane region, and intracellular signaling domain with tyrosine kinase activity [58–60]. The erythropoietin receptor is activated as a homodimer with only a small number of ligand-receptor contact points, which account for the bulk of the binding energy [61, 62]. Peptide mimetics of

erythropoietin have been discovered with agonist activity in compounds containing as few as 13 amino acids [63–66]. However, the major drawback of the peptide mimetics is that they are unlikely to be active if administered orally because of their relatively large molecular weights and instability. Non-peptide molecules, which mimic erythropoietic factors in vitro, have activation sites independent of the hormone-binding domain [67, 68]. A large proportion of erythropoietin receptors naturally exist as homodimers on the cell surface even in the absence of the ligand erythropoietin [69]. The distal region and receptor tyrosines of the erythropoietin receptor are not essential for erythropoiesis in vivo [70], but the transmembrane domains are critical for signal transduction [71]. Erythropoietin mimetics presumably induce receptor activation by a conformational change in the homodimer [72].

The huge potential market for an orally active erythropoietin mimetic coupled with the expanding knowledge of the structural biology of the erythropoietin receptor will continue to drive the search for these compounds. Nephrologists, however, now have a therapeutic choice between two effective recombinant DNA-derived glycoproteins, rHuEpo or NESP, for the management of anemia in patients with chronic renal failure.

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston, Massachusetts, USA*): Peter, thanks for a superb review of NESP. Could you tell us how the degree of glycosylation of erythropoietin compares to that of other secreted hormones, such as insulin?

Prof. Maxwell: Hemopoietic growth factors/hormones are secreted as glycoproteins. The expressed proteins undergo post-translational modification with the addition of carbohydrate groups in the cytoplasm prior to secretion from the cell. The carbohydrate component of a glycoprotein is essential for its biologic action in vivo as it prevents rapid clearance of the protein from the circulation. To produce rHuEpo, the human erythropoietin gene is expressed in mammalian cells to ensure that the erythropoietin protein is correctly glycosylated following translation. Heavily glycosylated proteins have longer circulating half-lives. In contrast, insulin is not glycosylated and therefore recombinant human insulin production is possible via simpler manufacturing processes involving bacterial cells (which lack these glycosylation pathways).

Dr. Harrington: Are there studies on the bone marrow erythropoietin elimination pathway? Do we know enough about it to use that pathway as a method to increase its half-life, that is, by interfering with the degradation process?

Prof. Maxwell: The metabolism of erythropoietin has been studied by a number of groups [14–18] but there is no consensus on the predominant route of elimination of rHuEpo. It had been reported that the liver was the primary site for degradation of erythropoietin following uptake by asialoglycoprotein receptors [16]. Recent evidence suggesting that the bone marrow is the main site for erythropoietin clearance [19] requires further investigation to define the degradation pathway. At present the only way to increase the half-life of erythropoietin is by modifying the structure of the molecule itself by pegylation or adding sialic acid residues.

Dr. Peter Conlon (*Beaumont Hospital, Dublin, Ireland*): Weiss and colleagues reported that the same total rHuEpo dosage given once weekly was equivalent to divided doses thrice weekly [37]. What role is there for NESP compared to once-weekly rHuEpo?

Prof. Maxwell: At present that is difficult to answer conclusively. Most of the published literature relating to the frequency of rHuEpo administration indicates that more frequent dosing is most cost effective compared to once-weekly injections [31, 33–36]. Conversely, the study by Weiss and colleagues concluded that the same total weekly dosage of subcutaneous rHuEpo had equivalent efficacy whether rHuEpo was given once weekly or in divided doses two or three times per week [37]. By virtue of its longer half-life, NESP is a more potent molecule than rHuEpo. If an equivalent amount (in terms of the number of molecules) of NESP and rHuEpo were administered once weekly, I would expect NESP to have the greater efficacy. However, the relative costs and long-term safety of NESP in the treatment of renal anemia are not established yet.

Dr. John Harty (*Daisy Hill Hospital, Newry, Northern Ireland*): While the Weiss study has demonstrated no difference in hemoglobin levels despite variation in rHuEpo dosing frequency, these observations were in stable patients. Would you anticipate that in unstable or ill dialysis patients, reduced frequency rHuEpo regimes are inadequate to maintain hemoglobin, and does NESP have the potential to be more advantageous in this setting?

Prof. Maxwell: The essence of your question is, will NESP be able to maintain erythropoiesis more effectively than rHuEpo in hostile environments, for example, in patients with sub-optimal dialysis or in those with chronic inflammatory states. Understandably, these patients are usually excluded from clinical trials, so we have no evidence that NESP is more effective in ill dialysis patients. These ill patients are often said to be "resistant" to rHuEpo, but in practice this resistance might reflect inhibition of erythropoiesis in the bone marrow that is mediated by pro-inflammatory cytokines and uremic toxins. It might be that NESP has no advantage over rHuEpo in this setting.

Dr. George Mellotte (*St. James Hospital, Dublin*): One of the concerns with rHuEpo is the need for supraphysiologic doses of iron. Have you any evidence that iron demands are different with NESP?

Prof. Maxwell: I don't think there is any evidence that iron utilization in NESP-treated patients is fundamentally different than that in rHuEpo-treated patients. The clinical trials were conducted in patients who were iron replete, and additional intravenous iron was administered if the serum ferritin values fell below 200 μg/L. The total iron administered per year in NESP-treated patients was not compared to a matched group of rHuEpo-treated patients.

Dr. Henry Brown (*Antrim Area Hospital, Northern Ireland*): Are there any differences in the profiles of erythropoietin isoforms with respect to number of sialic acid residues between healthy and anemic individuals and the potency of rHuEpo in both groups?

Prof. Maxwell: Circulating erythropoietin exists as a heterogeneous collection of individual isoforms. When isolated, these isoforms have between one and 14 sialic acid residues per molecule of erythropoietin. Erythropoietin isoforms with a higher number of sialic acid residues have a longer half-life and are therefore more potent. A change in the relative proportions of these isoforms would be expected to affect the rate of erythropoiesis. I am not aware of any investigations demonstrating that anemic individuals have an isoform profile with relatively few sialic acid residues per erythropoietin molecule.

Dr. Philip Kalra (*Hope Hospital, Salford, United Kingdom*): You mentioned that erythropoietin increases hemoglobin by reducing apoptosis of early red cell forms. Is anything known regarding the molecular mechanism of this effect?

Prof. Maxwell: Apoptosis is a major component of normal erythropoiesis, and erythropoietin controls erythrocyte production by retarding DNA breakdown, which in turn permits the survival and differentiation of erythroid precursor cells [73]. Knockout studies in mice have confirmed that the absence of erythropoietin is lethal to embryos because of failure of the final stages of erythroid differentiation. Signal transduction from the erythropoietin receptor engages several distinct signaling pathways with activation of phosphatidylinositol 3-kinase (PI 3-kinase) [58, 59]. A downstream target of PI 3-kinase, the serine/threonine protein kinase Akt (protein kinase B), has a major anti-apoptotic role in erythropoietin-stimulated erythroid precursors [74].

Dr. Kottarathil Abraham (*Beaumont Hospital*): At what point is the equivalence of intravenous and subcutaneous NESP lost? In other words, would the first patient described have maintained her hemoglobin level with intravenous rather than subcutaneous administration of NESP every two weeks?

Prof. Maxwell: The equivalent efficacy of intravenous and subcutaneous NESP administration has only been demonstrated in patients receiving once-weekly injections. As the half-life of subcutaneous NESP is almost twice that of intravenous NESP, I would expect that once the dosing intervals were extended to every two weeks, the routes of administration would no longer have equivalent efficacy.

Prof. Francis Muldowney (*University College Dublin*): Is the increased red cell mass in thyrotoxicosis mediated by erythropoietin?

Prof. Maxwell: There is some evidence that tri-iodothyronine augments erythropoiesis indirectly by stimulating the release of soluble hemopoietic growth factors from bone marrow leukocytes [75]. This response appears to be independent of the direct mitogenic action of erythropoietin.

Dr. Liam Plant (*Cork University, Cork, Ireland*): Can you speculate as to the mechanism whereby continued erythropoietin exposure protects young red blood cells from neocytolysis?

Prof. Maxwell: Erythroid progenitor cells require erythropoietin for proliferation, differentiation, and continued viability. Erythropoietin both stimulates mitogenesis and prevents programmed cell death of these erythroid cells. The rapid decrease in red cell mass observed following descent from high altitude or in astronauts entering microgravity is associated with suppression of endogenous erythropoietin. Administration of exogenous rHuEpo can prevent the change in red cell mass [76]. It is difficult to explain the mechanism of destruction of newly formed erythrocytes, as these cells have already lost their nucleus and therefore should not be susceptible to apoptosis. Erythropoietin withdrawal might lead to rapid hemolysis by altering the interaction between newly formed erythrocytes and reticuloendothelial phagocytes [76].

Dr. Con Cronin (*Mid-Western Regional Hospital, Limerick, Ireland*): Are you surprised at the incidence of hypertension with rHuEpo and NESP, bearing in mind the experience with rHuEpo over a decade? Is it a dose adjustment, red cell mass problem, or a result of other alternative independent factors?

Prof. Maxwell: The mechanisms responsible for hypertension induced by rHuEpo or NESP therapy are not well understood. Multiple factors can contribute to the rise in blood pressure, including rapid increases in hematocrit and red cell mass, increased sensitivity to vasoconstrictors, decreased responsiveness to vasodilators, a direct vasopressor effect of erythropoietin, and remodeling of arterial resistance vessels [77]. The incidence of hypertension in NESP-treated patients is similar to that of rHuEpo-treated patients in the clinical trials I discussed.

Prof. Hugh Brady (*Mater Misericordiae Hospital, Dublin*): The Epo receptor is a member of the super-

family of cytokine receptors. Does high-dose rHuEpo activate other cytokine receptors? Are these interactions likely to contribute to its bioactivity profile?

Prof. Maxwell: Erythropoietin is highly specific for its cognate receptor at physiologic concentrations of the hormone [59]. Pharmacologic doses of rHuEpo should not activate other cytokine receptors because they have specific affinity for other ligands. Indeed, transgenic mice that overexpress the erythropoietin gene develop erythrocytosis but not thrombocytosis or leukocytosis.

Dr. John Donohoe (*Beaumont Hospital, Dublin*): In chronic renal failure, why is the degree of anemia discrepant from patient to patient or from disease to disease? For instance, why do patients with ESRD secondary to polycystic kidney disease often have higher hemoglobin levels than patients with other diseases? Is this due to a quantitative difference in erythropoietin production or possibly to a qualitative difference with a shift in the profile of erythropoietin isoforms toward "pauci-sialic acid" types?

Prof. Maxwell: The degree of anemia is primarily related to the amount of erythropoietin being secreted by the kidneys. In general, patients with polycystic kidneys have higher circulating erythropoietin levels compared to patients with other chronic renal diseases with equivalent glomerular filtration rates. Another factor might be variation in the use of ACE inhibitors. The hypothesis that ACE inhibitors interfere with erythropoiesis in chronic renal failure remains controversial despite widespread use of these drugs for post-renal transplant erythrocytosis [78]. No evidence supports the suggestion that different profiles of Epo isoforms exist in distinct renal diseases.

Dr. Donal Redden (*Duke University, Durham, North Carolina, USA*): In view of the adverse results from the normalization of hematocrit in hemodialysis trial, should we be similarly cautious with NESP in aggressive anemia management in patients with cardiovascular disease?

Prof. Maxwell: Your excellent question reminds us of the reported increased mortality in ESRD patients with cardiac disease when hematocrits were normalized [79]. The optimal hematocrit for patients with ESRD is still the subject of debate. I think we should be cautious treating anemia in patients with known cardiac disease irrespective of whether NESP or rHuEpo is the agent used.

Dr. Yvonne O'Meara (*Mater Misericordiae Hospital, Dublin*): What is the definition of iron replete? Do we need to be more aggressive in managing iron therapy by achieving ferritin values higher than 100 μg/L and transferrin saturation >20%, as in the two patients under discussion?

Prof. Maxwell: For the reported clinical trials, a patient was defined as being iron replete if the ferritin level was >200 μg/L and the transferrin saturation was >20%. Treatment with NESP could be initiated provided the ferritin level was >100 μg/L and additional intravenous iron therapy was provided. In our own hemodialysis unit, we aim to maintain ferritin levels between 200 and 800 μg/L with transferrin saturation >20%.

Dr. Damian Fogarty (*Antrim Area Hospital, Northern Ireland*): What accounts for the earlier onset of renal anemia in patients with diabetic nephropathy?

Prof. Maxwell: The perception is that individuals with diabetic nephropathy develop anemia earlier than other patients with chronic renal disease with comparable glomerular filtration rates. It has been suggested that hyporeninemia is linked to erythropoietin deficiency [80]. It is difficult to explain it on the basis that diabetics have a unique tubulointerstitial pathology that leads to an earlier decrease in peritubular or tubular cell erythropoietin synthesis. The more aggressive use of ACE inhibitors and angiotensin II receptor blockers in these patients might blunt erythropoiesis, but this hypothesis is unproven [78].

Prof. Brian Keogh (*Tallagh Hospital, Dublin*): Is there likely to be any innovation in the route of delivery of erythropoietin or NESP?

Prof. Maxwell: The bioavailability of rHuEpo or NESP is maximal when administered intravenously, that is, all of the administered dose enters the circulation. Subcutaneous administration of these compounds is more cost-effective since the prolonged half-life of the compound, reflecting slow absorption from a subcutaneous depot, outweighs the lower bioavailability. Administration by intraperitoneal injection or inhalation is inefficient and not cost-effective. As both NESP and rHuEpo are complex glycoproteins, they are inactivated by digestion following oral administration. In the future it is possible that mimetic compounds that are orally active will be developed for clinical use.

Dr. Peter Garrett (*Tyrone County Hospital, Omagh, Northern Ireland*): Are any trials in progress that directly compare once-weekly subcutaneous rHuEpo versus once-weekly subcutaneous NESP?

Prof. Maxwell: I am not aware of any reported or published trial that has analyzed this important question. I believe it is still unproven which compound and dosing frequency is the most cost-effective.

Dr. Harrington: Could I ask you a cost-benefit question? It seems to me that the practicing nephrologist is likely to more interested in the cost of achieving a target hemoglobin rather than being concerned about the mass of protein being administered.

Prof. Maxwell: I agree entirely that cost will be the bottom line for a practicing nephrologist. If, in routine clinical practice, NESP proves to have a similar safety profile to rHuEpo, then we need an urgent answer to the cost-benefit question. A large trial comparing once-weekly subcutaneous NESP versus once-weekly subcutaneous

rHuEpo should be undertaken. I suspect, however, that if either NESP or rHuEpo clearly emerged as the most cost-effective compound, the market price of the "losing" compound would be decreased to compensate.

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