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CLINICAL IMPLICATIONS OF PEGYLATION: ADVANTAGES AND OPPORTUNITIES IN RENAL ANEMIA

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ABSTRACT

Pegylation is a complex process which includes attachment of Polyethylene glycol chain to the target molecule giving it certain advantages in terms of Pharmacokinetics and Pharmacodynamics. Various molecules have been modified with this process rendering them more suitable for administration as well as increasing their therapeutic efficacy. Its usefulness in Chronic Kidney disease (CKD) has also been explored. Anemia is a common comorbidity associated with CKD. The commonest causes include Erythropoetin and iron deficiency. Therefore the management relies on iron supplementation and Erythropoesis stimulating agents (ESAs). There are various ESAs available including short and long acting, depending on their half lives. The long acting ESAs include Methoxy Polyethylene Glycol EPO (MPG-EPO), which is 3rd generation ESA. The half life of this ESA is prolonged by addition of polyethylene glycol chain which gives it certain advantages. This review discusses about the process of pegylation and how it has become helpful for the management of patients suffering from renal anemia.

Anemia is one of the common complications of chronic kidney disease (CKD).[1] The prevalence of anemia increases with CKD stage. The major causes for anemia in CKD is attributed to iron deficiency and decreased erythropoietin production. Iron deficiency in CKD can be either absolute or functional. Absolute iron deficiency usually results due to dietary iron deficiency, decreased absorption and blood loss, while functional iron deficiency occurs due to inflammatory block of iron because of raised hepcidin level.[2] Erythropoietin deficiency is the most important cause of anemia in CKD and has been shown to occur in all stages of renal failure. Because the kidneys are the only source of erythropoietin (EPO) synthesis in adults, loss of kidney mass, such as in progressive CKD, often leads to a decrease in EPO production, leading to anemia.[3] Currently the management of anemia in CKD lies with iron supplementation and Erythropoiesis Stimulating agents (ESAs).[4] Recombinant Human EPO had been approved by FDA in 1989 for renal anemia management.[5] They are referred to as first-generation and usually have a shorter half-life, requiring regular dosing 1-3 times a week. Darbepoetin alfa, considered a second-generation ESA, is modified by EPO to give it a longer half-life and can usually be given weekly or biweekly. Recently, a third-generation ESA modified from EPO by adding a large pegylation chain called continuous EPO receptor activator (CERA) was approved to be marketed.[6] It acts on the EPO receptor differently than other ESAs, giving it a much longer half-life and can usually be given once every two weeks or once a month. Among the longer acting ESAs, PEGylated Erythropoietin has been approved by FDA for usage in CKD population.[7,8]

What is Pegylation

Polyethylene glycol (PEG) are amphiphilic polymers composed of repeating subunits of ethylene oxide, each with Molecular weight (Mw) 44 Da and whose number is expressed as a whole integer 'n'. The molecular weight of PEG is equal to n × 44 Da.[9] Pharmaceutical PEGs are inert, non-toxic and contains two terminal hydroxyl groups which can be chemically activated.[10] Structure of PEGs include branched and multi-branched PEGs.[11] They are useful for conjugative addition of small molecules drug loading capacity and PEG-based hydrogels.[9]

PEGylation is the chemical reaction between one or more activated polyethylene glycol molecules and a biomolecule, typically a protein, peptide, or small molecule or an oligonucleotide, leading to the development of a potential novel molecular entity (NME) with enhanced pharmacokinetic and pharmacodynamic properties, allowing it to reach its highest therapeutic potency.[9,12] In its most common form, PEG is a linear or branched polyether diol with

many useful properties such as biocompatibility, solubility in aqueous and organic media, lack of toxicity, very low immunogenicity, antigenicity, and good excretion kinetics.[13] PEGylation produces alterations in the physicochemical properties of the parent molecule, including changes in conformation, steric hindrance, electrostatic binding properties and hydrophobicity. Each ethylene glycol subunit in PEG associates with two to three water molecules making PEGylated molecules about five to ten times larger than a soluble protein of a similar molecular mass.[14] The process was introduced by Abuchowski and colleagues in 1977. They reported the superior immunogenic properties of bovine serum albumin (BSA)–PEG conjugates compared to the unmodified biomolecule.[15] Later it was shown that PEGylation increased circulation lifetimes and decreased immunogenicity when compared to native proteins. Since then, numerous researchers have shown that PEGylation enhances the pharmacological characteristics of proteins. Many types of proteins have been approved for clinical use including enzymes, hormones, cytokines, blood factors, antibody fragments and antibodies. Protein-based medicines include vaccines, therapeutic proteins (and peptides) and blood products (e.g., albumin). The FDA has approved PEG for use as a vehicle or base in foods, cosmetics and pharmaceuticals, including injectable, topical, rectal and nasal formulations.[16] Due to the increased weight of the PEGylated molecule and masking of the protein surface, PEGylation is able to promote therapeutic efficacy by slowing clearance through glomerular filtration, Reticulo-Endothelial System, or proteolytic destruction.[17] Additionally, the ability of PEG to cover the protein surface significantly reduces the immune response triggered by heterologous proteins, minimizing both the risk of anaphylactic reactions from repeated administrations as well as the production of antibodies recognizing and inactivating the foreign protein. Lastly, PEGylation technology is adaptable enough to enable customized modifications of each protein to meet the needs of various applications.[18]

When compared to native, unmodified proteins, the clinical uses of therapeutic PEGylated proteins represent significant improvement due to their high stability and very low immunogenicity, which generate sustained clinical outcomes with low doses and less frequent administration, enhancing patient's quality of life.[17] PEGylated therapeutic proteins, for example, are administered once a week as a subcutaneous injection, and their blood concentration always remains close to the optimal therapeutic level because the PEG modification ensures a sustained absorption from the injection site that serves as depot. Non-PEGylated therapeutic proteins are typically administered every one or two days, with the consequence that blood concentration fluctuations with a negative impact on clinical activity.[18,19]

Pegylation conjugation techniques can be classified into two categories- first and second generation.[17] The first PEGylated product- pegademase bovine was approved by FDA in 1990.[20] Since then, the advancements have happened in this field. From random to site-specific PEGylation, from many linear PEGs to a single, branching PEG, and from modest 5 kDa PEGs to 40 kDa PEGs, PEGylation technology has advanced.[21] Also this technology has advanced towards other therapeutic agents like peptides, aptamers and small molecules.[14] Several first generation PEGylated products are still in use like pegademase, a PEGylated form of the enzyme adenosine deaminase for the treatment of severe combined immunodeficiency disease (SCID), and pegaspargase, a PEGylated form of the enzyme asparaginase for the treatment of leukaemia.[16] Both these drugs which are non-human enzymes, depend on PEGylation to reduce the immune response that limits their therapeutic usefulness. They use a PEGylation strategy that involves the non-specific attachment of multiple, relatively small, 5-kDa PEG molecules. This non-specific attachment strategy, under the proper experimental conditions, does not significantly disrupt the active site of the enzyme but does suppress the undesired immune response.[22] 2nd generation PEG products are more specific conjugates compared to the 1st generation. Pegylating site-specifically can minimize the loss of biological activity and reduce immunogenicity. Most PEG conjugation techniques seek to extend the circulatory half-life without compromising activity. It must be noted that the development of PEG conjugation techniques and the variety of PEGs used for the process have led to the increase in demand for PEGylated Pharmaceutical Products.[23] PegInterferon alfa 2a and 2b, two commercially available PEGylated derivatives of Interferon (IFN), serve as an instructive illustration of how various PEGylation techniques can have varying effects on the pharmacokinetics, pharmacodynamics, and biodistribution of PEGylated derivatives.[18]

Major advantages of PEGylated products.[24]

Reduced renal clearance	PEGylation increases the circulating half-life of proteins by decreasing proteolytic degradation and renal clearance
Increased solubility	Decrease the susceptibility for proteins to aggregate in solution
Reduced immunogenicity	By reducing the frequency of dosing, PEGylation can reduce the chances for immunotoxicities to occur. PEGylation may also prevent the formation of antibodies by masking immunogenic sites on proteins
Reduction in biological activity	This is a consequence of steric shielding where the PEG

	molecule partially blocks interactions between the therapeutic protein and its target receptor. PEGylation does not, however, change the function of the protein, so while the steric shielding by PEG can also decrease initial interaction with the target of the protein, once the interaction between the protein and its target occurs, the biological outcome is the same as for the non-PEGylated protein

The first protein derivative, PegFilgrastim received approval in 2002.[26] The N-terminal methionyl residue of G-CSF underwent site-specific pegylation to generate it, which enhanced its pharmacokinetics without impairing the biological action.[18] Pegfilgrastim remains in plasma long enough to allow a single subcutaneous injection per chemotherapy treatment, rather than the multiple injections needed of the unconjugated protein.[12]

PegIFN-2 alfa2b and 2a are available for therapeutic use. Peg-2 alfa avoids the common issues with PEGylation in terms of binding to the receptor, drug metabolism, and renal excretion since the linkage is hydrolytically unstable in plasma and the free protein is released slowly after injection.[12] For 2b, the pegylation bond has greater stability, it circulates intact and itself reacts with the receptor than the native protein.

Pegvisomant is the first specific growth hormone receptor antagonist and is first in class therapy for treatment of Acromegaly. It has been approved for use in 2003.[27] Although PEGylation decreases the effectiveness of the GH receptor antagonist, it dramatically reduces immunogenicity and the rate of elimination from the body, making it a potent treatment for acromegaly. The recommended dosage for patients begins with a 40 mg loading dose administered subcutaneously. The patient will subcutaneously self-administer 10 mg of Somavert daily. Serum IGF-I concentrations should be measured every four to six weeks, with adjustments to the dosage of Somavert in 5 mg increments depending on the elevation or decline of insulin growth factor-1 (IGF-I) levels.[15]

PEGylated Erythropoetin in CKD

Continuous erythropoiesis receptor activator or methoxy polyethylene glycol-epoetin beta (MPG EPO) is the 3rd generation ESA introduced in the market. Pegylation significantly increases the half life of circulating ESA compared to the other ESAs. It is referred to as 3rd generation ESA after Darbepoetin and rHuEPO.[28]

PEGylated rhEPO molecules typically contain mixtures of molecules in which PEG is linked to various reactive amines, each which can have different effects on activity and protein folding.[29] During synthesis, a PEG - conjugated amino acid is introduced in place of the unconjugated amino acid, allowing targeting of particular amino acid positions for PEG attachment, such as the glycosylation sites, and reducing the potential for loss of in vitro activity. This might extend the serum half life.[29]

Methoxy polyethylene glycol epoetin beta has been approved by the United States Food and Drug Administration (USFDA), European Medicines Agency (EMA) and Drugs Controller General of India (DCGI) for management of anemia in CKD. The average half life of MPG EPO is 134 hours when it is given intravenous and 139 hours when given subcutaneously[130]. SC bioavailability is 52%.[30] The initial dose of the drug is 0.6 µg/kg of body weight administered as a single i.v. or s.c. injection once every two weeks. If Hb is maintained between 10-12 g/dl, C.E.R.A. can be administered once a month using a double dose of 12 µg/kg. C.E.R.A. dosing is based on the total weekly dose of ESA at the time of conversion and ranges from 120 to 360 mcg monthly or 60 to 180 mcg every two weeks.[31] When starting or changing therapy with C.E.R.A, Hb should be monitored every two weeks until stabilization, and every two to four weeks thereafter.[32]

The increased size and altered clearance of PEG-EPO also impact its distribution in the body. Compared to conventional erythropoietin, which primarily targets the bone marrow to stimulate red blood cell production, PEG-EPO exhibits a more extensive distribution profile. The PEGylated form reaches various tissues and organs, ensuring a sustained presence of erythropoietin and promoting its therapeutic effects beyond hematopoiesis. This broader distribution contributes to the overall efficacy of PEG-EPO in managing anemia and related conditions.[33]

Furthermore, the prolonged bioavailability of PEG-EPO is another advantage derived from its pharmacokinetic properties. As a result of the pegylation process, PEG-EPO achieves a more controlled release of erythropoietin over an extended period. This sustained release allows for a stable and continuous stimulation of red blood cell production, maintaining hemoglobin levels within the target range. By avoiding rapid fluctuations in erythropoietin levels, PEG-EPO reduces the risk of adverse events associated with erythropoietin therapy, such as high blood pressure or the need for frequent dose adjustments.[29]

Advantages of PegEPO

1. Prolonged half-life: PEGylation of erythropoietin significantly increases its half-life compared to rhEPO. This means that peg-EPO can stay in the

body longer, reducing the frequency of administration. This convenience can improve patient compliance and reduce the burden of repeated injections.

2. Lower dosing frequency: Peg-EPO can be administered less frequently than rhEPO due to its longer half-life. Instead of requiring multiple injections per week, peg-EPO can be administered once every few weeks or even once a month. This reduced dosing frequency can improve patient comfort and reduce the need for frequent doctor appointments, particularly in Non dialysis patients
3. Better stability: Pegylation increases the stability of erythropoietin, making it more resistant to degradation. This improved stability allows the drug to be stored and transported more easily without compromising its effectiveness. It also reduces the risk of waste or the need for cooling, which can be useful in resource-constrained settings.
4. Smoother Hemoglobin rise: Peg-EPO has been shown to support hemoglobin levels in chronic kidney disease (CKD) dialysis patients. Its long-lasting effects help prevent fluctuations in hemoglobin levels, which can improve symptom control and reduce transfusion dependency.
5. Reduction of side effects related to injection frequency: Erythropoietin injection may be associated with reactions, discomfort or pain. Due to the extended dosing intervals of Peg-EPO, the frequency of injections is reduced, which can minimize injection-related side effects and improve patient tolerance.
6. Simplified regimen: The less common dosing regimen of Peg-EPO simplifies the regimen for patients. They may need to visit healthcare facilities less often and require fewer injections, improving comfort and quality of life.

CONCLUSION

PEGylation is a clinically proven method that is both safe and generally applicable to different structural classes of proteins used to treat a wide range of diseases (eg, infections, oncology, inflammation). PEGylation is most commonly used to extend the half-life of protein-based drugs. It is used in many clinical settings today. Especially in CKD, PegEPO has clear advantages over short-acting ESAs, including lower dosing frequency and improved patient compliance. In addition, it causes less hemoglobin variability, which improves the control of anemia in these patients. Compared to traditional, frequently administered ESAs, PegEPO is convenient for the simplified treatment of anemia with a monthly dosing schedule.

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