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A review on impact of pegylation on biopharmaceuticals

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***Abstract*---**Covalent conjugation of polyethylene glycol (PEG) molecules to biopharmaceutical molecules is known to increase the pharmacological and medicinal characteristics of proteins and other big molecules and has been utilized effectively in 12 authorized medications. PEG reagents with straight and branched chains up to 40 kDa were utilized with a variety of PEG derivatives with varied linker chemistries. This article discusses the characteristics of PEG, the history and evolution of PEGylation chemistry, and examples of PEGylated pharmaceuticals with a proven track record. They prefer to employ bigger PEG polymers and complicated PEG structures, although they use extremely pure and well-characterized PEG reagents. The preclinical toxicity data for PEG in PEGylated biologics

that have been authorized are summarised. Microscopically detected cell vacuolization in phagocytes, which is connected to the biological function of absorption and elimination of particles and macromolecules from blood and tissues. It's possible. Side effects in toxicity tests typically relate to the active moiety of the medicine, not the PEG moiety, according to experience with commercially available PEGylated pharmaceuticals.

Keywords---pegylation, biopharmaceutical, covalent, conjugation, PEG reagent.

Introduction

PEG (polyethylene glycol) molecules are synthetic, highly water-soluble, inert polymers available in a wide variety of molecular weights and "A long chain, a linear synthetic polymer composed of ethylene oxide units, $HO(CH_2CH_2O)_nCH_2CH_2OH$, in which n can vary to provide compounds with molecular weights from 200-20,000. It is non-toxic and has been administered orally and intravenously to humans (PEG-adenosine deaminase for severe combined immunodeficiency disease; PEG-asparaginse for acute lymphoblastic leukemia; PEG-superoxide dismutase for oxygen toxicity. PEG can be coupled to proteins following appropriate derivatization of the OH groups of the PEG. The NH_2 groups of lysine side chains are particularly accessible sites and either a few or many sites can be modified. Given adequate technology for their production, PEG-modified proteins have numerous therapeutic and other applications". PEGs of various molecular weights are widely utilized in consumer items such as laxatives, toothpaste, and hair shampoos, as well as in biopharmaceuticals over the past 20 years. PEGylation is a chemical modification that is used to improve the pharmacological qualities of small molecule and biotherapeutic medicines. PEGylation can extend a protein's or nucleic acid's circulation time, improve drug ingredient water solubility, protect against in vivo biological inactivation by proteolysis, and minimize immunogenicity in some biopharmaceuticals. The major reason that PEGylation technology is utilized in parenteral medications that are otherwise removed by the kidney or another organ is to extend the half-life.

The use of proteins and peptides as therapeutics has a long and successful history. Starting with recombinant insulin in 1982, protein-based therapies have become an important tool in combating disease and illness with over 130 proteins and peptides approved for clinical use by the Food and Drug Administration (FDA) Due to their large size and specific conformation, proteins have the advantage of being highly specialized for their binding and/or activity. This means that there is less of a chance for cross-reactivity, which can cause potentially fatal side effects. In addition, since most protein therapeutics are based on endogenously expressed proteins, there is less of a chance of developing an immunogenic reaction to them. In addition, because of their vast diversity, proteins can be used to treat a number of different illnesses: from treating endocrine disorders to combating various cancers to alleviating autoimmune diseases, to being the active agent in many vaccines. This diversity makes proteins attractive options for researchers to

use in developing novel therapeutics.Pegylation can be accomplished using a variety of chemical reagents in a number of ways. Pegylated prodrugs are made up of degradable links.

Based on balanced risk/benefit analyses, twelve PEGylated biopharmaceuticals have been authorized. The toxicological effects of these items were generated from the active component of the medication rather than the PEG moiety, according to nonclinical safety assessments. In nonclinical toxicity tests, the sole impact attributable to PEG was cellular vacuolation, which was detected with 5 of the 12 authorized PEGylated biopharmaceuticals (for 2 drugs toxicology information is not available). Vacuolation is mostly found in phagocytic cells, however, it can also be detected in nonphagocytic cells. Larger PEGylated biopharmaceuticals are likely cleared by phagocytic cells, whereas tiny PEG molecules and PEG conjugates are removed via kidney and liver due to their hydrophilicity and size. For a more in-depth look into tissue vacuolation and the nonclinical safety of PEGylated biotherapeutics.

The chemistry of PEG reagents, PEGs, and linker chemistry employed in 12 authorized medications is described in this paper. A brief review of the cellular vacuolation found in nonclinical toxicological research is provided. Additional information and evaluations on the safety of PEGylated biopharmaceuticals are available. PEGylation is a process in which one or more units of chemically activated polyethylene glycol react with a biomolecule, usually a protein, peptide, small molecule, or oligonucleotide, creating a putative new molecular entity possessing physicochemical and physiological characteristics that are distinct from its predecessor molecules. In recent years, PEGylation has been used not only as a drug delivery technology but used also as a drug modification technology to transform existing biopharmaceuticals clinically more efficacious than before their PEGylation. PEGylation bestows several useful properties upon the native molecule, resulting in improved pharmacokinetic and pharmacodynamic properties, which in turn enable the native molecule to achieve maximum clinical potency. In addition, PEGylation results in a sustained clinical response with a minimal dose and less frequency of dosing, leading to improved quality of life via increased patient compliance and reduced cost. During the development of various pegylated protein therapeutics, several new insights have been gained. This review article focuses on the approaches, strategies, and the utilization of modern PEGylation concepts in the design and development of well-characterized pegylated protein therapeutics.

Pegylation Chemistry

PEG reagents, both linear and branched-chain, are employed in recently marketed medications. Two PEG molecules are connected to a central core in branched PEG reagents, from which a tethered reactive moiety will bind to the drug molecule. When using branched-chain PEG reagents, products with a greater PEG density per modification site are produced. Multiarm reagents have not yet been authorized by the Food and Drug Administration (FDA). If the target is a mono-PEGylated product, branched or multiarm reagents are favourable.

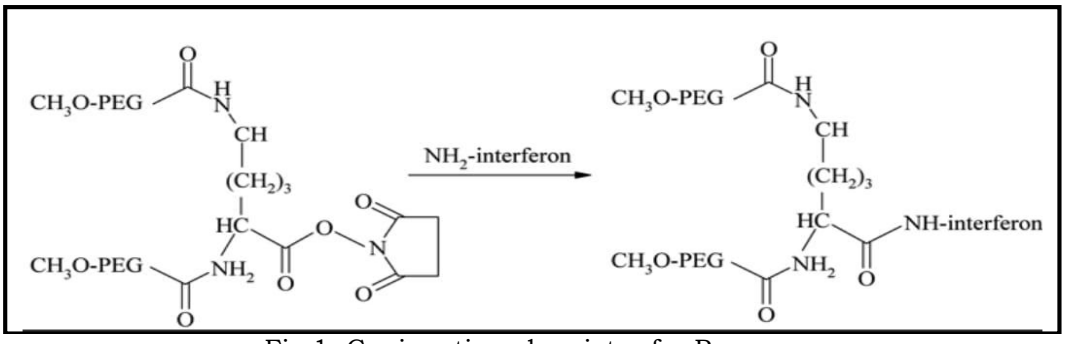

Fig 1. Conjugation chemistry for Pegasys

A polyethylene glycol-modified human GM-CSF, namely chemically modified protein according to the present invention, may be purified from a reaction mixture by conventional methods which are used for purification of proteins, such as dialysis, salting-out, ultrafiltration, FPLC (fast protein liquid chromatography protocol), ion-exchange chromatography, gel chromatography, and electrophoresis. Ion-exchange chromatography and Fast Protein Liquid Chromatography are particularly effective in removing unreacted polyethylene glycol and human GM-CSF. A polyethylene glycol-modified human GM-CSF may also be purified from a reaction mixture by partitioning the PEG GM-CSF in a PEGcontaining aqueous. Monofunctional PEG reagents having a functional group connected to one end of the PEG polymer are used to PEGylate proteins, permitting interaction with N-terminal amine, lysine, cysteine, and other amino acids. The amino acids that are solvent accessible vary depending on the protein. Lysines are nearly always found on the surface of proteins, although naturally occurring free thiols are very reactive and are consequently "hidden."

Conditions that allow reaction with a specific reagent may not be compatible with the protein's natural structure, hence suitable controls must be performed to confirm that the PEGylation conditions are compatible. There have been descriptions of mono-PEGylation, site-directed modification, and random modification techniques. Depending on the chemistry used, random PEGylation can produce a range of positional isomers. If functionally reactive areas are compromised, this might result in a loss of particular activity. Limited PEGylation with many attachment sites, on the other hand, might increase pharmacologic qualities while decreasing functional and effective impacts.

Chemical Approaches for Site-Selective Pegylation

For the selective modification of specific amino acids in peptides and proteins, the knowledge of some characteristics of their primary structure is needed. An important physicochemical feature in proteins is the difference in pKa between the amino group of an N-terminal amino acid residue (~7.6) and the amino groups in the side chains of lysine (~10.5) and arginine (~12). This difference allows the selective N-terminal modification of proteins based on pH control and the use of reductive agents like sodium cyanoborohydride. A useful strategy for the specific conjugation of peptides and proteins is based on the amino acid ratio in a protein being variable. Moelbert et al. reported the accessibility index on the

surface of the 20 essential amino acids, which makes it possible to know the expression of these amino acids in different areas of the proteins about their natural abundance. It has also been reported that short peptides/proteins (less than 50 residues) tend to over-represent glutamine and cysteine in the N-terminal region. It is well known that single-chain proteins possess only one N-terminal residue, having a uniquely reactive site for chemical modification. Therefore, as virtually all proteins present these functional groups, several valuable reactions have been developed for their selective modification.

The use of potassium ferricyanide as an oxidizing agent in o-aminophenol-performing N-terminal PEGylation has also been shown. In 2016 Song et al. described an alternative strategy for PEGylation at the N-terminus of several proteins as well as two peptides based on the chemoselectivity of catechol. More recently, Rosen and Francis described classical methods for the selective modification of N-terminal amino group under pH control. These methods include the selective acylation and alkylation of N-terminal amines at low-to-neutral pH and also transamination using pyridoxal-5′-phosphate aldehyde, which undergoes condensation with ε-amines from lysine side chains and N-terminal α amines to form imines. Chen et al. demonstrated the ability of benzaldehyde to selectively modify native peptides and proteins on their N-termini. Preservation of the positive charge on the N-terminus of the human insulin A-chain through reductive alkylation instead of acylation leads to a 5-fold increase in bioactivity. They showed that under mild conditions, aldehyde derivatives and carbohydrates can site-specifically react with peptide and protein N-termini, providing a universal strategy for site-selective N-terminal functionalization in native peptides and proteins.

PEG-isocyanate is in the group of PEG reagents used for the site-specific modification of different proteins. The reaction takes place *via* the amine group to produce a stable thiourea linkage. For example, in 2009 Cabrales et al. generated PEGylated human serum albumin (PEG-HSA) by conjugating PEG-phenyl-isothiocyanate 3 and 5 kDa at primary amine groups of the HSA, enhancing the hydrodynamic volume of the protein and restoring intravascular volume after hemorrhagic shock resuscitation. Furthermore, Chen and He reported in 2015 the achievement of nanophosphors coated with PEG-isocyanate and polylactic acid (PLA) for paclitaxel delivery, resulting in a significant improvement and serving as a platform in the field of drug development. Lee et al. synthesized a dual functional cyclic peptide gatekeeper attached to the surface of nanocontainers by using PEG-isocyanate as a linker to enhance dispersion stability and biocompatibility. This allowed the active targeting of cancer cells with high CD44 expression together with the ability of triggered drug release. It is important to note that specific PEG-reagents like isocyanates have a short half-life in aqueous solutions; thus, a stoichiometric excess of these reagents is necessary, causing difficulties in the removal of the remaining PEG.

A relevant report for one-step N-terminus-specific protein modification showed the stable and selective imidazolidinone product at the N-terminus, with 2-pyridine carboxaldehyde (2PCA) derivatives. The main basis of this reaction is the nucleophilic attack of the neighboring amide nitrogen on the electrophilic carbon of the initially formed N-terminal imine. As an example, a 2PCA-functionalized

polyacrylamide-based hydrogel has been developed for the immobilization of extracellular matrix proteins through the N-terminus to study their biochemical and mechanical influence on cells.

Effects of Pegylation on Protein Stability and Folding

When comparing PEGylated proteins to their non-PEGylated counterparts using circular dichroism (CD), ultraviolet absorption, or nuclear magnetic resonance (NMR) spectroscopy, it is commonly concluded that the addition of PEG does not appreciably modify the protein structure. The effects of PEGylation on the physical characteristics of the modified protein are described in many cases in the literature. Always do a mock reaction without active PEG to determine whether any effects are attributable to the reaction circumstances or are actually due to the PEG reagent's alteration. This control analysis is especially crucial for pharmaceutical development because poorly folded protein material is more susceptible to proteolysis and is more likely to contribute to immunogenicity development.

The charge is reduced when lysine is modified to form an amide bond, as discussed in the chemical portion of this page. The pI will alter as a result of this. Other chemical changes will be charge neutral, such as reductive aldehyde PEGylation at an amine (reductive amination). Surface charges can modify the effective pI, therefore more shielding by the PEG may disguise them. Because of the hydrophilic nature of the PEG, general noncovalent surface interactions may be changed. Depending on the protein and the quantity of PEG, these interactions may change. As a result, the precise examples given here may not apply to every protein. A few instances are presented below to show how PEGylation of peptides and proteins did not cause a correctly folded structure to be disrupted. As evaluated by NMR and molecular dynamics, two lysine-specific conjugates (Lys12, Lys21) of growth-regulating hormone (hGRF 1-29) peptide PEGylated with a single 5 kDa PEG did not reveal structural deformation due to the PEG attachment33. The peptide's intrinsic inclination to form a -helix was unaffected by the presence of PEG.

The scientists determined that steric hindrance and PEG orientation relative to the ligand-binding sites were responsible for the discrepancies in biological activity. Based on the crystal structure, the secondary structure of a bigger peptide, insulin (molecular weight 6.6 kDa), is 53 percent -helix, 8% sheet, 18 percent turn, and 21% random coil. The findings of a CD comparison of four distinct monoinsulin conjugates revealed that there were no structural variations due to PEG size or location. PEG reagents from the first generation were typically little more than 5 kDa in size. Early PEG reagents were not lysine-specific, and some had degradable bonds. Davis first employed cyanuric chloride (2,4,6-trichloro-s-triazine) to make activated PEG, which was then reacted with catalase and bovine serum albumin to produce conjugates with a longer circulation duration and lower immunogenicity than the original proteins. PEG dichlorotriazine reacts with serine, tyrosine, cysteine, and histidine and is not specific for lysine. The cyanuric chloride derivatives are inappropriate for commercial goods due to their lack of specificity and toxicity.

Although the second chloride in PEG dichlorotriazine is less reactive than the first, it still has enough reactivity to produce di-PEGylated species. Enzymatic site-specific modification is a major growth area in the PEGylation field. While there are numerous site-specific enzymatic labeling methods including or tagging, biotin ligase tagging, lipoic acid ligase labeling, form glycine generating enzyme labeling, sialylation, phosphopantetheine transferase labeling, galactose oxidation, prenylation, transglutamination and myristoylation, only a handful of these methods have been employed to prepare site-specific PEG conjugates. Currently, there are no approved products that use enzymatic PEGylation with one major drawback being the difficulty in scaling up many of these reactions for commercial production. However, given their mild reaction conditions, availability of substrates, and rapid kinetics, enzymatic labeling methods appear primed to enhance the field of protein PEGylation for the development of therapeutic proteins with new and improved properties.

Lack of specificity, degradability, decreased conjugate activity, water hydrolysis, the requirement for greater molecular weight PEGs, and conjugate heterogeneity all led to the need for more selective next-generation PEG reagents. Second-generation PEG reagents have increased lysine selectivity and are N-terminal or thiol selective.The availability of higher molecular weight raw PEGs with low diol content allowed for the manufacture of high-quality conjugates with a molecular weight of less than 20 kDa, allowing for the creation of branching reagents and higher molecular weight linear PEGs suited for protein conjugation.

Poly (ethylene glycol) modified human GM-CSF with increased biological activity

Recombinant human GM-CSF is known in the art and used to treat several disorders including neutropenia following radiotherapy or chemotherapy. As with most cytokines, human GM-CSF has a short halflife in circulation, requiring repeated injections. Additionally, recombinant human GM-CSF is immunogenic and antigenic, leading to the generation of inhibitory antibodies. In rare instances, PEGylation may result in altered biological properties. After PEGylation, GM-CSF exhibits differentiation of neutrophil priming activity and colony-stimulating activity. Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is a cytokine active as a white blood cell growth factor that stimulates stem cells to produce granulocytes and macrophages. In its human mature form, it is O-glycosylated at multiple sites (Ser5, Ser7, Ser9, and/or Thr10) [27, 28], but can be produced as a non-glycosylated protein by recombinant expression in E. coli. Recombinant GM-CSF is usually administered to patients after a bone marrow transplant or chemotherapy.

The prior art teaches three commercial varieties of recombinant human GM-CSF

- Regramostim, produced in CHO cells, has the fulllength native human GM-CSF peptide sequence and has the most complete (closest to human) glycosylation profile. As a consequence of its full glycosylation, Regramostim has increased halflife but a significantly reduced bioactivity compared to other GM-CSF products (Hussein et al, 1995).

- Sargramostim (Leukine™), produced in the yeast Saccharomyces cerevisiae, differs from the native human GM-CSF amino acid sequence by substitution of Leucine at position 23. This change ameliorates a protease degradation problem in the yeast production system (US 5,393,870). Even a single amino acid change, however, can be implicated in increased immunogenicity of proteins in general and is proposed as a causative factor in yeast GM-CSF immunogenicity in particular (Rini et al, 2005). Sargramostim, by its production in yeast, is somewhat glycosylated. As a consequence of its glycosylation, Sargramostim has significantly reduced bioactivity in vivo compared to nonglycosylated GM-CSF (Hussein et al, 1995).
- Molgramostim (Leucomax™), produced in E.coli, differs from the native human GM-CSF amino acid sequence by deletion of the six Nterminal amino acids, as well as the addition of a Nterminal methionine residue. As a bacterial protein product, Molgramostim is unglycosylated, contributing to its superior bioactivity compared to Regramostim and Sargramostim (Hussein et al, 1995).

Molgramostim's sideeffect profile is claimed to be worse than that of Sargramostim (Dorr, 1993), but it is suggested that this is due to unnecessarily high doses of the more active nonglycosylated form. In light of different levels of immunogenicity seen in humans with two nonglycosylated GM CSF products with differing sequences, it is suggested that even a single amino acid change from the native human sequence in nonglycosylated GM-CSF can influence immunogenicity; Molgramostim has much more significant differences from the native human sequence than this, that may account for its observed levels of immunogenicity (Wadhwa, 1999). Such prior art demonstrates that a glycosylated GM-CSF produced in yeast or mammalian system behaves differently than a non-glycosylated truncated GM-CSF produced in E. coli, both in culture and in clinical trials.

Several groups have demonstrated that PEGylation of cytokines improves the pharmacokinetic profile (and thus activity) and reduces immunogenicity by shielding immunogenic epitopes from immune scrutiny. The prior art also teaches PEGylated human GM-CSF. Specifically, US Pat, No 6,384,195 teaches a human GM-CSF protein that is PEGylated at multiple sites with PEG molecules in the range of 5 to 6 kDa molecular weight. There is no teaching as to the source of the human GM-CSF (mammalian, yeast, or bacteria). Finally, the biological activity that was noted with the PEGylated GM-CSF is from a radiolabeled (iodinated) human GM-CSF protein. As such, the observed activity may be the result of the cells reacting to exposure to radiation, lodination is a harsh treatment that often results in modification of a protein's secondary structure.

Thus, any results described in the '195 patent are difficult to interpret as being specific to PEGylation of the GM-CSF protein. Sherman et al teach the conjugation of 19 kDa PEG to human GM-CSF by pNitrophenyl Carbonate to produce monoPEGylated and diPEGylated human GM-CSF. Additionally, Sherman et al conjugated either a 5 kDa or 42 kDa PEG to human GM-CSF by a PEG aldehyde to produce a monoPEGylated human GM-CSF. Sherman states

that human GM-CSF is from Immunex, which produces Sargramostim (Leukine™) in a yeast expression system.

As described above, glycosylated GM-CSF behaves differently than non-glycosylated GM-CSF. Additionally, Sherman discloses PEGylated murine GM-CSF. However, human GM-CSF does not bind to mouse cells and murine GM-CSF does not bind to human cells, and there is no crossreactivity between these GM-CSFs. Additionally, murine GM-CSF behaves differently in a mouse than human GM-CSF behaves in a human (O'Reilly, R., J., et al, 1990). Publications by Malik, F. et al 1992 and Knusli, C et al 1992, describe a PEGylated human GM-CSF protein having conserved biological activity compared to non PEGylated GM-CSF in stimulating replication of GM-CSF responsive cells. Both Malik and Knusli's publications describe the recombinant human GM-CSF as obtained "from Biogen, Amgen or Hoechst".

However, there is no description of the sequence of the human GM-CSF used or its glycosylation state. Neither Malik nor Knusli describes the molecular weight of the PEG molecule that was conjugated to human GM-CSF or the position(s) of the PEG molecule. And finally, neither Malik nor Knusli isolated their PEGylated GM-CSF protein, and thus all reported test results are for a mixture of non PEGylated GM-CSF and GM-CSF PEGylated to various degrees. Furthermore, the effects of a given cytokine in vivo do not necessarily reflect the in vitro situation (O'Reilly, R., J., et al, 1990). DeFrees et al describe a sitedirected PEGylated human GM-CSF prepared by expressing nonglycosylated GM-CSF in E. coli, followed by enzymatic GalNAcglycosylation at specific serine and threonine residues, followed by enzymatic transfer of sialic acid conjugated with linear 2OkDa PEG to the introduced GaINAc residues (primarily at Ser7 and Ser9).

However, a nonhomogenous mixture of singly, doubly, and triplyPEGylated GM-CSF was produced by this method, and neither in vitro nor in vivo experiments to demonstrate bioactivity or pharmacokinetic parameters were performed (DeFrees et al, 2006). Doherty et al created analogs of human GM-CSF engineered to contain additional cysteine residues, which were then PEGylated with cysteine-reactive 5kDa, 1OkDa, or 2OkDa linear PEG or 4OkDa branched PEG. The 5, 10, and 2OkDa PEG modifications did not substantially change the in vitro bioactivity of human GM-CSF, while the 4OkDa PEGylation decreased bioactivity. The PEGylated cysteinebearing GM-CSFs demonstrated increased halflives in rats correlating with the size of the PEG molecules; since human GM-CSF does not function in rodents, no in vivo efficacy data was presented (Doherty et al, 2005).

Protein PEGylation and its pharmaceutical significance

In the late 1970s, Professor Frank Davis and his colleagues covalently linked methoxy PEG (mPEG) to bovine serum albumin[15] and bovine liver catalase[16], using cyanuric chloride as an activating agent. Their studies showed that “hanging a bit of PEG onto a protein” markedly improved the overall properties and stability of the protein. Most PEGylation chemistry is designed to create a conjugate that contains a stable linkage to the protein. In most cases having a stable linkage to the protein is beneficial because of the suitability for long-term storage, easier purification, and availability of prefilled syringes. It is also generally observed that stable linkages to a protein can reduce the activity,

possibly due to the presence of the PEG chain at the active or binding site of the protein or steric crowding at the active or binding site. Also, the PEG molecular weight has a direct impact on the activity; higher molecular weight PEG conjugates tend to have lower in vitro activity but have higher in vivo activity due to the improved pharmacokinetic profile. The objective of most PEG conjugation techniques is to increase the circulation half-life without altering activity. This technique is now well established and is known as "PEGylation". The applications of PEGylation can be extended to peptides, enzymes, antibody fragments, nucleotides, and even small organic molecules.

PEGylation can impart several significant and distinct pharmacological advantages over the unmodified form, including improved drug solubility, reduced dosage frequency, toxicity and rate of kidney clearance, an extended circulating life, increased drug stability, enhanced protection from proteolytic degradation, decreased immunogenicity and antigenicity, and minimal loss of biological activity. The reduced kidney clearance of PEGylated proteins can be attributed to an apparent shielding of protein surface charges and an increased hydrodynamic volume of the conjugated product due to the ability of PEG molecules to coordinate with two to three water molecules per monomer unit25. Furthermore, these hydrated polymer chains provide a protecting mask for the protein, becoming more effective with an increase in the number and molecular weights of the attached PEGs, reducing the phagocytic uptake by parenchymal cells, preventing opsonization and increasing residence time in systemic circulation.

In addition to these pharmacological advantages, PEGylation can substantially alter the physicochemical properties of the parent protein, including electrostatic and hydrophobic properties. PEGylation significantly influences the elimination pathway of the molecule, by shifting from a renal to a hepatic pathway. The tissue-organ distribution profile of the molecule is also greatly influenced by PEGylation, wherein PEGylated proteins preferably follow a peripheral distribution. The pharmaceutical value of PEGylation is now well accepted, with many FDA-approved drugs already launched in the market and many in clinical trials. Table 1 shows some examples of approved PEGylated therapeutics.

Table 1
Examples of FDA approved PEGylated drugs

Name	Company	Year	Indication	Therapeutic area
Cimzia®	Nektar/UCB	2008	Crohn's disease	Immunology
Mircera®	Nektar/Roche	2007	Anemia in chronic kidney disease (CKD)	Nephrology
Macugen®	OSI (previously EyeTech)	2004	Age-related macular degeneration (AMD)	Ocular
Somavert®	Pfizer	2003	Acromegaly	Immunology
Pegasys®	Roche	2002	Hepatitis C	Immunology
Neulasta®	Amgen	2002	Neutropenia during chemotherapy	Oncology
PEG-Intron®	Schering-Plough/Enzon	2001	Hepatitis C	Immunology
Oncaspar®	Enzon	1994	Lymphoblastic leukemia	Oncology

Adagen®	Enzon	1990	Severe combined immunodeficiency disease (SCID)	Oncology

Discussion

An attempt to PEG-modify GM-CSF to convey the reported benefits of modification (improved circulation time, reduced immunogenicity and antigenicity, improved solubility), using a new coupling method, yielded PEG-protein adducts with an unprecedented increase in one of two biological activities of the molecule. In both fractionated and unfractionated material, colony-stimulating activity was unchanged or slightly reduced by PEG-modification whereas priming of human neutrophils was dramatically increased. PEG-modification by other methods typically reduces biological activity by 5-75% depending on the method used (Harris et al, 1989; Katre et al, 1987: Ashihara et al, 1978; Beauchamp et al, 1983). Only occasionally amongst the many proteins modified has there been a reported increase in biological activity for a PEG-protein adduct (Rajagopalan et al, 1985; Abuchowski& Davis, 1979: Katre et al, 1987) and this has usually been very sensitive to coupling ratios, polymer length and substrate (particularly favoring small substrates). Intriguingly two of these proteins were streptokinase and trypsin (Rajagopalan et al, 1985; Abuchowski & Davis, 19 79) suggesting that PEG'S interference with proteolysis may explain this observation (autoproteolysis can occur). With PEG-IL-2 a modest increase in activity (1.4 times control) was reported, but evidence of the statistical significance of this difference was not given (Katre et al. 1987). This was limited to substitution by one or two PEG molecules and activity was decreased at higher degrees of substitution (Katre et al, 1987).

As per the literature review, GMCSF is a key proinflammatory cytokine. In human patients, reactions at GMCSF injection sites, such as swelling, redness, and tenderness, are common side effects. Subcutaneous injection of GMCSF or 20, 30, or 4OkDa PEGGMCSF resulted in localized inflammation in a primate model of myelosuppression. Surprisingly, while the 2OkDa PEGGMCSF produced mild inflammation that was easily treatable with antihistamine (diphenhydramine), the 30 and 40 kDa PEGGM-CSF molecules caused more severe inflammation. The unexpected finding suggests that 2OkDa PEG GMCSF is the optimal choice of PEGylated GMCSF.

Conclusion

Twelve biopharmaceuticals have been authorized as a result of Pegylation. Although original Pegylation consisted mostly of random attachment of relatively tiny PEG molecules (5 or 10 kDa), bigger chain Peg molecules have recently been utilized that are targeted to unique and precise places in a protein or aptamer through a linker. Cleavable linkers have been utilized more recently to give pegylated proteins a "prodrug" quality because the PEG is cleaved off the drug molecule during metabolism, resulting in a physiologically active drug. The recovery of animals treated with the 20 kD PEGGMCSF is surprising given the concentration of PEGGMCSF in the serum. Following the first dose, the serum levels of 40 kD PEGGMCSF reached about 650,000 pg/ml compared to about

280,000 ρg/ml for the 30 kD PEGGMCSF and about 250,000 pg/ml for the 30 kD PEGGMCSF (Figure 7). Additionally, the serum levels for the 30 kD and 40 kD PEGGMCSF molecule appeared to remain at their peak for about 2 to 3 days after the first injection, whereas the levels of 20 kDa PEGGMCSF peaked on the day of injection and rapidly decreased. The fact monkeys treated with 20 kDa PEGGM-CSF recovered from neutropenia sooner than the 2OkDa and 3OkDa PEGGMCSF treatment groups, despite lower concentrations of 20 kDa PEGGMCSF is even more surprising given that the results summarized. suggest there is little to no difference in in vitro potency amongst the 2OkDa, 3OkDa, and 4OkDa PEGGM-CSF. Inflammatory Response to PEGGMCSF is Reduced with 2OkD PEGGMCSF

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