PEGYLATED PROTEIN DRUGS: A PROMISING NEW APPROACH FOR DRUG DELIVERY

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Summary

Pegylation is a process in which polyethylene glycol (PEGs) is covalently attached to a drug or therapeutic protein. Polyethylene glycol is a non toxic non immunogenic, hydrophilic highly flexible molecule. Addition of polyethylene glycol to drugs has various advantages like reduced immunogenicity against therapeutic drug or protein, increased solubility, increase in duration of action, reduced frequency of administration, optimized pharmacokinetics etc. Pegylation of a drug or therapeutic protein can be done by Chemical reaction between PEG and target molecule, Genetic engineering combined with chemical methods, Use of enzymatic technologies such as GlycoPEGylation or Transglutaminase reactions and Size-Exclusion Reaction Chromatography(SERC). Recently various pegylated drugs have been introduced in to the market which include PEG – bovine adenosine deaminase, PEGylated interferon alpha, PEGylated L-asparaginase , Pegfilgrastim, Pegylated liposomal doxorubicin and may more. Because of various advantages, pegylation of drugs can provide a promising new approach for drug delivery in years to come.

Key Words: Pegylated protein drugs, Pegylation, Pegylated interferons, Pegvisomant

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Introduction

Revolution in Biotechnology has produced novel peptides and proteins that have become important new drugs. Polypeptide drugs are produced in large quantities by using Recombinant DNA in Escherichia coli and other organisms.

Polypeptide drugs have several shortcomings that limit their usefulness which

1. Include susceptibility to destruction by proteolytic enzymes,
2. Short circulating half-life, short shelf life,
3. Low solubility,
4. Rapid kidney clearance and
5. Their propensity to generate neutralizing antibodies. [1]

Researchers have attempted to improve the clinical properties of polypeptides by

1. Altering amino-acid sequences to reduce degradation by enzymes and antigenic side effects,
2. By fusing them to immunoglobulin's or albumin to improve half-life, and
3. By incorporating them into drug-delivery vehicles such Liposomes.

But all these methods failed to provide desired benefits; Pegylation is an alternative method that overcomes these deficiencies. [2]

Pegylation is a process in which polyethylene glycol (PEGs) is covalently attached to a drug or therapeutic protein. PEGs are prepared by polymerization of ethylene oxide, they are also known as Polyethlene oxide (PEO) or Polyoxy ethylene (POE).[3]

Structure of PEG

$HO\text{-}Ch_2\text{-}(CH_2\text{-}O\text{-}CH_2\text{-})_n\text{-}CH_2\text{-}OH$. [4]

The technology of pegylation was developed from pioneering work of Frank F. Davis in 1970. The first PEG-protein company was Enzon, founded in 1981, and the first approved PEG-drug product was PEG-adenosine deaminase, approved in 1990.[5]

Properties of Polyethylene Glycol

Poly ethylene glycol has several chemical properties that make it especially useful in various biological, chemical and pharmaceutical settings:

- Non-toxic and non-immunogenic – can be added to media and attached to surfaces and conjugated to molecules without interfering with cellular functions or target immunogenicities.
- Hydrophilic (aqueous-soluble) – attachment to proteins and other biomolecules decreases aggregation and increases solubility.
- Highly flexible – provides for surface treatment or bioconjugation without steric hindrance. [4]

Advantages of Pegylation

1. Reduce immunogenicity and antigenicity by shielding receptor-mediated uptake by the reticuloendothelial system (RES), and preventing recognition and degradation by proteolytic enzymes.
2. Increased solubility.
3. Increased blood circulation of the drug by reducing the renal filtration and altering biodistribution.
4. Decreased frequency of administration.
5. Provide water solubility to hydrophobic drugs and proteins.
6. Optimized pharmacokinetics.[1]

Pegylation process

PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target macromolecule. The production of PEGylated proteins can be achieved through

1. Chemical reaction between PEG and target molecule

PEG Compounds that contain a reactive or targetable functional group at one end undergo covalent modification by combining with a drug.

The simplest method to PEGylate proteins, which are rich in surface primary amines, is to use a PEG compound that contains an NHS ester group at one end. This is the basis for the MS(PEG)n Reagents, which are available in four discrete PEG lengths (n = 4, 8, 12 and 24).

TMS(PEG)n is a branched form of MS(PEG)n, containing three methyl-PEG12 arms.

SAT(PEG)4 contains the amine-reactive NHS-ester group at one end and a protected sulfhydryl group (S-acetyl) at the other end. The reagent is most often used as part of a crosslinking or immobilization strategy.

MM(PEG)n and TMM(PEG)n are linear and branched reagents for PEGylating sulfhydryl groups. MM(PEG)n is available in two PEG lengths (n = 12 and 24). TMM(PEG)n contains three methyl-PEG12 arms.

MA(PEG)n and CA(PEG)n are polyethylene glycol compounds of discrete length (n = 4, 8, 12 and 24) that contain methyl-and-amine or carboxyl-and-amine ends. While these functional groups are not spontaneously reactive, they are easily targeted by various crosslinking and immobilization reagents for construction of peptides, manipulation of surface chemistries and other uses.[4]

2. Genetic engineering combined with chemical methods

For example in the introduction of unnatural amino acid - introduction of para-azidophenylalanine by the use of a genetically engineered yeast (E. coli tyrosyl tRNA/tRNA synthetase pair) and the reaction of an azido group specifically with a PEG-alkylene derivative. [5]

3. Use of enzymatic technologies such as

A. GlycoPEGylation - PEGylation of glycosylation sites using a specific sialyltransferase or

B. Transglutaminase reactions - PEGylation of glutamine using a specific transglutaminase. [5]

4. Size-Exclusion Reaction Chromatography(SERC)

This combines control of the PEGylation reaction with simultaneous purification of the product.[6]

PEGylated Pharmaceuticals on the market

1. PEG – bovine adenosine deaminase

Manufactured by Enzon Pharmaceuticals, was the first pegylated protein approved by the U.S. Food and Drug administration in March 1990.

It is used to treat X – linked severe combined immunogenicity syndrome, as an alternative to bone marrow transplantation and enzyme replacement by gene therapy. [7]

2. PEGylated interferon alpha

Used in the treatment of chronic hepatitis B and Hepatitis C infection

A. Pegylated interferon alpha 2a is a 40 kDa protein used as antiviral drug.

Currently it is approved for the treatment of chronic hepatitis B and Hepatitis C infection (including patients HIV co-infection, cirrhosis) . Patients with genetic polymorphism near IL28B gene encoding for interferon lambda 3 are associated with significant differences in response that is more possible to achieve sustained virological response after treatment.[8] Now it is conclusively demonstrated the same genetic variants are also associated with the natural clearance of the hepatitis C virus. [9]

Pegylated interferon alpha 2a is combined with ribavirin and administered for 48 weeks. If this regimen fails then 72-week regimen is the preferred strategy for optimizing sustained response rates.[10]

B. Pegylated interferon alpha 2b is commonly used in the treatment of Hepatitis C infection; it has also been tried in the treatment of malignant melanoma[11] and neurofibromatosis.[12]

Like Pegylated interferon alpha 2a patients with genetic polymorphism near IL28B gene encoding for interferon lambda 3 are associated with significant differences in response[8] and same genetic variants are also associated with the natural clearance of the hepatitis C virus.[9]

It has been noted that peginterferon alfa-2b or peginterferon alfa-2a in combination with ribavirin for chronic hepatitis C virus (HCV) infection have shown same efficacy.[13]

3. PEGylated L-asparaginase (Oncaspar)

For the treatment of acute lymphoblastic leukemia in patients who are hypersensitive to the native unmodified form of L-asparaginase (Enzon).

It is also found to be useful in chronic lymphocytic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma and plasma cell leukemia.[14]

4. Pegfilgrastim (Neulasta)

Pegfilgrastim is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol.

It is an immunostimulator, act on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

Indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia.[15]

5. Pegylated liposomal doxorubicin (DOXIL/CAELYX)

For the treatment of multiple myeloma.[16]

6. **Pegvisomant (Somavert)**

Pegylated form of human growth hormone antagonist developed for the treatment of acromegaly.[17]

7. Recombinant form of a natural inhibitor of TNF-α {sTNF receptor type I (TNF-RI)}has been attached to a high-molecular-mass PEG to treat rheumatoid arthritis (RA) and Crohn's disease.[18]

8. **Pegylated nanoparticles for brain delivery**

Many drugs have limited entry in to the brain because of the presence of blood–brain barrier (BBB). Injecting drugs directly into the brain or disrupting the BBB carries high risks for patients.

These drugs can be combined with polymer nanoparticles, such as n-hexadecylcyanoacrylate (PHDCA), which help in transport of drugs across BBB. Animal studies show that PEG–PHDCA penetrates into the brain to a significantly greater extent than PHDCA alone.[19]

Conclusions

Combining a drug with a nontoxic, nonimmunogenic, hydrophilic, highly flexible polyethylene glycol provides advantages like reduced immunogenicity, optimized pharmacokinetics etc. Various drugs are now being pegylated and released in to the market. Pegylated drugs have clearly shown significant benefit when compared to their nonpegylated form. Thus pegylation can provide a promising new approach for drug delivery in years to come.

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