PEGYLATION – A NOVEL APPROACH FOR DRUG DELIVERY

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ABSTRACT: Pegylation is a process of attaching the strands of polymer PEG to molecules most typically peptides,proteins and anti-body fragments, can help to meet the challenges of improving the safety and efficacy of much therapeutics. It produce alteration in the physicochemical properties including changes in the confirmation, electrostatic binding, hydrophobicity etc. These physical and chemical changes increase systemic retention of therapeutic agent. Also it can influence the binding affinity of the therapeutic moiety to the cell receptorsand can alter the absorption and distribution patterns.

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PEGYLATION

In 1970's pioneering research by Davis, Abuchowsky and colleagues foresaw the potential of the conjugation of polyethylene glycol (PEG) to proteins. This technique is now well established and is known as pegylation. Polyethylene glycol is highly investigated polymer for the covalent modification of biological macromolecules and surfaces for many pharmaceutical and biotechnical applications.

Pegylation is the process of covalent attachment of polyethylene glycol (PEG) chains to another molecules, normally a drug or therapeutic protein. Pegylation can help in improving the safety and efficacy of therapeutics by altering the physicochemical properties including changes in the confirmation, hydrophobicity etc. It also alter the absorbtion and distribution patterns by influencing the binding affinity of the therapeutic moiety to the cell receptors.

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Pegylation now plays an important role in drug delivery, enhancing the potentials of peptides and proteins as therapeutic agents [1]

Pharmacological and commercial advantages of pegylation includes

- Enhanced drug stability
- Improved stability
- Increased biovailability
- Increased circulation time
- Provide protection against proteolytic enzymes
- Provide opportunities for new drug delivery formats

Disadvantages

- At high molecular weight PEG accumulate in the liver, leading to macromolecular syndrome.[3]
- Negative effect on distribution properties

Unpredictable pharmacokinetics

PEG may significantly reduce target receptor binding for drug conjugate due to stearic clouding.

Pharmaceutical applications

Pegylation, a newer technology which involves the covalent coupling of non toxic, hydrophilic PEG to active ingredients[4]. This modification of a pharmaceutical ingredient with PEG has several benefits when compared to the unmodified form and this technology enables us to find out newer method for the development of therapy.

In Cancer therapy

Eg:- PEG-asparaginase [5]

L-asparaginase which has been used clinicallyfor the therapy of acute lymphocytic leukemia and lymphosarcoma has some disadvantages like short circulation time in blood and immunological side effects ranging from mild allergic reaction to anaphylactic shock[6]

To overcome this drawback, L-asparaginase was modified with PEG-2 to form PEG-2 asparaginase.

Antibiotics

The use of antibiotics is associated with the problem such as low solubilities . The solubility, biodistribution, permeability etc can be increased by the conjugation of a drug to macromolecules. PEG doxorubicin conjugates linked by an amino acid or a peptide is an example [7]

Anti-inflammatory

Superoxide dismutase (SOD) and catalase

PEG-SOD and PEG- catalase have been used as novel anti inflammatory protein. Catalase converts hydrogen peroxide produced by SOD to oxygen and water, hence they elevate many of the inflammatory symptoms[6]

Antithrombosis proteins

Streptokinase which has been used as an intra venous agent for the therapy of deep vein thrombosis and pulmonary embolism have long half life in plasma and high resistant to proteolysis when it is conjugated with PEG[8]

Pegylated liposomes

Pegylation of liposomes brings an eightfold increase in plasma half life compared to unmodified liposomes[10]. Pegylated liposomes improve the delivery of encapsulated drugs,such as anticancer agent, doxorubicin[11]
Pegylated nanoparticles for brain delivery

Studies show that the PEG-PHDCA(n-hexadecylcyanoaceylate) penetrates in to deeper areas of brain including striatum, hippocampus and hypothalamus without causing any damage to the BBB or other brain structure. This method promises the development of drug carrier for brain delivery[12]

PEG as a diagnostic carrier

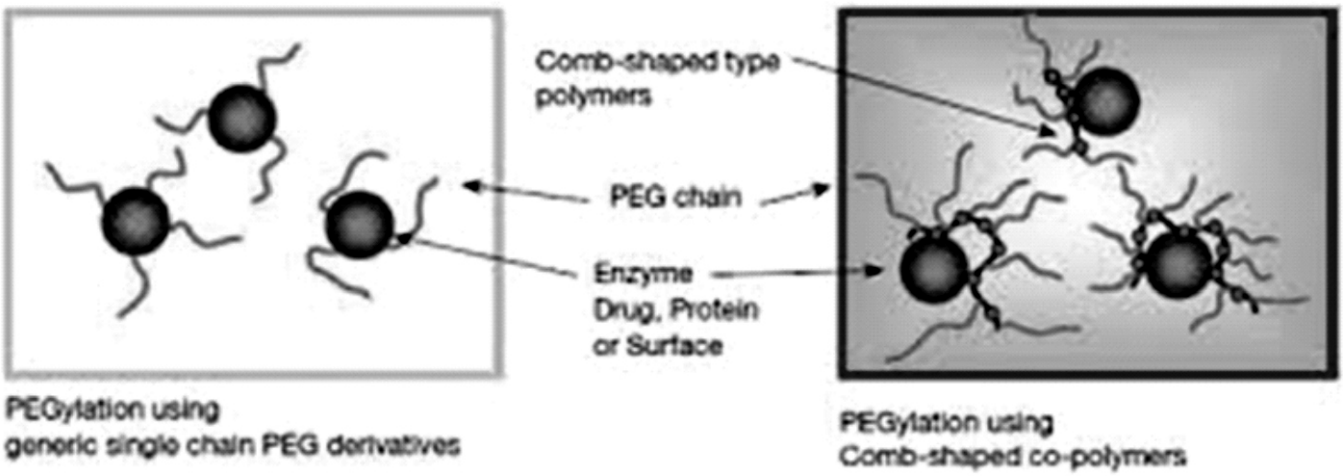
Pegylation increases the body resistance time of paramagnetic chelates that will be cleared more slowly than the unmodified molecules through the kidney or liver, and thereby allowing more detailed images by magnetic resonance[13]

Scope of pegylation

The revolution in the biotechnology field helps in the production of several novel peptides and protein that have become important new drugs[14]. The use of these polypeptide drugs have been limited because of

their disadvantages such as they are succeptability to destruction by proteolytic enzymes, short circulation, low solubility and they must be delivered by injection[15]

Various researches has been conducted in the medical field inorder to eliminate the problem associated with the use of peptide as drug[16]. As a result of these research, in 1970 Frank F.Davis, a professor of biochemistry at Rutgers university discovered that the attachment of an inert and hydrophilic polymer might extend half life and control immunogenicity [17]. They chose poly ethylene glycol as the polymer, since it lack



immunogenicity, non toxic, amphipathic behavior and readily cleared from the body [18].

The use of PEG was a successful strategy in biopharmaceutics which is termed as pegylation. Pegylation is defined as the modification of protein peptide or non peptide molecule by the linking of one or more PEG chains. Pegylation leads to the development of newer drug molecule and organic biocatalyst [3]

Pegylation process

The development of pegylated proteins utilizes a wide range of technologies such as traditional peptide chemistry, genetic engineering and other chemical method[4]

The first step of pegylation is the functionalization of PEG polymer. PEG is formed by linking/attaching repeating units of ethylene glycol to form polymers. The chemically activated PEG molecule are then attached to the pharmaceutical ingredient[16]. PEG which are activated by the same reactive moiety on each terminal is termed as "homobifunctional", whereas activated with different functional group are termed as "heterobifunctional". The α or ? amino group of lysine or the N-terminal amino acid group of other amino acids are the common reactive sites on polypeptides for the attachment of PEG polymer[19].

The commonly adopted batch process for pegylation involves mixing of reagents together in a suitable buffer solution at a temperature between 4-6 ? , followed by separation and purification of the product using a suitable technique based on its

physicochemical properties including size exclusion chromarography, ion exchange chromatography , membrane or aqueous two phase systems[20]

First generation pegylation process

In first generation pegylationtechnique , the PEG polymer are generally attached to the ? amino group of lysines. As a result mixture of PEG isomer with different molecular masses are obtained [8]. Linear PEG polymer with molecular masses of 12KDa or less are mainly used in first generation methods.

The first generation pegylation method was attended with some difficulties such as, the presence of PEG isomers can contribute to the antigenicity of the drug , have poor clinical outcome and it is difficult to produce the drug batches[21]. Even though several first generation pegylated drugs received regulatory approval are still in use such as pegademase, pegaspargase etc. [16]

Second generation pegylation process

The second generation pegylation chemistry make efforts to overcome the limitation associated with the first generation pegylation process.

In second generation pegylation process more efficient functional groups such as aldehyde, esters, amides etc made used for conjugation.

Another feature of second generation PEG polymer is the use of branched structures, instead of linear structure used in first generation PEG[22]. Branched PEGs are of increased molecular mass up to 60KDa or more. These branched PEGs reduces the antigenicity and protect the drug from destruction by proteolyticenzymes[23].

There was a considerable development in the procedure adopted for pegylation and nowadays wide range of chemical and enzymatic methods are used for conjugation [24]. The choice of a better reactive PEG allows the modification of only the wanted amino acids in the sequence. Amino groups were the first target of PEGylation by acylation or alkylation reactions, but nowadays conjugation of PEG to thiol, hydroxyl or amide groups were possible by using several specific chemical or enzymatic method.

These chemical and enzymatic pegylation technologies have their own advantage and disadvantages. Advantages of chemical method includes high yield reaction and broad applicability,

Sl. No	Name	Application
1	PEG methyl maleimide	Thiol specific pegylation of antibodies, viruses, peptides and proteins.
2	1) PEG butyraldehyde 2)PEG amidopropionaldehyde 3) PEG urethanopropionaldehyde	N- terminal specific pegylation of protein
3	6- arm PEG	More reactive component in hydrogel formulation

where as its disadvantages are pegylation may be incomplete, occurance of side reaction and these reactions are not highly specific.

Enzymatic processes are highly specific and have few side effects. The major disadvantages of these process is that the enzyme has to be separated at the end of the process[4]

Reagents used for pegylation

TableNo:1: pegylation reagents

Effect of pegylation on pharmacokinetic and pharmacodynamic properties

Simple modification of peptide and protein drug with polyethylene glycol (PEG) improves the pharmacokinetic and pharmacodynamic properties by increasing water solubility, reducing renal clearance and limiting toxicity[25]

The pharmacokinetic properties of a pegylated product depends upon the length of the PEG and the structure of the link between the PEG moiety and the protein. Pegylation increases the size and molecular weight of the molecule. It also produces alteration in the physicochemical properties of the parent molecule such as change in confirmation steric hindrance, change in electrostatic binding properties, hydrophilicity etc[26]

Pegylation differ from traditional formulation in different ways. For formulated products such as tablet, liquids and capsules the formulation process is reversible , the drug become active after getting released from the formulation and the API remains unchanged , where as in pegylated product the API can be chemically modified and the drug is not released from the formulation but it has a permanent

Protein	Pharmacokinetic effects	Pharmacokinetic effects
Interferon- α2a	Sustained absorption Increased half life (from 3.8h to 65 h) Decreased volume of distribution Decreased systemic clearance (from 6.6-29.2 to 0.06-0.10 l/h)	Invivo antiviral activity increased 12to 135 times Antitumour activity increased 18 fold
Interleukin-6	Increased half life (from 2.1-206 min)	Thrombopoietic potency increases 500 times
Tumour necrosis factor	Increased half life (from 3 to 45-136 min)	Antitumour potency increased 4 to 100 times

action[4]

Table 2: shows the influences of pegylation on pharmacokinetic and pharmacodynamics of some proteins compared with corresponding native proteins[10]

Pegylation, the process by which polyethylene glycol chains are attached to protein and peptide drugs can result in drugs that are often more effective and safer, and it shows improved patient convenience and compliance[16]. The first PEG protein company was Enzon, founded in 1981 and the first product was PEG-adenosine deaminase, approved in 1990[4]. Now a days many pegylated products are approved and are

Table 3 shows the approved pegylated products

Sl.No	Compound	Name	Company	Use
1	Pegylated Doxorubicin	Doxil/Caelyx	Schering-ploughortho biotech	Treatment of cancer
2	Pegylated Interferon alpha 2a	PEGASYS	Roche	Treatment of chronic hepatitis C and B
3	Pegylated Interferon alpha 2b	Pegintron	Pflizer	For Acromegaly
4	Pegylateduricase	Pegloticase	Savient	Treatment of gout
5	Pegylated L-asparaginase	Oncaspar	Enzon	Treatment of acute lymphoblastic leukemia
6	Pegylated Recombinant methionyl human granulocyte colony stimulating factor	Neulasta	Amgen	For cancer chemotherapy
7	PEG –adenosine deaminase	Adagen	Enzon	Severe combined immunodegficiency disease(SCID)
8	PEG –growth hormone receptor antagonist	Pegvisomant	Pflizer	For Acromegaly

successfully established in the market and many other are under clinical trial.

CONCLUSION

Pegylation has become a well-accepted method for the delivery of biopharmaceuticals . The market for biopharmaceutical product is rapidly expanding and the pegylated drug shows promise in treating a wide range of disease . Moreover many more application can still be exploited. Pegylation can be applied to any biopharmaceutical drug and therefore it is mandatory to think about pegylation when developing a new drug. Pegylated protein therapeutics is a multibillion dollar market and recent advances in pegylation technology create novel competitive products.

PEG based researches have been conducted to produce blockbuster result in area of therapeutics such as the pegylation of cells to create new drug delivery system in body bioreactor, or non immunogenic cells for safer transplantation. Pegylation of red blood cells(RBCs) at the level of membrane proteins ,carbohydrate or lipid head group was deviced mainly for transfusion purpose. Because of all this, there is great scientific and commercial interest in improving present methodologies and in introducing innovative process variations. In short the pegylation technologies have a great impact in biopharmaceutical development.

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