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PROTEIN THERAPEUTICS: AN UPDATED REVIEW

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ABSTRACT

Therapeutic protein are one of the prime option of biologicals as per their clinical uses. In recent times, uses of therapeutic protein increases day by day. Protein therapeutics are used extensively to treat various diseases like cancer, AIDS etc. Due to recent advancement in pharmaceutical biotechnology the interest towards therapeutic proteins are augmenting nowadays. Various clinical research are going on in this field to treat different diseases and pharmaceutical industries are also make interest on therapeutic proteins. Among the various treatment options therapeutic protein will provide highest chance of clinical success. Some recent clinical trials demonstrate that therapeutic protein may provide the safe and potential option to treat various diseases, but there are some drawbacks also like some immunogenic issues, safety, stability problem of protein, degradation of protein in various conditions.

Keywords: Therapeutic protein, Uses of therapeutic protein, Clinical research, Advantages of therapeutic protein, Drawbacks.

INTRODUCTION

Proteins are the organic compound with higher molecular weight. Whereas peptides are short string of amino acid comprising 2 to 50 amino acids. This two have therapeutic effect against various diseases like cancer, AIDS and various infectious diseases. When protein used as a therapeutic purpose then it known as a therapeutic protein[1,2]. With the development of Pharmaceutical biotechnology using therapeutic protein and it also increases various Pharmaceutical industries investing on the production of therapeutic proteins. Recombinant therapeutic protein, another Prime option of therapeutic protein treatment. Use of Recombinant therapeutic protein increase the Prophylaxis, diagnosis of different diseases. Protein therapeutics as a vaccine is another Prime option and it can increase body immune system as well as body defence mechanism[2-3].

In 1978 first genetically engineered Recombinant therapeutic protein has been developed which is human insulin. US-FDA approved total 239 therapeutic protein and peptide for clinical uses, many of therapeutic proteins are on clinical trial. Protein therapeutics are very much eminent on different fatal diseases like diabetes, cancer, AIDS etc[4-6]. Some proteins are not naturally occurring includes different antibodies and Fc fused proteins[7-10].

CLASSIFICATION OF THERAPEUTIC PROTEIN [3,11]

1. CLASSIFICATION BASED ON PHARMACOLOGICAL ACTIVITY

Group-I: Protein therapeutics with enzymatic or regulatory activity

(a) Replacement of protein that is deficient or abnormal.

Example: Increlex

(b) Augmentation of an existing pathway

Example: Ovidrel, Neupogen

(c) Provides a proper function or activity

Example: Myoblock

Group- II: Protein therapeutics with special targeting activity

(a) Interfere with a molecule or organisms

Example: Avastin

(b) Delivers other compound or protein

Example: Ontak

Group-III: Protein vaccines

(a) Protection against a deleterious foreign agent.

Example: Engerix

(b) Treating an autoimmune disease

Example: Rophylac

Group-IV: Protein diagnostics

Example: Geref

2. CLASSIFICATION BASED ON MOLECULAR TYPES

Antibody based drug, Anticoagulant, Blood factor, interferon, growth factor, hormones, interleukin, Fc fusion.

3. CLASSIFICATION BASED ON MOLECULAR MECHANISM

(a) Binding non covalently to target.

Example: mAbs

(b) Affecting covalent bonds.

Example: Enzymes

(c) Exerting activity without specific interaction.

Example: Serum albumin

PRODUCTION OF THERAPEUTIC PROTEIN

Due to investment on pharmaceutical biotechnology, usage of therapeutic protein increases day by day. In production methodology, those proteins which have lower molecular weight formulated chemically; whereas those proteins which have a large number of amino acids are formulated using living cell. In case of production of many Recombinant therapeutic protein, Recombinant DNA technology are used[12-14]. Escherichia coli (E.coli) acts as a microbial host used for production of different therapeutic proteins like interleukin 1 receptor antagonist, interferon-alpha, interferon beta, interferon-gamma, insulin etc. Saccharomyces cerevisiae used as a microbial host for production of hepatitis-B vaccine which is only one type of therapeutic protein, bacillus sp., Pseudomonas sp. ,these are also act as a microbial host in the production of different therapeutic protein[15-17].

In some cases animal cell cultures are also used because it can accomplish post-translational modifications and biologically active protein are produced. Transgenic technology is also used in the production of different therapeutic protein such as 'Arabidopsis', a transgenic host used to produce enkephalins, which is a therapeutic protein used as an anti-hyper-analgesic. Tobacco is an another type of host which is used to produce therapeutic proteins like protein c, epidermal growth factor, chloroplast, erythropoietin etc[18-20].

Expression cells	Type of host	Therapeutic proteins	Application
Chinese hamster (ovary cell)	Microbial host	Factor –viii Interferon beta	Haemophilia
Escherichia coli	Microbial host	Interleukin-1 receptor antagonist Insulin Interferon (alpha, beta, gamma)	Autoimmune disease Diabetes Sclerosis
Tomato	Transgenic plant	Angiotensin converting enzyme	Hypertension
Tobacco	Transgenic plant	Epidermal growth factor Erythropoietin Lactoferin	Wound repair Anaemia HIV Gaucher's disease Anticoagulant

PURIFICATION OF THERAPEUTIC PROTEIN

To inhibit the allergic reactions in the patients, proper purification of the therapeutic proteins and peptides must be done because they can affect the structural integrity and functionality of the proteins. In the process of choosing a right purification process for the proteins, some factors must be maintained including yield, immunogenicity, purity, structural integrity, functionality etc. There are several processes but reverse phase chromatography is one of the most widely used process for the purification of the protein but in this process organic solvents are used which can denature some proteins. So different processes may be used for different proteins[21].

PHARMACOKINETIC PARAMETERS

Pharmacokinetic factors are very important for the development of therapeutic proteins. Stability of the therapeutic proteins should be considered and confronted precisely. There are several chemical and physical instabilities for the therapeutic proteins which can lead to the degradation of the proteins via different processes including aggregation, denaturation, hydrolysis, racemization and oxidation. Assessment of the pharmacokinetic parameters is very important and a very critical step for the formulation and delivery of these protein compounds. Apart from the different kinds of instabilities, there are some major problems associated with the development of the therapeutic proteins including large molecular size. rapid elimination, hydrophobic nature, enzymatic degradation etc[22-24].

Following are the main four steps of pharmacokinetics

ABSORPTION

To overcome the problems of large size, hydrophobicity and enzymatic degradation, the therapeutic proteins are mainly administered by parenteral route. Subcutaneous route (SC) is the most widely used route for the administration of therapeutic proteins and following this SC injection the time required for the maximum systemic circulation for protein is about few hours and for monoclonal antibodies the time is measured in days[25,26]. In case of subcutaneous or intramuscular route of administration the drug may face some differences in terms of blood flow and lymph flow at the site of administration. Blood circulatory system absorb the peptide therapeutics with smaller molecular size and the lymphatic system is known to absorb the greater molecular sized therapeutic proteins. Some therapeutic proteins such as insulin and glucagon like peptide 1 (GLP-1) should be administered orally because they are meant to target liver and intestinal cells. Oral route of administration of these therapeutic proteins have some major issues like large molecular

weight or low intestinal permeation[27-29]. To overcome this hurdles, nowadays absorption enhancers are suggested to increase the permeability of this protein. Apart from the absorption enhancers there are some more significant advances made for the proper delivery and absorption of the therapeutic proteins. The pulmonary root has drawn a great attention for the delivery of this therapeutic proteins because the lungs have large surface area which can provide a closer interaction between the alveoli and the circulation resulting in high rate of absorption of this therapeutic molecules. It can also bypass the first pass effect[30-32].

DISTRIBUTION

Distribution is another important factor to impart the therapeutic effects. Molecules having smaller size can be distributed readily through the blood capillaries via efficient diffusion process[33,34]. There are some factors which can determine the rate and extent of drug distribution including size, lipophilicity and the mode of transportation of the molecule[35]. Smaller molecules can be transported through passive diffusion but the larger molecules are transported through active or connected transportation process. Protein binding plays an important role for the bioavailability and the distribution of the molecules[36]. The drugs which are independent from plasma protein binding can be distributed efficiently and impart their therapeutic effects in the body. The distribution of the drug may be affected by the existence of the receptors[23, 37].

METABOLISM

Therapeutic proteins usually are excreted after being degraded or bio-transformed via different pathways and broken into fragments of amino acids.[22] There are various enzymes involved for the metabolism process of the drugs and they are also present throughout the body. Therapeutic proteins should be given with caution to the patients having hepatic impairment due to their extensive hepatic metabolism characteristics[38,39]. There are several proteins which can affect the metabolising enzymes like cytochrome p450 enzyme which can be affected by IL-1β, IL-6 and TNF-α. Several pathways including formation of immune complex cells followed by F-γ receptor-mediated clearance, non-specific endocytosis are responsible for the removal of the therapeutic proteins from the blood. There are some other factors as well which affect the metabolism of the therapeutic proteins including the size, charge, distribution, structure and hydro or lipophilicity of the therapeutic proteins[40, 41].

ELIMINATION

Elimination of therapeutic proteins or clearance of therapeutic proteins from the systemic circulation starts with the passage across the capillary endothelia. This elimination of protein involves renal excretion, hepatic elimination and biliary elimination process[42, 43].

Renal elimination: After glomerular filtration of the protein, it can be excreted unchanged in the urine or degraded to such compounds and then excreted in the urine or active reabsorption may occur by the proximal tubules by a process known as luminal endocytosis[11].

Hepatic elimination: In the removal of proteins from the systemic circulation liver plays a very important role. There are several mechanism for the hepatic elimination including receptor-mediated endocytosis, non-selective pinocytosis, and receptor-mediated uptake[44, 45].

Biliary elimination: Some proteins can be excreted from the systemic circulation by biliary excretion as well including insulin, epidermal growth factor etc. They usually entered from plasma to the bile by a specific transport process[46, 47].

CHALLENGES OF THERAPEUTIC PROTEINS

When proteins used as a therapeutic purpose, in such cases some challenges or limitations are also present. Although recent advancement on pharmaceutical biotechnology and genetic engineering helps in the formulation of therapeutic proteins and that's create a huge impact on health care management. There has been several challenges in past but in recent times researchers overcome most of the challenges. But some challenges are present and have to be addressed in near future[20, 22].

There are some challenges as follows:

(I) Due to high production cost, protein therapeutics are much expensive, that's why a limited number of patients can access this therapy[22, 49].

(II) Safety and Immunogenicity are the prime factors on protein therapeutics. There are some factors on safety such as patient’s condition, disease state, half-life of that molecule, route of administration etc[50].

Whereas immunogenicity have another big challenges like small amount of protein in protein therapeutics can affect immunogenicity. In some cases therapeutic proteins may induce neutralizing or non-neutralizing of antibodies. There are some factors in immunogenicity such as changes in protein structure, denaturation and aggregation, route of administration, genetic characteristics of patients etc[51, 52].

(III) Stability of protein is another challenge in protein therapeutics. There are some factors which effects the stability of protein such as (a) Temperature, protein must be stored in less than 4-5°c temperature, and excess temperature may affect the stability of protein. (b) Changes in amino acid sequences may affect the stability of protein (c) Thermo-sensitive polymers also increase the stability of protein[53, 54].

(IV) Degradation of proteins is another prime challenges in protein therapeutics. Due to some unavoidable conditions, proteins are got degraded. Due to aggregation, suboptimal buffer conditions, proteolysis protein lose its therapeutic properties. There are some factors in degradation such as oxidation, fragmentation, deamidation, chemical cross linking etc[55, 56].

(V) There are some pharmacokinetic and pharmacological challenges such as time, route of administration and site of

action of the protein sometimes create challenges on the therapy. Hepatic first-pass metabolism, rapid eliminations, short biological half-life of protein are some prime challenges in pharmacokinetics of protein therapeutics[22, 11].

IMPLEMENTATION OF THERAPEUTIC PROTEIN IN CLINICAL HEALTH

Therapeutic proteins have done revolutions for several diseases and it is believed that in near future they will be available in a wide range. These therapeutic proteins and peptides are believed to induce cellular immune and humoral responses in patients. Therapeutic proteins having immune reactions can have life-threatening consequences including anaphylaxis or reduction in efficacy or can induce autoimmunity[48].

CONCLUSION

In this review, we have discussed about therapeutic protein, its classification, implementation in health care system, pharmacokinetics, and different challenges of therapeutic protein. Therapeutic proteins are drawing tremendous interest in clinical health now a days due to the growth of pharmaceutical biotechnology and genetic engineering. Many of the therapeutic proteins are used clinically to treat different diseases like cancer, diabetes, AIDS etc. and more therapeutic proteins are in the clinical trial. Several researches are going on this field. As we have mentioned different challenges like chemical changes in protein structure or temperature may lead to some change in the potency of that therapeutic protein, not only that but also some other challenges are there as well. In some recent advancement and research have reported that we can overcome all the challenges and therapeutic proteins can take the centre stage in near future.

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