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Review Article

A NOVEL THERAPEUTIC APPROACH USING BIOTECHNOLOGY ON PHARMACEUTICAL PRODUCTS

P.MOUNIKA*[1], MOHIT KUMAR[2], KISHOR .M.R[2], SANJIT[2], ABISHEK PANDEY[2]

[1]Lecturer, [2]Department of pharmaceutics, Gautham College of pharmacy, Bengaluru.Email: mounikareddypharma@gmail.com

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ABSTRACT

Objective: The present review focus on biotechnology medicines, therapeutic agents obtained from genes, recombinant DNA, developments in protein drug delivery, regulatory aspects of biotechnology-based pharmaceuticals, formulation approaches to protein stabilization, methods used to evaluate protein pharmaceuticals, Production of Biopharmaceuticals from Recombinant DNA Technology. **Conclusion:** For treatment of disease, therapeutic agents are derived from biotechnology have been approved for human use. Recombinant protein products are developing as a part of pharmaceutical development. The advancement in technology mapping or the genome, explosive growth of the targets identified.

Keywords: Antibodies, Hormones, Hybridoma Technology, Chromatography.

INTRODUCTION

Since 1980, more recombinant drugs approved for use in the United States, recombinant protein products have become a part of pharmaceutical development [1]. Biotechnology medicines." These medicines, are recombinant versions of proteins found in vivo. For some, these protein agonists activate specific receptors located in defined target tissues. They are used either as replacement therapy, e.g., insulin and growth hormone, or as a supplement to in crease the activity of endogenous proteins. They are also used as therapeutic antibodies and site-specific carriers of toxic drugs for therapy or as imaging agents [2].The use of therapeutic proteins to replace or supplement endogenous protein molecules has been a long established treatment for diseases such as diabetes, growth hormone deficiency, and hemophilia [3].The first successful treatment of diabetic patients with animal-derived insulin [4].

Table 01: Table 1 Examples for Biotechnology products

Vistide	Cidofovir injection	Gilead Sciences	Cytomegalovirus retinitis in AIDS patients
Wellferon	Interferon alfa-nl	Glaxo Wellcome	Treatment of chronic hepatitis C
Zenapax	Daclizumab	Hoffman-La Roche	Prevention of kidney transplant rejection

Recombinant DNA and hybridoma techniques are capable of providing mole clues of a well-defined chemical composition and producing them in cell culture media that can be carefully controlled. The critical advances in techniques offer development and clinical usefulness of therapeutic protein molecules.

Production of Biopharmaceuticals from Recombinant DNA Technology

Recombinant DNA technology, often used synonymously with genetic engineering, involves the isolation of cellular DNA fragments that code for proteins of therapeutic interest [5, 6]. The DNA fragments are inserted into cellular hosts that, by normal replication, make multiple copies of the original sequence.

The products of biotechnology are typically proteins, herein distinguished arbitrarily from peptides as molecules having in excess of 30 amino acid residues. By using biochemical purification techniques, the protein may be isolated in a highly purified form [7].

Protein Structure

Proteins are condensation polymers of amino acids, joined by peptide bond. The primary structure refers to the sequence of amino acids and the location of any disulfide bonds. Secondary structure is derived from the steric relations of amino acid residues that are close to one another [8]. The alpha-helix and beta-pleated sheet are examples of periodic secondary structure. Tertiary structure refers to the overall three-dimensional ar-chitecture of the polypeptide chain.

The native, biologically active form of a protein molecule is held together by a delicate balance of Noncovalent forces: hydrophobic, ionic, van der Waals interactions, and hydrogen bonds [9].

The term *denaturation* is applied to both reversible and irreversible disruption of the native, biologically active conformation [10]. Denaturation involves changes in Noncovalent interactions, such as hydrogen bonding, hydrophobic interactions, and electrostatic forces.

Mechanisms and Causes of Protein Destabilization

A. Covalent Protein Destabilization

Hydrolytic Reaction

Oxidation

Racemization

Disulfide Bond Exchange

B. Noncovalent Protein Destabilization

Aggregation Surface adsorption

Precipitation

Methods Used To Evaluate Protein Pharmaceuticals

The development of biopharmaceutical formulations supported by a combination of several analytical approaches in order to provide a comprehensive characterization of the protein being

tested[11].The high complexity of proteins requires that biopharmaceutical development scientists use physicochemical methods but also immunological and biological methods to detect for chemical, physical, and functional changes, which may result from degradative processes. In addition to stability-indicating methodologies, analytical methods can be developed that assist in the determination of the compatibility of formulation excipients with the protein [12]. Quality control and stability to monitor identity purity and potency. Assays that are used to support quality and stability of pharmaceuticals used in human studies are required to undergo more extensive validation than during early preclinical development[13].Extensive validation than during early preclinical development. The process of assay validation involves the experimental demonstration of method parameters including assay specificity, dose-response profile and range (including detection and quantitation limits), accuracy (including recovery of spiked sample and ability to detect degradation), precision (repeatability and reproducibility), and robustness (reliability in re-sponse to assay variations). Assay validation guidelines published by regulatory agencies are available to pharmaceutical scientists [14].

Liquid Chromatography

This method is used to assess the purity and degradation of biopharmaceutical proteins. HPLC column technology al lows the biopharmaceutical scientist to choose the type of chromatographic separation depending on the properties of the protein. Three modes of HPLC are commonly used in biopharmaceutical development: reverse-phase HPLC, size exclusion HPLC, and ion exchange HPLC. The separation of protein molecules in each mode is based on hydrophobicity, size, and charge [15].

Reverse-Phase HPLC

The mechanism of separation for RP-HPLC is based on hydrophobic interactions between the protein molecule and the column stationary support. The mobile phase in RP-HPLC is typically prepared with a buffer at a specified pH mixed with an organic solvent like acetonitrile or n-propanol. In many cases, low percentage trifluoroaceticacid or similar reagents added to the mobile phase to act as an ion pair with the protein to increase hydrophobicity, thereby enhancing the interaction with the stationary phase. Elution of the protein sample through the column takes place as a result of changes in polarity of the mobile phase by use of shallow gradients, which can be programmed into the pump controller [16].

Size Exclusion HPLC

SEC, therefore, can separate proteins based on the molecular weight and can yield information on the levels of aggregation and fragmentation in a protein formulation. The size of the test protein is determined by running a calibration set of proteins of known molecular weight. The use of high performance size-exclusion chromatography to ex amine the aggregation of interferon-y and interleukin-2 after storage at elevated temperature, after mechanical agitation, and following rapid freeze-thaw.

Ion-Exchange Chromatography

Ion-exchange chromatography (IEC) is based on the selective retention of the protein sample based on the charge on the protein at the pH of the mobile phase and a corresponding counter-ion covalently bonded to the stationary phase in the column packing. The development scientist can choose stationary resins with cation or anion functional groups and can select the elution of the protein sample using a gradient in ionic strength [17].

Optical Spectroscope

Optical spectroscopic techniques used to evaluate protein pharmaceuticals include UV and visible absorption spectroscopy, optical rotatory dispersion (ORD) and circular dichroism (CD), fluorescence, and infrared (IR) and Raman spectroscopy [18].The technique is useful in determining changes in secondary structure as a function of stability, thermal treatment, or freeze-thaw.

Electrophoresis

Electrophoretic techniques are based on the differences in migration of a protein through a sieve-like gel, de pending on the molecule's size or net charge, in response to an applied electric field [19].

Immunoassays and Biological Activity Assays

Bioassays are analytical in vitro or in vivo procedures, which measure the specific ability or capacity of a product to effect a desired biological response. The role of bioassays in the development of biopharmaceutical products has become increasingly important. Bioassays may be used during the early stages of formulation development, if available, and they are required as part of an Investigational New Drug (IND) and Biologics License application (BLA). Regulatory guidelines comment on the need for an assessment of biological potency for products of recombinant DNA and hybridoma technology [58]. Bioassays are used for monitoring lot-to-lot consistency and are increasingly relied upon to predict biological equivalency of a product when manufactured using different processes [20].

Functional assays to determine potency may be conducted using established animal models or using cell-based in vitro assays. Biological assays conducted using animal models tend to be more time consuming, variable, and difficult to control as compared to cell-based in vitro assays. For this reason, cell-based bioassays are sometimes preferred over in vivo assays [21].

If the required in vivo response is binding of the protein to a target receptor or antigen (soluble or membrane bound), then enzyme-linked immunoassays (ELISA). ELISA tests are typically included to monitor the quality of monoclonal antibodies being developed for human use. In the quality control of protein formulations, ELISA tests may also be used to quantify proteins compared with a known amount of standards or to detect specific protein contaminants, such as host cell proteins [21].

Formulation Approaches to Protein Stabilization

The formulation development of proteins. Principally it consists of determining the principal routes of degradation and then formulating the protein to arrest these pathways. A variety of approaches exist for stabilizing proteins, for example, chemical modification, immobilization, and site-directed mutagenesis [22].The principal formulation strategy is to stabilize the protein using clinically acceptable additives (excipients) or through the use of suitable pharmaceutical-processing technologies.

Protein Stabilization in Solution Using Additives

Proteins are generally most stable in solutions that mimic their natural environment. This includes a wide range of conditions. For example, mature insulin precipitates under conditions of pH and salt concentration at which serum proteins are soluble. The protein formulation program may begin with an assessment of the effect of pH, ionic strength, and oxygen on the stability and solubility of the protein.

Protein Stabilization in the Dried Solid State

Lyophilization and spray drying are two such processes. Both technologies may be used to dehydrate heat sensitive molecules and, thereby, inhibit the degradative reactions that may be observed when proteins are formulated in solution. Stabilization by freeze-drying could potentially allow the therapeutic product to be stored at room temperature for distribution into markets that do not guarantee refrigerated delivery [23].

Lyophilization and Protein Formulation Development

Stabilization of proteins against those degradative processes with retention of structure and function through removal of water requires an understanding of the process of Lyophilization or freeze-drying. Lyophilization is a process in which water is removed form a product after it is frozen and placed under vacuum, allowing the ice to change directly from solid to vapor without going through a liquid phase. The three steps in the process are: (a) freezing of the solution, (b) primary drying where

sublimation of ice water takes place, and (c) secondary drying where tightly bound water is removed (desorption). Excipients useful for stabilization of freeze-dried powders for long-term storage include sucrose, trehalose, dextrans, polyvinylpyrrolidone (PVP), sorbitol, polyethylene glycol, and mannitol.

Spray-Drying of Protein Pharmaceuticals

Several techniques have been employed for this purpose, including spray-drying, spray-freeze drying, milling of lyophilized powders, supercritical fluid precipitation, and co-precipitation with polymers. Spray-drying produces particles that have good flow properties and narrow size distribution, and the process can be adapted to produce particles of a range of sizes, dependent on the application .Disaccharides like sucrose and trehalose have been shown to preserve protein structure and activity in the solution state by pre ferential exclusion effect. Surfactants like polysorbate 20 and polysorbate 80 have been shown to protect the proteins from harmful hydrophobic interfacial inter actions [24].

Regulatory Aspects of Biotechnology - Based Pharmaceuticals

All of these products are classified as "biologics." these products require licensing under the Public Health Service Act and must comply with the regulations set forth in the Code of Federal Regulations, Title 21 and Parts 600--680. Regulatory control and review of biotechnology-derived biologics, except for those considered to be medical devices, is administered by the Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA). Regulatory approval for biologic products is based on the submission of a biological license application (BLA) to CBER, in contrast with a New Drug Application (NDA) sub mitted for small molecule products to be reviewed by the Center for Drugs Evaluation and Research (CDER) branch of the FDA. Regulatory authorities and industry associations have undertaken several important initiatives to promote international harmonization of regulatory requirements.

Developments in Protein Drug Delivery

Protein therapeutics are prepared as sterile products for parenteral administration, and also in pulmonary, oral, transdermal, and controlled-release injectable formulations have been made. Approaches to alter the therapeutic potential of proteins by modification of the pharmacokinetic pro file and/or reduction of the immunogenicity have been explored. Plasma half-life extension may be obtained through chemically modifying the molecule to inhibit its pharmacological clearance or by controlling the rate at which the protein is delivered to the bloodstream. Of the chemical-modifying technologies explored to date, polyethylene glycol (PEG) modification appears to be the most promising [155]. PEG modification (PEGylation) increases the plasma half-life of protein and reduces the immunogenicity of proteins dramatically. In addition, the proteins remain biologically active [156]. A possible mechanism for the altered behavior of the PEGylated proteins may be the increased [25].

Possible noninvasive routes for delivery of proteins include nasal, buccal, rectal, vaginal, transdermal, ocular, oral, and pulmonary. For each route of delivery there are two potential barriers to absorption: permeability and enzymatic barriers. However the pulmonary and nasal routes appear to hold the greatest promise. The oral route would be by far the most popular. The nasal route possesses higher permeability and presents less of an enzymatic barrier than does the oral route. The nasal route has been successful for a number of polypeptide drugs. Nasal formulations for lutem1zmg hormone-releasing hormone (LH-RH) analogs (desmopressin, oxytocin, and calcitonin) have reached the marketplace. Notably, however, this route of delivery has not been as successful for larger proteins with molecular weights greater than 10kDa and may be associated with local irritation and toxicity with long-term administration [25].

CONCLUSION

Proteins and peptides can be formulated into biological products and these are stable in solution form due biological nature of the formulation. The advancement and breakthroughs in the stabilization and formulation of proteins, advancement in bio engineering and specialized and novel delivery systems makes the therapeutic drug in biological product, which gives good response to the human's body. The result leads to development of novel therapeutics

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