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Protein and Peptide Drug Delivery: By Noninvasive Technology.

Vaibhav Agarwal*, Vansh Khattry, Gopa Roy Biswas, and Sutapa Biswas Majee.

NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata - Group of Institutions
124, B. L. Saha Road Kolkata-700053.

ABSTRACT

Protein and peptide based pharmaceuticals are a predominant class in the field of therapeutic and clinical application. Parenterals are an orthodox choice for this which has some drawback related to patient acquiescence and formulation. The evolution of new macromolecule drugs, i.e. proteins and peptides can be registered by non-invasive technology like pulmonary drug delivery, which surpasses oral, intranasal, and transdermal surrogates. This drug delivery offers supremacy including large surface area, highly vascularized mucosa, porous endothelial membrane, lower enzymatic activity relative to GIT and avoidance of first-pass metabolism. Due to the physiochemical vulnerability and enzymatic impediment of proteins and peptides, there are several obstructions for the development of suitable formulation. So there is need of penetration enhancers, enzyme inhibitors and suitable vehicles to accrue the bioavailability. The absorption chemical enhancers, which augment the permeability of drugs through the epithelial membranes without triggering any tissue damage, are principally useful for the delivery of peptide and protein drugs.

Keywords: non-invasive route, nanoparticles, protein and peptide, liposome, penetration enhancer

Corresponding author

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INTRODUCTION

Proteins are polymers are consisted of amino acids which are covalently linked by peptide bonds. Peptides are small proteins made up of a few dozen amino acids. The parenteral route is a well established choice of protein and peptide delivery but it has some drawback related to patient compliance and formulation.Those demerits include lack of drug reversal, risk of infection and emboli, risk of hypersensitivity reactions, and cost. The non-invasive routes which can be used for the development of such drugs are the GI tract including colon, intra-mucosal routes, nasal and pulmonary routes growing attention has been given to the potential of a pulmonary route as a non-invasive administration for systemic delivery of therapeutic agents (mainly peptides and proteins) due to the fact that the lungs could provide a large absorptive surface area. The drugs can be administered by pulmonary route utilizing two techniques: aerosol inhalation and intra-tracheal instillation. The future of pulmonary drug delivery, whether for macromolecules or small molecules, appears to be broadening. Delivery of macromolecules to the lung periphery offers many advantages over injection and other non-invasive methods. One of the first barriers for absorption through pulmonary route is the permeation across a cell layer. Being charged, large and hydrophilic, proteins show poor bioavailability. Penetration enhancers are the one of the most important component formulation and are responsible for the disruption of the mucosal barriers and applicable to improve the membrane permeations of large macromolecular substances like proteins and peptides. The enzyme inhibitors are the enzymatic approach of the protein and peptide drug delivery systems. GIT and liver play an important role in metabolization of the protein and peptides into smaller fragments. The drug delivery system is important for the delivery of protein and peptides and can be successfully achieved by using various carrier systems like: dry emulsion, microspheres, liposomes and nanoparticles. The drugs can be well administered by pulmonary route by utilizing two techniques. They are aerosol inhalation and intra-tracheal instillation. [1]

PROTEINS AND PEPTIDES

With the discovery of insulin (1922), there has been a remarkable increase in the identification, commercialization and research of potential protein and peptide drugs research efforts have followed two basic pathways: One path focused on non-invasive means of delivering proteins to the body and the second path has been primarily aimed at increasing the biological half-life of the therapeutic molecules. The needle and syringe still remains as the primary administration of protein delivery since the oral route of delivery is a challenging task. Major hurdles i.e. the combined natural barriers of drug stability, drug permeability, pharmacokinetics and pharmacodynamics of protein therapeutics are still round the corner, inspite of which considerable progress has been made and work has been carried out over the past few years. One of the challenges in working with peptide therapeutics is their small size, which typically equates to a short circulating life. As a result, macromolecule therapeutics often quickly loses their effectiveness that requires frequent dosing. Proteins easily get denatured by heat or by agitation and are therefore kept at refrigerated temperatures, along with stabilizing agents for long-term storage. These factors impact not only the cost of therapy, but also patient acceptance and compliance, thus affecting their therapeutic usefulness. Nowadays R-DNA technology and hybridoma techniques are also used. [2] Table 1 shows few examples of protein/peptides applied to the specific diseased state. [3]

Table 1: Protein/peptides applied to the specific diseased state

Disease State	Protein/Peptide
Anti-TB vaccine	Muramyl dipeptide
Asthma	IL-1R, Anti-IgE Mab
Cancer	Interferon-γ, Interleukin-2
Emphysema	Secretory leukoprotease inhibitor, Alpha-1-antitrypsin
Multiple sclerosis	Interferon-β
CVS acting drugs:	Angiotensin 2 antagonist, Bradykinin Captopril
CNS active drug	Cholecystokinin, B-endorphin
Immunomodulators	Bursin, Cyclosporin, Interferon.

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DISADVANTAGES OF CONVENTIONAL ROUTE

Despite being the route of choice for protein and peptide based drugs parenteral route have its own downfalls like: poor patient compliance, the pain and discomfort experienced to inject in the same site again and again, lack of drug reversal, risk of infection and emboli, risk of hypersensitivity reactions, cost and the inconvenience with paediatric patients. Some of the marketed proteins/peptides for parenteral application are tabulated in Table 2. [2]

Table 2: Some marketed protein/peptides for parenteral application

PRODUCT	FORMULATION	ROUTE	INDICATION
Metrodin	FSH 75IU	i.m.	Induction of ovulation
Pergonal	FSH and LH	i.m.	Infertility
Profasi	HCG	i.m.	Infertility
Elspar	Asparginase	i.m., i.v.	Leukaemia
Glucagon	Glucagon	i.m., i.v., s.c.	Hypoglycaemia

DISADVANTAGES OF SEMI-CONVENTIONAL ROUTES

Oral - There are two formidable problems, the first one being protection against the metabolic barrier in GIT, and the second being absorption of a carrier system for absorption of peptides with more than three amino acids.
Nasal - It alters the nasal environment during diseased state and also mucosal toxicity on prolonged use.

Transdermal - It has low rate of permeation for most of protein drugs due to large molecular weights and also has high intra- and inter-patient variability. [3]

NEED FOR DEVELOPMENT OF AN UNCONVENTIONAL ROUTE: PULMONARY DELIVERY

Clearly the disadvantages outweigh the advantages of the conventional route of drug delivery and the promising future of the unconventional non-invasive route to substitute if not completely replace the conventional route poses a dire need to develop and research the unconventional route. Of the unconventional non-invasive routes present the pulmonary route of delivery has better scope than the others.

PULMONARY DRUGS AS AN ALTERNATIVE

Pulmonary administration is a promising route of proteins and peptides comparing with other alternative routes of administration. The lungs provide a large surface area for drug absorption. It is approximately 80-140 m^2. The alveolar epithelium is approximately 0.1–0.5 mm thick thereby rapid drug absorption may occur. [4] The alveoli can be effectively targeted for drug absorption by delivering the drug as an aerosol. Furthermore, the first-pass metabolism of the GIT can be avoided. Some metabolic enzymes are found in the lungs but their metabolic activities differ from those found in the GIT this makes the pulmonary administration of many peptides and proteins very promising. Concomitantly proteins were processed in controlled release devices. [5,6]

Aerosols preparations are stable dispersions or suspensions of solid materials and liquid droplets incorporated in a gaseous medium. The drugs delivered by this is deposited in the airways in which larger particles are deposited by gravitational sedimentation and inertial impaction while the smaller particles get their way into the peripheral region of the lungs by diffusion.

Pulmonary delivered drugs are rapidly absorbed except large macromolecular drugs which yield low bioavailability because of enzymatic degradation and/or low mucosal activity. Pulmonary bioavailability of these drugs can be enhanced by incorporating penetration enhancers and deliver drugs to diseased lungs by using perfluorocarbon fluid dispelling the oedematous fluid by drug dissolved perfluorocarbon liquid which

results in distribution of drug throughout the lung providing higher local tissue concentration. A dosage form is sought for retinoids which is effective against lung cancer but produces harsh side effects in the pill form. [7]

Latest developments: Many new agents are now under investigation for pulmonary delivery, both for targeted lung as well as systemic delivery. These include growth hormones (for growth hormone deficiencies), -1 antitrypsin (for emphysema and cystic fibrosis), interferons (for multiple sclerosis and hepatitis B and C), and para thyroid harmone (PTH) and other peptides (for osteoporosis). Inhalation delivery methods may also be applied to gene therapy via tissue targeting and organ targeting, as well as vaccines. In collaboration with Amylin Pharmaceuticals, Inc., Alkermes has developed a once-a-week Medisorb® formulation of BYETTA® (exenatide) for the treatment of type 2 diabetes known as exenatide LAR. In collaboration with Eli Lilly and Company, Alkermes is using the AIR® (Advanced Inhalation Research) pulmonary drug delivery technology to develop inhaled formulations of insulin and recombinant parathyroid hormone. Aradigm has developed AERx pulmonary technology, which would help in delivering morphine and insulin into the lungs. Nektar Therapeutic in collaboration with Pfizer began dosing first diabetic patients for phase III clinical trial for inhalable insulin Exubera®. Several drugs are currently investigated for potential systemic absorption through pulmonary system which includes insulin, calcitonin, luteinizing-hormone releasing hormone (LHRH) analogs, granulocyte colony stimulating factor (rhG-CSF) and human growth hormone (hGH).

Although it was known that inhalation of insulin aerosol was efficacious, a number of challenges to systemic delivery of insulin via inhalation remained unresolved for several years. First, there was the need to deliver a substantially higher dose of insulin to the lung than was needed via subcutaneous delivery to achieve the same systemic effects. Aerosol devices that were avail- able in the early 1990s were too inefficient to deliver these large doses, since a large proportion of the drug was either retained in the device or was never delivered past the oropharyngeal region. In addition, conventional devices that were available at the time required compressors and electricity to generate the aerosol particles. Clearly, a portable device that didn't require electricity and delivered a high percentage of the drug to the lung was needed to increase patient acceptance and compliance. The solution to this challenge led to the development of portable, more efficient inhalation devices and new drug formulations. Devices and formulations that are furthest along in development of inhaled insulin include the Nektar, Aradigm, and Aerogen products. Each of these products delivers aerosol containing a high percentage of 1–3m particles, which are considered optimal for targeting the alveolar lung region, and incorporate methods for controlling breathing parameters that are known to influence aerosol deposition in the lung (eg, inspiratory flow rate and lung volume at the time of inhalation). The Nektar device (Nektar Therapeutics, San Carlos, California) uses com- pressed air to disperse dry powder insulin into a spacer before inhalation. The patient then inhales insulin from the spacer during a slow, deep breath. The Aradigm device (Aradigm, Hayward, California) is a breath actuated, aqueous mist inhaler. Liquid insulin aerosol is delivered electronically by means of mechanical extrusion when the patient's inspiratory flow rate and lung volume are appropriate. The Aerogen device (Aerogen Inc, Mountain View, California) is a breath-actuated, liquid aerosol in- haler that delivers insulin to the patient during inspiration by means of vibrating mesh technology. Nektar has completed phase III testing and has applied for Food and Drug Administration approval. Aradigm is in early phase III testing. Aerogen is in phase II development. [4,8]

SUITABLE VEHICLES FOR FORMULATION OF PROTEINS AND PEPTIDE DRUGS

Chemical modification (Prodrug approach): The chemical modification of protein and peptide drug delivery system of drugs is important to improve the enzymatic stability as well as membrane permeations. It is applicable for the reducing the immunogenicity.

The Chemical Modification is Includes in Two Types of Modifications as Follows:

1. Amino acid Modification
2. Hydrophobization

1. Amino acid Modifications: The Modification of amino acid is one of the important approach in which the substitution of the D- amino acid and L- amino acid is important to alter the physiological properties of protein and peptide drug delivery systems.

Example: Desmopressin and Deaminovasopressin are the two important analogs of vasopressin; former involves deamination of first amino acid and replacement of last L- arginine D-arginine to give deaminovasopressin.
Application: The amino acid modification is important to enhance the membrane permeability and maintain the enzymatic stability.

2. Hydrophobization: It is having an important approach for the Lipophilic Moieties.

Example: NOBEX INSULIN by the Palmitoylatios. [9]

Enzyme Inhibitors (Protease): The enzyme (protease) inhibitors are the enzymatic approach of the protein and peptide drug delivery systems. GIT and liver play important role in metabolization of the protein and peptides into smaller fragments of the two to ten amino acids with the help of the variety of proteolytic enzymes. These protease inhibitors are coadministered with protein and peptide to alter the environment for the enzyme stability to supress the proteolytic activity. The enzyme proteases inhibiters are divided into four types they are Aspartic Proteases (Pepsin, Rennin), Cystinyl Proteases (Papain, Endopeptidase), Serinyl Proteases (Thrombin, Trypsin), and Metallo Proteases (Carboxypeptidase). [10]

PENETRATION ENHANCERS

Penetration enhancers are the one of the most important component of protein and peptides formulation is responsible for the disruption of the mucosal barriers and applicable to improve the membrane permeations of large macromolecular substances like proteins and peptides. The several classes of compounds are mainly used has a permeation enhancers are such as surfactant (Polysorbate, SLS, Pluronic F-68), chelating agent (EDTA), fatty acids (Sodium Carprate), mucoadhesive polymeric systems (Thiomers, Cellulose derivatives), phospholipids (PC). The basic mechanism of penetration enhancers are the, detergent and surfactant molecules are the increases the transcellular transport of the drug material is responsible to disrupting the structure of the lipid bilayer of lipid membrane are having more permeability. Another mechanism is the calcium chelates are the responsible for the action of complex formation of the calcium ions and they are passing through the tight junctions and they are facillated the paracellular transport of the hydrophilic drugs materials. Fatty acids are the important for the improving the paracellular absorption by phospholipases C activations and upregulation of intracellular calcium ions, is leading to the contraction of actine myosin. [5]

FORMULATIONS

The protein and peptide drug delivery system is important for the oral delivery of protein and peptides can be successfully achieved by using various carrier systems are like **Dry Emulsion, Microspheres , Liposomes and**

Nanoparticles.

Dry Emulsion: It is important application in drug delivery system s to prevent the instabilities of the long term storage of multiple emulsions. Dry Emulsion is prepared by the spray drying, lyophollization and evaporation techniques. In dry emulsion preparation application of the PH responsive polymers like HPMCP, is important for the emulsions are the enteric coated and site specific achieved.

Microspheres: The uniform distribution of drug in oral drug delivery in Protein peptides drug is known as Microspheres. The pH responsive microspheres are the mainly used in oral delivery for the protection of the stomach from proteolytic degradations and Protection upper portion of small intestine from proteolytic degradations.

Liposomes: Liposomes are the small microscopic vesicles in which aqueous volume is entirely enclosed by the membrane composed lipid molecules. Liposomes in drug delivery system, the encapsulation of the insulin with sugar chain portion of mucin and PEG completely suppressed the degradation of the insulin molecules in intestinal fluid. The uncoated from of liposomes are suppressed it on partially surface coating of the liposomes molecules in PEG or mucin gained resistances against dagestion by salts and increased the stability of GI tract.

Nanoparticles: Nanoparticles are Nano sized colloidal structure having size is 10-1000nm. The particles in nanometric sized range of the particles are absorbed intact by the intestinal epithelium and they are the less prone towards the enzymatic degradations. The particle size surface charges are the influencing the uptake of nanoparticle system in GI tract.

Growing attention has been given to the potential of a pulmonary route as an noninvasive administration for systemic delivery of therapeutic agents (mainly peptides and proteins) due to the fact that the lungs could provide a large absorptive surface area (up to 100 m^2) but extremely thin (0.1 µm – 0.2 µm) absorptive mucosal membrane and good blood supply. However, recent advances show great promise, but pulmonary delivery of peptides and proteins is complicated by the complexity of the anatomic structure of the human respiratory system and the effect of disposition exerted by the respiration process.

FORMULATIONS

The drugs can be administered by pulmonary route utilizing two techniques: aerosol inhalation (also used in intranasal applications) and intratracheal instillation. By applying aerosol technique, we could achieve more uniform distribution with greater extent of penetration into the peripheral or the alveolar region of the lung, but this costs more and also faced with difficulty in measuring the exact dose inside the lungs. In contrary to this, instillation process is much simple, not expensive and has non-uniform distribution of drugs.

The Future of Inhalable Therapeutics

The future of pulmonary drug delivery, whether for macromolecules or small molecules, appears to be broadening. Delivery of macromolecules to the lung periphery offers many advantages over injection and other non-invasive methods. As the fields of biotechnology, genome research, and protein therapeutics continue to burgeon, the opportunities for aerosol delivery of these compounds expand accordingly.

Interest is also focused on formulating certain small molecule pharmaceuticals for pulmonary delivery. Although many of these drugs are readily administered in oral preparations, pulmonary delivery would offer substantial advantages over oral delivery, including rapid onset of the action, in a matter of seconds, and avoidance of the problems that can accompany gastrointestinal absorption, such as low solubility and low bioavailability of the drug, possible gastrointestinal upset caused by drug irritability, unwanted metabolites resulting from gastrointestinal metabolism, and the interaction of the drug and certain foods.[2]

CONCLUSION

The Protein and peptide based pharmaceuticals are rapidly becoming very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future. Although oral and injectable drug formulations still dominate the market its disadvantages have started to outweigh its advantages. This poses as an urgent challenge to the pharmaceutical industry to develop viable delivery systems for the efficient delivery of this complex therapeutics. In times like these the pulmonary route is like an exemplary ray of sunshine that will definitely enlighten the pharmaceutical therapeutics towards a brighter future.

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