Review Article

PHARMACEUTICAL APPROACHES RELATED TO SYSTEMIC DELIVERY OF PROTEIN AND PEPTIDE DRUGS: AN OVERVIEW

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ABSTRACT

In new era of medication, recently under development of biotechnology and genetic engineering, the industry is capable of producing number of therapeutic peptides and proteins commercially. The research efforts to formulate the immediate and sustained release formulation of protein and peptide based pharmaceuticals which is given to body directly or using polymeric carriers. Disease related to CVS, CNS, GIT, hormones and cancer can be effectively treated by this new class of therapeutic agents. We have primary aimed to improve bioavailability, half life, penetrability and stability. Generally, protein and peptide drugs are administered by parenteral route. Many peptide based pharmaceuticals can't accomplish their full range of therapeutic benefits when delivered by parenteral route because they are limited by extremely short duration of their biological function. Alternate non-parenteral routes like oral, nasal, pulmonary, ocular, buccal, rectal, vaginal and transdermal have been tried with varying degree of success. Various problems associated with administration of protein and peptide drugs are needed to overcome by different pharmaceutical approaches. Several approaches available for maximizing pharmacokinetic and pharmacodynamic properties are chemical modification, formulation vehicles, mucoadhesive polymeric system, use of enzyme inhibitors, absorption enhancers, penetration enhancers etc. This review summarizes recent approaches in protein and peptide drug delivery system.

Keywords: Protein, Peptide, Parenteral, Non-Parenteral, Pharmaceutical approaches.

INTRODUCTION

Proteins and peptides are the most abundant components of biological cells.[1] They exist functioning moieties such as enzymes, hormones, structural element and immunoglobulin. Also, take part in metabolic process, immunogenic defense mechanisms and other biological activities.[2] Each peptide or protein molecule is a polymer chain with α-amino acid linked together by peptide bonds in a sequential manner, formed by interaction between the α-carboxyl and α-amino groups of the adjacent amino acids, resulting polymers are generally called peptides.[3] A polypeptide containing 50 to 2500 or more units of amino acids in a peptide chain is called protein. For contribution of three- dimensional structure of the protein, regularly repeating backbone with distinctive side chains of polypeptide interact with each other.[4] Basically, they are macromolecules with the high molecular weight. Molecules referred to as polypeptides generally have molecular weights below 10,000 Dalton and those called proteins have higher molecular weight.[1]

Diffusion of drugs through the epithelial layer greatly affect by high molecular weight and size of protein.[5, 6] Difficulties arise in formulation and delivery of proteins due to its unique physical and chemical properties.[7] Proteins are very prone to physicochemical degradation and easily get denatured by heat or by agitation, so kept at refrigerated temperatures along with stabilizing agents for long-term storage.[8]

The discovery of numerous hormones and peptides those have found applications as a biopharmaceuticals, role of regulatory proteins and peptides in pathophysiology of human disease, increasing importance of proteins and peptides.[9] Peptide based pharmaceuticals are well accepted in medical practice and research activities because proteins serve as significant role in the integration of life processes and act with high specificity and potency.[10]

Due to tremendous growth in molecular biology and genetic engineering, the large-scale production of polypeptides is possible by using recombinant DNA and hybridoma techniques.[11] More than 200 proteins and peptides have received US Food and Drug Administration approval for treating a variety of human diseases.[12] The total global market for protein drugs was about $47.4 billion in 2006. The market will reach $55.7 billion by the end of 2011, an average annual growth rate of 3.3%.[13] The 2006 "Biotechnology Medicines in Development" identifies 418 new biotechnology medicines for more than 100 diseases, including cancer, infectious diseases, autoimmune diseases, AIDS and related conditions, which are in human clinical trials or under review by the Food and Drug Administration.[14]

Protein and peptide drugs most commonly administered by parenteral route. Other routes such as oral, intranasal, transdermal, buccal, intraocular, rectal, vaginal, pulmonary route etc have been tried with varying degree of success.[15-19]

Table 1: Some examples of peptide based pharmaceuticals and their potential function/biomedical applications

Protein & peptide drugs	Function/applications
Angiotension II antagonist	Lowers blood pressure
Bradykynin	Vasodilation
Cholecystokynin (CCK-8 or CCK-32)	Suppress appetite
β-endorphin	Relieves pain
Bursin	Selective B cell differentiating hormone
Interferons	Enhance activity of killer cell
Gastrin antagonist	Reduce secretion of gastric acid
Pancreatic enzyme	Digestive supplement
Human growth hormone	Treats dwarfism
Insulin	Treats diabetes mellitus
Vasopressin	Treats diabetes insipidus

DELIVERY OF PEPTIDE BASED PHARMACEUTICALS FOR SYSTEMIC MEDICATION

A) PARENTERAL SYSTEMIC DELIVERY

General consideration

A wide variety of delivery systems exist for drug substances but currently, peptide and protein drugs are marketed almost exclusively for parenteral administration. Parenteral administration is believed to be the most efficient route and also the delivery method of choice to achieve therapeutic activity for protein and peptide drugs. Traditional drug delivery of proteins has relied on parenteral injections of liquid formulations to overcome first pass metabolism[20]. Delivery of proteins and peptides is limited by the extremely short duration of their biological functions. Due to short half-life of proteins, frequent injections are required so, patient compliance is poor. Controlled release system can extend exposure after a single administration and it drastically reduces the number of injections[21]. Amino acids and small peptides are not themselves immunogenic, but macromolecular proteins are often recognized as "foreign" by the body, which may respond with the production of a specific antibody.

Parenteral drug delivery mainly consists of three major routes - IV, IM & SC.

Currently method of choice for parenteral systemic delivery of therapeutic protein and peptide is intravenous administration, mainly for the drugs which are either excessively metabolized by and/or bound to tissues at the site of IM or SC. Usually the intravenous injections are not well tolerated by patients. Treatment of primary immune deficiency disease cannot be achieved with IM administration of gammaglobulin because of limitations in muscle mass and volume [22]. In contrast, IM administration of gammaglobulin has been found beneficial to provide long-term protection from hepatitis infection.

Approaches

Controlled-release drug delivery system produces prolongation of biological activity. For developing controlled release delivery system of proteins and peptides various parenteral route based approaches have been undertaken which include polymeric depot injections, microspheres, liposomes, nanoparticles, hydrogels etc.

- **Microspheres**

Various biodegradable polymers have been investigated for preparation of microspheres as depot formulation. The biodegradable microspheres are used to deliver small molecules, proteins, and macromolecules. There are three manufacturing technique for protein containing microspheres, namely the w/o/w –technique, phase separation methods and spray drying. The microsphere release drug in a zero order fashion for 1 to 3 months into animals after intramuscular or subcutaneous injection.

Leuprolide (synthetic gonadotropin-releasing hormone) microsphere prepared by w/o/w emulsion-solvent evaporation method, which containing the LHRH superagonist leuprorelin acetate with polylactic-co-glycolic acid (PLGA-14000) and Polylactic acid (PLA-15000) [23]. Enzyme replacement therapy through prolidase loaded microparticulate systems have been prepared by the w-o-w double emulsion solvent evaporation method using PLGA polymer[24]. Other examples of PLGA microsphere containing protein or peptide drugs are glycoprotein (GP) IIb/IIIa antagonist, plasmid DNA and Interleukin-1α[25-27].

Table 2: List of marketed microsphere drug product

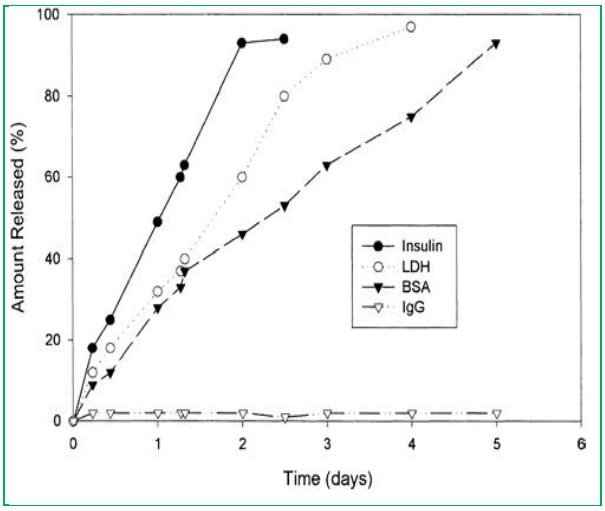
Drug	Commercial name	Company
Risperidone	Risperdal® Consta®	Alkermes
Naltrexone	Vivitrol®	Alkermes
Leuprolide	Lupron Depot®	TAP
	Enantone Depot®	Takeda
Somatropin	Nutropin®Depot	Genentech/Alkermes
Triptorelin	Trelstar™ Depot	Pfizer
Buserelin	Suprecur® MP	Sanofi-Aventis
Lanreotide	Somatuline® LA	Ipsen-Beafour
Bromocriptine	Parlodel LAR ™	Novartis

- **Hydrogels**

Hydrogels are three-dimensional hydrophilic polymeric networks having capacity to absorbing large quantities of water[28]. It offers a 'protein-friendly' environment and excellent biocompatibility. Dextran-based hydrogels are used for controlled release of pharmaceutically active proteins. To form a gel, it needs to undergo cross-linking, by either chemically or physically[29]. Gel formulation of cross-linked polyacrylamide-polyvinylpyrrolidone is used to achieve the prolong release of immunoglobulin, luteinizing hormone, bovin serum albumin, insulin etc. However, the reproducibility of release kinetics is poor and it improved by using a low-temperature solvent casting method[30, 31]. For albumin and haemagglutin as

model antigens, the amphiphilic lipid, sorbitan monostearate (SMS) organogels containing either w/o or vesicular in water in oil (v/w/o) emulsion were investigated in vivo as delivery vesicles. Intramuscular administration of the v/w/o gel yielded the long lasting depot effect (48hr)[32,33]. Photopolymerizable biodegradable hydrogel is used as a controlled release carrier. This system consisted of a PEG (polyethylene glycol)-oligo-glycol-acrylate with photo initiator, such as eosin and visible light. The controlled release of protein was observed over a period of several days. Protein release from photocrosslinked biodegradable hydrogel containing PEG-PLA (polylactic acid) is illustrated in figure-1.[34, 35]

Figure 1: Protein release from photocrosslinked biodegradable hydrogel



- **Liposomes**

Liposome is the most sophisticated way to prepared controlled release protein and peptide pharmaceuticals. Depo Foam® technology is used for sustained release liposome depot product, which consist of novel multivesicular liposomes characterized by their unique structure of multiple non-concentric aqueous chamber surrounded by a network of lipid membranes[36]. The most viable route for administration of drugs via Depo Foam® formulations are intrathecal, subcutaneous, intramuscular, epidural and intra-articular. Depo Foam® formulations have high drug loading, high encapsulation efficiency low content of free drug in the suspension, little chemical change in the drug caused by the formulation process, narrow particle size distribution and spherical morphology. Depo Foam® formulations of various protein and peptide have been developed and characterized, which include insulin, myelopoietin (Leridistim), leuprolide, enkephalin, octreotide etc.[37, 38] Another approach in liposomal technology is vesicular phospholipid gels (VPGs). Liposomes of protein such as erythropoietin and peptide such as Cetrorelix were prepared using VPGs technology and evaluated *in vitro*.[39, 40]

- **Solid lipid nanoparticles**

SLN are colloidal particles composed of a biocompatible/biodegradable lipid matrix that is solid at body temperature and exhibit size range in between 100 and 400 nm. SLN shows excellent physical stability, protection from degradation, controlled drug release, good tolerability and site specific targeting. One problem associated with solid lipid nanoparticle is low drug loading capacity but it can be improve by nanostructured lipid carriers (NLC) and the lipid drug conjugate nanoparticles (LDC). Techniques utilized for preparation of SLN are high-pressure homogenization (HPH), microemulsion, solvent emulsification-evaporation or diffusion, w/o/w double emulsion method and high-speed stirring and/or ultrasonication[41].

- **PEGylation**

Poly Ethylene Glycol (PEG) is non-toxic, linear or branched polymer and has been approved by the FDA for use in foods, cosmetics, and pharmaceuticals. Chemical conjugation with PEG (PEGylation) is currently considered one of the most successful techniques to prolong the residence time of protein drugs in the bloodstream. The advantages of PEGylation for protein molecules include enhanced bioavailability, decreased dosing frequency due to prolonged residence in the body, a decreased degradation by metabolic enzymes, optimized pharmacokinetics, increased efficacy, improved safety profile, a reduction or elimination of protein immunogenicity, improved drug solubility, and stability.

Several PEGylated proteins available in the market are PEG-adenosine deamidase (Adagen®, Enzon), PEG-L-asparaginase (Oncaspar®, Enzon), pegvisomant (Somavert®, Pfizer), PEG-alpha-interferon- 2b (PegIntron® etc.[42-44].

B) NONPARENTERAL SYSTEMIC DELIVERY

1. Oral delivery

General consideration

The understanding of physicochemical properties of protein and peptide drugs is necessary to development of effective oral delivery system. These properties include molecular weight, hydrophobicity, ionization constants, and pH stability, as well as biological barriers that restrict protein and peptide absorption from the gastrointestinal (GI) tract, including pH variability, enzymatic degradation, and membrane efflux.

Potential problems associated with oral protein and peptide delivery like extreme pH condition, protease degradation, epithelial cell barrier, short plasma half-life, low bioavailability, tendency to undergo aggregation, adsorption and denaturation. These problems create some challenge to pharmaceutical scientist[45]. Currently, only two proteinous drugs include Interferon alpha and human growth hormone are given orally in clinical development in the US[46].

Approaches

- **Chemical modification**

Chemical modifications include some strategies like olefenic substitution, carboxyl reduction dehydroamino acid substitution, d-amino acid substitution, thio-methylene modification, PEGylation and retro inversion modification. Result gives more stable prodrug with increase plasma half life [47, 48, 49].

a) Amino acid substitution

This is demonstrated by vasopressin analogs 1-deamino-8-D-arginine vasopressin called desmopressin in which L-arginine release by D-arginine. Desmopressin is twice as active at the 75th fraction of the dose, which is attributed to enhanced membrane permeation and enzymatic stability [50].

b) Nobex Conjugated Technology

In this technology, an amphiphilic protein conjugate is prepared by attaching short chain PEG and alkyl group to the amino groups of the protein molecule, which splits off in the blood circulation to release the parent protein. Nobex's conjugated insulin consists of a short chain PEG linked to an alkyl group which, in turn, is linked to LYS-29 of the β chain. It is found to be more absorbed and effective [51, 52].

- **Protease inhibitors**

Protease inhibitors are co-administered with protein and peptides for maximum enzyme stability. For example, enzyme degradation of insulin is to be mediated by the serine proteases trypsin, a-chymotrypsin and thiol metalloproteinase enzymes. The stability of insulin has been demonstrated by using these enzyme inhibitors like aprotinin, bestatin, FK-448, camostatmesilate, soyabean trypsin inhibitor etc. Another method to enzyme inhibition is to manipulate the pH to inactivate local digestive enzymes[53-55].

Table 3: Enzymes and their specific inhibitors

Enzyme	Specific Inhibitors
Acid Protease	Diazoacetyl DL-norleucine methyl ester; 1,2-epoxy-3(Q- nitrophenoxy) propane; Pestatine
Aminopeptidases	Bestain, Baccitracin
Chymotrisin	Chymostatin; N-Tosyl-L-phenylalanine chloromethyl ketone
Endoprotease	α_2-Macroglobulin
Metaloendoproteases	Phosphoramidon
Metaloproteases	Ethylenediamine tetra-acetic acid

- **Use of penetration enhancers**

Therapeutic agents must have to cross biological membranes and reach systemic circulation to exert their pharmacological effects. Due to large molecular size of protein, penetration enhancer and special type of carrier system are used. Some examples of penetration enhancers are surfactants, bile salts, Ca^{++} chelating agents, fatty acids, medium chain glycerides, acyl carnitine, alkanoyl cholines, chitosans, phospholipids etc. Protects the drug from the intestinal proteases and maximize their driving force for passive permeation a well designed carrier systems are used like lipid vesicles, particulate system, emulsions etc. Surfactant can stabilize a protein against denaturation by decreasing the self-association and absorption of protein on hydrophobic interface of delivery matrix. Sodium glycocholate, a penetration enhancer, inhibits leucine amino peptidase and protect insulin from proteolysis[56-58].

- **Formulation approaches**

a) Emulsions

Emulsion protects drug from acid and luminal proteases in the GIT. Enhance permeation through intestinal mucosa. S/O/W emulsions, a new type of oral dosage form of insulin, in which a surfactant-insulin complex is dispersed into the oil phase is recently developed[59]. Water/oil/water type of multiple emulsion formulation to deliver insulin orally to rabbits and diabetic rats via an indwelling catheter in the jejunum and observed a reduction in the urinary glucose level in the diabetic rats[60].

b) Microspheres

The pH responsive microspheres are used to prevents proteolytic degradation in stomach and upper portion of small intestine. *Lowman et al*. observed oral bioavailability of insuline in healthy and diabetic rats by using insulin loaded microspheres of poly (methacrylic-g-ethylene glycol)[61].

c) Liposomes

Liposome improves physical stability and increases membrane permeability but to improve the stability of liposome GI-resistant lipids or polymers are used. The oral delivery of an insulin-entrapped liposome prepared from phosphatidylcholine and cholesterol as the delivery system is effective in reducing the blood glucose level of diabetic animals, but its stability and effectiveness are rather unpredictable[62, 63].

d) Nanoparticles

Recently, nanoparicles are developed as particulate carriers for oral protein and peptide drugs. The proteins and peptides encapsulated in the nanoparticles are less sensitive to enzyme degradation and increases intestinal epithelial absorption[64]. Insulin was encapsulated using phase inversion nanoencapsulation. The insulin released over a period of approximately 6 hours and had 11.4% of the efficacy of intraperitoneally delivered insulin[65].

- **Other approaches**

Mucoadhesive polymeric system is used to achieve site-specific drug delivery and improve membrane permeation as well as increase of oral drug bioavailability of protein and peptide drugs[66]. Most useful bioadhesive polymers

are either polyacrylic acid or cellulose derivatives include carbopol, polycarbophil polyacrylic acid (PAAc), polyacrylate, poly (methylvinylether-co-methacrylic acid), poly (2-hydroxyethyl methacrylate), poly (methacrylate), poly (isobutylcyanoacrylate), carboxymethyl cellulose, hydroxyl ethyl cellulose, hydroxypropyl cellulose, sodium carboxymethyl cellulose, methylcellulose, methyl hydroxyethyl cellulose etc.

Azopolymer coating involved the coating of peptide with azoaromatic group of polymers and the cross-linking of azopolymers to form an impermeable film to protect the orally administered peptide or protein molecules from degradation and metabolism in the stomach and small intestine[67].

2. Nasal delivery

General consideration

When drug molecules absorbed nasally can directly enter to the systemic circulation before passing through hepatic circulation. Commonly, this route used for topically active drugs to alleviate histaminic symptoms in nasal cavity[68-70]. The nasal absorption of peptides is found to achieve a lower bioavailability than that of its structural component, amino acids. This low bioavailability is shown due to size of peptide molecule, absorption via passive diffusion susceptibility to hydrolytic degradation in the nasal cavity[71].

Protein and peptide with a molecular weight less than 1000 Da are adequately absorbed with an average bioavailability of 70 ± 26%. For example, thyrotropin-releasing hormone (MW 362 Da) achieved a transnasal bioavailability of 40 ± 1.5%, but if molecular weight greater than 1000 Da, percentage of dose absorbed decline as the molecular weight increased according to the relationship[72].

$$\%\, absorption = \frac{100}{1 + a(MW)^{-b}}$$

Where a = 0.001 and b = 1.35 for human nasal mucosa; a = 0.003 and b = 1.3 for rat nasal mucosa.

There are some challenges for pharmacist to develop better delivery of protein and peptide drugs include person to person variation in absorption, less bioavailability, protease degradation, possibility of nasal mucosal irritation, pathological changes on prolonged administration etc[45].

Nasal spray dosage form has been developed for synthetic vasopressin analogs such as phenylalanyl-lysine vasopressin (PLV-2) and demopressin. Nasal absorption of horseradish peroxidase is recently investigated in mouse, rat, squirrel and monkey. The result indicated that horseradish peroxidase passes freely through the intracellular junctions of olfactory epithelium and reach to the CNS within 45 to 90 minutes [73, 74]. Several investigations have been carried out to evaluate the nasal route systemic delivery of LHRH analogs to determine their efficacy as contraceptive agents[75, 76]. lypressin and desmopressin, two more peptides have been successfully developed and marketed as nasal pharmaceutical products in the United States: oxytocin (Syntocinon, Sandoz) and nafarelin acetate (Synarel, Sytex).

Approaches

- **Viscosity Modification**

For instance, the half time of clearance could be increased significantly by using solution with higher viscosity because the clearance time from the nasal cavity can be delayed. For intense, 0.6 % of hydroxypropyl methylcellulose is used.

- **pH Modification**

Usually, the lowest solubility of peptides and proteins is found at their isoelectric point. Solubility can be increased by adjusting the pH farther away from the isoelectric point of a particular peptide. Hirai *et. al*. have been demonstrated that insulin is capable of crossing the nasal membrane in an acidic medium[77].

- **Increased Nasal Blood Flow**

Enhancement in nasal peptide absorption has been reported with an increase in local nasal blood flow[78]. Histamine[79], prostaglandin E_1[80] and β-adrenergic agonists[81] are the vasoactive agents, which are known to enhance nasal blood flow.

- **Dissociation of aggregation**

Proteins are prone to form higher order aggregates in solution. In case of insulin, in solution at pH 7.0 it exists as hexameric aggregates so fails to cross the nasal membrane. However, satisfactory nasal absorption of insulin was observed with sodium deoxycholate[82]. On the bases of this report, it is assumed that dissociation of insulin hexamer to dimmers and monomers by sodium deoxycholate is partly responsible for better transport of insulin across the nasal epithelium.

- **Membrane transport and enzyme inhibition**

Penetration enhancers like bile salts, surface active agents and chelating agents are reported to increase the nasal absorption of proteins and peptides. Thus, the bile salts affect both the permeability of nasal mucosa and inhibit the activity of leucine aminopeptidase, and there by enhance the absorption of insulin[77].

3. Pulmonary delivery

General consideration

Lungs are an attractive site to obtain effective, non-systemic delivery alternative to injections for the targeted disorders of the lung. The lungs have enormous surface area ($70m^2$) for absorption of therapeutic protein and peptide. Pulmonary delivery is used in case of diseases which could not be treated by using oral medications and which require macromolecule drug therapies. This route has many advantages over oral, intranasal, and

transdermal alternatives[83]. In case of asthma, pulmonary drug delivery is most commonly used.

Pulmonary bioavailability not the same for all peptide and protein drugs but it is largely depends on the physical properties of the delivered protein. Major challenges in delivery of protein and peptide by this route are to achieve reproducibility in the deposition site of the applied dose, alveolar macrophages, epithelial cell barrier, rapid rate of absorption, limits on the amount of protein delivered per dose, permeability challenge in smokers etc[45]. Chemical stability, uniformity, morphology, dispersibility and particle size which are managed by particle engineering and formulation methods, used to manufacture drug powders for inhalation, created opportunities to expand the applications for pulmonary delivery of proteins and peptides like many therapeutic molecules.

Examples of proteins and peptides for targeted lung as well as systemic delivery include insulin, growth hormones, -1 antitrypsin, interferons, para thyroid harmone (PTH), Leuprolid and albumin. The pulmonary absorption of leuprolide was investigated in human volunteers and found to be absorbed to the extent of 18%[84].

Approaches

- **Liposomes**

For drug delivery system, Liposomes have been used for many years. Liposomal aerosols are also one of good formulation due to its several advantages like, local irritation is prevent, sustained release, reduced toxicity improved stability in the large aqueous core and also chance to manipulate release and targeting by changing the technique of preparation and altering the bilayer constituents. The lipid composition, size, charge, drug/lipid ratio and method of delivery are important because some of parameters are dependent on it such as, release rate, drug carrying capacity and deposition of liposomes in the lungs[85-87].

Table 4: Liposomal Formulations of Proteins/Peptides for Respiratory Delivery

Drug	Effect	Ref
Cyclosporin	The lung rapidly and preferentially absorbed the liposomal cyclosporine; the drug was retained for 120 minutes in a dog model.	89
Insulin	Liposomal formulation facilitated pulmonary absorption and enhanced the hypoglycemic effect.	90
Catalase	Liposome formulations conferred resistance to pulmonary oxygen toxicity.	91
Interleukin-2	Local delivery of liposomal IL-2 to the lungs facilitated bioactivity and reduced toxicity.	92

Insulin liposomes are one of the recent advances in the controlled release of aerosol preparation[88]. Potential elicitation of an immune response and its safety issue, associated with pulmonary delivery of protein and peptide pharmaceuticals. The lung has decreased proteolytic activity compared with the oral delivery and avoidance of first-pass metabolism of systemically delivered drugs. Hypoglycemic effects have been significantly enhanced by the intratracheal delivery of insulin liposomes (dipalmitoylphosphatidyl choline:cholesterol, 7:2).

- **Lipid-based Microparticles**

Lipid-based hollow-porous microparticles named pulmospheres, were loaded with human immunoglobulin (IgG) delivered into the upper and lower respiratory tract of mice, which triggered local and systemic immune responses [93].

- **Microspheres**

Microspheres are physically and chemically more stable than liposomes and allow for higher drug loading. They are, therefore, a useful carrier system for proteins and peptides[94, 95]. The delivery of microspheres in the lungs is dependent on the preparation technique, the delivery device and polymeric material chosen.

Devices for Delivery

Metered-dose inhaler (MDIs), nebulilizer and dry powder inhaler (DPIs) are mainly used devices for pulmonary drug delivery system. The choice of device will depend on the drug, the formulation, the site of action, and the pathophysiology of the lungs. For example, liposomes do not form in conventional MDIs and would therefore be better suited for nebulization or drying to form a DPI. Potential limitation of nebulization is the stability of proteins and peptides because many biopharmaceuticals are unstable in aqueous solutions. MDI is not choice of the delivery method for proteins/peptides because when they come into contact with the propellants or with the large air-liquid interface which create susceptibility to penetration[96]. The unit-dose dry powder inhalers are most suitable for stability reasons of protein and peptide delivery to the lungs. The newer devices are developed to overcome this drawback which include AERx (Aradigm, Hayward, CA), Respimat (Boehringer, Germany) and AeroDose (Aerogen Inc, Mountain View, CA) that have been used successfully to deliver proteins to the lungs[97-100]. An important factor in the formulation design is selection of device for delivery of proteins to the lungs.

4. Transdermal delivery

General consideration

Transdermal delivery is more successful for several decades. It becomes frontline of research with a focus on the development of the systemic administration of peptide and protein therapeutics. Skin contains aminopeptidases, which exhibit less enzymatic activity

then other protiolytic enzymes present in GIT so, bioavailability of the peptide drug delivered is increase than oral administration but they cannot permeate the skin's outer stratum corneum layer to achieve significant therapeutic effect, although mechanical abrasion and chemical enhancers increase drug permeation[101, 102]. Transdermal systems are well accepted for hormone replacement therapy, smoking cessation, and pain management.

Approaches

- **Iontophoresis**

It is a novel noninvasive method specially investigated for local and systemic delivery of proteins and peptides through the transdermal delivery. In this technique, the membrane transport facilitates of charged molecules, depending on their ionic characteristics. To undergo iontophoresis, the peptide/protein molecules must carry a charge and this can be achieved by controlling the pH and ionic strength of solution. The heat generated during the process of iontophoresis can also cause denaturation.

Table 5: Transdermal delivery of proteins and peptides by iontophoresis

Drug	Result	Ref
Insulin	Improved delivery	103
TRH	Rate of permeation increased 2-10 fold	104
Salmon calcitonin (sCT)	In vivo study in rats. Induced hypoglycemic effect	105
Delta sleep inducing peptide (DSIP)	Degraded by skin enzyme, o-phenanthroline (enzyme inhibitor) enhanced iontophoretic delivery of DSIP	106

- **Phonophoresis**

It is another strategy used to enhanced transdermal delivery. In this method, ultra sound is applied via a coupling-contect agent to the skin. The drug absorption is enhanced via thermal effect of ultrasonic waves and subsequent temporary alterations in physical structure of skin. It may be presumably due to fluidization of biomembrane above its Tc. Thus protein/peptide molecules may become permeable to the biomembrane resulting into their improving absorption. But the limitation is denaturation of the protein/peptide bioactives at elevated temperature or mechanical disruption of its structure.

Table 6: Transdermal delivery of proteins and peptides by phonophoresis

Drug	Result	Ref
Insulin		
INF-γ	Low frequency ultra sound used. Improvement in transdermal delivery was found.	107
Erythropoietin		

- **Penetration enhancer**

Use of penetration enhancer holds promise in topical delivery of protein and peptide also. Penetration enhancers like oleic acid, dimethyl sulfoxide, surfactants and azone have been used but considerable skin irritation has been reported with penetration enhancer thus it is likely to limit its utility.

➢ **Transferosomes™**

It is the carrier aggregates of phosphatidylcholine, which in aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle. By addition of at least one bilayer softening component such as a biocompatible surfactant or an amphiphile drug, lipid bilayer flexibility and permeability are greatly increased. The transport through transferosome is very efficient (>> 50%) and reproducible for both lipophilic and hydrophilic agent.[108] Insulin has been delivered successfully using this delivery system.

➢ **Macroflux® technology**

It is a transdermal patch technology, which has been developed to deliver therapeutic peptides, proteins and other macromolecules in a controlled, reproducible manner that optimizes the bioavailability and efficacy without significant discomfort to the patient. Dose delivery is controlled by the patch size and drug loading on the microprojections. The system is minimally invasive and well tolerated. Drug-coated Macroflux® microprojections penetrate the skin and deliver the drug into the epidermal layer for rapid dissolution and absorption that yield high drug utilization and bioavailability. Macroflux® transdermal technology provided controlled and sustained delivery of an antisense oligodeoxy- nucleotide (7 kDa) achieving a delivery of 15mg, over a 24-hour period, from a 2cm ^{2} patch[109, 110].

5. Buccal delivery

General consideration

It has been practiced for many years as evidenced by development of pharmaceutical dosage form. Because of anatomic and physiological differences among various oral mucosal tissues, the location for buccal delivery should be carefully selected. The buccal peptide absorption is assumed to be via a passive absorption mechanism[111]. Various parameters that influence the extent of buccal peptide absorption are molecular weight, polarity, conformation, dissociation and enzymatic & chemical stability.

Approaches

- **Formulation composition**

The systemic delivery of insulin through the buccal mucosa is significantly affected by the formulation composition used. Insulin could not be effectively absorbed when using a simple disk shaped dosage form

prepared by the direct compression of insulin in a mixture of hydroxypropylcellulose (HPC) and carbopol 934 (CM). buccal absorption was achieved by using a dome shaped two phase mucosal adhesive device prepared by dispersing insulin crystals with sodium glycocholate, an absorption promoter[112].

- **Self-adhesive buccal patch**

It is feasible to deliver peptide based pharmaceuticals such as buserelin (synthetic LHRH), oxytocin, vasopressin analogs (DDAVP and DVDAVP) and insulin[113]. Protirelin is also readily absorbed through the buccal mucosa with hydroxyethylcellulose (HEC) patches but its pharmacodynamic efficiency through the buccal route is about 200 times less than that of *i.v.* protirelin[114].

6. Ocular delivery

Ocular route may also be utilized for systemic delivery of protein and peptide based pharmaceuticals. Drugs instilled in to the precorneal cavity can reach the systemic circulation via blood vessels underlying the conjunctival mucosa or via overflow of drug solution in to the nasolacrimal drainage system followed by absorption through the nasal mucosa. Some physiological and toxicological factors like tear dilution, lachrymal drainage and protein binding affect the ocular absorption of peptides and protein.

When insulin and glucagon delivered as eye drop formulation to anesthized rabbit, pharmacodynamic responses was found[115, 116]. The systemic bioavailability of insulin was improved to 4-13% with the incorporation of absorption promotors, such as bile salts (the sodium salt of glycocholic, taurocholic and deoxycholic acid) and nonionic surfactant (polyxyethylene 9-lauryl ether). In other studies, systemic delivery of topical applied [D-Ala^2]-met-enkephalinamide, in fully awake rabbits and reported the attainment of peak plasma concentration within 15-20 minutes following a solution instillation, with a bioavailability of around 36%[117, 118].

7. Rectal delivery

The use of rectum for the systemic delivery of peptide based pharmaceuticals is relatively recent idea. In the rectum, the upper venous drainage system is connected to the portal system, whereas the lower venous drainage system is connected directly to the systemic circulation by the iliac veins and the vena cava. In contrast to the oral delivery, the rectal route may provide in addition to the possibility of bypassing hepatic first pass elimination, the advantage of reduced proteolytic degradation for peptide and proteins and thus improve systemic bioavailability, especially when they are coadministered with absorption promoting adjuvants. It is beneficial to the chronic administration of peptide and protein pharmaceuticals for systemic medication.

The rectal absorption of insulin from a microenema was reported to be significantly promoted when co-administered with sodium 5-methoxy salicylate (SMS). The addition of 4% gelatin was observed to have a synergistic effect on the enhancement of rectal absorption of insulin by SMS[119, 120]. On the other hand, in addition of the lecithin to the suppository base was observed to prolong the hypoglycemic effect of insulin because of the slowed release of sodium salicylate[121].

Table 7: Rectal delivery of protein and peptide based pharmaceuticals

Drug	Testing model	Formulation	Bioavailability (%) OR response	Ref
Insulin	Human	Rectal enema with Sodium cholate	50% reduction in BGL within 30 min.	122
Pentagastrin	Rats	Rectal enema with SMS	23-33 %	123
Gastrin	Rats	Rectal enema	11-25 %	123
Calcitonin	Rats	Rectal enema with polyacrylic acid gel base	2-3 %	124

8. Vaginal delivery

For long term systemic medication, vaginal route having some advantages like- feasibility of self administration, possibility of prolong retention, minimization of proteolytic degradation. These are particularly beneficial to chronic administration of protein and peptide pharmaceuticals but vaginal permeability is strongly influenced by variation in the serum estrogen level. Vaginal route is useful for administration of LHRH & its synthetic analogs, especially when a low but long lasting release of gonadotropins is required[125].

SUMMARY

Protein and peptide based pharmaceuticals are rapidly becoming a very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in a very near future. Systemic delivery of protein and peptides is extremely challenging. Different drug delivery systems, technologies, still have to be developed for enhancing exposure of protein therapeutics. The new and relevant approaches are also developed to make protein and peptide pharmaceuticals commercially viable and therapeutically useful.

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