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Protein and peptide based drug delivery: pharmaceutical approaches

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Article Info :	Abstract
<p>Article History Received on: 17-02-2021 Revised on : 10-03-2021 Accepted on : 15-03-2021</p> <p>Keywords: Proteins, Peptides, Approaches, oral delivery, formulation vehicles, absorption enhancers.</p>	<p>Protein and peptide are last three decades therapeutic peptides and proteins have risen in prominence as potential drug of future. Polymers of protein consist of amino acids covalently linked by peptide bonds. Peptides are small proteins composed of up to a couple of dozen amino acids proteins are rapidly degraded by digestive enzymes. Till recently, injections remain the foremost common means for administering these protein and peptide drugs. In the other routes that have been tried with varying degrees of success are the oral, buccal, intranasal, pulmonary, transdermal, ocular and rectal. In this review, the aim is to specialise in the varied routes and approaches for delivery of Peptide and protein drugs. The Continuous efforts are focussed for formulation of this therapeutics into safe and effective delivery systems. In this review briefly describes the possible methods for the delivery of protein and peptide drugs through various routes.</p>

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Introduction

Protein and peptides are increasingly recognized as potential candidates for the development of new therapeutics for variety of human ailments. The relatively specific mode of action, proteins and peptides can be administered at comparatively low doses for therapeutic effects. This potent therapeutics is indicated for several chronic conditions such as cancer, hepatitis, diabetes, rheumatoid arthritis and leukemia [1]. The advent of biotechnology and modern analytical tools has promoted discovery and largescale production of protein and peptide drugs. Peptide are short chain of amino acid residues with a defined sequence (e.g., Leuprolide). Protein are polypeptides which have naturally nature and have a defined sequence of amino acids and a three-dimensional structure (e.g., Insulin). Parental

administration is the major route for administration of therapeutic proteins and peptides [2]. Protein and Peptide are the largemolecular size, charge, hydrophilic nature and low stability also contribute to their poor bioavailability. Therefore, the continuous research for the development of formulation for the delivery of protein and peptide drugs [3]. Chemical and structural complexities of protein and peptide involved demand an effective delivery system in which the physicochemical and biologic properties, including molecular size, conformational stability, solubility, sensitivity of light, moisture, heat, biological half-life, immunogenicity, dose requirements, susceptibility to break down in both physical and biological environments, requirement for specialized mechanisms for transport across biological membranes are to be considered [4].

Peptide and protein structure

Protein and peptide are essential to have an idea about structure of protein and peptide in order to deal with various problems encountered while developing drug delivery system. The peptide chains in peptides and proteins are seldom linear and adapt a variety of specific folded three-dimensional patterns and conformations. Peptides and proteins are polymers of

amino acids connected via amide linkages referred to as peptide bonds.

- Primary structure: Number and specific sequence of amino acids
- Secondary structure: Arrangement of individual amino acids along the polypeptide backbone
- Tertiary structure: Three-dimensional arrangement of a single protein molecule
- Quaternary structure: Proteins that contain two or more polypeptide chains associated by non-covalent forces [5].

Barriers to peptides and protein delivery [6,7]

The successful delivery of peptide and protein-based pharmaceuticals is primarily determined by its ability to cross the various barriers presented to it in the biological milieu. Various barriers encountered are-

- Enzymatic Barriers
- Intestinal Epithelial Barriers
- Capillary Endothelial Barrier
- Blood Brain barrier (BBB)

Stability of proteins:

Proteins are only marginally stable under physiological conditions. Forces like hydrophobic, electrostatic interactions and hydrogen bonding act more as stabilizing factors. Protein degradation pathways such as chemical, physical and biological as shown in Table-1 presents a challenge to formulation scientists, for the development of stable pharmaceutical preparations. The measures to improve chemical and physical stability are summarized in Table 1. Biological stability of protein and peptide can be improved by co-administration of enzyme inhibitors or by altering 3D structural orientation.

Table 1: Stability of protein and peptide

S. No	Stability	Problem	Overcome /Prevention
1	Chemical Instability		
A	Deamidation	Spontaneous degradation and loss of amino acid sequence homogeneity Denaturation, increase the immunogenicity	Buffer composition, lowering of Ph
B	Oxidation	Air, residual peroxide content, or intense fluorescent	Lyophilization, Use of antioxidants, chelating agents,

		light, may lead to the decomposition of protein and peptide	protection from light.
C	Proteolysis	Exposure to Harsh conditions such as high pH, high temperature or proteolytic enzyme	Storing at cold and sterile conditions
D	Disulfide exchange	It alters its 3dimensional structures and results in its biological activity.	By thiol scavengers such as P-mercuribenzoate, N-ethylmaleimide, Copper ions.
E	Racemization	Racemization may form peptide bonds that are sensitive to proteolytic enzymes.	Addition of thiol scavengers such as P-mercuribenzoate, N-ethylmaleimide, Copper ions, may prevent susceptible Sulphur and disulphide.
F	β-elimination	β-elimination of cystine residue leads to destruction of disulphide bonds of protein, due to high temperature.	Blocking thiol group
2	Physical Instability		
A	Aggregation and precipitation	Ionic complexation, salting out, charge neutrality close to the isoelectric pH, and results in limiting solubility of the molecule. Increase in thermal motion of the molecule due	Use of nonionic surfactants to prevent aggregation and sugars to stabilize the proteins

		to agitation.	
B	Denatured protein	It may lead to the decrease in solubility, alteration in surface tension, loss of crystallizing ability, changes in constituent group reactivity and molecular profile, vulnerability to enzymatic degradation, loss of antigenicity and loss of specific biological activity.	Maintaining pH, ionic strength and temperature.
C	Adsorbed protein	If the peptide and protein drug entities are adsorbed at interface there may reduction in concentration of drug available to show its function	Use of Surfactants, smooth glass walls and reduces in excess agitation

Delivery of peptide and protein drugs

Different routes include -

1. Oral route
2. Buccal route
3. Nasal route
4. Transdermal route
5. Pulmonary route
6. Rectal route
7. Parenteral route

Oral route [8-10]

Oral route is that the hottest route of delivery from the patient's point of view. Most important advantages of this route are convenience, acceptability and high patient compliance. The main barriers to successful oral delivery of protein and peptides are almost like that of traditional drug candidates, but these are more pronounced within the case of peptide/protein moieties.

The main barriers to effective oral delivery are-

- Protein and Peptide have poor intrinsic permeability of across biological membranes.
- Protein and peptides are susceptibility to enzymatic attack by intestinal.
- Rapid post-absorptive clearance.
- Physical instability like aggregation and adsorption

Buccal route[11-14]

The buccal membrane has numerous elastic fibers within the dermis, which is another barrier to the diffusion of drugs across the buccal membrane.

The barriers to efficient drug absorption are:

- Mucus layer covering the oral epithelium.
- Epithelial barriers.
- The Peptides in the saliva and the mucus layer and microbial flora.

The buccal peptide absorption is assumed to be via a passive absorption mechanism. The various parameters that influence the extent of buccal peptide absorption are relative molecular mass, polarity, conformation, dissociation, and enzymatic and chemical stability. They do not allow drinking and the patient sometimes is even a handicap for speaking. Administration time is restricted with these formulations and thus controlled release can't be achieved. To overcome these drawbacks self-adhesive systems have been designed which are capable of being in intimate contact with the mucous, viz. Buccal, sublingual, or gingival. The different types of adhesive polymers include water-soluble and insoluble hydrocolloid polymers from both the ionic and the nonionic types. Some of the polymers are sodium carboxymethylcellulose, hydroxyl propyl methylcellulose, polyvinyl pyrrolidone, acacia, calcium carbophilic, gelatin, and polyethylene glycol.

The strategies employed for Buccal Delivery are

-

- Adhesive tablets
- Adhesive gels
- Adhesive patches
- Absorption promoters

Nasal route [15,16]

Generally, the intranasal route is suited for the intermittent delivery of highly potent peptide/protein drugs having low molecular weight. Peptide drug moieties like calcitonin, ACTH, insulin and interferon are reported to have appreciable absorption through nasal mucosa. Nasal route is chiefly use to delivery of protein drug. For achieving a systemic effect, the nasal route is the most efficient one after the parenteral route.

Types of dosage form

- Nasal spray
- Nasal drops
- Aerosol

Transdermal route[17]

Advantages of Transdermal Route for peptide/protein Delivery are:

- Better and improved patient compliance
- Elimination of hepatic first pass phenomenon
- In transdermal route in controlled administration is possible and thereby avoidance of toxic effects. Also drugs with shorter half-life can be administered.

Limitations of Transdermal Route for peptide/protein Delivery are:

- Low rate of permeation for most protein drugs in this route due to their large molecular weight and hydrophilicity and lipophilic nature of the stratum corneum
- High intra and inter patient variability

Various approaches for Transdermal delivery Route of peptide drugs are:

- Iontophoresis
- Phonophoresis
- Penetration enhancers
- Prodrugs

Pulmonary route[18-20]

Its particle that reaches the alveoli can be absorbed into the systemic circulation, avoiding first pass metabolism and the harsh conditions of the gut. This Particle characteristics such as aerodynamic diameter can be engineered to deliver particles to different areas of the lung. Aerodynamic diameter is derived from Stoke's Law and is defined by:

$$da = (\rho_p / \rho_0) 0.5 dg$$

Advantages of Pulmonary Route for peptide/protein Delivery are:

- Provides a direct route to the circulation.
- Reduction in dose requirement up to 50-fold and thus a cost-effective option.
- Fast absorption.
- Safe route for drug entry even in patients with lung diseases.
- No triggering of immune function.
- Increased patient compliance with a minimum of discomfort and pain

Rectal route[21-23]

Advantages of Rectal route are:

- It is very vascularized.
- It avoids to an outsized extent the primary pass or pre-systemic metabolism.

- It is suitable for drugs which will cause nausea/vomiting and irritate the Gi mucosa on oral administration.
- In case of adverse reaction or drug overdose, the drug absorption is often interrupted.
- A large dose of drug is often administered.
- Drug are often targeted to the systema lymphaticum.

Factors affecting absorption from the rectal route are:

- Amount of liquid present within the rectum.
- This route pH and buffer capacity of the rectal fluid.
- This route Surface tension and viscosity of the rectal fluid.
- Luminal pressure exerted by the rectal wall which reinforces rectal absorption.
- Solubility, partition coefficient, pKa of the drug.

Parenteral routez [24]

Parenteral mode of drug delivery has been the main route of choice for protein/peptide, due to their poor absorption and metabolic instability when given by other alternative routes. Potent nature of those moieties demands their targeting to specific receptors to enhance therapeutic index of a drug. If peptides are presented at high dosage levels, there stands the likelihood of generation of immune responses and other undesirable deleterious side effects and interactions. This drug Targeting protects both the drug and body from these contraindicative manifestations. Parenteral drug delivery system includes Intravenous, intramuscular, subcutaneous, intraperitoneal, intrathecal use.

Pharmaceutical approaches

Chemical Modification

Protein modification can be done either by direct modification of exposed side-chain amino acid groups of proteins or through the carbohydrate part of glycoproteins and glycol-enzymes[25]. Modifications of individual amino acids combined with the substitution of 1 more L-amino acid with D-amino acids can significantly alter physiological properties. While the natural vasopressin is orally active within the water-loaded rat at large doses, desmopressin is twice as active at the 75th fraction of the dose, which is attributed to enhanced membrane permeation and enzymatic stability. Since the liver may be a significant participant within the control of blood sugar, it's believed that successfully activating the liver with oral insulin may provide a mechanism to potentially reestablish normal glucose control in the diabetic patient and turn on a number of metabolic activities that can help mitigate complications of diabetes

[26]. Another example of hydro-phobization to extend lipophilicity of insulin is palmitoylation. Insulin was conjugated to 1,3-dipalmitoylglycerol at the free amino groups of glycine, phenylalanine, and lysine to form mono and dipalmitoyl insulin [27]. This facilitated the transfer of insulin across the mucosal membranes of the massive intestine and improved its stability against intestinal enzymatic degradation. To decrease binding to albumin, Brader et al. recently synthesized octanoyl-N-Lys β -29, co-crystallized with human insulin, and determined pharmacokinetic and insulin release profiles after injection in beagle dogs [28].

Enzyme Inhibitors

The choice of protease inhibitors will depend upon the structure of those therapeutic drugs, and therefore the information on the specificity of proteases is important to ensure the steadiness of the drugs within the GI tract [29]. The steadiness of insulin has been evaluated within the presence of excipients that inhibit these enzymes. Inhibitors of insulin degrading enzymes include 1,10-phenanthroline, p-chloromeribenzoate and bacitracin reported the utilization of a mixture of an enhancer, sodium cholate and a PI to realize a tenth increase in rat intestinal insulin absorption. They found a robust reduction of albumin degradation by a mix of proteases within the presence of Carbopol 934P. As a result of the covalent attachment of cysteine to polycarbophil, the inhibitory effect of the polymer towards carboxypeptidase A, carboxypeptidase B and chymotrypsin might be significantly improved. Another approach to enzyme inhibition is to control the pH to inactivate local digestive enzymes. A sufficient amount of a pH-lowering buffer that lowers local intestinal pH to values below 4.5 can deactivate trypsin, chymotrypsin and elastase [30].

Absorption Enhancers

In order for therapeutic agents to exert their pharmacological effects, they need to cross from the biological membranes into the circulation and reach the location of action. Absorption enhancers are the formulation components that temporarily disrupt the intestinal barrier to enhance the permeation of those drugs. Numerous classes of compounds with diverse chemical properties, including detergents, surfactants, bile salts, Ca²⁺ chelating agents, fatty acids, medium chain glycerides, acyl carnitine, alkenoylcholine's, N-acetylated α -amino acids, N-acetylated non- α -amino acids, chitosan's, mucoadhesive polymers, and phospholipids have been reported to enhance the intestinal absorption of large polypeptide drugs [31,32]. However, permeation enhancers often induce toxic side effects. Dodecylphosphocholine and quillaia saponin, dipotassium glycyrrhizinate, 18 β -glycyrrhetic acid, sodium caprate, and taurine also increases the permeability of hydrophilic compounds across Caco-2 cells [33-36]. Among the recent absorption enhancers displaying this principle and

exhibiting the safest and best promising leads to enhancing drug delivery is Zonula Occludes toxin. In vitro experiments in the rabbit ileum demonstrated that Zot reversibly increased intestinal absorption of insulin by 72% and immunoglobulin G by 52% in a time dependent manner they further observed an encouraging 10-fold increase in insulin absorption in both rabbit jejunum and ileum in vivo with ZOT [37]. Karyekar et al. has recently reported that Zot increases the permeability of relative molecular mass markers and chemotherapeutic agents across the bovine brain micro vessel endothelial cells during a reversible and concentration dependent manner and without affecting the transcellular pathway as indicated by the unaltered transport of propranolol in the presence of Zot [38]. Kotze et al. have evaluated the transport enhancing effects of two chitosan salts, chitosan hydrochloride and chitosan glutamate (1.5% w/v), and the partially Qatariized chitosan derivative, N-trimethyl chitosan chloride (TMC) (1.5 and 2.5% w/v), in vitro in Caco-2 cell monolayers [39-42].

Formulation Vehicle

A primary objective of oral delivery systems is to guard protein and peptide drugs from acid and luminal proteases within the GIT. To beat these barriers, several formulation strategies are being investigated. Here, we discuss the utilization of enteric-coated dry emulsions, microspheres, liposomes and nanoparticles for oral delivery of peptides and proteins. Drug absorption enhancement depends on the sort of emulsifying agent, particle size of the dispersed particles, pH, solubility of drug, sort of lipid phase used etc. the lipid phase of microemulsions consists of medium chain fatty acids triglycerides increasing the bioavailability of muramyl dipeptides analog. Torisaka et al. have recently prepared a replacement sort of oral dosage sort of insulin, S/O/W emulsions, during which a surfactant-insulin complex is dispersed into the oil phase [43]. To beat this drawback, it's formulated into dry emulsion. Dry emulsion formulations are typically prepared from O/W emulsions containing a soluble or an insoluble solid carrier within the aqueous phase by spray drying, lyophilization or evaporation. Dry emulsions are considered lipid-based powder formations from which an O/W emulsion are often reconstituted. the discharge behavior of encapsulated insulin was found to be aware of external pH and therefore the presence of lipase under the simulated GI conditions. Supported the results obtained during this study and therefore the incontrovertible fact that any water-soluble drug is often complexed with surfactants, the new solid emulsion formulations might be extensively applicable to oral delivery of pharmaceutical peptides and proteins [44-48]. The influence of pH variability through the stomach to the intestine on the oral bioavailability of peptide and protein drugs could also be overcome by protecting them from proteolytic degradation within the stomach and upper portion of the tiny intestine using pH-responsive microspheres as oral delivery vehicles. It's

stated that particles within the nanosized range are absorbed intact by the intestinal epithelium, especially, through Peyer's patches and visit sites like the liver, the spleen and other tissues [49]. The proteins and peptides encapsulated within the nanoparticles are less sensitive to enzyme degradation through their association with polymers. The factors affecting uptake include the particle size of particulate, the surface charge of the particles, the influence of surface ligands and therefore the dynamic nature of particle interaction within the gut. There are several reports on the intact liposomal uptake by cells in *in vitro* and *in place* experiments [50-51]. Attempts are made to enhance the steadiness of liposomes either by incorporating polymers at the liposome surface, or by using GI-resistant lipids. These results demonstrated that surface coating of liposomes with PEG or mucin gained resistance against digestion by bile salts and increased the steadiness within the alimentary canal. Consequently, the surface coating should be the potential way to add desirable functions to the liposome for oral drug delivery [52].

Mucoadhesive polymeric systems

Mucoadhesive polymeric systems are the foremost promising approach among several approaches. Mucoadhesive properties can provide an intimate contact with the mucosa at the location of drug uptake preventing a pre-systemic metabolism of peptides on the thanks to the absorption membrane within the alimentary canal. Additionally, the duration of the delivery system at the location of drug absorption is increased. Mucoadhesive polymers are ready to adhere to the mucin layer on the mucosal epithelium and thus leads to the rise of oral drug bioavailability of protein and peptide drugs. Most of the present synthetic bio-adhesive polymers are either polyacrylic acid or cellulose derivatives. Samples of polyacrylic acid-based polymers are Carbopol, polycarbophil, polyacrylic acid, polyacrylate, poly(methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly(methacrylate), poly(alkyl cyanoacrylate), poly(isohexylcyanoacrylate) and poly(isobutyl cyanoacrylate). A new gastrointestinal mucoadhesive patch system (GI-MAPS) has been designed for the oral delivery of protein drugs [53]. The system consists of 4 layered films contained in an enteric capsule. The surface layer was attached to the center layer by an adhesive layer made from carboxyvinyl polymer. After oral administration, the surface layer dissolves at the targeted intestinal site and adheres to the tiny intestinal wall, where a closed space is made on the target site of the gastrointestinal mucosa by adhering to the mucosal membrane. However, recent advances in microfabrication technology within the semiconductor industry have made it possible to supply many micron-size GI-MAPS. Carbopol

polymers are shown to inhibit luminal degradation of insulin, calcitonin, and insulin-like growth factor-I (IGF-I) by trypsin and chymotrypsin. In contrast, cationic polymers adhere to the charged mucus mainly thanks to electrostatic forces [54-56]. As both anionic and cationic mucoadhesive polymers exhibit a high buffer capacity, a demanded microclimate regarding the pH is often adjusted and maintained over numerous hours within the polymeric network. On the contrary, the strong mucoadhesive properties of thiomers are believed to be supported additional covalent bonds between thiol groups of the thiomers and cysteine-rich subdomains of mucus glycoproteins [57]. Hussain et al. have showed that surface conjugation of the bio-adhesive molecule-tomato lectin increases the uptake of orally administered inert nanoparticles in rats. Improved intestinal absorption of 9-desglycinamide, 8-arginine vasopressin (DGAVP) was observed in rats *in vitro* also as *in vivo* using the weakly cross-linked poly(acrylate) derivative polycarbophil dispersed in physiological saline (Haas and Lehr). The authors suggested that chitosan-EDTA conjugates protect peptide and protein drugs from enzymatic degradation across the alimentary canal [58-60].

Conclusion

In this conclusion, delivering proteins and peptides by the oral route is extremely challenging. The nature of the digestive system is designed to breakdown these polypeptides into amino acids prior to absorption. Low bioavailability of drugs remains to be an active area of research. GIT site has been investigated by researchers, but no major breakthrough with broad applicability to diverse proteins and peptides has been achieved. Progress has been made over the past few years in developing innovative technologies for promoting absorption across GI and numbers of these approaches are demonstrating potential in clinical studies. The chemical modification and use of the mucoadhesive polymeric system for site-specific drug delivery seen to be promising candidates for protein and peptide drug delivery.

Author Contribution

All authors are contributed equally.

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