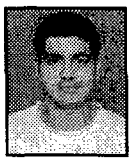
Enhancing the Oral Delivery of Proteins and Peptide Drugs



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Abstract

Many peptides and proteins possess biological activity that makes them potent therapeutics – particularly anticancer agents and hormones. Oral delivery of peptide drugs is preferred over other routes of administration due to better patient compliance. However, the use of many polypeptides as therapeutic agents is hampered by their rapid elimination from the circulation because of enzymatic degradation in the gastrointestinal tract (GIT), renal filtration, uptake by the reticuloendothelial system (RES) and accumulation in non-targeted organs and tissues. This therefore requires large doses of polypeptide administration, which frequently results in non-specific toxicity and increased cost of the treatment. This review focuses on the challenges posed by the GI system and how different pharmaceutical approaches can be used to make oral delivery of protein and peptide drugs more feasible. It also covers paracellular transport of the drug following oral delivery. The properties of the colon such as high pH conditions, presence of microbial flora etc are being exploited to use the colon as a specific site for delivery of peptide drugs. The review throws light not only on this area but also encompasses current approaches adopted by pharmaceutical companies to produce orally administered peptide drugs.

Keywords: Peptides, Gastrointestinal tract, Paracellular transport, Colon-specific drug delivery.

1. Introduction

1.1. Obstacles encountered in oral delivery of peptide drugs

Structural features of protein and polypeptide drugs, together with the natural anatomical and physiological features of the gastrointestinal (GI) tract, have made oral delivery of this class of compounds extremely challenging. The relatively large size and hydrophilic nature of peptides severely limit the absorption of these molecules in the gastrointestinal tract (GIT). Peptides are susceptible to degradation in the strongly acidic gastric environment. Pepsin in the stomach can break down peptide bonds and inactivate the peptide. They are also susceptible to cleavage by intestinal proteases that are secreted from pancreas or localized on the membranes of microvilli of intestinal epithelial cells. Another impediment to oral absorption is the mucus layer in the GIT that binds the charged molecules such as peptides and prevents their absorption through the lumen of the intestine. Hence the overall bioavailability of orally delivered peptides of molecular size greater than two or three amino acids is extremely poor. The use of many polypeptides as therapeutic agents may also be hampered by their rapid elimination from circulation because of renal filtration, uptake by the Reticuloendothelial System (RES) and accumulation in non-targeted organs and tissues in the body. This necessitates larger quantity of the Active Pharmaceutical Ingredient (API) to be administered because of such inherently low bioavailability of orally delivered peptides.

1.2 Advantages of oral route

The conventional route of therapy involving protein or peptide drugs is via parenteral administration (i.e., by injection). This is primarily due to the lack of absorption of such drugs through the gastrointestinal tract when administered orally. However, injections are painful and sometimes difficult to administer relative to other dosage forms. Further, patient compliance is problematic where these drugs may require frequent administration, especially to juvenile or geriatric patients. Accordingly, oral delivery is preferable to injections for patient acceptance since it is less painful and more convenient. The oral delivery of therapeutics for the treatment of disease and pathological conditions is preferred since the approach is associated with high patient compliance and comfort, self-administration, reduction in administration costs and the facilitation of the use of chronic treatment regimens.

2. Strategies to design orally active peptide formulations

Attempts to improve the oral bioavailability of peptide drugs have ranged from changing the physicochemical properties of peptide molecules to the inclusion of functional excipients in specially adapted drug delivery systems. A few approaches have been discussed below.

2.1 Conversion of active peptide to prodrug

Initial methods to address problems associated with peptide and protein drugs have included altering amino acid sequences and

chemistries to reduce degradation by enzymes and antigenic side effects. Alternatively, the activity of proteolytic enzymes that degrade the peptide drugs can be modulated. One of the strategies includes the use of protease inhibitors which interact with digestive enzymes to reduce inactivation of the orally delivered drug. They include sodium glycocholate, camostat mesilate, bacitracin.

The conversion of active peptide to pro-drug by covalent linkage of an inert group is another option available to improve the degree of protein absorption. Such modifications aid the release of the biologically active moiety upon hydrolysis in vivo. The modification of terminal carboxy and/or amino group of the peptide drug for the protection of the active part against enzymatic degradation, introduction of lipophilic group for passive trans-membrane transport and the attachment of active peptide transporter subsrate for cytoplasmic delivery using intracellular transport mechanism, have been explored to a significant success.

2.2 Increasing the half-life of the peptide in circulation

The half-life in circulation needs to be increased for those peptide drugs requiring sustained presence for therapeutic efficacy. Hence the conjugation of peptides with water-soluble polymers is carried out so as to slow down renal filtration thereby increasing their residence time in circulation.

2.2.1 PEGylation

One of the most commonly employed processes is polyethylene glycol (PEG)-ylation technology which enlarges the size of an active molecule by attaching a web-like shield of hydrated PEG polymer chains around the molecule. Polymer molecules attached to the protein globule creates steric hindrance, hence protecting it and increasing its half life for improved bioavailability.

2.2.1.1 Advantages of PEGylation

The advantages of advanced PEGylation for therapeutic molecules include enhanced bioavailability, prolonged duration of action, optimized pharmacokinetics, increased efficacy, decreased dosing frequency, improved safety profile, and improved drug solubility and stability. PEG is non-toxic and has been approved by the FDA for use in foods, cosmetics, and pharmaceuticals. Advanced PEGylation can also be used to create prodrugs, where active drugs are released by degradation of more complex molecules (prodrugs) under physiological conditions, providing a powerful method of drug delivery.

2.2.1.2 Successful commercial PEGylated drugs

PEGylation is now frequently considered as the method of choice for modifying protein therapeutics to achieve greater bioavailability, sustained duration, less toxicity, and in many cases, enhanced efficacy. For example, two approved PEGylated interferon alfa products are now available for treatment of hepatitis C: PEG interferon alfa-2b (PEG-INTRON® – Schering-Plough) and PEG interferon alfa-2a (PEGASYS® –Roche). PEGylated interferons typically only need be administered weekly (as opposed to three times per week for non-PEGylated) and maintain more constant levels of interferon in the blood. In fact, the superior performance of these products has led the National Institutes of Health to declare PEG-interferon to be the standard of treatment for hepatitis C. Another highly successful PEG drug in the marketplace is Neulasta® (pegfilgrastim) from Amgen, which is the second generation of Amgen's highly successful Neupogen® product. Neulasta has a longer biological half-life and increased bioavailability over Neupogen, allowing for significantly reduced dosing frequency to once per chemotherapy cycle.

2.2.2 Conjugation with SMA

In addition, certain polymers that are not large enough to prevent renal clearance, but attach themselves, together with the conjugated drug, to long-circulating blood plasma components have been used to prepare derivatives to extend circulation longevity. For example, conjugation with poly(styrene-co-maleic acid anhydride(SMA)) with a molecular weight as low as 1500 Daltons increases the circulation time of anti-cancer polypeptides via binding to plasma albumin. Also Neocarzinostatin-SMA conjugate is currently approved in Japan for the treatment of hepatoma.

2.3 Stabilizing the peptide by conjugation to a carrier molecule

As an alternative to the complexation approach microparticulate carriers such as liposomes and micelles are recommended to be used. These carriers allow higher drug load and provide greater protection against enzymatic degradation.

2.3.1 Liposomes

Liposomes are being currently researched extensively since they are biologically inert, biocompatible and have very little toxic and/or antigenic effect. Surface modification of the liposomes with hydrophilic polymers such as PEG is also another approach being evaluated. These conjugates will not be affected by RES.

For example, insulin-loaded polymer microparticles (ILP) composed of cross-linked poly(methacrylic acid) and poly(ethylene glycol) are multi-functional carriers showing high insulin incorporation efficiency, a rapid insulin release in the intestine based on their pH-dependent complexation properties, enzyme-inhibiting effects and mucoadhesive characteristics. Thus, they are potential carriers for insulin delivery via an oral route.Also, biodegradable polymers such as FDA-approved poly(lactic acid) (PLA) and poly(lactide-co-glycolide acid) (PGLA) may be potentially useful as materials for oral delivery vehicles. Such microparticles can be easily prepared with high protein encapsulation efficiencies (>50%) as well as with the capability to effectively protect the encapsulated proteins from degradation in the GIT.

2.3.2 Hydrogels

Complexation of the drug with hydrogels is also being explored. New biodegradable hydrogels containing both acidic co-monomers and enzymatically degradable azoaromatic cross-links are suitable for site-specific delivery of peptides into the colon. In the low pH range of the stomach, the gels have low equilibrium degree of swelling and the drug is protected against digestion by enzymes. The degree of swelling increases as the hydrogel passes down the GIT due to increasing pH. In colon, the hydrogels have reached a degree of

swelling that makes the cross-links accessible to azoreductases. The rate of degradation depends on the structure of the hydrogel.

2.4 Intracellular delivery of peptide drugs using vectors

Most macromolecular peptides exert their therapeutic action extracellularly by surface receptor interactions, however, some other have their targets within the cell. The low permeability of cytoplasmic membranes to macromolecules presents an additional obstacle for the development and delivery of peptide-based systems. Here an active transport mechanism is hence needed. Specific vector molecules promote the intracellular delivery of associated drug carriers via receptor-mediated endocytosis. This process involves the attachment of vector molecules with associated drug carriers to specific ligands on target cell membranes followed by the formation of endosomes. Although the delivery of intact proteins is compromised by their insufficient endosomal escape followed by lysosomal degradation, enhanced endosomal escape can be achieved through of lytic peptides, pH-sensitive polymers or swellable dendritic polymers. These agents have provided encouraging results in overcoming the limitations of endocytosis-based cytoplasmic peptide delivery, but there is still an exciting opportunity for further research.

3. Paracellular Transport

Paracellular transport refers to the transfer of substances between cells of an epithelium. Paracellular transport of therapeutic proteins and polypeptides following oral administration is being studied with increased intensity today.

3.1 Penetration Enhancers

Paracellular transport of peptide drugs largely involves the use of penetration enhancers (or absorption enhancers) which interact with the cell membranes. Common enhancers are chitosan, bile salts, anionic detergents, non-ionic detergents, medium chain glycerides, salicylates, acyl amino acids, acylcarnitines, lysolecithin, ethylenediaminetetraacetic acid and particulate carriers.

3.1.1 Chitosan

Chitosan is a naturally occurring, non-toxic, biocompatible and biodegradable polysaccharide. Chitosan has been shown to be a potential penetration enhancer. In particular, chitosan showed penetration enhancement properties towards either monostratified or pluristratified epithelia. In particular, it is able to enhance drug absorption of hydrophilic high molecular weight molecules through intestinal mucosae (monostratified and endowed with tight junctions). Tight junctions, or zonula occludens, are the closely associated areas of two cells whose membranes join together forming a virtual impermeable barrier to fluid. In those epithelia that are rich in tight junctions, the mechanism of penetration enhancement by chitosan is mainly due to a transient widening of the junctions between cells while the mechanism of penetration enhancement across pluristratified epithelia, which lack tight junctions, has still to be clarified . The effectiveness of chitosan as penetration enhancer is impaired by its insolubility at pH above 6.An attempt has been made to solve the chitosan solubility problems by synthesizing the partially quaternized derivative *N*-trimethyl chitosan chloride (TMC), which is soluble irrespective of pH and has proved a potent penetration enhancer of hydrophilic and/or high molecular weight drug molecules across the intestinal epithelium. Chitosan derivatives, such as *N*-trimethyl chitosan chloride, increase the paracellular transport by the following mechanism: The interaction between the positive charges of cationic chitosan and anionic glycoproteins on the surface of the epithelial cells can displace specific cations necessary for the coordinated closing of tight junctions (Kotze et al., 1999). It has also been shown that chitosan derivatives can redistribute actin filaments and reorganize the protein structure of tight junctions, thus enhancing the paracellular transport of various paracellular marker molecules (Dodane et al., 1999).

3.1.2 Zonula Occluden Toxin (ZOT)

Among these recently introduced penetration enhancers, zonula occluden toxin (ZOT) has proven to be the most attractive (Fasano and Uzzau, 1997; Fasano, 1998b; Salama et al., 2003). ZOT is neither cytotoxic nor damaging to intestinal epithelial cells ex vivo (Fasano, 1998b). It increases the paracellular permeability of a variety of compounds by reversibly expanding tight junctions by interacting with specific receptors located primarily within the small intestine.

3.1.3 Thiolated polymers

Thiolated polymers, or thiomers, are another recently introduced category of permeation enhancers that may potentially increase the paracellular transport of a variety of drug compounds. Recently, a new enhancer consisting of a thiolated polymer [poly(acrylic acid)-cysteine, chitosan-4-thio-butylamidine] and reduced glutathione (GSH) has been shown to increase the paracellular transport of insulin, and heparin across rat intestinal epithelium in vivo.

3.2 Methods to Increase Paracellular Drug Permeation

3.2.1 Physicochemical modification of the permeant

This approach takes advantage of chemical and/or physical modifications of the drug molecule. The partial or total substitution of the l-isomer form of amino acids with the d-isomer form has been used to overcome enzymatic degradation by gastrointestinal luminal enzymes. Several peptides synthesized with the d-isomer have been shown to be potent substitutes for naturally occurring peptide hormones or antibiotic of clinical interest.

3.2.2 Modulating the tight junctions associated with the paracellular pathway

An alternative to physicochemical modification of the permeant is manipulation of the structure of tight junctions to expand the pore diameter and increase the amount of the candidate macromolecule that undergoes paracellular absorption. Compounds that modulate tight junctions, such as calcium chelating agents, surfactants, medium-chain fatty acids, bile salts, phosphate esters, and some cationic polymers, have been extensively described in the literature. These compounds act through three major mechanisms to modulate the paracellular pathway; namely, their effect(s) on the (1) mucous layer, (2) membrane components, and/or (3) tight junctions.

The strategy of reversibly expanding tight junctions to increase the

paracellular transport of drug molecules is not without safety concerns because the expansion of tight junctions introduces the potential for absorption of unwanted toxins/bacteria/immunogens into the systemic circulation. Thus, the importance of modifying the physicochemical properties of the permeant may represent a more practical and safer strategy for increasing the paracellular absorption of therapeutic proteins.

4. Colon as a Site of Peptide Drug Delivery

The colon has always attracted attention as a potential site for systemic absorption of peptide drugs on account of its lower proteolytic activity compared to the upper GIT. Colon-specific drug delivery has gained increased importance not only for the delivery of drugs for the treatment of local diseases associated with the colon such as Crohn's disease, ulcerative colitis, irritable bowel syndrome etc. but also a potential site for the systemic delivery of therapeutic peptides and proteins.

Due to the distal location of the colon in the GIT, a colon-specific drug delivery system should prevent drug release in the stomach and small intestine and effect an abrupt onset of drug release upon entry into the colon. Such a system can be formulated by utilizing some specific conditions existing in the colon in comparison to other parts of the GIT. Overall, the physiological changes along the GIT can be generally characterized as a continuum, with decrease in enzymatic activity, motility and fluid content and an increase in pH as we move from esophagus to rectum. Another challenge in developing therapeutically effective products for the treatment of colonic pathologies is the impact of diseased condition on the delivery system. Hence, prior to development of any system, due consideration must be given to the physiological changes that occur in diseased state that might affect the performance of such a system.

Colon delivery system can be improved in several ways. The use of timed-release system, pH-dependent coating, microbially degradable polymers, intestinal pressure-controlled system and redox-sensitive polymer are common.

Recently much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability; reduced risk of systemic toxicity and local irritation; predictable gastric emptying; no danger of alteration in drug release profile and formulation behavior due to unit to unit variation, change in gastroluminal pH and enzyme population; more uniform drug dispersion and absorption; less inter and intra subject variability because of smaller particle size. Most commonly used multiparticulate systems for colon-specific drug delivery include pellets, granular matrices, beads, microspheres and nanoparticles. Presence of specific bacterial populations in the colon and an increasing pH gradient have been extensively explored as triggering mechanisms to initiate colon-specific drug release.

4.1 pH dependent systems

The pH in the terminal ileum and colon (except ascending colon) is higher than in any other region of the GIT. Thus a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region. Formulation of enteric coated granules is one of the simplest approaches based on this principle. Enteric coating has traditionally been used to prevent drug release in the upper GIT. Most commonly used pH-dependent coating polymers for oral delivery are methacrylic acid copolymers, Eudragit L100 and Eudragit S100 which dissolve at pH 6.0 and 7.0 respectively. The combination of these two polymers in various ratios makes it possible to manipulate drug release within 6.0 to 7.0 pH range. In vitro release studies of indomethacin pellets coated with a particular ratio of Eudragit L100 and Eudragit S100 showed that the pellets released no drug within the pH range 1.2 (stomach) to 6.5 (proximal small intestine); release was slow at pH 6.8 (distal small intestine); and release was rapid at pH 7.2 (terminal ileum).

4.2 Microbially controlled systems

This approach is most appealing as it relies on the unique enzymatic ability of the colonic microflora which causes biodegradation of the drug carrier and enables a more specific targeting irrespective of pH variations along the GIT. Many natural polysaccharides such as chondroitin sulfate, pectin, dextran, guar gum etc have been investigated for their potential in designing colon-specific drug delivery systems. For example, a multiparticulate system consisting of chitosan microspheres coated with Eudragit L100 or Eudragit S100 was used for the colonic delivery of metronidazole for the treatment of amoebiasis. In order to prevent early loss of drug from microspheres, the chitosan was cross-linked with glutaraldehyde. It was shown that this multiparticulate system prevented release of the drug until it entered the colon. Cross-linking of chitosan with glutaraldehyde reduces the solubility of chitosan, thereby resulting in less protein release during upper GI transit. At the same time, the cross-linking and reduced solubility does not affect the susceptibility of chitosan matrix to colonic enzyme digestion.

4.3 Nanoparticulate systems

Nanoparticle size colloidal carriers composed of natural or synthetic polymers have also been investigated for colon targeting for the delivery of protein and peptide drugs. For colonic pathologies, nanoparticles tend to accumulate at the site of inflammation because a strong cellular immune response occurs in inflamed regions due to increased presence of neutrophils, natural killer cells, macrophages which efficiently take up nanoparticles. This results in prolonged residence time in the desired area. For covalent attachment, the nanoparticle surface has to show free functional groups like carboxylic or amine residues. Nanoparticles have large specific surface area which is indicative of high interactive potential with biological surfaces.

Drug	Trade Name	Formulation	Dose
Mesalamine	Asacol	Eudragit S tablets (dissolves at pH 7.0)	0.8 – 2.4 g/day
Mesalamine	Salofac	Eudragit L tablets (dissolves at pH 6.0)	1.0 – 4.0 g/day
Mesalamine	Mesazal	Eudragit L coated tablets	1.0 – 2.0 g/day
Budesonide	Entocort	Eudragit L coated beads	9 mg/day

Table 1. Marketed drug products for the treatment of inflammatory bowel disease.

5. Approaches of Pharmaceutical Companies

5.1 Emisphere Technologies Ltd.

Emisphere's broad-based oral drug delivery technology platform,

Technique employed	Polymer(s) used	Drug used
pH dependent	Eudragit L100 and Eudragit S100	Mesalazine
	Eudragit L100 and Eudragit S100	Diclofenac sodium
	Eudragit S, Eudragit FS, Eudragit P4135 F	Prednisolone
	Eudragit L 30 D-55 and Eudragit FS 30 D	Paracetamol
Time dependent	Hydroxypropyl methyl cellulose acetate succinate	Diltiazem HCl
	Lactose/ Behinic acid	Indomethacin
	Hydroxyethyl cellulose, ethyl cellulose, microcrystalline cellulose	Theophylline
Colonic bacteria dependent/ Polysaccharide based	Chitosan	Diclofenac sodium
	Pectin	Indomethacin
	Guar gum	Dexamethasone
	Amylose	5-Acetyl salicylic acid
	Alginates	5-Acetyl salicylic acid

Table 2. Examples of colon targeted formulations based on conventional techniques:

known as the eligen® technology, is based on the use of proprietary, synthetic chemical compounds, known as EMISPHERE® delivery agents, or "carriers." These delivery agents facilitate or enable the transport of therapeutic protein macromolecules across biological membranes such as those of the gastrointestinal tract, allowing the therapeutic molecules to exert their desired pharmacological effect. The delivery agents have no known pharmacological activity themselves at the intended clinical dose levels. Emisphere's eligen® technology makes it possible to orally deliver a therapeutic protein molecule without altering its chemical form or biological integrity.

5.1.1. Delivery Agent Mechanism

Drug molecules exist in many different shapes, or "conformations". Some conformations can be transported across the cell membranes while others are too large or too charged to do so. The eligen® technology uses the body's natural passive transcellular transport process to enable large or highly charged molecules to cross cell membranes. Once the drug molecule crosses the membrane, the EMISPHERE® delivery agent dissociates from the drug molecule, which then reestablishes its natural conformation and returns to its therapeutically active state. Studies have shown that this process does not involve chemical modification of the drug molecule and the integrity of cell membrane and cytoskeletal structure are maintained.

Emisphere's eligen® technology overcomes several major obstacles to effective oral delivery including degradation of the drug in the GIT and poor absorption of the drug across biological membranes. The eligen® technology stabilizes the drug molecule in a conformation that reduces degradation. Also, EMISPHERE® delivery agents interact with the drug molecule to create an entity with significant absorption properties. The rapid rate of transit through the biological membrane also limits degradation from digestive enzymes.

6. Conclusion

The biopharmaceutical industry is increasingly looking for new ways to create higher value pipelines through highly differentiated therapeutics, to lower development risk for lead candidates entering the clinic, and to reduce product development time and cost. Innovative drug delivery technologies are emerging as a key component of the pharmaceutical development process by allowing companies to create superior and in some cases, breakthrough therapies, as well as extend pharmaceutical product and patent life.

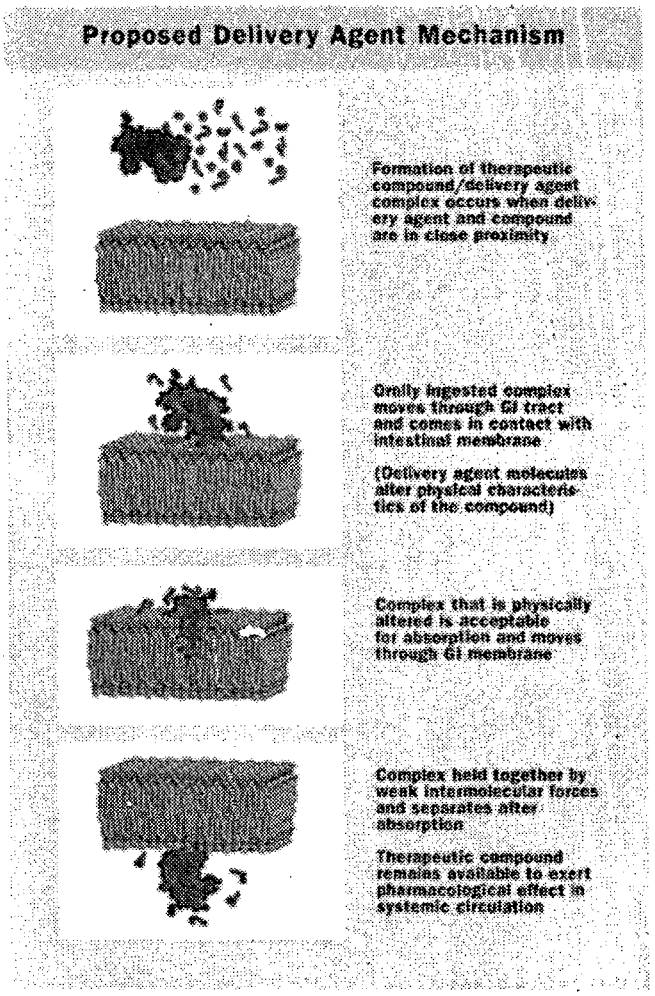

Figure 1. Proposed delivery agent mechanism

One may be optimistic that there would be not only more significant studies advancing the cause of enhancing oral delivery of peptide drugs but also their positive results in the ever-expanding pharmaceutical industry.

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