Cell-penetrating Peptide-biodrug Strategy for Oral and Nasal Delivery: Review of Recent Findings

El-Sayed Khafagy ¹, Noriyasu Kamei ², Mariko Takeda-Morishita ²*

¹ Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
² Laboratory of Drug Delivery Systems, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Japan

1. Introduction

In the next few decades, novel biotherapeutic agents, such as peptides and proteins, will occupy a significant portion of the drug marketing in the near future to treat several diseases.¹² However, their noninvasive absorption is limited because of their poor delivery through the epithelial membrane.³ Problems arising from the unfavorable physicochemical and biological properties of therapeutic peptides and proteins, affect their noninvasive absorption. So far, the parenteral route is the most common route of delivery for such biodrugs.⁴

Over the last several decades, many approaches have been used to overcome the inherent uptake barriers for therapeutic peptides and proteins across the gastrointestinal (GI) tract as well as transmucosal and transdermal routes.⁵,⁶ The oral delivery route remains the most convenient system because it is non-invasive, patient-friendly and allows self-administration, but it is the most challenging for peptide and protein delivery regimens.⁷ The oral administration of biotherapeutic agents is notoriously difficult because of their poor permeability through the intestinal mucosa, due to their high molecular weights, hydrophilicity, and susceptibility to enzymatic degradation.⁸ To yield therapeutic activity, orally administered biodrugs must be absorbed efficiently from the intestinal lumen to the circulation without being metabolized extensively in the intestine. Low permeability across epithelial mucosa of the GI tract and lack of stability in the luminal environment remain two main causes of the poor oral bioavailability of biodrugs.⁹

2. Strategies for enhancing oral biodrug delivery

To increase the oral bioavailability of biodrugs such as peptides and proteins, a strategy involving permeation enhancement or protease inhibitors as additives can be effective and provide higher reproducible bioavailability. Although such approaches can be successful in the laboratory, they still represent a challenge for widespread acceptance by clinicians and regulatory bodies. The use of enzyme inhibitors in long-term therapy remains questionable because of possible absorption of unwanted proteins as well as disturbing the digestion of nutritive proteins and the stimulation of protease secretion as a result of feedback regulation.¹⁰
Because of the complexity and poor success in the oral administration of biotherapeutic peptides and proteins, other routes of administration offer an interesting option to conventional parenteral routes. However, we believe that novel approaches are needed to improve the permeability of biodrugs through epithelial membrane of the GI tract through further research of new delivery systems.

Structural modification of biodrugs provides several opportunities to improve not only membrane permeability but also proteolytic stability. For example, first, the strategy of producing prodru

On the other hand, other strategies such as liposomal, micellar, and protein nanostructures have been explored for oral delivery of peptides and proteins. These approaches have shown promise in improving the permeability of biodrugs through the GI tract. However, further research is needed to optimize the pharmacokinetic properties of the macromolecules, as well as the delivery systems based on these carriers.

To increase intestinal transport, researchers have investigated the possibility of drug modulation by conjugation of novel bio-functional moiety to peptides and proteins with transport-carrier molecules that are recognized by endogenous cellular-transport systems in the GI tract. This approach might represent a more practical and safer strategy for increasing intestinal absorption of peptides and proteins. The associated transport mechanisms are membrane transporters and receptor-mediated endocytosis, recognizing and internalizing specific ligands attached to macromolecules. Receptor-recognition ligands, such as lectins, toxins, viral hemagglutinins, invasins, transferrin, and vitamins (vitamin B12, folate, riboflavin, and biotin), can be tethered to a drug substance to improve the specificity of the intracellular delivery systems to specific target cells. Receptor-mediated endocytosis of vitamin B12 has been demonstrated for oral delivery of peptides and proteins. Transferrin is also useful as a carrier for oral delivery of protein drugs such as insulin and granulocyte colony-stimulating factor (G-CSF). Recently, biologic activity of functionally active G-CSF-transferrin fusion protein following oral administration to mice has been demonstrated to have similar to that of subcutaneous G-CSF administration. With high expectations, this new recombinant fusion protein technology will be useful for the future development of orally effective biodrugs.

The most promising oral delivery strategies for biodrugs based on particulate carriers have been developed to circumvent the barriers to oral peptide delivery. They efficiently protect protein and peptide drugs against enzymatic degradation in the harsh environment of the GI tract, provide high transfer of drugs across the epithelial mucosa, control the release rate, and target drug delivery to specific intestinal sites. Certain particles can be taken up by epithelial cells or the lymphoid tissues in Peyer patches without using additional penetration enhancers. So far, polymeric drug delivery systems based on hydrogels, nanoparticles, microspheres, and lipid-based drug delivery systems (e.g. microemulsions, liposomes, and solid lipid nanoparticles) have been developed and used for oral macromolecular drug delivery.

Lipid-based particles generally do not entrap hydrophilic macromolecular drugs with high efficiency. In addition, they have low stability in the GI tract. Conventional liposomes and microemulsions have not met with much success in the mucosal delivery of hydrophilic macromolecular drugs. Fusogenic liposomes equipped with the envelope glycoprotein of Sendai virus or coated with a mucoadhesive polymer have already shown significant improvement of hydrophilic macromolecular drug absorption from the intestine. But solid particles overall are better than lipid-based particles for oral delivery because Peyer patches are follicles of lymphoid tissue covered by a specialized epithelium containing M cells. These M cells are responsible for particle uptake, and surface charge and size of particles are the important factors governing the uptake of particulates by the M cells. In general, nanoscale dimensions favor transport of particles across the mucosal epithelium. Desai and colleagues reported that 100 nm poly (lactic-co-glycolic acid) (PLGA) particles diffuse throughout the submucosal layers, whereas 10 mm particles are predominantly localized on the epithelial lining of the tissues. Taken together, nanoscale carriers composed of biocompatible polymers are thought to be promising for the development of an oral delivery system for macromolecules.

Recently, a layer-by-layer self-assembly technique has been applied to chitosan and sodium alginate micro-encapsulation. Alginate-chitosan microcapsules provide a simple method for controlling the loading and release of protein molecules within these polysaccharide microcapsules. Nanocapsules based on poly (ethyl 2-cyanoacrylate) containing insulin to form biocompatible microemulsions represent a convenient method for the entrapment of bioactive peptides. Research in this area have also shed new light on the potential use of chitosan microspheres in orally and other mucosally administered protein and peptide drugs because they show excellent mucoadhesive and permeation-enhancing effects across biologic surfaces. Control of the size and size distribution of chitosan microspheres is necessary to improve their reproducibility, bioavailability, and repeatable release behavior. But chitosan–coated nanoparticles clearly reduce the transepithelial resistance of a Caco-2 cell monolayer. Therefore, their potential use for clinical applications is questionable.

3. Cell-penetrating peptide-biodrug strategy for oral and nasal delivery

3.1. Background of cell-penetrating peptide-biodrug strategy

Recently, developed cell-penetrating peptides (CPPs), such as HIV-1 Tat and oligoarginine, are considered a useful tool for the intracellular delivery of therapeutic macromolecules. Because of their ability to increase the absorption of biodrugs through the epithelial membrane, the major barrier to their non-parenteral delivery, CPPs have potential as tools to overcome the low permeability of therapeutic peptides and proteins. A further advantage of this promising strategy is that this successful non-invasive absorption could be achieved by more convenient methodology, such as coadministration of CPPs with drugs through intermolecular interaction. Hereafter, the further establishment of delivery system based on CPPs is required to develop the noninvasive forms of therapeutic peptides and proteins.

As shown in Figure 1, Morishita and coworkers have been recently exploring the potential of CPPs as a modulator that enhances the intestinal uptake of biodrugs molecules. The intestinal permeability of insulin coadministered with oligoarginine composed of six (R6), eight (R8), or 10 arginine (R10) residues applied into a rat intestinal loop shows that D-R8 and D-R10 enhance insulin absorption from the intestine more markedly than D-R6.
indistinguishable with that of the drug alone. Conversely, D-penetratin improves the ileal absorption of insulin (a peptide possessing negative charge at physiological pH) significantly by coadministration of oligoarginine or penetratin; however, the absorption of IFN-β (a large protein possessing weak positive charge at physiological pH) and neutralized FITC-dextran is only slightly affected by coadministration of R6. In addition, in an in vitro permeation study using isolated rat ileal membrane, the ileal permeation of leuprolide, a peptide possessing positive charge, is unchanged in the presence of R6.

The approach using CPPs has achieved significant improvement in intestinal and nasal absorption by coadministration of biodrugs and CPPs as a physical mixture, as distinct from the conventional method using CPPs for intracellular delivery in which CPPs are covalently linked to drugs and carriers. The ability of a CPP to enhance drug absorption has been shown to be different depending on the kind of biodrugs. Based on the physical properties of drugs, it has been proposed that the electrostatic interaction between each drug and a positively charged CPP may be associated with the absorption-enhancing efficiency of CPP coadministration. Therefore, to elucidate the characteristics of the binding between macromolecular drugs and CPPs and to establish their relationship with the absorption-enhancing effect of CPP coadministration have been demonstrated in our laboratory. Based on these results, the absorption-enhancing effect of coadministration of bioactive drugs and D-R8 and L-penetratin corresponds with intermolecular binding between the bioactive drug and CPPs, implying that this binding is an important factor governing the enhancing effect of CPPs on the absorption of macromolecular drugs.

The electrostatic interaction between each drug and CPP was evaluated with the ability of the CPP to enhance intestinal drug absorption and the intermolecular interaction of drug and CPP. First, the binding characteristics between D-R8, a typical oligoarginine, and several peptide drugs possessing different isoelectric points (pIs) were analyzed by surface plasmon resonance (SPR)-based measurement. The sensorgram response is increased depending on injected peptide concentration after gastrin or insulin has been injected into a D-R8-immobilized-flow cell at pH 6.0. By contrast, no increase of the sensorgram response has been observed after other peptide drugs including exendin-4 and calcitonin were injected. Because gastrin and insulin have low pIs, they are negatively charged at pH 6.0, and so it is proposed that their binding to positively charged D-R8 was observed. By contrast, most peptide drugs that cannot bind to D-R8 have high pIs and are positively charged at pH 6.0. Therefore, it is suggested that no binding was observed between these peptide drugs and D-R8. However, exendin-4, oxytocin, and a few peptides cannot bind to D-R8 at this pH despite their low pIs. It is proposed that factors other than electrostatic characteristics, such as hydrophobicity and tertiary structure, may be associated with intermolecular interactions between peptide drugs and D-R8. The effect of coadministration of D-R8 on the intestinal absorption of these peptide drugs was examined in an in situ loop absorption study. In this study, gastrin and insulin were used as peptide drugs that bind to D-R8,
and exendin-4 and calcitonin were used as peptide drugs that do not bind to D-R8. The absorption of gastrin and insulin is increased in the presence of D-R8. By contrast, the absorption of exendin-4 and calcitonin is not affected by coadministration of D-R8. Based on these results, the absorption enhancing effect of coadministration of drugs and D-R8 corresponds with intermolecular binding between the drug and D-R8, implying that this binding is an important factor governing the enhancing effect of D-R8 on the intestinal absorption of macromolecular drugs.

The intestinal absorption of macromolecular dextran, FD-4, is not enhanced by coadministration with L- or D-R6 at a dose (25.0 mg/kg) sufficient to increase insulin absorption. Because FD-4 is a hydrophilic compound and a marker of the paracellular pathway, these results exclude the possibility of involvement of opening the paracellular pathway on the insulin absorption-enhancing effect of oligoarginine. CPPs are thought to be taken up mainly by cell-mediated macropinocytosis through the interaction between the positive charge derived from arginine residues and the negative charge derived from proteoglycans on the cell surface.

Therefore, the hypothesis of this proposed mechanism could explain how the molecular complex of the drug and oligoarginine is introduced into epithelial cells. Oligoarginine enhances the intestinal absorption of insulin but does not enhance the absorption of other macromolecules such as IFN-β and FD-4. Insulin has a negative charge in neutral conditions, and insulin may associate with oligoarginine electrostatically. If so, these molecular complexes could be taken up by the interaction between oligoarginine and the surface of epithelial cells. By contrast, IFN-β has a relatively positive charge in neutral conditions, which may set up a repulsive force or prevent intermolecular interaction with oligoarginine. Similarly, uncharged macromolecules, such as FD-4, may not interact with positively charged oligoarginine.

5. Pharmacokinetic parameters of CPP-biodrug absorption

Recent developments in molecular imaging methodology have been applied to study the function of endogenous molecules and the pharmacokinetics of therapeutic agents. Among such imaging methodologies developed to date, the imaging technique using positron emission tomography (PET) is of particular interest because it is a noninvasive and highly sensitive method to quantify the time profile of drug accumulation in specific tissues and the drug concentration in the blood. PET-based examinations of intestinal absorption and the subsequent pharmacokinetic behavior of peptides and proteins as biodrug models would be a revolutionary approach to understanding the intestinal absorption behavior of such agents in the intact animal.

The intestinal absorption and subsequent tissue distribution of 68Ga-labeled insulin following the ileal administration of 68Ga-labeled insulin with or without CPPs are visualized by PET imaging of rats. Coadministration of L- and D-R8 and L-penetratin increase the intestinal absorption of 68Ga-DOTA-insulin. Among the CPPs used in this study, L-penetratin has accelerated the insulin absorption, and D-R8 shows a prolonged 68Ga-DOTA-insulin absorption and hypoglycemic effect. In addition, the coadministration of D-R8 and L-penetratin induces a larger increase in the AUC of 68Ga-DOTA-insulin than L-R8; the 4.14- or 3.02-fold increase in the AUC of 68Ga-DOTA-insulin has been observed by coadministration of D-R8 or L-penetratin, respectively. The hepatic and renal distribution of 68Ga-DOTA-insulin after absorption is quantified based on these imaging results. These results show negligible accumulation of 68Ga-DOTA-insulin or its metabolites into the liver and kidney after the administration of insulin solution with 68Ga-DOTA-insulin. By contrast, coadministration of CPPs increases the accumulation of 68Ga-DOTA-insulin or its metabolites into both the liver and the kidney. In particular, 68Ga-DOTA-insulin passes rapidly through the liver and accumulated in the kidney. Although the renal accumulation mediated by L-penetratin is saturated 60 minutes after administration into the ileal segment, the accumulation by D-R8 is not saturated within 60 minutes after administration. In addition, the increase in the amount of 68Ga-DOTA-insulin in the liver and the kidney is accompanied by an increase in blood 68Ga-DOTA-insulin concentration.

6. Conclusion

To develop and improve oral delivery systems with such properties, the focus should be on developing superior materials and delivery carriers for oral bioactive macromolecular drug delivery systems. Therefore, development of improved oral delivery devices for peptides and proteins will require continuous comparison of the in vitro and cellular studies with in vivo studies. CPPs are likely to become powerful tools for overcoming the low permeability of therapeutic biologics such as peptides and proteins through the epithelial cell membrane safely, and may be a viable alternative to existing parenteral therapies. Further advantage of this promising strategy is that this successful intestinal and nasal absorption could be achieved by more convenient methodology and the coadministration of CPP with drugs through the intermolecular interaction among them. Those findings of recent research are already useful to establish fundamental guidelines for developing non-invasive delivery systems of biologics using CPP as a carrier.

References


