The oral delivery of protein and peptide drugs

A variety of strategies can be used to overcome the barriers to protein/peptide drug delivery by the oral route.

Dr Richard Soltero and Dr Nnochiri Ekwuribe, Nobex Corporation

Currently, only two biotechnology drugs that can be given orally are known to be in clinical development in the US

The use of polypeptides and proteins for systemic treatment of certain diseases is now well accepted in medical practice. The role that these drugs play in replacement therapy is so important that many research activities are being directed towards the synthesis of large quantities by recombinant DNA technology. Many of these polypeptides are endogenous molecules that play an important role in maintaining organ function and metabolic homeostasis. Research institutions and pharmaceutical companies around the world have implemented strong research programmes to identify these proteins, their use and methods of producing them.

The availability of biotechnology products to develop is no longer an issue; there are over 350 peptide and protein drugs currently under development. However, delivering therapeutically active proteins and peptides by the oral route has been a challenge and a goal for many decades. Currently, only two biotechnology drugs that can be given orally are known to be in clinical development in the US. For such drugs to be absorbed through the intestinal tract, they must be protected from enzymes and must pass through the luminal barriers into the bloodstream. This article will present a review of strategies used to overcome the barriers to protein drug delivery by the oral route.

Routes of administration

The most common route of administration for proteins and peptides is by injection, although many other routes have been tried with varying degrees of success (1). Routes such as intranasal (2, 3), transdermal, buccal (4), intraocular and pulmonary will deliver the drug to the systemic circulatory system while avoiding transit through the digestive system. A major factor that limits the usefulness of these substances for their intended therapeutic application is that they are easily metabolised by plasma proteases when they reach the peripheral circulation. In addition, adverse effects associated with applying these drugs to the pulmonary or other mucosal surfaces may be limiting.

The oral route of administration for these substances is even more problematic because, in addition to proteolysis in the stomach, the high acidity of the stomach destroys them before they reach the intestine for absorption. Polypeptides and protein fragments, produced by the action of gastric and pancreatic enzymes, are further cleaved by exo- and endopeptidases in the intestinal brush border membrane to yield di- and tripeptides, and even if proteolysis by pancreatic enzymes is avoided, polypeptides are subject to degradation by brush border peptidases. Any of the peptides that survive passage through the stomach are further subjected

to metabolism in the intestinal mucosa, where a penetration barrier prevents entry into cells. On the other hand, oral and colonic delivery have the advantage of delivering drugs through the intestinal tract to the hepatic portal vein, and then to the systemic circulation. The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides by mouth.

In spite of the obstacles to gastrointestinal survival, there is substantial evidence in the literature to suggest that nutritional and pharmaceutical proteins are absorbed through the intestinal mucosa, although in minute amounts (5). Small amounts of peptide drugs can be absorbed by the action of specific peptide transporters in the intestinal mucosa cells; this suggests that properly formulated protein or peptide drugs may be administered by the oral route with retention of sufficient biological activity for their therapeutic use.

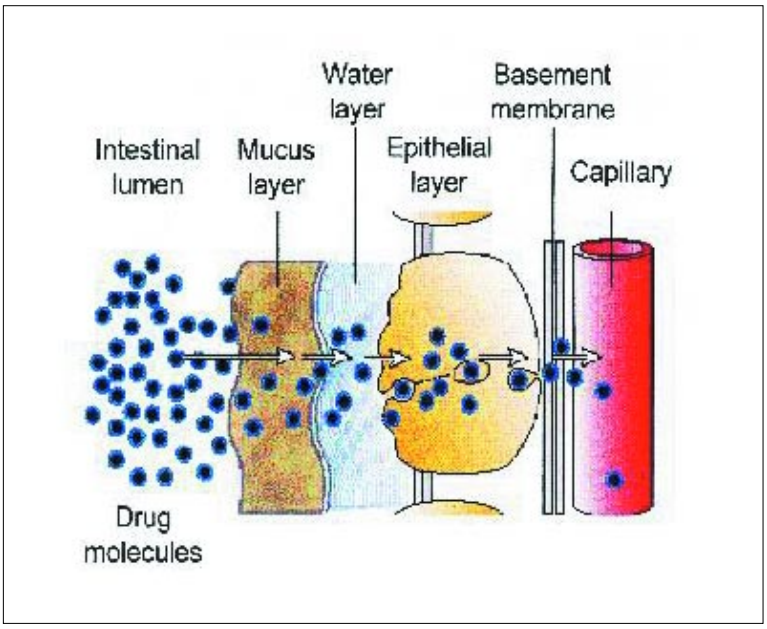

Figure 1. Barriers to the absorption of a drug in the intestine.

Strategies for oral delivery

Formulating for delivery through the gastrointestinal (GI) tract requires a multitude of strategies. The dosage form must initially stabilise the drug, while making it easy to take orally. Once delivered to the stomach or intestinal tract, the protein has to be protected from enzymatic degradation since digestive processes are designed to break down proteins and peptides without any discrimination in favour of therapeutically active compounds.

One strategy for overcoming the body's natural processes is to alter the environment for maximum solubility and enzyme stability of the protein by using formulation excipients such as buffers, surfactants and protease inhibitors. If the enzyme attack can be defeated or delayed, the proteins can be presented for absorption. Earlier work by Abuchowski and Davis (6) showed that proteins and peptides could be derivatised with polyethylene glycol (PEG) to achieve properties such as retention of activity, prevention of immunogenicity and prevention of excessive enzymatic degradation. More recently, Nobex has introduced the use of oligomers with both hydrophilic and lipophilic properties (7); these oligomers confer the enzymatic stability necessary for proteins to survive the digestive processes in the gut.

If a protein drug has the stability and enzyme resistance necessary to survive transit through the stomach and into the intestine, it is then faced with a number of lipophilic and hydrophilic barriers to cross (Figure 1). The drug must first dissolve in the contents of the intestinal lumen if it is not already in solution; then there is a mucus layer and a water layer protecting the surface of the epithelial cells. The protein or peptide drug must have sufficient water - and lipid-solubility to pass through these layers. The epithelial tissue represents the next barrier. There are cases where a protein can be absorbed into these cells by endocytosis, and then transported to the basement membrane on its way to the capillaries.

Another strategy for oral delivery, therefore, is to promote absorption through the intestinal epithelium. Absorption may be enhanced when the product is formulated with acceptable safe excipients (8). A typical transport mechanism for proteins across the epithelial boundary is paracellular transport. There are tight junctions between each of the cells in the epithelium that prevent water and aqueous soluble compounds from moving past these cells. A number of absorption enhancers are available that will cause these tight junctions to open transiently, allowing water-soluble proteins to pass. Fatty acids, surface-active agents, EDTA, glycerides and bile salts have all been shown to be effective in opening these tight junctions.

Most of the data showing the successful oral delivery of model proteins, such as insulin and calcitonin, has been generated in animal studies. These compounds have been combined with a variety of excipients and formulated in unique delivery systems to attain measurable systemic levels after oral delivery. In many cases, the formulation approaches would not be appropriate for human delivery; however, one can learn much from these model systems. For example, adding sodium cholate (9) or soybean trypsin inhibitor (10) was effective in delivering insulin to mice and dogs. Fatty acids also help, and are thought to

...there is substantial evidence in the literature to suggest that nutritional and pharmaceutical proteins are absorbed through the intestinal mucosa, although in minute amounts

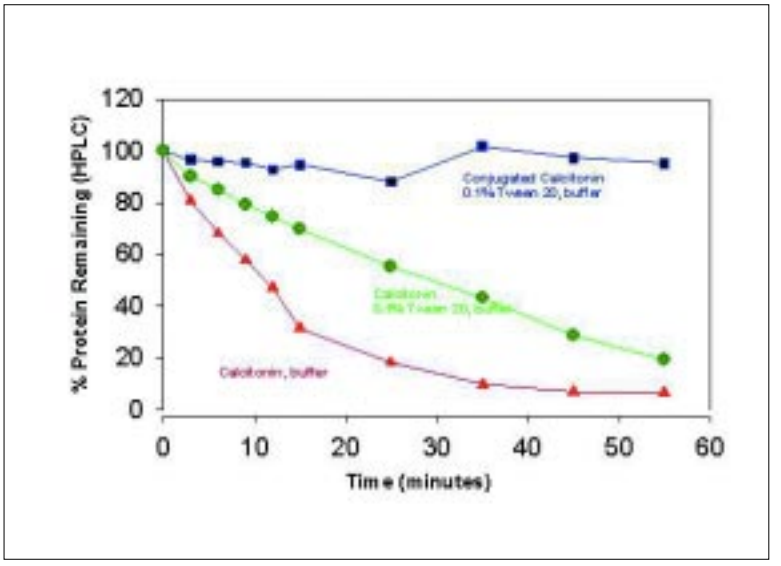

Figure 2. Enzyme stability of calcitonin incubated in a buffer with chymotrypsin at 37°C. Adding a surfactant to the buffer protects the compound and enzyme stability is improved. An ACE form of conjugated calcitonin in the same medium becomes inherently stable, and shows no loss of protein even after one hour.

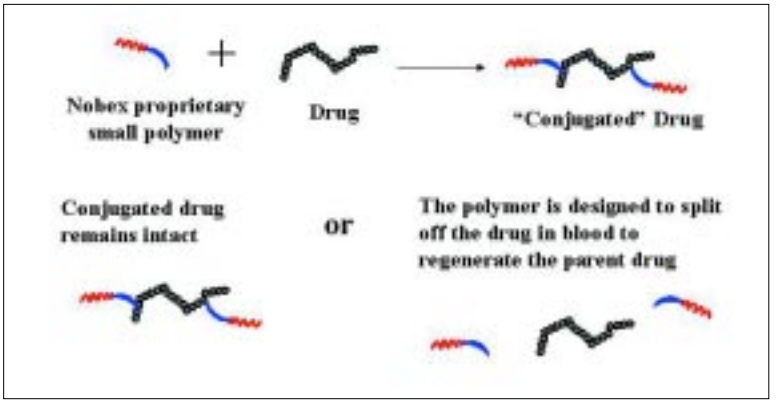

Figure 3. Nobex conjugation technology.

A compromise may lie in the balance of hydrophobic and hydrophilic characteristics of the oligomer used in the modification of protein drugs ...

interact with the lipid layer of epithelial cells causing a temporary disruption that can allow certain proteins to be absorbed in animals (11).

One more way to enhance absorption is to deliver the protein to the lower GI tract where absorption of proteins is more easily facilitated. To achieve this end, the formulator can apply standard drug delivery techniques, such as enteric coatings or sustained release.

Altered chemical entities

Drug molecules or their formulations must facilitate both aqueous and lipid layer penetration for proteins to be absorbed. Results indicate that simply increasing lipophilicity is not sufficient to improve transcellular or paracellular transport to the point where therapeutic levels of a drug can be absorbed. On the other hand, increasing the hydrophilicity of protein drugs does not increase paracellular transport, nor does it facilitate transcellular transport. A compromise may lie in the balance of hydrophobic and hydrophilic characteristics of the oligomer used in the modification of protein drugs, resulting in an altered chemical entity (ACE). This technique has worked successfully for several proteins at Nobex.

The Nobex technology involves the bonding of polyethylene glycol (PEG) and alkyl groups or fatty acid radicals to produce desired amphiphilic oligomers. These oligomers are conjugated to proteins or peptides to obtain desired amphiphilic products that can traverse the aqueous and lipid layers of the mucosa, and can resist excessive degradation of protein or peptide drugs. In the case of protein drugs, the attachment of the oligomers prevents the product from existing in multiple self-association forms; reduced self-association ACEs enable the product to penetrate through the epithelial walls. Nobex oligomers, being amphiphilic, enable the protein drugs to maintain close interaction with the mucosal wall, and are more compatible with formulation excipients than non-conjugated proteins.

An example of how this technology improves enzyme stability is shown in Figure 2. The enzyme stability of calcitonin when incubated in a buffer with chymotrypsin at 37°C is poor. Adding a surfactant to the buffer protects the compound and enzyme stability is improved. An ACE form of conjugated calcitonin in the same medium becomes inherently stable, and shows no loss of protein even after one hour. The activity of the compound was not affected by this conjugation.

Nobex has created a series of modified proteins that provide high water- and lipid-solubility and are resistant to enzyme attack. The technology consists of conjugating one or more oligomers to selected sites on the protein or peptide. As indicated in Figure 3, these conjugations can be either covalently bound to form an ACE conjugated drug, or loosely bound so they hydrolyse off in the plasma to regenerate the parent drug.

This conjugation technology has been applied to several proteins, including insulin. Historically, insulin has been a model compound for oral delivery techniques, and many investigators have tried and failed to deliver the native compound using formulation and drug delivery techniques. 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 beta chain. Biological activity is retained and the compound is readily absorbed from the GI tract.

Oral delivery of insulin

This conjugated protein is known as hexyl insulin monoconjugate 2 (HIM2), and is the first successfully delivered oral dosage form of insulin to show good oral bioefficacy in humans. This ACE has been studied in Phase I and II clinical trials in Europe, and in five Phase II clinical trials in the US. The pharmacokinetic profile mimics insulin secreted from the pancreas, since the drug is delivered through the GI tract and through the hepatic portal vein. The oral form of insulin goes directly to the liver and is believed to stimulate normal biochemical pathways, including glucose control.

HIM2 was given to fasted Type I diabetic patients in two sequential doses as shown in Figure 4. Type I patients are distinguished from Type II patients in that Type I have no ability to produce their own endogenous insulin, whereas Type II have some insulin production. In the absence of insulin control, a Type I diabetic's blood glucose level will increase to a point where the patient becomes ketoacidotic with serious and even fatal consequences. When HIM2 was administered to fasted patients, their glucose levels stabilised and remained at normal levels for over four hours. Additional clinical trials in both Type I and Type II populations have substantiated these findings.

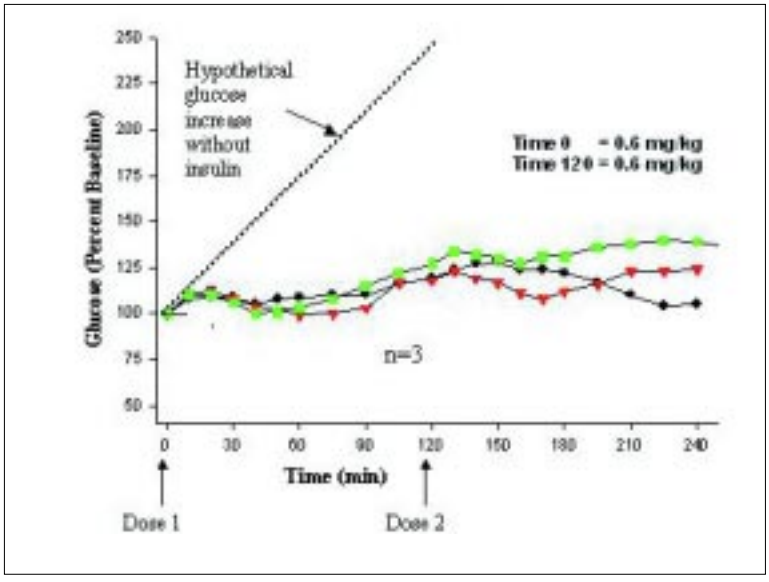

Figure 4. Fasted Type I diabetics' glucose response to sequential oral doses of conjugated insulin.

Human clinical results with conjugated insulin are a clear demonstration that a protein can be developed into a therapeutically viable product. With the conjugated form of insulin and an appropriate formulation, a product can be developed which has the attributes required for oral delivery. It survives the enzyme-rich environment of the intestinal tract, and crosses the various boundaries of the lumen to be absorbed into the bloodstream. Once into the circulation, it demonstrates the desired effect of controlling blood glucose

Conclusion

Human clinical results with conjugated insulin are a clear demonstration that a protein can be developed into a therapeutically viable product

Oral delivery of peptides and proteins for the therapeutic treatment of disease is now possible. These drugs can make the passage through the intestinal tract, through the luminal barriers and into the bloodstream. Strategies used to overcome these barriers include employing proper excipients for enzyme resistance and absorption enhancement. Common drug delivery techniques - such as sustained release and targeted delivery - may also assist. The most successful technique is the altered chemical entity, which is designed to work in combination with formulation strategies to achieve a protein product that is orally available in humans.

Dr Richard A Soltero is currently Executive Director of Pharmaceutical Development at Nobex Corporation. Prior to this position, he spent 25 years in Pharmaceutical R&D at Ciba-Geigy, Berlex and J&J, and five years in contract research organisations. Previous positions included Vice President of Laboratory Operations at Magellan Laboratories and at AAI, and Head of Worldwide Analytical, Microbiology and Stability R&D at SmithKline Beecham Consumer Healthcare.

Dr Nnochiri Ekwuribe received a PhD degree in organic medicinal chemistry from the University of California, San Francisco. His most recent work has been in the area of drug delivery systems and particularly in the oral delivery of protein/peptide drugs, and he is the inventor of the amphiphilic polymer technology licensed to Nobex. He is currently Vice President of Chemistry Innovations and Drug Discovery at Nobex.

References

1. Wearley LL (1991). Recent progress in protein and peptide delivery by noninvasive routes. Crit Rev Ther Drug Carrier Syst, 8, 4, 331-94.
2. Torres-Lugo M and Peppas NA (2000). Transmucosal delivery systems for calcitonin: a review. Biomaterials 21, 12, 1191-6.
3. O'Hagan DT and Illum L (1990). Absorption of peptides and proteins from the respiratory tract and the potential for development of locally administered vaccine. Crit Rev Ther Drug Carrier Syst 7, 1, 35-97.
4. Sayani AP and Chien YW (1996). Systemic delivery of peptides and proteins across absorptive mucosae. Crit Rev Ther Drug Carrier Syst, 13, 1-2, 85-184.
5. Lee YH and Sinko PJ (2000). Oral delivery of salmon calcitonin. Adv Drug Deliv Rev, 42, 3, 225-38.
6. Abuchowski A and Davis FF (1979). Preparation and properties of polyethylene glycol-trypsin adducts. Biochim Biophys Acta, 578, 1, 41-6.
7. Radhakrishnan B, Rajagopalan J, Anderson WL, *et al.* (1998). Structure-activity relationship of insulin modified with amphiphilic polymers. Pharm Sci 1, 1, S-59. (American Association of Pharmaceutical Scientists, San Francisco, CA.)
8. Sinko PJ, Lee YH, Makhey V *et al.* (1999). "Biopharmaceutical approaches for developing and assessing oral peptide delivery strategies and systems: *in vitro* permeability and *in vivo* oral absorption of salmon calcitonin (sCT). Pharm Res, 16, 4, 527-33.
9. Hosny EA, Ghilzai NM, *et al.* (1997). Promotion of oral insulin absorption in diabetic rabbits using pH-dependent coated capsules containing sodium cholate. Pharm Acta Helv, 72, 4, 203-7.
10. Ziv E, Kidron M, *et al.* (1994). Oral administration of insulin in solid form to nondiabetic and diabetic dogs. J Pharm Sci, 83, 6, 792-4.
11. Muranishi S (1997). Delivery system design for improvement of intestinal absorption of peptide drugs. Yakuga.