Oral Insulin – Fact or Fiction?

Possibilities of Achieving Oral Delivery for Insulin

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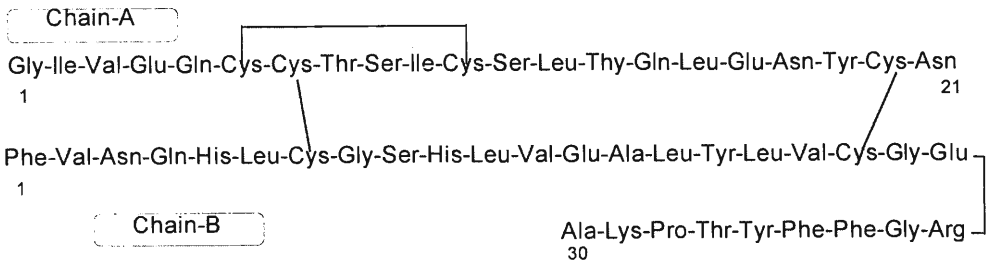
Oral insulin, penetration enhancers, protease inhibitors, microspheres, nanoparticles, bioadhesive delivery.

Insulin is a major protein hormone secreted by the β-cells of the pancreas and is important for the control of diabetes. Insulin is usually administered to diabetic patients through subcutaneous injection. This mode of therapy has certain inherent disadvantages such as local pain, itching and insulin lipodystrophy around the injection site. Hence, pharmaceutical scientists have been trying to design an oral delivery system for insulin. Many challenges are associated with the oral delivery of insulin, relating to the physical and chemical stability of the hormone, and its absorption and metabolism in the human body. Here we discuss various strategies for the oral delivery of insulin that are being tried out, as well as methods used to improve the absorption of orally consumed insulin and to reduce its degradation by digestive enzymes.

Before the discovery of insulin (*Box* 1) by Banting and Best in 1921, a diagnosis of diabetes (*Box* 2), especially in young age, was tantamount to a death sentence. During the first decade of the 'insulin era', only an acid solution of an impure form of the hormone was available for therapy. The introduction of Zn^{2+} crystallization in 1934, as well as the development of recrystallization methods, made it possible to crystallize insulin. This substantially improved the purity and, hence, the biological activity of the hormone. Insulin recrystallized several times was better tolerated by patients suffering from allergic reactions to relatively impure preparations of insulin. Until the late 1960s, recrystallized insulin was considered to be extremely pure, but the introduction of new analytical methods such as disc electrophoresis and gel filtration made it possible to detect the presence of significant amounts of protein impurities even in recrystal-

Box 1. Insulin

Insulin is a protein hormone secreted by the β-cells of the islets of Langerhans in the pancreas. It is secreted in response to elevated blood glucose and amino acid levels, and promotes the efficient storage and utilization of these fuel molecules by controlling the transport of metabolites and ions across the cell membrane. Insulin promotes the entry of glucose, fatty acids and amino acids into cells and enhances glycogen, protein and lipid synthesis. Insulin is made up of two polypeptide chains namely, chain-A (21 amino acids) and chain-B (30 amino acids), which are held together by two disulfide bonds (*see the structure given below*). Insulin deficiency causes a disease called diabetes mellitus and, hence, administration of exogenous insulin is used to control this disease. The various preparations of insulin available in the market include bovine insulin (from cattle), porcine insulin (from pigs), a mixture of both, or recombinant human insulin. Since insulin has a very short life in the plasma, insulin products that last longer are also available. In these products, insulin is complexed with zinc, globin, protamine or a combination of any of these.



Structure of porcine insulin.

lized insulin. The use of chromatographic purification in the 1970s reduced the impurities to less than 0.5% and the immunologic complications of insulin treatment (allergy and insulin resistance) were essentially eliminated. Since then, the use of insulin has given diabetic patients many years of life, albeit at the expense of having to take daily injections.

Drawbacks of Repeated Insulin Injection

The most commonly used mode of administering insulin is by subcutaneous (i.e. under the skin) injection. However, there are several drawbacks of this method such as local discomfort, inconvenience of multiple injections, and occasional hyperinsulinemia due to overdose. In diabetic patients, who require a daily dose of subcutaneous insulin, pain, itching and allergy often occur at frequently used sites of injection. Continued

Box 2. Diabetes mellitus

Diabetes mellitus is one of the most common medical disorders, affecting people of all ages. This disease cannot be cured. It can, however, be managed and controlled through medication. Diabetes affects almost 40 million Indians, and studies suggest that one in every 12 Indians above the age of 40 may be diabetic.

Diabetes is a condition where an excess of glucose (sugar) occurs in the blood. Though glucose is required to provide energy for the body, a surplus of it creates problems. In a normal person, the food is digested and converted into glucose, which is carried by the blood to all the cells of body. The cells use glucose as fuel to produce energy. In the absence of insulin, or when insulin levels are low, glucose cannot enter body cells and this increases the level of glucose in the blood. This excess glucose is then removed from the body through urine.

Diabetes can be of two types, namely, Type-I and Type-II. Type-I diabetes is known as 'insulin-dependent diabetes mellitus' (IDDM) or juvenile diabetes. This disorder affects people quite early in life and thereafter progresses rapidly. Type-I diabetics do not produce enough insulin in their pancreas and are dependent on insulin injections throughout their lives.

Type-II diabetes is known as 'non-insulin dependent diabetes mellitus' (NIDDM) and is more frequent than Type-I diabetes. Type-II diabetes affects people later in life, usually after the age of 40, and accounts for over 90% of all cases of diabetes.

Heredity plays an important role in diabetes that shows up during old age. The stronger the family history, the greater are the chances of developing diabetes. Other factors contributing to diabetes include obesity and overnutrition, lack of exercise, mental stress and tension, side effects of diabetogenic drugs such as steroids and diuretics, and also repeated infections and old age.

No cure has yet been found for diabetes. However, it can be controlled using insulin or oral hypoglycemic agents, or a combination of drugs, diet and regulated exercise. Oral hypoglycemic drugs are medicines that stimulate the pancreas to produce more insulin. They also have an extrapancreatic effect, which increases the uptake of glucose by the cells by acting on receptors present on cell surface.

administration of insulin at these sites may further lead to a condition called 'insulin lipodystrophy', which results in atrophy of fats at the frequent sites of insulin injection. This can be observed as irregular depressions on the skin. The absorption of insulin from such sites is affected, and high doses of insulin are required at these sites for producing the required pharmacological effect of hypoglycemia. These high doses of insulin, unfortunately, may cause other harmful effects. Due to the many drawbacks of the subcutaneous route for administration of insulin,

alternative modes of administering insulin continue to attract considerable research interest. Nearly all available orifices of the human body have gained attention as possible non-invasive sites for insulin absorption. Among them, the oral and nasal routes have yielded some promising results in delivering insulin without pain. The possibilities of oral delivery of insulin and methods followed for it are discussed below.

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Formulation Development of Oral Insulin

The oral bioavailability (the fraction of the amount consumed that is actually available to the body after absorption in the gastrointestinal tract) of most peptides and proteins is less than 1%. The reasons for this are poor absorption of the drug in the gastrointestinal tract and the high likelihood of degradation by proteolytic (protein digesting) enzymes like pepsin. Moreover, any peptide or protein drug that is absorbed intact may undergo first pass metabolism in the liver. The food and drugs we consume often contain traces of toxic materials. Hence, before entering into blood circulation, the absorbed food and drugs from small and large intestines are drained into the liver, through the portal vein, and the process of detoxification then takes place in the liver. This is called first pass metabolism or first pass effect. A major portion of the drugs get metabolized through this first pass effect, and this leads to reduced bioavailability and, therefore, a reduced pharmacological action of the drug.

Ever since insulin was introduced into the field of medicine, the possibility of oral administration of insulin has excited great interest. The oral route is considered to be the most acceptable and convenient route of drug administration for chronic therapy. However, for the reasons explained above, insulin delivery by this route is not as efficient as by the subcutaneous route. Absorption of proteins and peptides from different regions of the intestine is not uniform. The cellular morphology of the intestines changes from region to region, and the proteolytic activity of protease gradually decreases from the duodenum to large intestine. This suggests that there may be an optimal site

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for insulin administration in the small intestine, and that the selective release of insulin directly into the mid-jejunum (in the small intestine) would help to protect the insulin from the gastric and pancreatic enzymes. In the last three decades or so, hundreds of research papers on the possibilities of oral administration of insulin have been published, and the more recent attempts have explored the following options, either singly, or together:

- Protecting insulin from enzymatic degradation by using antiproteolytic agents.
- Promoting the gastrointestinal absorption of insulin through simultaneous use of a multitude of different penetration enhancers.
- Chemical modification of insulin to improve its stability.
- Bioadhesive delivery systems for enhancement of contact of the drug with the mucous membrane lining the gastrointestinal tract.
- Carrier systems such microspheres and nanoparticles which can improve the bioavailability of insulin.

Peptidase Inhibitors and Penetration Enhancers

Peptidase or protease inhibitors promote oral absorption of therapeutic peptides and proteins by reducing their proteolytic breakdown by enzymes in the gastrointestinal tract. Administration of insulin via microspheres (see section on 'Carrier systems'), together with the protease inhibitor aprotinin, has been found to be the most efficacious combination involving protease inhibitors.

Penetration enhancers can increase the absorption of peptides and proteins in the gastrointestinal tract by their action on transcellular and paracellular pathways of absorption. Penetration enhancers include substances like surfactants, fatty acids, bile salts and citrates, as well as chelators like ethylene diamine tetra acetate (EDTA). Surfactants and fatty acids affect the

transcellular pathway by altering membrane lipid organization and thus increase the absorption of drugs consumed orally. Bile salt micelles, EDTA and trisodium citrate have been reported to increase the absorption of insulin. Cyclodextrins have also been used to enhance the absorption of insulin from lower jejunal and upper ileal segments of rat small intestine. A significant increase in the bioavailability of insulin can be achieved by the co-administration of protease inhibitors and penetration enhancers.

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Chemical Modification

Modifying the chemical structure of a peptide or protein is another approach to enhance its bioavailability by increasing its stability in the face of possible enzymatic degradation and/or its membrane permeation. However, this approach is more applicable to peptides rather than proteins, because of the structural complexity of proteins. For example, it is found that substitution of D-amino acids for L-amino acids in the primary structure can improve the enzymatic stability of peptides. A diacyl derivative of insulin has been seen to maintain its biological activity and also have increased absorption from the intestine.

Bioadhesive Drug Delivery Systems and Carrier Systems

Bioadhesive drug delivery systems anchor the drug to the gastrointestinal tract, and have been widely investigated to prepare sustained release preparations for oral consumption of drugs. The anchoring of the drug to the wall of the gastrointestinal tract increases the overall time available for drug absorption because the delivery system is not dependent on the gastrointestinal transit time for removal. Moreover, a drug administered through this method does not need to diffuse through the luminal contents (the partially digested food, etc.) or the mucus layer in order to reach mucosal epithelium lining the gastrointestinal tract. Because of intimate contact with the mucosa, a high drug concentration is presented for absorption, and there is also the possibility of site-specific delivery if bioadhesion can

be targeted to occur at a particular site in the gastrointestinal tract. Bioadhesive polymers such as polycarbophil and chitosan have been reported to improve the oral absorption of insulin.

Carrier systems such as nanoparticles, microspheres and liposomes (*Box* 3) can also be used to improve the oral absorption of peptides and proteins. The use of different carrier systems has been reported to improve the absorption of insulin in rats. The introduction of liposomes as a drug delivery system in the late 1980s renewed interest in the oral administration of insulin. In the following years, many investigators tested the ability of liposomes to fulfill the dual role of (1) preventing the degradation of insulin in the upper gastrointestinal tract, and (2) enhancing insulin absorption from various regions of the small intestine. The strategy of utilizing insulin loaded microparticulate systems (nano/microcapsules or particles) to circumvent the gastrointestinal enzymatic barrier, and to improve absorption by the intestinal mucosa has also been tested a number of times.

Limitations of Oral Delivery of Insulin

A sobering fact is that in most of the above described approaches, the uptake of insulin via oral route, despite all the precautions, was less than 0.5%. A few studies performed in humans also revealed wide variation in responses to massive doses, meaning that accurate dosing is not possible. The different approaches that have been used to enhance the oral absorption and to reduce the enzymatic degradation of insulin are not infallible, and each approach has its own disadvantages and limitations.

Formulations of insulin with protease inhibitors such as aprotinin have typically shown inconsistent effects, with *in vitro* and *in vivo* effects often being different. With penetration enhancers, the limitation is lack of specificity, which may lead to long-term toxic implications. Surfactants can cause lysis of mucous membrane and may thus damage the lining of the gastrointestinal

Box 3. Different Carrier Systems used for improving the Oral Bioavailability of Insulin

Various particulate systems that can be used as carriers for controlled release and targeting of peptide and protein drugs are described below. These can be targeted passively (according to their particle sizes) or actively (by conjugating with monoclonal antibodies).

Liposomes: These are tiny spheres formed when phospholipids are combined with water. The structure of liposomes resembles that of cell membranes. They are vesicles made up of either one or more lipid bilayers alternating with aqueous compartments. Liposomes are of the following types:

Small Unilamellar Vesicles (SUV): They have a single lipid bilayer of the size of 20-70 nm (*Figure* 1).

Large Unilamellar vesicles (LUV): size 100-400 nm.

Multilamellar Vesicles (MLV): 200 nm to several microns size and contain two or more concentric bilayers (*Figure* 2).

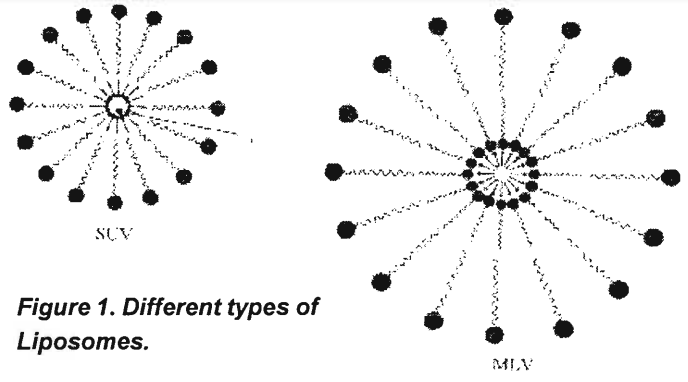

Figure 1. Different types of Liposomes.

Various molecules can be incorporated into liposomes to modify their properties. For example, incorporation of cholesterol into the liposomes results in a more stable membrane. Liposomes can be passively targeted to liver. SUVs can be targeted to parenchymal cells of liver due to their small size. They can also be targeted to other organs by attaching antibodies to them. They are being tested for controlled release of genetically engineered peptides and proteins such as macrophage stimulating agent, muramyl dipeptide and enzymes. The use of liposomes for targeting has some disadvantages. They are relatively less stable, the entrapped drug may leak and their drug carrying capacity is low.

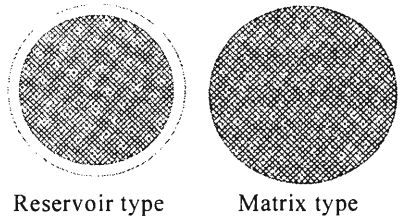

Figure 2. Types of microspheres used for insulin delivery.

Microspheres: These are solid spherical particles with a size range of 1 to 600 mm and contain dispersed drugs. They are prepared by emulsification and either natural biodegradable polymers (gelatin or albumin) or synthetic polymers (polylactic or polyglycolic acid) are used. The dispersed phase consists of droplets of polymer-drug solution. Entrapment, ionic or covalent bonding can incorporate the drug. Two types of microspheres are possible. In the first type, the drug is encapsulated in a microcapsule and in the second type, the drug is dispersed in a polymer matrix (*Figure* 2). The microspheres can be passively

Box 3. continued...

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targeted to lung capillaries or to RES. Active targeting is also possible if they are attached to targeting moieties. Albumin microspheres mixed with iron oxide can be used as magnetic microspheres and can be targeted to specific organs by the use of an external magnet.

Nanoparticles: These are similar to microspheres but have a particle size ranging between 10 and 1000 nm. These can be made from biodegradable material such as albumin, or non-biodegradable material like methylmethacrylate. Tagging the nanoparticles with targeting moieties such as monoclonal antibodies increases their specificity of targeting.

Suggested Reading

[1] V H L Lee, *Protein and Peptide Drug Delivery*, Marcel Dekker Inc, New York, 1991.

[2] Y W Cho and M Flynn, *Oral delivery of insulin*, Lancet, 1518-1519, 1989.

[3] M Saffran, Targeting of drugs, *Life Sciences*, Plenum Press, New York, Vol.238, 1992.

[4] A Kelkar, Needle free injection systems, *Pharmatimes*, Vol.33, 12-13, June 2001.

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tract. Similarly, chelators such as EDTA cause depletion of Ca^{2+} ions, which may in turn cause disruption of actin filaments and thus damage the cell membrane. Chemical modification does not always lead to improved oral absorption. For example, diacyl derivatives of insulin exhibited a higher proteolysis than native insulin in the small intestine of rat under *in vitro* conditions.

The bioadhesive systems may be affected by the mucous turnover of the gastrointestinal tract, which varies based on site of bioadhesion. Moreover, directing a delivery system to a particular site of adhesion in the gastrointestinal tract is yet to be achieved. The nanocapsules of insulin prepared using polyisobutylcyanoacrylate as polymeric carrier showed initial low plasma concentration followed by higher plasma concentration after sometime, with no significant net enhancement of absorption. Hence, from carrier systems, insulin gets released slowly into intestinal lumen, with small amounts being absorbed.

Thus, although techniques for developing more efficient insulation formulations are actively being pursued, it has not yet been possible to design an efficient oral delivery system for insulin. Successful delivery of insulin by oral route needs a succession of events to bypass the various penetration or enzymatic barriers. Often, to achieve this goal, a combination of two or more approaches may be required. At the present time, it is unlikely that in the short future any oral preparation that can achieve consistent, efficient and economic insulin delivery will be forthcoming.