ABSTRACT: Diabetes mellitus is a serious pathologic condition that is responsible for major healthcare problems worldwide and costing billions of dollars annually. Insulin replacement therapy has been used in the clinical management of diabetes mellitus for more than 84 years. The present mode of insulin administration is by the subcutaneous route through which insulin is presented to the body in a non-physiological manner having many challenges. Hence novel approaches for insulin delivery are being explored. Challenges to oral route of insulin administration are: rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity. Liposomes, microemulsions, nanocubicles, and so forth have been prepared for the oral delivery of insulin. Chitosan-coated microparticles protected insulin from the gastric environment of the body and released intestinal pH. Limitations to the delivery of insulin have not resulted in fruitful results to date and there is still a need to prepare newer delivery systems, which can produce dose-dependent and reproducible effects, in addition to increased bioavailability.

KEYWORDS: Insulin; Oral delivery; Challenges; Approaches; Market status
Insulin if administered via the oral route will help eliminate the pain caused by injection, psychological barriers associated with multiple daily injections such as needle anxiety (Korykowski M, 2002) and possible infections (Lin YH et al., 2007). In addition, oral insulin is advantageous because it is delivered directly to the liver, its primary site of action, via the portal circulation, a mechanism very similar to endogenous insulin; subcutaneous insulin treatment however does not replicate the normal dynamics of endogenous insulin release, resulting in a failure to achieve a lasting glycemic control in patients (Morishita M, 2006; Agarwal V et al., 2001).

**Challenges to Oral Insulin Delivery**

Generally, peptides and proteins such as insulin cannot be administered via the oral route due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen, and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity (Nakamura K et al., 2004; Sajeesh S et al., 2006; Jain D et al., 2005). The oral bioavailability of most peptides and proteins therefore is less than 1%. The challenge here is to improve the bioavailability to anywhere between 30 – 50% (Lee VH, 1991).

**Enzymatic Barrier**

The harsh environment of the gastrointestinal tract (GIT) causes insulin to undergo degradation. This is because digestive processes are designed to breakdown proteins and peptides without any discrimination. (Tuesca A et al., 2006) Insulin therefore undergoes enzymatic degradation by pepsin and pancreatic proteolytic enzymes such as trypsin and $\alpha$-chymotrypsin (Agarwal V et al., 2001; Patki VP et al., 1996). Overall, insulin is subjected to acid-catalyzed degradation in the stomach, luminal degradation in the intestine and intracellular degradation. The cytosolic enzyme that degrades insulin is insulin-degrading enzyme (IDE) (Chang LL et al., 1999). Insulin is however not subject to proteolytic breakdown by brush border enzymes (Agarwal V et al., 2001). Insulin can be presented for absorption only if the enzyme attack is either reduced or defeated.

**Intestinal Transport of Insulin**

Another major barrier to the absorption of hydrophilic macromolecules like insulin is that they cannot diffuse across epithelial cells through lipid-bilayer cell membranes to the blood stream (Lin YH et al., 2007). In other words, insulin has low permeability through the intestinal mucosa (Torisaka E et al., 2005). There is no evidence of active transport for insulin (Schilling RJ et al., 1999). It has been found however that insulin delivery to the mid-jejunum protects insulin from gastric and pancreatic enzymes and release from the dosage form is enhanced by intestinal microflora (Schilling RJ et al., 1999; Koosapur H et al., 1999). Various strategies have been tried out to enhance the absorption of insulin in the intestinal mucosa and in some cases; they have proven successful in overcoming this barrier.

![Fig. 1 Barriers to absorption of drug in the intestine (Soltero et al., 2001).](Image)

**Dosage form Stability**

The activity of proteins depends on the three-dimensional molecular structure. During dosage form development, proteins might be subject to physical and chemical degradation. Physical degradation involves modification of the native structure to a higher order structure while chemical degradation involving bond cleavage results in the formation of a new product (Agarwal V et al., 2001). Proteins must be characterized for change in conformation, size, shape, surface properties, and bioactivity upon formulation processing. Changes in conformation, size, shape can be observed by use of spectrophotometric techniques, X-ray diffraction, differential scanning calorimetry, light scattering, electrophoresis, and gel filtration (Pearlman R et al., 1991).
Approaches for oral Insulin

Attempted Oral Insulin Delivery Systems
Most peptides are not bioavailable from the GIT after oral administration (Cho YW et al., 1989). Therefore, successful oral insulin delivery involves overcoming the enzymatic and physical barriers (Tuesca A et al., 2006) and taking steps to conserve bioactivity during formulation processing (Agarwal V et al., 2001). In developing oral protein delivery systems with high bioavailability, three practical approaches might be most helpful: (Morishita M et al., 2006)

1. Modification of physicochemical properties such as lipophilicity and enzyme susceptibility.
2. Addition of novel function to macromolecules.
3. Use of improved carrier systems.

The various oral delivery systems which have been attempted to deliver insulin orally either singly or in a synergistic approach can be categorized as follows:

Enzyme Inhibitors
Insulin is degraded in the GIT by pepsin and other proteolytic enzymes. Enzyme inhibitors slow the rate of degradation of insulin which increases the amount of insulin available for absorption (Agarwal V et al., 2001). The earliest studies involving enzyme inhibitors were carried out with sodium cholate along with aprotinin which improved insulin absorption in rats (ziv E et al., 1987). Significant hypoglycemic effects were also obtained following large intestinal administration of insulin with camostat mesilate, bacitracin (Yamamoto A et al., 1994). Other inhibitors which have shown promise are leupeptin (Liu H et al., 2003), FK-448 (Fujji S et al., 1985), a potent and specific inhibitor of chymotrypsin and chicken and duck ovomucoid (Agarwal V et al., 2001). In one study, polymers cross-linked with azoaromatic groups formed an impervious film to protect insulin from digestion in the stomach and small intestine (Saffran M et al., 1986). The use of enzyme inhibitors in long-term therapy however remains questionable because of possible absorption of unwanted proteins, disturbance of digestion of nutritive proteins and stimulation of protease secretion (Shah RB et al., 2002).

Penetration Enhancers
Hydrophilic molecules like insulin are adsorbed to the apical membrane and are internalized by endocytosis (Agarwal V et al., 2001). Another theory suggests absorption via paracellular transport. Tight junctions between each of the cells in the epithelium prevent water and aqueous soluble compounds from moving past those cells. Hence, approaches for modulating tight-junction permeability to increase paracellular transport have been studied (Salamat-Miller N et al., 2005). A number of absorption enhancers are available that cause these tight junctions to open transiently allowing water-soluble proteins to pass. Absorption may be enhanced when the product is formulated with acceptable safe excipients (Soltero R et al., 2001). These include substances like bile salts, surfactants, trisodium citrates, chelating agents like EDTA (Li CL et al., 2004), labrasol (Eaimtarakam S et al., 2002). The drawbacks with penetration enhancers include lack of specificity, i.e., they allow all content of the intestinal tracts including toxins and pathogens the same access to the systemic bloodstream (Rieux A et al., 2006), and risk to mucous membranes by surfactants and damage of cell membrane by chelators (Gowthamarajan K et al., 2003). Mucoadhesive polymers have been proven to be safe and efficient intestinal permeation enhancers for the absorption of protein drugs (Thanou M et al., 2001; Plate NA et al., 2002).

Chemical modifications
Modifying the chemical structure of a peptide or protein is another approach to enhance bioavailability by increasing its stability against possible enzymatic degradation or its membrane permeation. However, this approach is more applicable to peptides rather than proteins because of the structural complexity of proteins. For example, 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 maintains its biological activity and also increases absorption from the intestine (Giriraj KG et al., 2003).

Carrier Systems
Hydrogels
These are cross-linked networks of hydrophilic polymers, which are able to absorb large amounts of water and swell, while maintaining their three-dimensional structure (Yupeng R et al.). Complexation hydrogels are suitable candidates for oral delivery of proteins and peptides due to their abilities to respond to changes in pH in the GI tract.
and provide protection to the drugs from the harsh environment of the GI tract (Nakamura K et al., 2004).

**Liposomes**

Insulin-entrapped liposomes cause dose-dependent hypoglycemia. Researchers have prepared liposomes with varying composition by two methods: solvent evaporation hydration and solvent spherule evaporation (Choudhari KB et al., 1994). Liposomes containing lecithin 100 mg, cholesterol 20 mg, insulin 150 units, and Tween 1% v/v were found to be most effective. The effect of insulin-liposome was prolonged in diabetes-induced rabbits than that of normal rabbits. The pharmacodynamics of the insulin-liposome system was comparable with the action of 1 U/kg of insulin administered subcutaneously.

**Erythrocytes**

Human red blood cells have been developed as oral carrier systems for human insulin. In a study by Al-Achi et al., male Wistar rats were made diabetic by a single intraperitoneal injection of streptozocin (100 mg/kg) (Al-Achi A et al., 1998). Rats received orally one of the following (100 U, 2 mL): an insulin solution, a ghosts-insulin suspension, a vesicles-insulin suspension, a liposomes-ghosts-insulin suspension, or a liposomes-vesicles-insulin suspension. Free-carrier suspensions or sodium chloride solution (0.9%) were given orally as controls. Blood glucose concentration was determined just before administration and at 1, 2, 3, 4, 5, 6, and 7 h post administration. Results showed that all treatment groups, except liposomes-ghosts-insulin, were significantly different statistically from their respective controls (i.e., the free carriers).

**Nanospheres**

Damge et al. prepared insulin-loaded nanospheres by polymerization of isobutyl cyanoacrylate (IBCA) in an acidic medium (Damge C et al., 1997). These nanospheres displayed a mean size of 145 nm and an association rate of 1 U of insulin per milligram of polymer. These nanospheres were dispersed in an oily medium (Miglyol 812) containing surfactant (Polox-amer 188 and deoxycholic acid) and evaluated for in vitro and in vivo degradation. No degradation due to proteolytic enzyme was observed in vitro. When these nanospheres (100 U per kilogram of body weight) were administered perorally in streptozotocin-induced diabetic rats, a 50% decrease in fasted glucose levels from the second hour up to 10-13 days was observed. This effect was shorter (2 days) or absent when nanospheres were dispersed in water. Using 14C-labeled nanospheres loaded with (125I) insulin, it was found that nanospheres increased the uptake of (125I) insulin or its metabolites in the gastrointestinal tract, blood, and liver while the excretion was delayed when compared to (125I) insulin nonassociated to nanospheres.

**Nanocubicles**

A liquid formula that can be easily dispersed in water to produce particles named "Nanocubicles" was developed by Chung et al. (Chung H et al., 2004). These nanocubicles containing insulin were administered to fasted streptozotocinduced diabetic rats. For comparison, an aqueous solution of insulin in water was also administered. Nanocubicles without insulin and insulin in phosphate buffer saline (PBS) were administered as controls. Blood glucose concentration and insulin concentration were measured 1, 2, 3, 4, and 6 h after the administration of the insulin formulations. In vitro experiments showed that the particles were taken up by the Caco-2 cells at a high ratio. It was observed in these studies that the serum glucose concentration was controlled for more than 6 h after oral insulin administration but returned to the basal concentration in 3 h when 1 IU/kg of insulin was injected, intravenously.

**Other Approaches**

**Tablets**

**Thiolated chitosan insulin tablets:** The efficacy of orally administered insulin has also been improved using thiolated chitosan. 2-Iminothiolane was covalently linked to chitosan and the resulting chitosan-TBA (chitosan-4-thiobutylamidine) conjugate exhibited 453.5 ± 64.1 µmol thiol groups per gram of polymer (A.H. Krauland et al., 2004). Two enzyme inhibitors Bowman-Birk-Inhibitor (BBI) and Elastatinal were covalently linked to chitosan. Chitosan-TBA conjugate (5 mg), insulin (2.75 mg), the permeation mediator reducer glutathione (0.75 mg), and the two inhibitor conjugates (in each case 0.75 mg) were compressed to make chitosan-TBA-insulin tablets. Control tablets were also prepared using chitosan and insulin. Chitosan-TBA-insulin tablets showed a controlled release of insulin over 8 h. In vitro mucoadhesion...
studies showed that the mucoadhesive/cohesive properties of chitosan were at least 60-fold improved by the immobilization of thiol groups on the polymer.

**Microemulsions**

Cho and Flynn (Cho YW et al., 1989) developed water-in-oil microemulsions in which the aqueous phase is insulin and oil phase is lecithin, non-esterified fatty acids and cholesterol in critical proportions. *In vivo* studies showed substantial reduction in blood glucose. Recent studies have focused on enteric-coated dry emulsion formulations prepared from solid-in-oil-in-water emulsions. These responded to changes in external environment suggesting potential application for oral insulin delivery (Torisaka E et al., 2005).

**Oral insulin pills**

Insulin administration in the form of a pill has always been an attractive concept in research. Due to numerous limitations of this mode of insulin administration, efficacy has been hard to demonstrate. Research has focused on overcoming these limitations by stabilising the degradation, improving the permeability, and adding absorption promoters to protect the insulin as it passes through the stomach.

**Oral spray**

An alternative to injected insulin that is currently being explored by researchers is a mouth spray containing insulin that would be absorbed through the lining of the mouth and throat. The liquid formulation allows the insulin to be absorbed by the mucus membranes in the cheeks, tongue, and throat. The benefit from oral spray is identical to an insulin injection in its ability to lower blood glucose levels.

**Pulmonary or inhaled insulin**

The inhaled insulin system delivers a dose of insulin, either in liquid or dry powder form, through the mouth, directly into the lungs, where it enters the blood circulation as rapid-acting insulin. With inhaled insulin, the highly permeable alveolar epithelium and large surface area of the lungs provide an effective, efficient portal for macromolecular delivery.

**Market Status of Oral Insulin Products**

**IN-105 (Biocon, Bangalore)**

Biocon is developing the IN-105 conjugated insulin molecule, administered as a tablet. This oral insulin pill has polymers added at specific locations in the B chain of the insulin to prevent insulin from getting destroyed in the stomach (insulin is made up of two polypeptide chains namely, chain-A with 21 amino acids and chain-B with 30 amino acids, which are held together by two disulfide bonds). Biocon's R&D group has successfully developed a carefully selected formulation to give consistent absorption through the intestines, delivering the glucose-lowering effect. In the clinic, this molecule has completed phase I trials and is expected to enter phase II in India later this year to illustrate proof of concept. The encouraging results of the phase Ia and Ib studies represent a pivotal hurdle crossed in the development of IN-105 as a product. IN-105 will enter phase I trials in Europe towards the end of the year.

**Oral-lyn (Generex Biotechnology, Canada)**

Oral-lyn is the company's proprietary oral insulin spray product. The liquid formulation is absorbed into the body by the lining of the inner mouth using the company's proprietary RapidMist device. Since it is buccally absorbed, no insulin is deposited in the lungs by the Oral-lyn RapidMist. August 2007 saw the commercial launch of Oral-lyn in the Indian market. Generex Biotechnology entered into Master Product Licensing and Distribution Agreement of Oral-lyn with Shreya Life Sciences, the fourth largest distributor of insulin in India. In April 2008, Generex entered into a similar agreement for the distribution of Oral-lyn in China, Hong Kong, and the following additional countries: Indonesia, South Korea, Malaysia, the Philippines, Singapore, Thailand, and Vietnam. Presently, Generex Oral-lyn is in phase III clinical trials at several sites around the world—US, Canada and Ukraine.

**Transgene (Biotek, Andhra Pradesh)**

Transgene has developed an oral delivery technology which combines several oral delivery approaches into a single drug delivery system. Unique in its approach, this technology involves using biodegradable novel polymeric nanoparticles loaded with insulin as a new carrier to ferry the insulin across the intestinal epithelial tissues. Nanoparticles are solid spherical particles with a size range of 10 and 1,000 nm containing dispersed drugs. Transgene has attempted to improve the intestinal absorption of insulin and other peptides. The technology has been well proven in animal models, and human clinical studies are in progress. Drug companies are obviously interested in
the potential of oral insulin to net a massive share of the market, and therefore, investment in research is substantial and ongoing. Biocon, Transgene Biotek and Generex Biotechnology have proven to be insightful in the race to enhance the treatment of diabetes, and are definitely ahead of the pack.

Conclusion
Attempts have been made to achieve oral insulin delivery using various systems. It has been proved that insulin is subjected to acid catalyzed degradation in stomach, luminal degradation in intestine, and intracellular degradation. Scientists have been able to protect the insulin delivery systems from acidic environment of the stomach and target it to the intestine. The maximum bioavailability of the insulin has been reported to be very low because of the poor absorption of insulin from the intestine. Attempts have been made to increase the absorption of insulin from intestine using absorption enhancers such as aprotinin (protease inhibitor), tween, oligoarginine, sodium glycol-cholate, deoxycholic acid, and taurodeoxycholate.

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