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Oral Delivery of Insulin for Treatment of Diabetes: Classical Challenges and Current Opportunities

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Oral dosage form is the most common form of delivery systems due to several benefits such as ease of manufacturing, ease of administration, better formulation stability and patient compliance. Recently many proteins and peptides have been investigated for their usefulness in therapy, but mainly as parenterals due to the associated inherent problems, for instance its rapid degradation, low permeability and absorption in gastrointestinal tract. These problems must be solved or minimized to an extent that would be clinically significant before the oral delivery of proteins and peptides becomes a reality. Among the proteins, oral delivery of insulin has been attempted extensively but without much success so far and yet no formulation with oral delivery of insulin could be marketed. Several approaches have been developed to enhance oral absorption of insulin, such as inhibition of acidic and enzymatic degradation, enhancement of membrane permeability or widening of tight junctions, enhancement of insulin uptake and development of novel insulin carriers. This review article mainly focuses on the classical challenge of oral delivery of insulin and different strategies to overcome the related issues. Moreover, the current drug delivery technologies adopted in an attempt to develop practicable oral insulin have been discussed.

Key words: Diabetes, insulin, oral, protease inhibitor, permeability, absorption, bioavailability

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INTRODUCTION

Diabetes is a group of metabolic disorders of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from a defect in insulin secretion or action or both (American Diabetes Association, 2006). It is a global epidemic with devastating humanitarian, social and economic consequences affecting more than 230 million people worldwide and this figure is expected to rise to 366 million by the year 2030 (WHO., 2005). It is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy) and increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. It is the fourth leading cause of death in most of the developed countries. At least 50% of all people with diabetes are unaware of their condition. In some countries, this figure may be as high as 80%. By the time diabetes is diagnosed, many have already started to develop the complications of diabetes: visual impairment, kidney failure, heart disease, stroke and nerve damage (American Diabetes Association, 2009).

Type 1 diabetes that accounts for 5-10% of cases is characterized by beta cell destruction leading to absolute insulin deficiency. Autoimmune destruction of beta cell has multiple genetic predisposing factors and directly related to the surrounding environment (Wahl *et al*., 1998). Type 2 diabetes is characterized by predominant insulin resistance and relative insulin deficiency or vice versa. This is the most common form of diabetes mellitus forming 90-95% of diabetic cases. It is highly associated with a family history of diabetes, older age, obesity and lack of exercise (American Diabetes Association, 2009; Lisa, 2007).

Keeping in view of devastating consequences associated with diabetes, an extensive effort have been made to overcome this health disorder through insulin administration. So, far practical purposes insulin therapy is the most common and acceptable therapy. However, due to ease of administration and several advantages associated with oral dosage forms, oral delivery of insulin is also being attempted as an alternative but has experienced several technical problem and minimal success. This review article focuses on challenges associated with oral insulin delivery and different strategies to overcome. Moreover, current developments and trends have also been discussed.

INSULIN INJECTION THERAPY: ADVANTAGES AND DISADVANTAGES

For the treatment of diabetes, nothing better than insulin has been discovered as insulin therapy is known to improve insulin receptor sensitivity, reduces glucotoxicity and lipotoxicity (Scarlett *et al*., 1982; Zhao *et al*., 2009). Insulin injection therapy has advantages such as prompt and precise monitoring of blood glucose level as no absorption and other confounding factors are involved. Moreover, it reduces the risk of long-term diabetes complications and produces sustained tight glycaemic control provided that patients start it early and adhered well to the treatment (Turner *et al*., 1999; Li *et al*., 2004; Ryan *et al*., 2004; Weng *et al*., 2008). However, using insulin injections as long-term therapy has several disadvantages such as pain at the site of injection, inconvenience of multiple injections, allergic reactions, hyper-insulinemia and insulin lipodystrophy around the injection site (Gowthamarajan and Kulkarni, 2003; Funnell, 2007; Vardar and Kizilci, 2007). Lipodystrophy is known to reduce further insulin absorption as much as 25% thus compromising glycemic control (Johansson *et al*., 2005). Weight gain and hypoglycemia are other common adverse reactions of insulin therapy (Bott *et al*., 1997; Allen *et al*., 2001). This review article reveals that at least one third of the diabetes patients fail to take their insulin as prescribed or intentionally skip their doses which may be related to adverse effects associated with insulin injections. Fear of insulin or fear of injection is another factor which is known to be associated with reduced compliance and adherence to the treatment and hence poor glycemic control, clinical complications, psychological co-morbidities, poor health status and increased risk of mortality for diabetes patients (Pamnani, 2008).

ORAL DELIVERY OF INSULIN: POSSIBLE ADVANTAGES AND DISADVANTAGES

The oral delivery of drugs is considered as the most acceptable and convenient route of drug administration especially for chronic diseased conditions like diabetes. Orally administered insulin would eliminate side effects, compliance problems to treatment adherence and other complications associated with insulin injection therapy such as pain caused by injection, psychological barriers associated with multiple daily injections such as needle anxiety (Korytkowski, 2002) and possible infections (Lin *et al*., 2007a). Current subcutaneous insulin treatment does not replicate the normal dynamics of endogenous insulin release, resulting in a failure to achieve a lasting glycemic control in patients (Agarwal and Khan, 2001; Morishita *et al*., 2007). Oral insulin delivery would be advantageous because it would be delivered directly to its target organ, liver, through the portal circulation, a mechanism very similar to endogenous insulin. Likely disadvantages of oral delivery of insulin would be slow onset of action and imprecise glycemic control as oral delivery systems usually have large number of excipients and suffer with dose dumping, release burst, variability in absorption and bioavailability due to several confounding factors such as fasting and fed states, concomitant administration and drug-drug interactions.

CLASSICAL CHALLENGES AND CURRENT OPPORTUNITIES TO THE ORAL DELIVERY OF INSULIN

Proteins and peptides including insulin may not be successfully delivered per-oral due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen and poor permeability across intestinal epithelium owing to its high molecular weight and lack of lipophilicity (Fix, 1996; Wang, 1996; Saffran *et al*., 1997). Pepsin, pancreatic proteolytic enzymes such as trypsin and α-chymotrypsin and cytosolic insulin is insulin-degrading enzyme causes degradation of insulin (Patki and Jagasia, 1996; Chang *et al*., 1997). Overall, insulin degrades very quickly by acid and enzymes and absorbs very slowly resulting in very low bioavailability that is clinically insufficient (Shah *et al*., 2002; Morishita and Peppas, 2006).

INSULIN PROTECTION IN GASTROINTESTINAL TRACT BY GASTRIC AND PANCREATIC ENZYME INHIBITORS

Removal of enzyme attack or protection from it may be helpful in successful oral delivery of insulin. For this reason several researcher have investigated enzyme inhibitors and fate of oral insulin. Enzyme inhibitors slow the rate of degradation of insulin, which increases the amount of insulin available for absorption. This review article revealed the use of different protease inhibitors resulting in significant hypoglycemic effects which include aprotinin (Ziv *et al*., 1987; Morishita *et al*., 1993; Laurenti *et al*., 1996; Kimura *et al*., 1996; Morimoto *et al*., 2000; Radwan and Aboul-Enein, 2002; Katayama *et al*., 2003; Cilek *et al*., 2005; Park *et al*., 2007; Jelvehgari *et al*., 2011; Boateng *et al*., 2014) bacitracin (Yamamoto *et al*., 1994; Kimura *et al*., 1996; Bernkop-Schnurch, 1998; Park *et al*., 2007; Su *et al*., 2012; Balabushevich *et al*., 2013; Jose *et al*., 2013) camostat mesilate (Yamamoto *et al*., 1994; Ogiso *et al*., 1997; Tozaki *et al*., 2001; Del Curto *et al*., 2009), leupeptin (Tasaka *et al*., 1989; Liu *et al*., 2003) and diethylene triaminepentaacetic acid (Su *et al*., 2012).

Concurrent administration of protease inhibitors or its incorporation in several drug delivery systems with insulin has resulted in improved absorption, pharmacological activity and bioavailability of oral insulin. However, its use in long-term therapy of diabetes is dubious as several undesired effects such as stimulation of protease secretion, disturbance of digestion of nutritive proteins and absorption of unwanted proteins along with insulin would be unavoidable.

ALTERNATIVE STRATEGIES TO PREVENT INSULIN DEGRADATION IN GUT

Micro encapsulation is a process by which core materials such as solids, liquids or even gases may be enclosed in microscopic particles of polymers or several other substances. Such processes isolate the core from its harmful external environments such as isolating vitamins from the deteriorating effects of oxygen, volatile core from evaporation and isolating a reactive core from chemical attack. The selection of appropriate coating material decides the physical and chemical properties of the final encapsulated product. Generally hydrophilic polymers, hydrophobic polymers or a combination of both are used for the microencapsulation process. The polymer should be capable of forming a film that is cohesive with the core material. It should be chemically compatible, non-reactive with the core material and provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability.

Different techniques may be employed for encapsulation to incorporate reasonably high concentrations of the drug stabile for sufficiently long period to be clinically useful. Degradation of oral insulin by proteolytic enzymes has been minimized by encapsulating it in coatings such as enteric coating (Hosny *et al*., 2002; Qi and Ping, 2004; Li *et al*., 2012) mucoadhesive coatings (Woitiski *et al*., 2011; Deat-Laine *et al*., 2013a; Li *et al*., 2013a).

LOW PERMEABILITY OF INSULIN AND UTILIZING PENETRATION ENHANCERS

Human skin provides a very efficient transport barrier to delivery of protein molecules like insulin, due to their large size and weakly hydrophobic nature. Intestinal permeation enhancement might be employed for oral insulin delivery so as to promote absorption through oral route. Hydrophilic molecules including insulin are adsorbed to the apical membrane and are internalized by endocytosis or via paracellular transport (Agarwal and Khan, 2001). Tight junctions between each of the cells in the epithelium prevent water and aqueous soluble compounds from moving through cells. Hence, approaches for modulating tight-junction permeability to increase paracellular transport have been studied (Salamat-Miller and Johnston, 2005). A number of absorption enhancers are available that may open these tight junctions transiently thus allowing water-soluble proteins to pass. These include substances like bile salts (Yamamoto *et al*., 1992; Uchiyama *et al*., 1999; Degim *et al*., 2004; Lane *et al*., 2005; Lane and Corrigan, 2006) surfactants (Touitou *et al*., 1980; Lane *et al*., 2005; Lane and Corrigan, 2006; Karamanidou *et al*., 2015), cell penetrating peptides (Liang and Yang, 2005; Morishita *et al*., 2007; Kamei *et al*., 2008, 2013; He *et al*., 2013; Nielsen *et al*., 2014; Zhu *et al*., 2014, 2015) Zonula occludens toxin (Fasano and Uzzau, 1997) and chelating agents like EDTA (Yamamoto *et al*., 1992; Uchiyama *et al*., 1999; Li and Deng, 2004).

Employing penetration enhancers or its incorporation in several drug delivery systems has resulted in improved absorption, pharmacological activity and bioavailability of oral insulin. However, its use in long-term therapy of diabetes is

harmful as these are not specific and are relatively toxic which may damage cell membrane. Moreover, absorption of undesired materials such as toxins and pathogens to systemic circulation along with insulin is highly likely which may prove dangerous.

ALTERNATIVE STRATEGIES TO INCREASE INTESTINAL PERMEABILITY OF INSULIN

Encapsulations of insulin in mucoadhesive microspheres or nanoparticles were found to enhance intestinal permeability of insulin. Several authors have reported increased permeation of insulin when encapsulated in Trimethyl chitosan-cysteine conjugate microsphere (Yin *et al*., 2009), thiolated polymethacrylic acid-polyethylene glycol-chitosan based hydrogel microparticles (Sajeesh *et al*., 2010a), multilayered nanoparticles of alginate and dextran sulfate (Woitiski *et al*., 2011), lauroylsulphated chitosan microparticles (Shelma and Sharma, 2013), whey protein and alginate microsphere (Deat-Laine *et al*., 2013b).

Methyl-β-cyclodextrin (MCD) complexed insulin encapsulated in polymethacrylic acid (PMMA) hydrogel microparticles was evaluated for permeability of insulin using Caco-2 cell monolayers and excised intestinal tissue with an Using chamber (Sajeesh *et al*., 2010b). The MCD complexation was found effective in enhancing insulin transport across Caco-2 cell monolayers, when applied in combination with the PMAA hydrogel system.

DRUG DELIVERY SYSTEMS REPORTING ENHANCED ABSORPTION OF ORAL INSULIN

Several drug delivery systems have been developed and evaluated in an attempt to achieve clinically sufficient bio availability of insulin after oral delivery. These systems either protected insulin degradation or enhanced intestinal permeability or uptake of insulin resulting in enhanced absorption and hence bio availability of oral insulin. Some drug delivery systems have combined both the strategies.

Liposomes developed for oral delivery of insulin: Liposomes are phospholipids vesicles with aqueous cavity which are formed when phospholipids are combined with water (Gowthamarajan and Kulkarni, 2003). These phospholipid vesicles can encapsulate both hydrophobic and hydrophilic drug. These are non toxic, non-immunogenic, biocompatible and biodegradable. The drugs encapsulated in liposomes are sufficiently protected from external harsh environment. The lipid bilayer of liposomes releases the drugs entrapped by fusion with other bilayers such as the cell membrane thus making them useful for delivery of poorly soluble and unstable drugs (Vemuri and Rhodes, 1995). This review revealed a huge number of conventional as well as modified liposomes for enhanced absorption and bioavailability of oral insulin (Dapergolas and Gregoriadis, 1976; Rowland and Woodley, 1981; Arrieta-Molero *et al*., 1982; Das *et al*., 1988; Petkowicz *et al*., 1989; Choudhari *et al*., 1994; Takeuchi *et al*., 1996; Kisel *et al*., 2001; Katayama *et al*., 2003; Wu *et al*., 2004; Degim *et al*., 2004; Zhang *et al*., 2005, 2014a, b; Park *et al*., 2011; Manosroi *et al*., 2011; Niu *et al*., 2011, 2012, 2014; Agrawal *et al*., 2014; Cui *et al*., 2015). The hypoglycaemic effect of insulin encapsulated in liposomes has been found to depend on the lipid composition, physical state and number of phospholipid bilayer and surface charge (Choudhari *et al*., 1994).

Physical instability, short shelf-life, low drug loading, leakage of entrapped drug and high production cost make it impractical for commercialization especially for treatment of chronic diseases such as diabetes (Gowthamarajan and Kulkarni, 2003).

Nanoparticles developed for oral delivery of insulin: Nanoparticles are spherical microscopic structures having at least one dimension less than 100 nm which may be either solid (nanospheres) or hollow (nanocapsules). Recently nanoparticles have been proposed as colloidal drug carriers with advantages such as improved bioavailability due to enhanced aqueous solubility, improved drug stability, targeted drug delivery. Thus over all, improved therapeutic index and decreased unwanted effects are the major benefits provided by such colloidal drug delivery carriers (Irving, 2007). The nanoparticle releases the entrapped drug either by controlled diffusion or by erosion from the core across the polymeric membrane (Mohanraj and Chen, 2006). Nanoparticles have advantage over liposomes in term of better physical stability, better drug loading capacity and longer duration of action. These are prepared from natural or synthetic polymers. Natural polymers studied for preparation and evaluation of oral insulin nanoparticles include chitosan (Lin *et al*., 2007b; Sadeghi *et al*., 2008; Rekha and Sharma, 2009; Yin *et al*., 2009; Avadi *et al*., 2010; Su *et al*., 2012; Fonte *et al*., 2012; Chuang *et al*., 2013; Li *et al*., 2013b; Mansourpour *et al*., 2015), alginate (Kadir *et al*., 2013; Wong and Sumiran, 2014), gelatin, albumin (Rieux *et al*., 2006; Woitiski *et al*., 2011) and lectin (Ghilzai, 2003). Synthetic polymers used for nanoparticle formulation include acrylates and its derivatives (Sajeesh and Sharma, 2006; Damge *et al*., 2010; Perera *et al*., 2009; Socha *et al*., 2009), polylactic-co-glycolic acid derivatives (Carino *et al*., 2000; Shi *et al*., 2008; Han *et al*., 2009; Zhu *et al*., 2015). The biological effect of insulin nanoparticles depends on the amount of both insulin and polymer. The nature of polymers strongly influences the nanoparticle size and release profile (Rieux *et al*., 2006).

Polymric nanoparticles have several disadvantages like low stability, low drug carrying capacity, leakage of entrapped drug and toxicity of the residual solvents and surfactants used (Gowthamarajan and Kulkarni, 2003). Solid lipid nanoparticles (SLNs) have been proposed as an alternative

liposomes and polymeric nanoparticles, due to various advantages, such as feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost, ease of scale-up and manufacturing (Muller and Peters, 1998; Kumar, 2000; Mukherjee *et al*., 2009). Several studies of insulin-loaded solid lipid-based nanoparticles have reported to enhance either oral bioavailability or pharmacological activity of oral insulin or both (Wang *et al*., 2009; Zhang *et al*., 2006, 2009, 2012; Sarmento *et al*., 2007; Liu *et al*., 2007; Fonte *et al*., 2012; Ansari *et al*., 2015).

Microemulsions and nanoemulsions investigated for oral delivery of insulin: Microemulsions are isotropic, transparent, thermodynamically stable liquids which are composed of oil, water and amphiphilic compounds like surfactant and co-surfactant (Lawrence and Rees, 2000). As size of the dispersed particles is much smaller than the wavelength of visible light, microemulsions are transparent and their structure cannot be observed through an optical microscope. Microemulsions have attracted increasing attention as potential drug delivery systems and as bioavailability enhancers for poorly water soluble drugs, due to their unique spontaneous energy-less formulation technique and capability of solubilizing drugs. These are known to protect water soluble drug molecules, in particular proteins and peptides from metabolism and to overcome physical barriers. Microemulsions enhance the bioavailability of poorly soluble drugs by maintaining them in molecular dispersion in the gastrointestinal tract and extending the absorption window available in the gastrointestinal lumen. Improved oral delivery of insulin from microemulsion systems have been reported by several authors (Cho and Flynn, 1989; Patel *et al*., 1991; Kraeling and Ritschel, 1992; Watnasirichaikul *et al*., 2000, 2002; Cilek *et al*., 2005; Graf *et al*., 2009; Sharma *et al*., 2010; Karamanidou *et al*., 2015; Rachmawati *et al*., 2015).

Self-microemulsifying drug delivery systems are are "Latent" microemulsions in the form of a stable, water-free combination of surfactants, co-surfactants and lipophilic phase, which creates a microemulsion when diluted in water or body fluids. These are developed to deliver sensitive drugs that would undergo hydrolysis in aqueous formulations. Hydrophobic drugs can also be delivered through these systems for oral administration as drug dispersed in gastrointestinal tract form a fine oil in water emulsion with mild agitations provided by gastric mobility which can subsequently be absorbed by lymphatic pathways (Tang *et al*., 2007; Kohli *et al*., 2010). Several self-microemulsifying or nanoemulsifying drug delivery systems have been developed and evaluated for oral delivery of insulin (Singnurkar and Gidwani, 2008; Sakloetsakun *et al*., 2013; Li *et al*., 2013a, 2014). These carriers have several demerits like low drug loading capacity and drug precipitation upon dilution.

Hydrogels and mucoadhesive drug delivery systems developed for oral delivery of insulin: Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. Hydrogels absorb large amounts of water and swell, while maintaining their three-dimensional structure. Hydrogels can be made from virtually any water-soluble polymer. The unique physical properties of hydrogels have sparked particular interest in their use in drug delivery applications. Their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network (Hoare and Kohane, 2008).

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 (Kavimandan *et al*., 2006; Nakamura *et al*., 2004). Hydrogels with improved delivery of oral insulin have been prepared by utilizing acrylates and their derivatives (Ichikawa and Peppas, 2003; Wood *et al*., 2010; Yin *et al*., 2010; Sajeesh *et al*., 2010a; Sonia and Sharma, 2013) and alginates (Woitiski *et al*., 2011; Deat-Laine *et al*., 2013a).

Hydrogels have several limitations such as non-homogeneity of drug loading, low tensile strength, low load-bearing, premature dissolution due to high water content and large pore sizes, flow away of the hydrogel from a target site (Hoare and Kohane, 2008).

Bio adhesive or mucoadhesive drug delivery systems are developed by incorporation of adhesive molecules into some kind of pharmaceutical formulation intended to stay in close contact with the absorption tissue, releasing the drug near to the action site, thereby increasing its bioavailability and promoting local or systemic effects (Woodley, 2001). Mucoadhesive drug delivery systems adhere to the mucous gel layer covering mucosal membranes establishing a high concentration gradient across intestinal epithelium leading to enhanced absorption of drugs (Ahuja *et al*., 1997; Andrews *et al*., 2009). Mucoadhesive delivery systems for improved insulin absorption includes chitosan (Pan *et al*., 2002; Rekha and Sharma, 2009, 2015; Fonte *et al*., 2012; Shelma and Sharma, 2013), acrylates (Whitehead *et al*., 2004; Sonia and Sharma, 2013) sodium salicylate and polyoxyethylene-9-lauryl ether (Hosny *et al*., 2001) have been proposed. The bioadhesive systems may however be affected by the mucous turnover of the GIT, which varies based on the site of absorption (Plate *et al*., 2002; Gowthamarajan and Kulkarni, 2003; Morishita and Peppas, 2006).

CONCLUSION

The development of drug delivery systems for oral administration of insulin continues to be pursued actively in academic institutions and pharmaceutical research centers. However, without much success so far, as no technique was able to deliver insulin orally with sufficient bioavailability.

The different approaches have been used to reduce the enzymatic degradation of insulin and to increase its uptake or permeability across intestine so as to enhance the oral absorption. However, each approach has its own advantage and disadvantages. Formulations of insulin with protease inhibitors have typically shown inconsistent results. Penetration enhancers are not specific hence cause toxic consequences. Surfactants cause lysis of mucous membrane and may damage the lining of the gastrointestinal tract. Chelators such as EDTA cause depletion of calcium ions, which may be dangerous for the cell membrane. Release of insulin from colloidal carriers is inconsistent and slow causing slow and insufficient absorption. The hydrogels and mucoadhesive systems are affected by the mucous turnover of the gastrointestinal tract showing high variability in results. Despite of extensive research being perused for the sake of developing oral insulin, it has not yet been possible to come up with an efficient delivery system which could provide clinically significant bioavailability of oral insulin. An oral delivery system of insulin if developed successfully would have several advantages such as better control of diabetes, better compliance on insulin treatment and avoidance of side effects associated with long term injection therapy of insulin.

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