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[Review article]

Nanotechnology based Oral Delivery of Insulin – A Retrospect

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ABSTRACT

Nanotechnology provides novel innovative means to detect, diagnose, and treat disease. In this regard, numerous nanoparticles-based approaches have been taken in an effort to develop an effective oral insulin therapy to treat diabetes. This paper gives the clear information on formulation of solid oral dosage form of insulin using different types of polymers in Nanotechnology platform. In non-invasive therapy for Diabetes Mellitus oral delivery is still a exciting job in Novel drug delivery system, Since degradation of insulin due to presence of enzymes, pH changes in the gastrointestinal tract and absorption through the GI mucosa is doubtful. In oral delivery Nanotechnology based insulin formulations which improves the bioavailability, absorption and protect the insulin from enzymatic degradation. In nanotechnology based oral insulin drug delivery with high bioavailability, various practical approaches might be most helpful like protecting insulin from enzymatic degradation, use of penetration enhancers, chemical modification, Bioadhesive delivery system, use of nanoparticales to improve bioavailability of insulin. Despite, various techniques each having its own limitation and advantages, the oral route scores over the others for the ease of comfort with which the therapeutic agents can be administered to the patients work on attempts to deliver insulin orally has definitely gathered momentum and is no longer considered with pessimism to develop the oral insulin drug delivery system.

KEYWORDS: Nanotechnology, Oral insulin, Polymers for nanotechnology.

INTRODUCTION

Resistance to injectable insulin has been identified as a major reason for clinical inertia and lack of achievement of target glycemic goals. Physicians as well as patients fear the complexity of insulin regimes, the risk of hypoglycemia, and the chances of weight gain, as well as the necessity of a needle prick, with insulin therapy. Insulin is perceived to have a high index of intrusion as the conventional insulins need to be given prior to meals .Patients anticipate the early development of an oral insulin, as it will be easy to administer, have a lower index of intrusion, be more convenient, and have more compliance or adherence from the patient, and finally lead to better glycemic control, and thus, prevention of complications of diabetes. Oral insulin may improve β-cell function by providing β-cell rest , and may help in preventing diabetes via induction of 'oral tolerance' or immuno modulation .Oral insulin is able to achieve a high porto-systemic gradient, as it is delivered to the liver from the gastrointestinal tract. This reduces systemic insulin exposure and may obviate the excessive weight gain sometimes seen with

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subcutaneous insulin.Oral insulin may also be able to correct the blunting of first-phase release of insulin, which is difficult with conventional subcutaneous insulins.

Challenges to Oral Insulin Delivery

The rapid enzymatic degradation in stomach, deactivation and digestion with the influence of proteolytic enzyme in the lumen of intestine and poor permeability across the intestinal epithelium due to high molecular weight with lack of lipophilicity are the major hurdle in designing the Insulin based oral formulations [1,2,3].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% [4].

Enzymatic Barrier [11]

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. Insulin therefore undergoes enzymatic degradation by pepsin and pancreatic proteolytic enzymes such as trypsin and α-chymotrypsin. 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). Insulin is however not subject to proteolytic breakdown by brush border enzymes[5]. Insulin can be presented for absorption only if the enzyme attack is either reduced or defeated.

Intestinal Transport of Insulin

The major hurdle in the absorption of hydrophilic insulin is that cannot diffused easily across epithelial cell membrane through lipid-bilayer cell membranes to the blood stream [6]. It is clearly known that insulin delivery to the mid-jejunum protects insulin from gastric and pancreatic enzymes. The amount of drug release is enhanced by intestinal microflora [7].

Dosage form stability

During the formulation development, proteins can undergo to physical or chemical degradation. The three dimensional structure plays a vital role in pharmacological and therapeutic activity.

During the physical and chemical degradation modification of the native structure, bond cleavage can occur this may leads to formation of novel product. If a protein needs to be survive transit through the stomach and intestine, knowledge and assessment of stability parameters during formulation processing is of utmost importance

Attempted Oral Insulin Delivery Systems

To improve the bioavailability of the insulin based oral formulation, modification of physicochemical properties such as lipophilcity and enzyme susceptibility of the peptide is necessary. Addition of novel function to macromolecules with the use of improved carrier systems are required.

Enzyme Inhibitors

Rate of degradation of Insulin depends on the enzyme inhibitors which is added which leads to better absorption. The earliest studies involving enzyme inhibitors were carried out with sodiumcholate along with aprotinin which improved insulin absorption in rats [8]. Significant hypoglycemic effects were also obtained following large intestinal administration of insulin with camostat mesilate, bacitracin. Other inhibitors which have shown promise are leupeptin, FK-448, a potent and specific inhibitor of chymotrypsin and chicken and duck ovomucoid. In one study, polymers cross-linked with azoaromatic groups formed an impervious film to protect insulin from digestion in the stomach and small intestine. Upon reaching the large intestine, the indigenous microflora degraded the polymer film, thereby releasing the drug into the lumen of the colon for absorption. 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.

Penetration Enhancers

The best strategy for oral insulin delivery is to promote absorption through the intestinal epithelium by permeation enhancement. Hydrophilic molecules like insulin are adsorbed to the apical membrane and are internalized by endocytosis. 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.

Various types of absorption enhancers are used to formulate the oral insulin which causes the tight junctions to open transiently and allows water-soluble proteins to pass.

Substances like bile salts, surfactants, trisodium citrates, chelating agents like EDTA, labrasol. Insulin transport across Caco-2 cells was shown to be dramatically increased by conjugation of insulin with TAT, a cell penetrating peptide (CPP).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, and risk to mucous membranes by surfactants and damage of cell membrane by chelators. Mucoadhesive polymers have been proven to be safe and efficient intestinal permeation enhancers for the absorption of protein drugs. The zonula occludens toxin, chitosan, thiolated polymers, and Pz-peptide have all demonstrated capacity to increase macromolecular drug absorption.

Combinational strategies involving

Enzyme inhibitors and absorption enhancers have been effective in increasing bioavailability of insulin. Combinations like sodium cholate and soybean trypsin inhibitor, sodium lauryl sulphate and aprotinin have resulted in reduction in blood glucose in dogs.

Approaches explored to overcome the obstacles

To improve the bioavailability of insulin, different approaches have been explored, including chemical modification, co-administration with absorption enhancers and/or enzyme inhibitors and incorporation into carriers, such as polymer based nanoparticles drug delivery system

Nanocarriers

These are carriers with a particle size of less than 1000 nm. Nanocarriers have received more attention recently due to their submicron size and their large specific surface area, both of which favour their absorption compared to larger carriers.

Types

Nanocarriers are categorized into: polymeric nanoparticles, nanovesicles and solid lipid nanoparticles. There are two types of polymeric nanoparticles: the matrix particles termed ‘nanospheres’ and the reservoir-type named ‘nanocapsules’. Vesicles have a hydrophilic core and hydrophobic bilayers.

Conventionally, liposomal vesicles were

Polymeric nanovesicles

Poly(lactic acid)-b-Pluronic-b-poly(lactic acid) block copolymers were synthesized [9]. This amphiphilic block copolymer aggregates in an aqueous solution to form vesicular nanoparticles. The oral administration of insulin-loaded vesicles to diabetic mice resulted in the reduction of blood glucose levels - 25% of the initial glucose level - which was maintained at this level for an additional 18.5 h [21].

Solid Lipid Nanoparticles (SLN)

Preparation of insulin-loaded cetyl palmitate solid lipid nanoparticles and it is demonstrated for its potential to deliver insulin orally. The drug loading capacity in solid lipid nanoparticles was improved by enhancing insulin liposolubility. Insulin was solubilized into mixed reverse micelles of sodium cholate and soybean phosphatidylcholine and transformed into SLN using a novel reverse micelle-double emulsion technique. Stearic acid and palmatic acid were used as a biocompatible lipid matrix [10]. The surface of the nanoparticles was modified by chitosan to enhance their penetration through GIT. In addition, chitosan was able to provide stealth properties to SLN, resulting in the absence of phagocytosis. Pharmacological availability values of 5.1–8.3% for SLN and 17.7% for chitosan-coated SLN were reported [11]. Lectins are proteins that bind sugar reversibly and are involved in many cell recognition and adhesion processes. They have been extensively adopted to target both absorptive enterocytes and M cells [12]. Wheat germ agglutinin binds (WGA) specifically to cell membranes and is taken up into cells by receptor-mediated endocytosis [13]. Zhang et al. utilized the advantages of WGA and formulated SLN modified with WGA to enhance the oral delivery of insulin. Insulin-loaded SLNs or WGA modified SLNs were administered orally to rats and elicited relative pharmacological bioavailability values of 4.46% and 6.08% and relative bioavailability values of 4.99% and 7.11%, respectively, in comparison with the subcutaneous injection of insulin.

Polymers used for the fabrication of insulin nanoparticles.

Both synthetic and natural polymers were investigated for the production of nanosystems. These polymers may be used alone or in combination to develop nanoparticles. Methods from the first category include: emulsification/solvent evaporation, solvent displacement and interfacial deposition, emulsification/solvent diffusion, salting out with synthetic polymers, ionotropic gelation, coacervation and polyelectrolyte complexation. Meanwhile, the methods of the second category are: emulsion polymerization, interfacial polymerization and interfacial polycondensation . These methods were thoroughly discussed by Reis et al.[14].

PLGA: The administration of insulin-loaded PLGA

nanoparticles for diabetes mellitus induced a rapid decrease in blood glucose levels for up to 24 h and increased insulin levels. The loading capacity was 78.35%. To facilitate loading efficiency, the lipophilicity of the insulin was increased by complexation with sodium lauryl sulphate or sodium oleate. Insulin encapsulation efficiency reached up to 90%. [15]. Mucoadhesive PLGA nanoparticles were prepared to enhance the oral bioavailability of the negatively charged PLGA nanoparticles.

Polylactides

Polylactides are more hydrophobic they degrade more slowly due to their crystallinity [16]. Enhanced insulin entrapment efficiency (up to 90%) in PLA nanoparticles, where insulin was complexed with phosphatidylcholine (SPC) to improve its liposolubility. An oral bioavailability of 7.7% relative to subcutaneous injection was obtained.

Poly ϵ caprolactone

PCL is semi-crystalline in appearance with greater viscoelastic property. PCL produce less acidic environment during degradation. The hydrophobic nature of PCL affects the encapsulation of the substances which are hydrophilic, such as peptides, enzymes and other proteins. Nanoparticles from a blend of a biodegradable polyester poly (ε-caprolactone) and a polycationic non-biodegradable acrylic polymer (Eudragit® RS). These nanoparticles were investigated as a carrier for the oral administration of insulin and demonstrated prolonged hypoglycaemic effect of insulin in both diabetic and normal rats.

Palkylcyanoacrylate

Insulin can be encapsulated by using polyalkyl cyanoacrylate emulsion or interfacial polymerization. Damge et al.[17] prepared an insulin-loaded poly (alkyl cyanoacrylate) nanocapsule. The oral administration of nanocapsules dispersed in Miglyol 812 to diabetic rats resulted in a 50% reduction of initial glucose levels from the second hour for up to 10-13 days. This effect was shorter (2 days) or absent when the nanocapsules were dispersed in water, whether with surface active agents or not.

Poly (Acrylic) acid

Anionic polymers, such as methyl acrylic acid (Eudragit L-100) and methyl methacrylate (S-100), have been used to formulate pH sensitive nanocarriers. Poly methacrylic acid– chitosan–polyethylene glycol nanoparticles were developed by Pawar et al. for the oral delivery of insulin. These nanoparticles displayed excellent binding efficiency on mucin from porcine stomach and elicited pH dependent release profiles in vitro [18]

Dextran

It is a nontoxic, highly water-soluble, biodegradable and biocompatible branched negatively charged polyion. A nanoparticle insulin delivery system was prepared by the polyelectrolyte complexation of oppositely charged natural polymers - dextran sulphate and chitosan in an aqueous solution. These pH sensitive nanoparticles released insulin in the intestinal medium [19]. The natural uptake processes of the intestine were utilized for the oral delivery of peptides and proteins. Vitamin B12 is an example of such carriers and was investigated for delivering different peptides [20]. Due to the susceptibility of vitamin B12/peptide conjugate to gastrointestinal degradation, dextran nanoparticles were coated with vitamin B12 and used as a carrier for the oral delivery of insulin [21]. These nanoparticles were found to be targeted at the systemic circulation through vitamin B12-intrinsic factor receptor ligand-mediated endocytosis via ileocytes of the intestine. The % pharmacological availability of nanoparticle conjugates containing 2, 3 and 4%

w/w insulin was 1.1, 1.9 and 2.6 times higher, respectively, compared with nanoparticles without VB12.

Alginate

Alginate is a naturally occurring polysaccharide obtained from marine brown algae. It is a linear copolymer composed of 1,4-linked-β-D-mannuronic acid and α-L-guluronic acid residues that gel in the presence of divalent cations. It is a nontoxic and biodegradable polyanion that forms polyelectrolyte complexes with polycations, such as chitosan. Insulin loaded nanoparticles were prepared by the ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan [22]. The pharmacological effect of insulin-loaded nanoparticles was evaluated in diabetic rats. The pharmacological availability was 6.8% and 3.4% for the 50 and 100 IU/kg doses, respectively [23] .

Alginate/chitosan nanoparticles form complexes with cationic β-cyclodextrin polymers. The nanoparticles protect insulin against degradation in simulated gastric fluid . Nanoparticle systems composed of alginate/chitosan cores coated with chitosan-polyethylene glycol-albumin. Protease attack on Insulin can be prevented by adding albumin and mucoadhesive property was improved by chitosan, while PEG served as a nanosphere stabilizer to improve the half-live of the insulin and increase the residence time along the intestine. Chitosan-PEG-albumin coated nanospheres demonstrated a more than 70% blood glucose reduction, increased insulinemia.

In contrast, nanospheres lacking albumin and PEG in the coating material were ineffective. Multilayer nanoparticles consisting of calcium cross-linked alginate, dextran sulphate, poloxamer 188, chitosan and an outermost coating of albumin were developed.

Chitosan

In ionotropic gelation method with Tripoly phosphate or polyelectrolyte between insulin and chitosan leads to formation of nanoparticles with better quality. The chitosan polyanion interaction leads to the spontaneous formation of nanoparticles without using the organic solvent or supplying the heat. A high level of drug entrapment is achieved with a good maintainance of protein structure and biological activity. In streptozotocin-induced diabetic rats nanoparticles were effective at lowering the serum glucose level at the dose of 50 U/kg and/or 100 U/kg. Chitosan based nanoparticles are coated with hydroxypropyl methylcellulose phthalate (HPMCP), in order to overcome the problem of intestinal degradation. HPMCP-chitosan nanoparticles showed a 2.8-fold increase in their hypoglycaemic effect when compared with chitosan nanoparticles without HPMCP [24].

The problem of the low solubility of chitosan in the neutral environment of the intestine was solved by synthesis of a partially quaternized derivative of chitosan - Trimethyl chitosan (TMC). TMC has good solubility and a permeation enhancing effect [25]. The significant internalization of insulin via clathrinand caveolae-mediated endocytosis on goblet cell-like HT29-MTX cells results in a better hypoglycaemic effect with a 1.5-fold higher relative bioavailability compared with unmodified TMC nanoparticles [26].

Mechanisms of the absorption of nanoparticles

The absorption of the nanoparticles was thoroughly reviewed by des Rieux [27]. A particle can traverse the intestinal epithelium by the paracellular (between cells) or transcellular route (through the cells). The transcellular route is the most common. With the transcellular transport of nanoparticles, the particles are taken up by cells through the endocytic process - which takes place at the cell apical membrane - transported through the cells and released at the basolateral pole. Two types of intestinal cells are important in nanoparticle transcytosis: the enterocytes lining the gastrointestinal tract and the M cells mainly located in Peyer's patches. The uptake of nanoparticles takes place by one of three endocytotic mechanisms: pinocytosis, macropinocytosis or clathrin-mediated endocytosis. Clathrin vesicles are for particles smaller than 150 nm while phagocytosis is for particulate matters of up to several μm. The uptake of particles, microorganisms and macromolecules by M cells occurs by fluid phase endocytosis, adsorptive endocytosis and phagocytosis.

Conclusion

Oral delivery of Insulin gives tremendous benefit in drug delivery technology platform, in which injections for diabetic patients can be stopped. Nanotechnology contributes to the success of oral

insulin delivery. Now a days researchers from both academic and industrial field work on oral Insulin.

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