Clinical and Experimental Health Sciences

Review / *Derleme*

Nanocarriers: Novel Approaches to Oral Delivery of Insulin

Nanotaşıyıcılar: Oral İnsülin Tedavisine Yeni Yaklaşımlar

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Cite this article as: Özer S, Kerimoğlu O, Uğurlu T. Nanocarriers: Novel Approaches to Oral Delivery of Insulin. Clin Exp Health Sci 2017: DOI: 10.5152/clinexphealthsci.2017; 7: 115-22

Abstract

Diabetes is among the major chronic diseases at present, and no medication has been developed that can replace the roles of endogenous insulin, especially for type 1 diabetes patients. However, insulin can be frequently administered by the subcutaneous route as a protein macromolecule because enzymatic and absorption-associated problems. It leads to immunogenic symptoms, adipose tissue complaints such as lipodystrophy, and hyperinsulinemia risks because of pharmacokinetic properties that do exactly overlap with those of endogenous insulin. In a remarkable number of patients, failure to attain permanent glycemic control by subcutaneous insulin treatment has shown by clinical trials based on noncompliance. Oral drug administration has always been the most preferred administration pathway for drugs with high patient compliance and convenience. Difficulties in the use of subcutaneous insulin have prompted scientists to find solutions for the oral administration of insulin. Similar to many other fields, nanotechnology has recently come to the fore in the pharmaceutical field. Compared with conventional systems, nanopharmaceuticals are drug delivery systems that enable promoted absorption, protection of the active ingredient from the external environment, lower dose applications, higher bioavailability, controlled release, and prolonged residence time. In vitro and in vivo studies have been performed with varied nanopharmaceutical systems in order to administer insulin orally for this purpose.

Keywords: Nanosystems, insulin, oral delivery, diabetes, bioavailability, oral peptides

Öz

Dünya üzerinde en fazla üyeye sahip kronik hastalıklardan biri olan diyabet için, özellikle tip 1 diyabetli hastalarda, vücutta üretilen insülinin görevlerini tam olarak karşılayabilen tedavi yöntemi henüz bulunamamıştır. Peptid yapılı bir makromolekül olan insülin, farklı uygulama yollarıyla verildiğinde enzimatik bozunmaya uğraması veya yeterince emilememesi sebebiyle çoğunlukla subkutan olarak uygulanır. Ancak rutin olarak subkutan ilaç uygulamak hastalarda birtakım immünolojik problemlere, yağ dokusu bozulduğundan lipodistrofi şikayetlerine sebep olabilmektedir. Ayrıca doğal olarak üretilen insülinle farmakokinetik özellikleri tam olarak uyuşmadığından hiperinsülinemi görülebilmektedir. Son yıllarda yapılan klinik çalışmalara göre hatırı sayılır oranda diyabet hastası insulinin enjeksiyonla yapılan tedavisine uyunç sağlayamadığından uzun vadede glisemik kontrol de sağlanamamaktadır. Oral dozaj şekilleri ile ilaç tedavisi uyunç ve uygulama kolaylığı sebebiyle en fazla tercih edilen sistemler olduğundan, subkutan insülin tedavisinin zorluklarına alternatif olarak oral insülin uygulanmasının yolu açılmaya çalışılmış ve formülasyonun uygulanması için öne çıkan problemlere çözüm aranmaya başlanmıştır. Nanoteknoloji, pek çok alanda olduğu gibi ilaç teknolojisinde de dikkat çekmeye başlamıştır. Nanofarmasötik sistemler geleneksel ilaçlara göre absorpsiyonu ve etkin madde için koruyuculuğu yüksek, terapötik seviyeye ulaşmak için gerekli doz miktarını azaltan, biyoyararlanımı artıran, kontrollü salım ve hedeflendirme yapabilme avantajlarını sağlayan, ilacın etki süresini azaltan sistemlerdir. Tüm bu avantajlar düşünüldüğünde, insülinin oral yoldan verilebilmesi için nanofarmasötiklerin uygunluğu *in vitro* ve *in vivo* deneylerle araştırılmaktadır.

Anahtar Kelimeler: Nano sistemler, insülin, oral uygulama, diyabet, biyoyararlanım, oral peptidler

INTRODUCTION

Negative results of the first study on orally administered insulin in diabetic patients indicated the challenges of oral delivery of a protein drug: poorer and more variable absorption and lower efficacy than those of subcutaneous injection. Over the past 2 decades, although oral delivery trials of macromolecules have been increasing in popularity, delivering substrates with a high molecular weight (MW) orally remains challenging (1, 2).

The quality of life of a diabetic patient who is treated with routine subcutaneous insulin may be significantly improved by alteration with oral delivery. In a remarkable number of patients, failure to attain permanent glycemic control by subcutaneous insulin treatment has been shown by clinical trials based on noncompliance (3). Oral insulin delivery offers many advantages over subcutaneous insulin delivery, such as better compliance, prevention of peripheral hyperinsulinemia, faster hepatic insulinization, avoidance of both hypoglycemia and weight gain. However, the oral absorption of insulin is limited (4).

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Received/Geliş Tarihi: 21.09.2016 **Accepted/Kabul Tarihi:** 21.01.2017 **Available Online Date/Çevrimiçi Yayın Tarihi:** 22.05.2017 **DOI:** 10.5152/clinexphealthsci.2017.143

DIABETES MELLITUS

Diabetes is a Greek word meaning "a compass" or "a siphon," and mellitus is a Latin word meaning "sweetened with honey" or "honey-sweet" (5). These 2 words refer to excessive glucose concentration in the blood and urine of diabetic patients. To date, almost 366 million diabetic patients have been diagnosed, and the number continues to increase. Besides other types of diabetes such as gestational, MODY type, or other specific types, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are frequently prevalent. T1DM is characterized with ß-cell destruction, resulting in absolute insulin deficiency, either idiopathic or autoimmune, and it is the most common chronic disease in childhood and is predominantly seen in people below 30 years of age. T2DM is associated with insulin secretion disorder of ß cells and insulin resistance, which is defined as the deterioration of normal biological response to exogenous and endogenous insulin, and many resultants can be regarded, such as genetic factors, cellular receptor mechanism, low birth weight, obesity, lifestyle, glucotoxicity, lipotoxicity, islet amyloid, adiposyte products, and inflammation (6).

Regular exercise, healthy diet, avoidance of excess stress, and tobacco are the main lifestyle modifications as the cornerstone of diabetes management (6). Insulin therapy is the primary approach for pharmacological management, particularly in patients with T1DM. Oral antidiabetics, however, may be essential for pharmacological management. These drugs are divided into several groups according to their mechanisms as secretogogues (sulfonylureas and meglitinides), insulin sensitizers (biguanides and thiazolidinediones), and α-glucosidase inhibitors (miglitol and acarbose) (7). Meanwhile, new agents for T2DM are under development.

INSULIN

After the discovery of isolated insulin in the 1920s and the beginning of clinical use, DM has ceased to be a fatal disease. Three new fields have been studied because of the importance of glycemic control in DM: the development of insulin analogs by the modification of insulin molecules to regulate the pharmacokinetic properties of subcutaneous insulin, transplantation of ß cells to the patient to provide endogenous insulin, and trials of different insulin delivery routes to regulate nonphysiological pharmacokinetics of subcutaneous insulin (6).

Insulin Structure

Human insulin consists of 51 amino acid residues. As illustrated in Figure 1, there are 2 polypeptide chains named as A and B, and the chains are linked to 2 disulfide bridges. The A chain contains 21 amino acids and carries an additional disulfide loop between the 6th and 11th amino acid residues. However, the B chain is longer and contains 30 amino acid residues. Primary structures of 50 different insulins from diverse animals have recognized so far. Despite variations in primary structures, the 3-dimensional conformations of all insulin molecules are essentially the same for different species. The A chain forms 2 almost antiparallel α-helices, from A2 to A8 and from A13 to A20. The B chain forms a single α-helix from B9 to B19, followed by a turn and a ß strand from B21 to B30 (8).

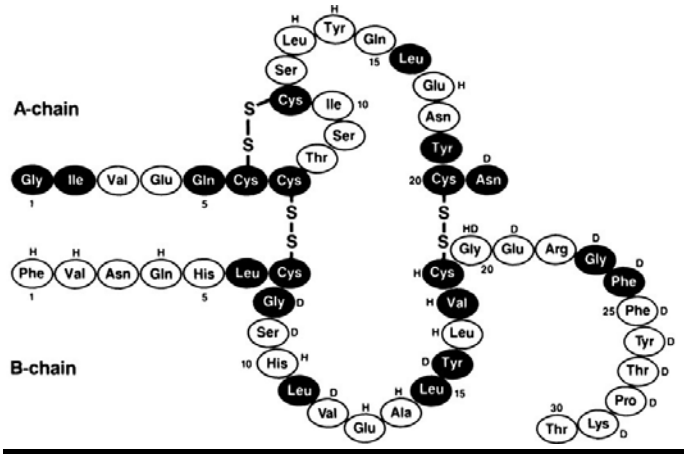

Figure 1. The primary structure of human insulin. The black residues designate the amino acids that are invariant among insulin species. The letters indicate the residues involved in the association of the molecule: D, dimer formation; H, hexamer formation. Porcine insulin only differs in position B30 (Ala instead of Thr). Bovine insulin has the same substitution and contains Ala in position A8 and valine in position A10 (8)

CLINICAL AND RESEARCH CONSEQUENCES

Pharmacokinetics of Insulin

Insulin administered by the subcutaneous route is directly absorbed into the bloodstream. The absorption rate is not constant, and the time for absorption of 50% of the insulin dose administered ($t_{\%50}$) may vary between individuals. This variability is generally a result of the diversity of the blood flow at the injection site. The absorption rate changes depending on the local blood flow in subcutaneous tissue; thus, the factors that increase blood flow will increase the absorption rate as well. Insulin added into the bloodstream is either free or in the form of antibody, as linked to IgG. The presence of insulin antibodies postpone the start of insulin activity, reduces the peak plasma concentration of free insulin in the blood, and prolongs the biological half-life of insulin (9). The kidneys and liver are the main organs responsible for the degradation of insulin. Under normal conditions, endogenous insulin released from pancreatic ß cells is rapidly metabolized mainly in the liver (60%) by the enzyme named glutathione insulin dehydrogenase and to a lesser extent in the kidneys and muscle tissue (9, 10). However injected insulin cannot directly enter the portal vein; therefore, the degradation and metabolism profile changes. The kidneys play the most important role in the degradation of insulin delivered subcutaneously (60%). Thus, in patients with renal disorders, insulin clearance decreases while the action time increases (9).

ORAL INSULIN DELIVERY

Pharmacokinetics of conventional subcutaneous insulin preparations cannot efficiently mimic natural the basal insulin secretion model; thus, subcutaneous insulin poses problems with regard to glycemic control (6). Physiological insulin that is secreted by the pancreas enters portal circulation and inhibits hepatic glucose production. The plasma insulin level in portal circulation is naturally twice that in peripheral circulation. The lower insulin level in portal circulation causes more glucose usage and the absence of hepatic glucose production, which causes the appearance of physiological hypoglycemia. On the other hand, subcutaneous insulin administration near-

ly equalizes the insulin levels in both plasma and portal circulation. Insulin has the hypoglycemia potential as a result of its action on peripheral tissues. Oral delivery of insulin can mimic the physiological fate of insulin and may provide better glucose homeostasis. Further, by reducing peripheral hyperinsulinemia, other relevant complications can be hindered (11).

The absorption of polypeptide drugs following oral administration is substantially precluded owing to high metabolic activity and inadequate penetration across the epithelial membrane of the intestine (12). This barrier presents new pursuits for polymers to deliver proteins orally. Proper polymers have been developed for oral insulin (13). Meanwhile, when insulin, which is a known mitogen, is administered orally, the risk of increasing the incidence of many cancer types, particularly colon cancer, constitutes a serious concern. Even if insulin is not a toxic compound, long-term toxicological and clinical studies of formulations should be completed for chemical contents, which are added as absorption enhancers or excipients. Besides, the absorption rate may be affected by diet. Thus, foods can specify how and when to administer oral insulin (14).

Barriers to Oral Insulin

Most polypeptide drugs are sensitive to pH variety in GIT, and pH variation results in pH-induced oxidation, deamidation, and hydrolysis reactions which constitute chemical barriers to absorption (15).

Gastrointestinal (GI) proteases digest 94%-98% of proteins taken orally and disintegrate them into peptide fragments and amino acids and reduce bioavailability. Even if a significant amount of insulin remains absorbed across the intestinal epithelium, the dose will be reduced by microsomal enzymes in the first pass effect (15, 16).

There are other barriers related to the absorption process as well. The mucus layer is the first barrier for such drugs. It is semipermeable; thus, after oral administration, the drug must infiltrate across the mucus layer to reach the surface of the intestinal epithelium. Meanwhile, the negative charge of the mucus layer results in an electronic repulsion between the layer and protein drug, which impedes close contact between absorptive epithelial cells and such drugs. Here, the intestinal epithelium plays an active role as the second barrier. Cell membranes mainly consist of phospholipid bilayers; therefore, absorption of lipophilic drugs via passive diffusion is quite limited, especially in case of drugs with MW below 700 Da. However, most protein drugs have a hydrophilic character and MW above 3000 Da, which is why it is difficult for them to traverse the cell membranes. Transport across the intestinal epithelium via the paracellular route is minimal due to tight junctions (TJ) between neighbouring cells (15). TJ or zonula occludens (ZO) are the first and the least permeable membrane for paracellular transport of proteins formed by several transmembrane proteins, and they connect intercellularly to one another between cells in the extracellular space. Adherens junctions, desmosomes, and gap junctions are another important barriers with less restricting features in the epithelial cell layer (Figure 2) (17).

Efflux systems such as P-glycoprotein (P-gp) may contribute to the poor availability of peptide drugs as well. P-gp is localized in the apical domain and actively pumps compounds from within the cell back into the intestinal lumen. Many clinical agents with diverse structures and functions have been found to be substrates, inhibitors, or inducers of P-gp (18).

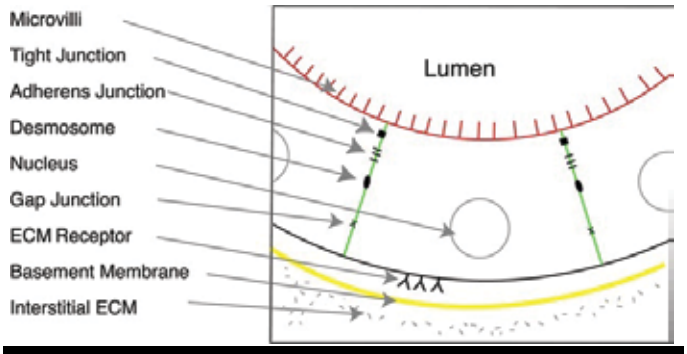

Figure 2. Epithelial cell organization in the context of a cyst (50)
ECM: extracelular matrix

The activity of proteins depends on the 3-dimensional molecular structure. During dosage form development, proteins may be degraded by chemical or physical factors. Physical degradation involves the modification of the native structure to a higher order structure, while chemical degradation involves bond cleavage and results in the formation of a new product (19).

Strategies to Overcome the Barriers

Successful oral delivery of insulin involves overcoming the barriers of enzymatic degradation, achieving epithelial permeability, and taking steps to conserve bioactivity during formulation processing; thus, many strategies have been attempted to overcome these barriers.

Enzyme inhibitors are being widely used to protect peptides against enzymatic degradation. Pancreatic inhibitor, soybean trypsin inhibitor, FK-448, camostat esylate, aprotinin, 1,10 phenanthroline, p-chloromercuribenzoate, and bacitracin are several inhibitors than can enhance bioavailability (11).

Permeation enhancers are another approach and are divided into several categories. Calcium chelators are stirring the cells up to extensive changes by inducing calcium depletion, e.g., decreased cell adhesion and disruption of actin filaments and/or adherent junctions. Surfactants act by their exfoliant properties on the intestinal epithelium by compromising its barrier functions. Sodium laurate and cetyl alcohol, sodium cholate, ethylenediaminetetraacetic acid (EDTA), ZO toxin (ZOT), cyclodextrin, dextran sulfate, azone, esters, crown ethers, sucrose esters, and phosphotidyl choline are other absorption enhancers usable for the oral delivery of proteins and peptides (11, 20).

The prodrug approach, structural modifications, and peptidomimetics, targeting the membrane transporters, receptors, and tissues, are the basic chemical modification approaches for protein drugs (18, 20). The use of the salt form of peptides or the covalent attachment of hydrophilic polymers to peptides can increase solubility and paracellular transport. Attractive hydrophilic polymers such as N-(2-hydroxypropyl) methacrylamide and polyethylene glycol (PEG) can be used to formulate polymer conjugates (17, 20). Peptides such as desmopressin, leucine encephalin analog, and insulin have been modified by certain fatty acids in various studies, and formulations have been achieved with a longer plasma half-life, better resistance to enzymes, and enhanced permeability by opening up the tight junctions or hybridizing with target molecules (17, 21).

Conjugating the peptide to small molecules such as cell membrane transporters or receptors can trigger endocytosis. Substituting L-amino acids with D-amino acids can alter physiological properties such as the improvement of enzymatic stability. By the modification of peptides to include more hydrophobic amino acids and covalent conjugation of hydrophobic moiety such as lipid or polymeric tail, peptides can gain more hydrophobicity. The lipophilic moiety option may result in membrane penetration or attachment via active or transcellular passive transport or may simply help to increase protein stability (17).

By single or combined usage with absorption modifiers such as enzyme inhibitors or permeation enhancers, polymer systems can help the drug overcome barriers. The combination of polymeric systems with absorption modifiers shows dual protection for the drug: polymer protection from enzymes and increased effect of absorption modifier for enzymatic stability or permeability. Basically, both systems can reveal enteric and additionally time dependent release of insulin. The dual controlled-release dosage form of insulin and inhibitor is another promising study topic. The inhibitor may be washed away from the release site of the protein provided to be released immediately alone. However, if the inhibitor is released together with the protein in a controlled fashion, it can enhance protein stability. Besides, polymer systems can be formulated alone. Nondegredable and biodegradable polymers have been used for nanosphere formulations (11).

NANOCARRIERS FOR ORAL INSULIN DELIVERY

In recent years, micro- and nanocarrier systems have become important in terms of better dissolution, stability, and bioavailability features of oral dosage forms. Nanocarriers, in particular, are designed to release the drug in the intestine for maximum bioavailability. Pharmacokinetics of the system is affected by physicochemical characteristics of both the active ingredient and nanocarrier. In particular, Peyer's patches (PPs), which are aggregated lymphoid nodules as part of the intestinal lymphoid tissue with immunologic duties, become the target site for oral peptides because of the natural function of direct transiting of polymer-based micro-/nanocarriers to the lymphatic system via M cells (Figure 3). Both the first past effect and P-gp efflux are avoided in this manner (22).

The present advantages of nanocarrier systems lead to novel formulations with potential success for oral insulin delivery.

Nanoparticles

Nanoparticles are natural or synthetic polymers, ceramic or inorganic element-originated solid colloidal drug carriers with a diameter between 1-100 nm, which is large enough to not leak from the blood vessel and small enough to not be caught by the mononuclear phagocytic system (MPS). The drug can be dissolved or kept in the nanoparticle, adsorbed, or linked to the surface. Nanoparticles are also suitable for active or passive targeting by surface modifications with hydrophilic agents such as PEG in order to avoid MPS and keep the drug in the bloodstream for a longer time, or they are designed to have a controlled release profile. Moreover, nanoparticles enhance drug solubility and protect the drug from enzymatic and hydrolytic degradation (22). These carriers are being widely researched because of the advantageous skills, and there are many nanoparticle studies in the literature about oral insulin delivery as well.

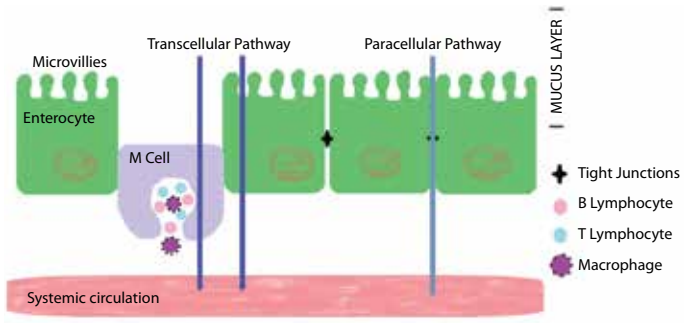

Figure 3. Pathways for insulin nanoparticle translocation through the intestinal epithelium (Modified from Fonte et al.) (4)

Polymeric nanoparticles prepared with biodegradable hydrophobic or hydrophilic polymers are remarkable carriers for oral insulin delivery. Poly (lactide-co-glycolide) (PLGA), polylactide (PLA), and poly (ε-caprolactone) (PCL) are the most studied hydrophobic polymers for oral insulin delivery. Chitosan (CS), meanwhile, is the notable hydrophilic polymer with excellent mucoadhesive properties, permeability capacity, and biocompatibility. Dextran, poly allylimine, and polyacrylic acid (PAA) are the other polymeric materials used in oral insulin studies (15).

Gold nanoparticles coated with chitosan were loaded with insulin and delivered to streptozocin-induced diabetic rats. In vivo evaluations indicated a significant decrease in the blood glucose level after the administration of oral gold nanoparticles compared with that of oral insulin (23).

Insulin-loaded alginate nanoparticles released 80% of insulin in the intestine-simulated buffer, and the amount of insulin released in the first 30 min was inversely proportional to the amount of alginate. The in vitro blood compatibility of nanoparticles was also investigated, and it was found that for a definite composition of nanoparticles, the protein adsorption and hemolysis percentage were the minimum, suggesting optimum blood compatibility of alginate nanoparticles of a definite composition (24).

Insulin-loaded alginate-dextran nanospheres were prepared, and it was found that alginate-dextran particles suppressed insulin release in acidic media and promoted sustained release at near neutral conditions. Nanoencapsulated insulin showed activity during both in vitro and in vivo examinations (25).

Pectin nanoparticles reduced the blood glucose level and increased the insulin level in animals as well. It is considered that pectin induces the protein kinase activity in the brain and pancreas while reducing it in the liver; consequently, insulin release and glycogenesis are enhanced, while glyconeogenesis is reduced (26).

In another study, the amount and release site of the drug was controlled by a copolymer, which was pH sensitive and coated. To that end, ABA triblock copolymers of PLGA-PEG were formed and insulin was encapsulated in it. It was found that a significant amount of insulin is released from nanoparticles at a pH condition similar to that of the intestine and the copolymer has the ability to protect insulin from enzymes in the gastric environment (27).

Insulin-loaded Eudragit® L100-L-systein conjugate nanoparticles showed better mucoadhesive properties than Eudragit nanoparticles. The released insulin ratio from thiolated Eudragit nanoparticles showed a significant increase when pH was changed from 1.2 to 6.8 at 37°C. A long-term (12 h) and high-level (28%) hypoglycemic effect was determined compared with that in the control group (28).

Mucoadhesive nanoparticles loaded with cell-penetrating peptide-linked insulin conjugates can overcome the problems and barriers when administered orally. The conjugation of insulin and Low-MW protamine (LMWP), which is a cell-penetrating peptide, demonstrated enhanced permeability through the intestinal mucus barrier and epithelial cells. Otherwise, after N-trimethyl chitosan chloride coating, which is a mucoadhesive polymer, PLGA nanoparticles were loaded with LMWP-insulin conjugates and enhanced retention was obtained with these mucoadhesive nanoparticles (MNPs) through the mucus layer in the intestine. With this strategy, released insulin conjugates could be divested from enzymatic degradation by a short distance to reach the epithelium and enhance permeation through it. Long-lasting and faster-onset properties could be gained for a hypoglycemic effect in rats, with 17.98%±5.61% pharmacological availability compared with that in case of a subcutaneous insulin solution (29).

Cubosomes

Some lipids and monoglycerides have conformations as bicontinious cubic phases, especially as isotropic bulk gels, which are composed of surfactants with proper hydrophilicity/hydrophobicity balance and can stand in equilibrium with excessive water and be dispersed into cubic nanoparticles (CNPs). CNPs can be fragmentized into smaller particles by lipases in the intestine but can still hold drugs in the lipid bilayer and avoid their precipitation in GI fluids by means of their lyotropic features. On the other hand, bioadhesiveness increases the contact between the drug and the GI barrier. CNPs are supposed to play important roles during lipid and drug absorption as secondary vehicles in the lipid digestion process. Similar mechanisms could be expected across the intestinal barrier (17).

Chung et al. (30) developed "nanocubicles," which can efficiently encapsulate insulin, and following the oral administration of insulin-loaded nanocubicles to fasted streptozotocin-induced diabetic rats, the serum glucose level was controlled for more than 6 h; the glucose level returned to the basal level in 3 h after intravenous injection of 1 IU/kg of insulin.

SLNs

SLNs are biodegradable and biocompatible colloidal systems with 50-1000 mm particle size, and they are obtained by the stabilization of solid lipids with surface active agents. They are alternative to nanoparticles and liposomes. Basically, SLNs comprise a solid lipid, surfactant, and hydrophilic phase. They are nontoxic owing to the lack of requirement of organic solvents for manufacturing and are suitable for controlled release and targeting with a high-loading capacity for both hydrophobic and hydrophilic drugs. On the other hand, they are affected by other colloidal structures in the medium and have disadvantages such as a high water content in dispersion and drug leakage (22).

Cetyl palmitate-based SLNs containing insulin were produced and characterized, and the potential of these colloidal carriers for oral administration was evaluated. The plasma glucose levels of rats after the oral administration of insulin-loaded SLNs were lower than those after the administration of an oral insulin solution and empty SLNs up to 24 h. The solid matrix of SLN was able to partially protect insulin against chemical degradation in GIT and to promote the intestinal absorption of insulin (31).

Lectin-modified SLNs containing insulin and their modified forms with wheat germ agglutinin-N-glutaryl-phosphatidylethanolamine (WGA-N-glut-PE) were formulated for comparison. After the oral administration of insulin-loaded SLNs and WGA-modified SLNs to rats, the relative pharmacological bioavailabilities were found to be 4.46% and 6.08% and the relative bioavailabilities were found to be 4.99% and 7.11%, respectively, in comparison with those after the subcutaneous injection of insulin. The oral bioavailability was still unacceptably low. The improvement of the drug entrapment efficiency and utilization of protease inhibitors were suggested to increase the bioavailability further (32).

Nanoliposomes

Liposomes are spherical vesicles consisting of 1 or more lipid bilayer and water in the center and between these lipid bilayers. The basic component, phospholipid, is an amphiphilic molecule. In addition to phospholipids, sterols [mainly cholesterol (CH)] are used to stabilize the membrane. Otherwise, nanoliposome is an expression for nanosized liposomes. Micro- and nanoliposomes have common chemical, structural, and thermodynamic features as well as differences such as a larger surface area, enhanced bioavailability and ability of targeting (22).

In a study aiming to evaluate the oral insulin bioavailability of nanoliposomes containing CH and bile salts (BS), nanoliposomes were formulated with sodium glycolate (SGC), sodium taurocholate (STC), and sodium deoxycholate (SDC). It was found that the hypoglycemic effect and oral bioavailability of the liposomes containing either BS or CH were in the order of SGC>STC>CH>SDC. BS-liposomes were found to be stable in GIT by showing a prolonged residence time. BS-liposomes were effective in terms of pharmacological activity, which was size and concentration dependent. It was concluded that BS-liposomes showed improved in vivo residence time and enhanced permeation across biomembranes (33).

Another study aimed to explore nanoliposomes modified with biotin (vitamin B_7) as novel carriers to enhance the oral delivery of insulin. Firstly, biotin-conjugated phospholipids incorporated into the membranes of liposomes and biotinylated liposomes (BLPs) were achieved. After the administration of BLPs to diabetic rats, a significant hypoglycemic effect and increased absorption were observed. The relative bioavailability ratios by the measurement of the pharmacological effect and insulin level in the blood were almost twice those of conventional liposomes. The significance of biotinylation was confirmed by the facilitated absorption of BLPs through receptor-mediated endocytosis as well as by the improved physical stability of the liposomes. Increased cellular uptake and quick GI transport further verified the ability of BLPs to enhance absorption (34).

Fear of animal-associated contaminants, demands of vegetarians, or religious preferences resulted in searching for botanical substituents instead of animal derivatives. To this end, liposomes were formulated with herbal sterols such as ß-sitosterol, stigmasterol, lanesterol, and ergosterol, which have a similar structure to CH. According to the study, β-sitosterol-, stigmasterol-, and lanesterol-containing lipo-

somes could not protect insulin effectively against degredation. Ergesterol liposomes (Er-Lips), conversely, were successful in protecting insulin, similar to the ones containing sodium glycocholate and superior to the ones containing CH. On the other hand, Er-Lips showed the best hypoglycemic effect in rats among other formulations. However, they exerted low toxicity to Caco-2 cells (35).

Nanoniosomes

Hydrating the mixture formulation of CH and nonionic surfactants such as alkyl ethers, alkyl esters, and alkyl amides is one alternative to phospholipids. After the alteration of the main constituents of liposomes, new vesicles are named niosomes or nonionic surfactant vesicles (NSVs), which have the advantages of better stability, lower cost, and ease of storage. A large number of available vesicle-forming nonionic surfactants also makes niosomes more attractive than liposomes in both pharmaceutical and cosmetic industries (22).

Nanostructured niosomes, which consist of Span 60/CH/N-trimethyl chitosan, loaded with insulin in an attempt of oral delivery were evaluated via in vitro and in vivo studies. Compared with insulin alone, insulin permeability was found to be 4-fold enhanced through the monolayer model of Caco-2 cells (36).

Nanoemulsions

Nanoemulsions are transparent or semitransparent systems with a large surface area and low surface tension, which are composed of nanoscale droplets of an immiscible liquid dispersed within another with the contribution of surfactants and auxiliary surfactants. Nanoemulsions are not affected by dilution or pH and temperature variety, unlike microemulsions. Little dimensions provide droplets to be kept suspended for a long time; therefore, flocculation and creaming are prevented for a long time. However, they end up with phase inversion; therefore, nanoemulsions are considered thermodynamically nonstable. The droplet size enables the system to be sanitized by filtration (22).

The self-nanoemulsifying drug delivery systems (SNEDDS), which are isotropic mixtures of oil, surfactants, co-surfactants, co-solvents, and drugs with an ability of spontaneously forming into nanoemulsions in GIT, have recently been highly remarkable for oral protein delivery (37). Studies in which protein drugs were directly dispersed into SNEDDS preconcentrate or were complexed with soy bean phospholipids (SPC) just before incorporation into SNEDDS indicated a promising potential of enhanced oral bioavailability and significantly reduced blood glucose levels (38-40).

Nevertheless, SNEDDS can be dispersed quite fast and expose insulin to degradation in stomach the stomach due to digestion. In order to avoid this, lyophilization was preferred to prepare insulin-SPC conjugates, following which conjugates were dispersed into SNEDDS and filled into gelatin capsules enteric coated with Eudragit L-100. The in vitro dissolution profile showed effective protection at pH 2 and gradual and steady release of insulin at pH 6.5, which are gastric and intestinal medium pH levels, respectively. A single oral dose pharmacodynamic/pharmacokinetic study conducted in healthy, fasted rats showed significantly increased bioavailability and a prolonged hypoglycemic effect of the enteric-coated SNEDDS capsules compared with those of an insulin solution (41).

Another technology is combining SNEDDS with polymers. For instance, thiolated chitosan-based SNEDDS were formulated. Compared with other control groups, the formulation began to release insulin in 30 min in pH 6.8 phosphate buffer with a burst effect and enhanced the release profile. The bioavailability of oral insulin could be significantly improved by the nanoemulsion system and thiolated polymer, as demonstrated by in vivo studies (42).

Nanogels

Nanogels are nanoscaled swellable networks composed of hydrophilic or amphiphilic polymer chains. Besides their main duties as drug carriers, they can be designed to spontaneously absorb biologically active molecules by salt bonds, hydrogen bonds, or hydrophobic interactions. Polyelectrolyte nanogels are attractive to oppositely charged drugs or biomacromolecules with low MW; thus, the loading capacity persists in comparison with that of other carrier systems (43).

Insulin absorption can be enhanced simply by reducing the size of P(MAA-g-MEG) microgels, which are pH responsive and designed by the polymerization of methacrylic acid and PEG-monomethyl ether monomethacrylate (PEGMA), but the in vivo performance of nanogels still needs to be improved (44, 45).

pH-sensitive nanogel preparations show appropriate skills to deliver insulin to the lower part of the intestine by taking the advantage of variance in GIT. Stimulus-sensitive ter-polymers containing N-isopropyl acrylamide (NIPAAm) (temperature-sensitive), butyl methacrylate (BMA), and acrylic acid (AA) (pH sensitive), reported by Ramkissoon-Ganorkar et al. (46), are also other smart nanogel formulations usable for insulin delivery.

Zhao et al. (47) synthesized hydroxypropyl methyl cellulose (HPMC) nanogels in order to provide controlled release of oral insulin. The obtained formulation was pH- and thermosensitive and the release profile could be controlled by such external stimuli. Moreover, the nanogel was synthesized through a surfactant-free method, preventing harmful effects of the solvent and surfactant residues.

CS-based nanogels with both negative and positive zeta potentials were loaded with insulin and compared with each other by an ex vivo experiment in the rat intestine. Negatively charged nanogels showed superior adhesion and permeation skills in the rat jejunum in comparison with positively charged ones. In accordance with that, according to an in vivo experiment in diabetic rats, the glucose-reducing ability of negatively charged nanogels was better as well (48).

Hoare and Pelton (49) designed amphoteric poly(N-isopropylacrylamide) hydrogels with submicron diameters and functionalized the structure with aminophenylboronic acide (PBA). These nanogels can swell/shrink in response to glucose depending on pH, concentration of PBA, and other anionic/cationic groups. The insulin uptake or release mechanism can be controlled owing to the switchable electrostatic charge of gels. However, an in vivo study is not performed.

CONCLUSION

Insulin is the essential element of replacement treatment in diabetes for the prevention of symptoms and complications. Difficulties related to subcutaneous insulin prompt researches to find novel insulin

preparations that are relevant to other delivery routes with the intention of better patient compliance. Oral delivery is the most common and reliable way known; nevertheless, oral insulin needs solutions in order to overcome enzymatic, absorption-related, and chemical degradation problems. Nanopharmaceuticals are novel promising systems with properties of increasing surface area and solubility, targeting to a specific area or receptor, and protecting insulin against degradation factors by encapsulation. Consequently, these systems are likely to become a solution for the present problems and can enhance bioavailability alone or with other enhancer excipients.

In this review, studies about nanopharmaceutical oral insulin preparations to overcome the problems caused by subcutaneous insulin have been evaluated. In conclusion, different studies have shown that insulin stability can be protected by encapsulation into nanocarriers against chemical and enzymatic factors. In order to protect the molecule against acidity in the stomach, enteric coating could be favoured to enhance bioavailability. Polymer systems, absorption enhancers, and enzyme inhibitors are effective for oral insulin preparations, which have better absorption rates than those of free insulin; however, toxicological analyses should be performed. Studies revealed that nanocarriers have significantly enhanced bioavailability but not to the extent desired. On the other hand, dual or multiple combinations of nanocarriers, polymer systems, absorption enhancers, and/or enzyme inhibitors may result in better bioavailability than solitary usage. Targeting PPs is considered to be another accurate approach. Novel nanosized oral insulin formulation trials and improvements have been ongoing.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.Ö., O.K., T.U.; Design - S.Ö., O.K., T.U.; Supervision - O.K., T.U.; Literature Search - S.Ö.; Writing Manuscript - S.Ö.; Critical Review - O.K., T.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - S.Ö., O.K., T.U.; Tasarım - S.Ö., O.K., T.U.; Denetleme - O.K., T.U.; Literatür Taraması - S.Ö.; Yazıyı Yazan - S.Ö.; Eleştirel İnceleme - O.K., T.U.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

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