AJPR
Avicenna Journal of Pharmaceutical Research
Avicenna J Pharm Res, 2021; 2(2):79-90. doi:10.34172/ajpr.2021.15
http://ajpr.umsha.ac.ir
UMSHA Press

Review Article

Hundred Years of Insulin: The Possibility of Development of Oral Dosage Form

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Article history:
Received: 1 Dec. 2021
Accepted: 10 Dec. 2021
ePublished: 30 Dec. 2021

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Abstract
Diabetes is one of the major medical problems in the world. The discovery of insulin was a milestone in the history of medical science. This legendary invention has completed 100 years of its journey. Through this 100-year journey, many important events have taken place, and many modifications have been made to make it convenient for commercialization and patient compliance. After the antidiabetic activity of the pancreatic extract was discovered, the bovine and pork insulin came into use. Then, recombinant human insulin was discovered. Now long-acting to rapid-acting insulin forms are available (e.g., insulin pens, insulin pumps, and the like), and different systems are in use to deliver them to the body, but all these insulins are in invasive dosage forms and have some disadvantages, too. As we are using the oral form of most medicines, we prefer the oral form of insulin. Therefore, the concern is now to develop the oral dosage form of insulin. Some proposed oral dosage forms are at different stages of the clinical trial such as classical dosage forms, enzyme inhibitors, unnatural amino acids, nanoparticles, cell-penetrating peptides, and so on. Since the oral forms of insulin create some limitations and confusion, further research is required to solve these problems. Accordingly, the aim of the review was to predict the possibility of developing marketable oral insulin.
Keywords: Oral insulin, 100 years of insulin , Diabetes, Current forms of insulin, Pancreas, Recombinant technology

Please cite this article as follows: Saha A, Sultana A, Yesmin F, Akter T, Sikder AA, Aktar F, et al. Hundred years of insulin: the possibility of development of oral dosage form. Avicenna J Pharm Res. 2021; 2(2):79-90. doi:10.34172/ajpr.2021.15

Introduction

Diabetes mellitus is described by the rise of blood glucose levels mediated by the lack of insulin generation or insulin tolerance as well as proportionate deficiency of insulin (1). Without insulin, diabetic ketoacidosis, unconsciousness, and death are possible. This is especially dangerous for individuals with type 1 diabetes who need insulin to survive (2). Diabetes has effects on a large proportion of the population around the globe and has affected rampant proportions. Diabetes afflicted 171.2 million people (2.8% of the world's population) in 2000, and it is expected to impact 366.2 million people (4.4% of the world's population) by 2030 (3-5). Diabetes was first documented by the ancient Egyptians followed by the traditional Greeks and Indians. It has historically been connected with a schedule of abstention from conventional foods and significant limitations on everyday activities, but the actual cause was unidentified until the mid-nineteenth century. Insulin discovery was a genuine game-changer in diabetes treatment. It was discovered at the end of the nineteenth and beginning of the twentieth centuries (6), characterized by contrasts, conflicts, major failures, disappointments, and hopes (7). Insulin is critical in the maintenance of type 1 diabetes and, at a later stage, type 2 diabetes (8). Insulin therapy effectively lowers the level of blood glucose in diabetic patients (9). The synthesis of human insulin analogs represents a watershed moment in this research. The insulin delivery systems currently available are syringes, jet injectors, infusion pumps, and pens. Subcutaneous injections are the most popular and dependable method of administration of insulin. The invasive nature of conventional insulin therapy with major discomforts such as pain at the injection site, lipodystrophy, and noncompliance by the patients has sparked a search for less intrusive and more comfortable means of insulin administration. Numerous non-invasive administration methods are being investigated (10). The final goal is to remove the requirement for exogenous insulin delivery and to restore patients' ability to synthesize and use insulin by their own. Newer explored methods include the artificial pancreas with a closed-loop system, transdermal insulin, and buccal, pulmonary, oral, ocular, nasal, and rectal routes (11,12). This review focused on the evolution of insulin over 100 years and the possibility of a sustainable oral dosage form of insulin.

Materials and Methods

To obtain the essential information, several published research and review articles were downloaded from Google Scholar, ScienceDirect, PubMed, Research Gate, and other web sources. Some Pharmacology books were also used to know about the history, evolution, and marketing of insulin. Information was obtained from these papers and compiled. Insulin, the discovery of insulin, history of insulin, oral insulin, and other keywords were utilized to find information.

History and Development of Insulin

Before the introduction of insulin, a variety of methods were used to regulate diabetes, some of which had no benefits or were extremely harmful. Pierre Adolphe Piorry, a French physician, used to recommend hypercaloric diets to treat the loss of calories in urine, among other things. However, some doctors made the proper choice such as consuming low-calorie meals including kale. John Rollo, the surgeon-general of the Royal Artillery, properly treated a patient with a food restriction (13). Treatments such as the Buchardat's treatment, low-calorie carbohydrate therapy, or the alien diet have been recommended as sugar-free diets (14). Elliott Proctor Joslin, the founder of the Joslin Diabetes Center, also developed the starvation diet (15,16). Furthermore, Karl Loening and Ernst Vahlen introduced miraculous tablets called metabolin and irrebolin in 1922 and 1924 (17,18).

The discovery and evolution of insulin is a remarkable story. Frederick Banting, Charles Best, J. J. R. Macleod, and J. B. Collip from the University of Toronto are credited with inventing the approach, while other researchers contributed crucial ideas and techniques. In 1869, Paul Langerhans, a German medical apprentice, found that the pancreas has two distinct cell types: acinar cells that produce the enzymes of the digestive system and cells organized in islands, or islets, that were suspected to have a secondary function. In 1889, Minkowski and Von Mering noted that pancreatectomized dogs suffer from a condition analogous to human diabetes mellitus, thereby proving its function. Numerous attempts have been made to isolate the pancreatic substance that controls blood glucose concentrations. Between 1903 and 1909, a Romanian scientist named Nicolas Paulesco observed that injecting pancreatic extracts reduced urine sugar and ketones in diabetic dogs and published findings indicating the discovery of a glucose-lowering chemical (19). Sharpey, an Edinburg Shafer, coined the term 'insulin' in 1916. In 1920, Moses Barron published a paper on the association between islets of Langerhans and diabetes, citing a case of pancreatic lithiasis. Dr. Frederick Banting and medical student Charles Best conducted studies on the pancreases of dogs in Toronto, Canada, in 1921 (20). Humans received their first injection of bovine insulin in 1922. The first human patient cured with pancreatic extract showed little effect on ketoacidosis, a minor effect on glycosuria, as well as the formation of a sterile abscess. They demonstrated normalization of all three disorders, glycosuria, hyperglycemia, and ketonuria in subsequent injection series (21). Crystalized form of insulin was invented in 1926 by John Jacob Abel (22). Frederick Sanger discovered the insulin amino acid sequence in 1951 and was awarded the 1958 Nobel Prize for this discovery (23,24). Dorothy Hodgkin (Nobel laureate in chemistry, 1964) and colleagues deduced the three-dimensional structure of insulin. Synthetic crystalline bovine insulin was invented by Chinese researchers (25). The three-dimensional structure of insulin was elucidated in 1969 (26). Further, Yalow and Berson were awarded the Nobel Prize in 1977 for inventing radioimmunoassay for the structure elucidation of insulin (19,27). Moreover, the first genetically engineered synthetic human insulin was formed by using *Escherichia. coli* in 1978 (28).

Human insulin synthesized by recombinant DNA technique is hydrophilic. The pH of the majority of preparations is neutral, which boosts insulin preparations stability and enables them to be stored at room temperature for short periods. Since the invention of human insulin, pork and beef insulin are no longer made. Other insulin formulations such as lente, protamine zinc, and ultralente are no longer available. International units are used to define the dosages and concentrations of insulin formulations that are therapeutically employed (19,29).

Table 1. Timeline of Insulin Discovery and Development

Year	Event	References
1869	Discovery of islets of Langerhans	19
1903-1909	Antidiabetic activity of pancreatic extract	19
1916	Introduction of the name of insulin	20
1920	Relation between islets of Langerhans and diabetes	20
1921	Experiments with pancreases of dogs	20
1922	First bovine insulin application in human	21
1926	Invention of crystalized form of insulin	22
1951	Establishment of amino acid sequence of insulin	23, 24
1965	Preparation of synthetic crystalline bovine insulin	25
1969	Three-dimensional structure of insulin elucidated	26
1978	First genetically engineered synthetic human insulin using *E. coli*	28
1923-Present	Being marketed by Novo Nordisk, Sanofi, Eli Lilly and Company, and 40 smaller manufacturers	30

Table 1 shows important events in insulin discovery and subsequent developments.

Commercialization of Insulin

Since the discovery of insulin in 1921, Eli Lilly and Company has been a pioneer in the development of insulin-based pharmaceuticals. Insulin was developed in conjunction with the University of Toronto by Josiah K. Lilly Sr. and George HA Clowes and was first marketed in 1923. The first human insulin, a rapid-acting insulin analog, and insulin analog combinations were developed and sold in the 1950s. Due to the development of basal insulin which is well-tolerated by the liver, Lilly has made substantial contributions to the field of science. Important insulin-related efforts include the manufacturing of the first densely packed rapid-acting insulin analog, clinical studies facilitating the use of highly concentrated human insulin, and a clinical production program for an ultra-rapid insulin analog that is currently in the advanced stages of development. In addition to collaborative research, creative product development, and investments in advanced technologies, Lilly's commitment to individuals with diabetes has been and will continue to be strong in the future (29).

Apart from the three largest global insulin manufacturers Novo Nordisk, Eli Lilly, and Sanofi, there are approximately 40 smaller manufacturers serving largely domestic markets, according to a comprehensive review of market intelligence information including market reports (Figure 1). Southeast Asia and Latin America have a higher concentration of small manufacturers than other areas (30).

Conventional Insulin Forms and Methods

There are five current forms of insulin based on how long it works in our body starting from rapid to long-acting. All five forms are invasive (32), and they are described briefly as follows:

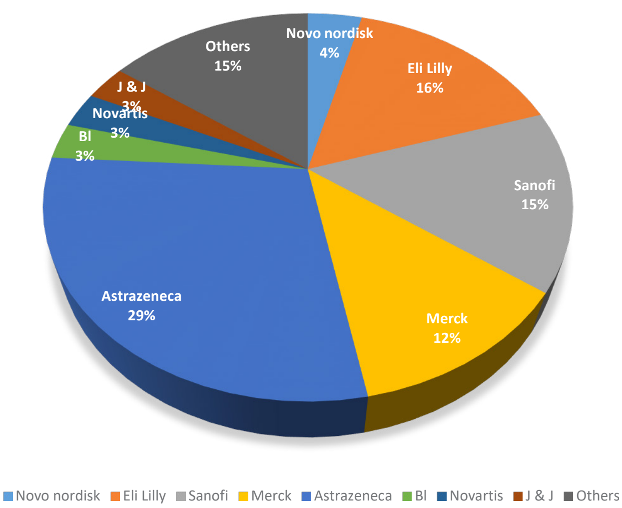

Figure 1. Insulin Market Share of Different Companies in 2017 (31).

Rapid-Acting Insulin

Rapid-acting insulins have a shorter and more predictable action. Since they function so quickly, they are usually consumed at the start of a meal. Their activity is similar to that of human insulin. The effects of lowering blood sugar levels are transient. Insulin deficiency can cause substantial blood sugar decreases in persons with type 1 diabetes. It is less potent and longer-acting compared to long-acting insulins. Short-acting insulin is designed to work well when used 30–60 minutes before a meal. It is transparent and does not split from the container/vial (e.g., Humulin R, Novolin ge Toronto).

Intermediate-Acting Insulin

Intermediate-acting insulin can be used for up to half of the day or overnight, depending on the requirements and tolerance. This type of insulin is generally used in association with rapid- or short-acting insulin to provide a longer duration of action (e.g., Humulin N, Novolin ge NPH).

Long-Acting Insulin

Long-acting insulins hold additional chemicals (buffers) that permit them to work for a longer period of time, causing the insulin to appear foggy. Whenever the buffered insulin is allowed to rest for even a few minutes, the buffered insulin falls to the base of the vial (e.g., Lantus, Basaglar, Levemir, and Tuojeo).

Premixed Insulin

Presumably, premixed insulins combine the exact proportions of short and intermediate-acting insulins. Intermediate-acting and quick- or short-acting insulins can be combined in the same syringe. It is impractical to blend all insulins. You can get insulin that is pre-mixed for immediate and intermediate action. Once injected, the combination of fastest-acting insulin will start working immediately. It peaks around the same time as each kind of insulin and lasts as long as the longest-acting insulin (e.g., 30% regular and 70% NPH, 50% lispro and 50% lispro protamine, and the like) (32). Different types of insulin are shown in Figure 2. Different systems are available for insulin delivery such as syringes, continuous subcutaneous insulin infusions, insulin pumps, insulin pens, and insulin jet injectors. Among them, insulin pens and insulin pumps are more preferred by the user (33,34) as illustrated in Figure 3.

Problems With Conventional Forms and Methods

Although the traditional methods are in use from the early stages of insulin development, they have some drawbacks, for example:

1. To simulate the normal production of basal and postprandial insulin, long-acting and short-acting insulin analogs created through recombinant DNA technology are also used. Since slow dissociation results in a gradual release of insulin into the bloodstream, the ability of short-acting and long-

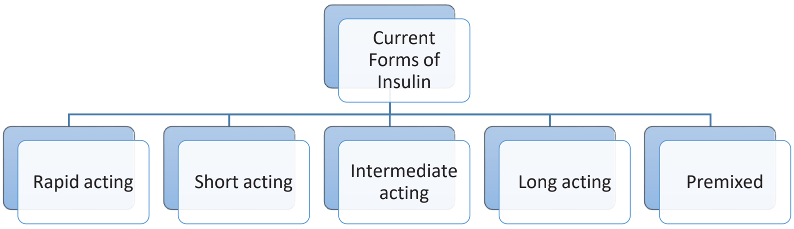

Figure 2. Current Forms of Insulin.

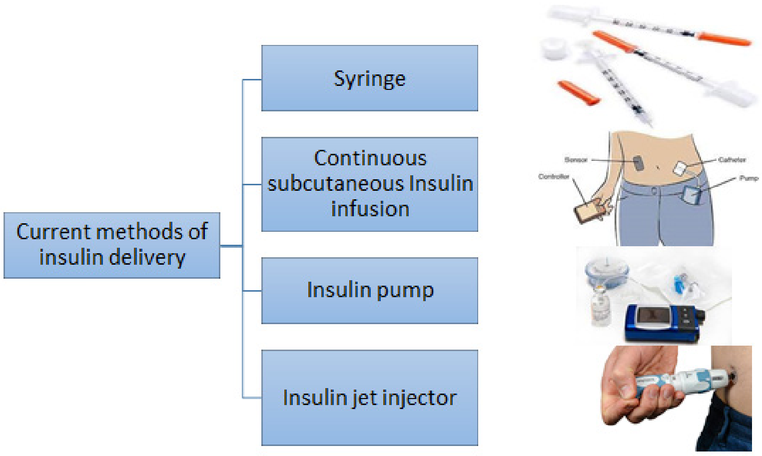

Figure 3. Current Methods of Insulin Therapy.

acting analogs to break hexamers into monomers in the subcutaneous tissue differs between the two classes (35,36).

2. Traditional syringes have several drawbacks, including their hefty structure and the time and effort required to properly use them. They are straightforward to use and ready for a wide range of commodities. However, the social syringe manipulations may have disadvantages. To suit their particular needs, patients may need to mix several insulin compositions in a single syringe, which can be painful and complex. In addition, people with weak dexterity may receive incorrect dosages, compromising their glucose control and overall health (37).
3. When compared to the usage of standard vials and syringes, insulin pump therapy is significantly more expensive (38).
4. Due to their size and cost, jet injectors are not extensively used in diabetic patients. One disadvantage of using jet injectors is that less insulin is absorbed over time. Another drawback is related to the sound that the injector makes while delivering the medication. Moreover, pressure is harder to regulate in young newborns, and adverse effects appear to be more common (38-40).
5. Patient education is critical to minimize operational errors, which are particularly prevalent when switching the cartridge in disposable pens (37).

Proposed Methods for Insulin Delivery

The present methods have been in use from the very beginning but they have some drawbacks; hence, some new methods should be introduced to solve these problems in the future. Some methods to use in the future include Injectable insulin as insulin degludec and human insulin Linjeta™, Artificial pancreas, Buccal delivery of insulin, inhaled insulin, transdermal insulin, and the most accepted oral insulin. Oral insulin has advantages in terms of patient compliance and physiological benefits by mimicking the natural fate of insulin via the initial transit to the liver and preventing hepatic glucose production directly and efficiently. Insulin is a protein that can be broken down by the low pH of the stomach and digestive enzymes in the stomach and small intestine. The gut epithelium limits insulin absorption. All of these factors contribute to low bioavailability and substantial inter- and intra-subject variability (41-48).

Development of Oral Insulin

For its noninvasive nature, oral administration remains the preferred way of administering medicine to patients. The gastrointestinal tract (GIT) epithelium may transfer peptides and proteins by transcellular or paracellular routes, depending on the circumstances. These compounds, on the other side, are hydrophilic and have a large molecular structure. This means that passive transport would not follow the transcellular absorption pathway as a result of these changes. Unlike the intracellular route, the paracellular route is an extracellular aqueous channel that may be useful for protein and peptide distribution caused by a lack of protease in the extracellular environment. A lack of effectiveness for absorption has been demonstrated in the paracellular route because proteins and peptides are unable to fit into these gaps when they are present. The pre-systemic enzymatic breakdown that occurs in the hostile environment of the GIT as well as pre-systemic elimination that occurs in the liver are two of the most critical factors leading to the extremely poor bioavailability of proteins and peptides. As a result, efflux transporters such as P-glycoprotein, a 170-kDa protein, may have a significant impact on protein and peptide bioavailability as this protein acts in the reverse direction of transcellular drug absorption (49-53). As a result, the following obstacles t prevent oral peptide delivery from being effective:

1. Low pH environment of the gastric media,
2. Enzymatic barrier,
3. Viscous mucous layer,
4. The intestinal epithelium cells (54-57).

The maintenance of insulin's biological stability in the GIT and the cytosol of enterocytes should always be considered across the formulation system to develop an adequate oral delivery mechanism for the medication. As a result, excipients may be utilized in delivery systems to inhibit insulin accumulation and enzymatic destruction, prolong insulin retention in the GIT, and improve intestinal absorption. Tablets, capsules, and intestinal patches have all been studied as insulin delivery systems to deliver insulin by paracellular and/or transcellular transport across the ileum and colon. Hydrogels, nanoparticles, and microparticles have been also investigated for use in delivering insulin through the ileum and colon as depicted in Figure 4 (58).

Table 2 illustrates different forms of oral insulin with their development method and current stage.

Classical Dosage Forms

Many research organizations across the world are attempting to create an oral delivery method, primarily in tablet or capsule form, mostly due to the ease and greater rates of patient satisfaction provided by tablets and capsules.

Chitosan-4-thiobutylamidine Tablets

Using chitosan-4-thiobutylamidine tablets, the researchers combined insulin, a Bowman-Birk inhibitor, and elastatinal as inhibitors of enzymes that were covalently attached to the chitosan surface. The enzyme blockers are consolidated in the tablet via the covalent bonding and thus are prevented from being released into the GIT. This reduces the likelihood of local and systemic side effects. More importantly, when chitosan and mucus glycoprotein are mixed, a mucoadhesive matrix is formed, which is instrumental in providing insulin and significantly decreasing glucose levels in blood in normoglycemic rats over 24 hours as presented in Figure 5 (59).

CODES™ Tablets

CODES™ is a colon medication delivery system that consists of a tablet in the core covered with three polymeric layers intended for colon drug delivery. Various insulin, meglumine, lactulose, citric acid, polyethylene oxide, and sodium glycocholate tablets have been created. As a result, lactulose is utilized to accelerate the release of medicine in the colon, while meglumine and citric acid are used to alter pH and insulin solubility, respectively, and sodium glycocholate is used to boost medication absorption. The gel barrier created by these chemicals and polyethylene oxide in the tablet core enables continuous insulin release in the intestines of dogs (64,65).

Eudragit S100 Enteric-coated Capsules

The anionic polymer Eudragit S1000 is utilized in the gut for pharmaceutical distribution since it is insoluble in acidic environments such as the stomach and aqueous solutions up to a pH of 6.11. Moreover, enteric-coated Eudragit S100 capsules packed with insulin and sodium salicylate as an absorption enhancer were studied in hyperglycemic beagle dogs. The capsules were prepared in polyethylene glycol (PEG) 4000 or Witepsol W35 bases and were given to the dogs. Clinical investigations revealed that a combination of insulin (1 g), sodium salicylate (50 mg), and hard gelatin capsules coated with Eudragit S100 was the most effective procedure (66-68).

Nanoparticles

Lipid-Based Insulin Nanoparticle

In terms of lipid-based insulin nanoparticles, the focus is on solid lipid nanoparticles (SLNs) and liposomes (the most often utilized systems). SLNs have been used as an alternative transport mechanism to polymeric nanoparticles since the 1990s due to their tolerability, biodegradability, and possibility for large-scale manufacturing. Major advantages of SLNs include the

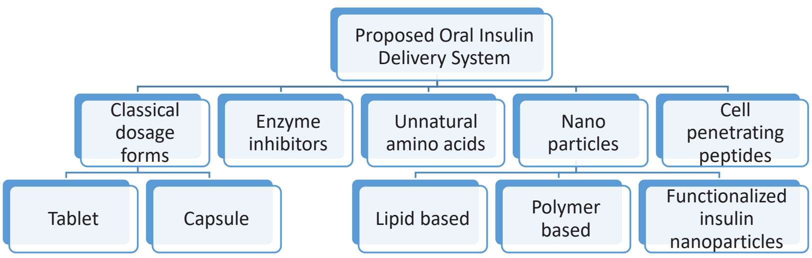

Figure 4. Proposed Oral Insulin Delivery System.

Table 2. Different Forms of Oral Insulin

Forms of Oral Dosage	Name	Method of Development	Stage of Development	Reference
Capsule	Capsulin	Enteric coated capsule containing insulin, an absorption enhancer, and a solubilizer	Phase IIa in type 1 DM & phase II in type 2 diabetes.	59
	ORMD	Enteric coated capsule containing insulin with an adjuvant to preserve the protein and promote its absorption in the gut	Phase IIa in type 1 DM & phase II in type 2 diabetes.	59
	ORA 2	Capsule which has insulin in dextran matrix	Phase 2 in type 2 DM	59
	Eligen	Insulin capsule with an absorption enhancer to promote passive transcellular transfer	Phase 2 in type 2 DM	59
Tablet	GIPET	Tablet containing an absorption enhancer to promote active micelle production and insulin delivery	Cancelled after phase II	59
	IN1953, IN1954	Tablet with long-acting insulin analog	Phase I in both type 1&2 DM	61
Recombinant Insulin	IN 105	Insulin modified with small PEG	Phase III	59,61
Nanotechnology	HDV-1	Liposomal insulin, hepatic directed vesicles insulin	Phase III	59
	CobaCyte	Polymer containing insulin, coated with vitamin B_{12} for targeted delivery	Phase I	59,69
	-	Insulin loaded bio-adhesive nanoparticles by NOD technology	Phase I	60

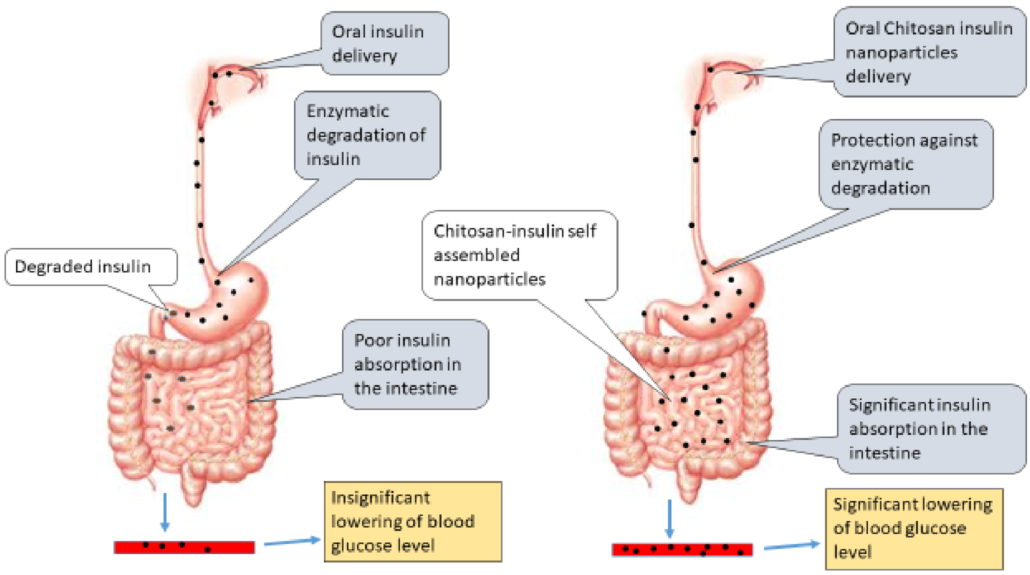

Figure 5. Oral Chitosan Insulin Nanoparticles Delivery.

reduced risk of acute and chronic toxicity as well as the ability to produce large quantities at a low cost (69-73).

Since the discovery of insulin-loaded liposomes by Patel and Ryman in 1976, several studies have demonstrated that they have a significant hypoglycemic effect. According to the findings of those studies, the composition of liposomes is significant for both the insulin sugar lowering effect via the oral ingestion and the stability of *in vivo* liposomes. Bio-adhesive dose formulations may be used to compensate for the low bioavailability of the drug in question. The features of a chitosan-coated liposome were studied both *in vitro* and *in vivo*, and the results were promising. It has been demonstrated that the usage of chitosan-coated liposomes can improve insulin absorption (74-80).

Polymer-Based Insulin Nanoparticles

Various experiments have been conducted so far to produce polymer-based nanoparticles for insulin oral administration. If the polymers are natural or synthetic, they may differ in their origin (81).

Chitosan has long been the polymer of choice from the natural origin for the development of nanoparticles because it can adhere to the mucus layer and temporarily open the strong interconnections between intestinal epithelial cells during digestion. It has been demonstrated that tripolyphosphate anions could produce gel chitosan and result in the formation of insulin-loaded chitosan nanoparticles in one study. It has also been utilized as a hydrophilic polymeric coating to aid insulin flow through the gut wall more rapidly, which has been found to be effective (82-86). In the encapsulation of insulin, N-trimethyl chitosan (a partly quaternized chitosan derivative), N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride, and chitosan-graft methyl methacrylate monomers have been employed to substitute chitosan as they give more stability by electrostatic interactions when they maintain mucoadhesive properties and permeation-increasing properties (87-89). After treating diabetic rats, nanoparticles synthesized with dextran and coated with chitosan revealed a sustained-release profile and dramatically increased the hypoglycemic impact of insulin. Insulin encapsulation in vitamin B_{12}-coated dextran nanoparticles has been proposed as a way to supplement diabetes treatment by using increased insulin absorption via vitamin B_{12} intrinsic factor receptor ligand-mediated endocytosis through leukocytes in the gut (90-92).

Alginate has also been employed to administer insulin orally in the past. Insulin embedded in alginate nanoparticles was shown to reduce baseline blood sugar levels by 40% in diabetic rats after being administered orally (93,94).

Synthetic polymers were used for insulin delivery because they have a longer release time than natural polymers. Polylactic acid, polylactic-co-glycolic acid (PLGA), and poly (-caprolactone) polymers are biodegradable and biocompatible; however, encapsulating insulin into hydrophobic polyester nanoparticles should be done using a water-in-oil-in-water double emulsion process. Other PLGA nanoparticle modifications include PLGA mannosamine, PLGA copolymer, and polyoxyethylene derivatives (i.e., PLGA: poloxamer and PLGA: poloxamine compositions), and PLGA with hydroxypropyl methylcellulose phthalate (102-105). A previous study showed that insulin encapsulated in poly (-caprolactone) nanoparticles and Eudragit decreased fasted glycemia in a dose-dependent way, which was mostly attributed to mucoadhesive properties of Eudragit (103,104). The use of insulin-loaded polybutylcyanoacrylate nanoparticles was shown to provide greater insulin protection against degradation by proteolytic enzymes when compared to the use of the same nanoparticles in an aqueous solution (105-108). Insulin encapsulated polyacrylic acid cysteine combined with polyvinyl pyrrolidone nanoparticles was found to be gastro-intestinally stable and to significantly reduce blood glucose levels when administered intravenously. Because of its mucoadhesive properties, polyacrylic acid cysteine seems to play a key role in the absorption of insulin (109).

Functionalized Insulin Nanoparticles

Various targeting approaches have been developed to boost the contact of insulin nanoparticles with intestinal absorptive cells as well as M cells in Peyer's patches, which have been found to improve glucose tolerance. Such approaches include the modification of surface properties and the attachment of a targeted molecule to the surface of nanoparticles, to name a few (110-112). To stabilize nanoparticles, one method is to coat them with a hydrophilic stabilizing agent or include them in the nanoparticle structure. Another method is to utilize bio-adhesive polymers such as chitosan and poly (methacrylic acid) or surfactant molecules as bio-adhesives. Fonte and colleagues revealed that insulin-loaded chitosan-coated SLN nanoparticles increased insulin entry in the Caco-2 cell monolayer, Caco-2/HT-29 coculture monolayer models, and Caco-2 cell coculture monolayers. When administered to diabetic rats, these nanoparticles were found to have a prolonged hypoglycemic effect that lasted for up to 24 hours (113,114).

Alternatively, grafting a ligand onto the plane of the nanoparticle can help precisely focus the nanoparticles to receptors on the surface of enterocytes or microglia (M cells). Lectins, for example, are involved in a variety of cell detection and adhesion functions that significantly increase the transit of nanoparticles through the digestive tract. The oral administration of lectin-modified SLN and wheat germ agglutinin glutaryl phosphatidylethanolamine-modified SLN containing insulin resulted in increased insulin bioavailability as well as protection against in vitro breakdown enzymes in both rats and monkeys (115,116).

Cell-Penetrating Peptides

Because of their ability to enhance the dispersion of proteins and peptides across the plasma membrane, cell-penetrating peptides (CPPs) have received attention in recent years, and as a result, CPP exhibits the potential for therapeutic applications (124). Peptides and CPPs have been combined in several recent studies, demonstrating that this combination is a feasible strategy for the oral delivery of these macromolecular medications. CPPs are short peptide sequences that are abundant in basic residues (i.e., arginine and lysine), allowing them to interact with negatively charged cell surface molecules through electrostatic interactions. CPPs are found in a variety of cell types including cancer cells (118-122).

Enzyme Inhibitors

When delivering peptides and proteins orally, one of the most difficult challenges is protecting them against degradation by a variety of endopeptidases (e.g., pepsin, chymotrypsin, and elastase) and exopeptidases (e.g., carboxypeptidases A and B) as they travel through the GIT. As a result, using enzyme inhibitors to increase the bioavailability of peptides in the oral cavity is one way to solve this problem. Many enzyme inhibitors (e.g., trypsin) or chymotrypsin inhibitors such as soybean trypsin inhibitor, FK-448, camostat mesylate, and aprotinin, were used to enhance the stability of oral insulin in the presence of enzymatic degradation (123-127). However, the use of enzyme inhibitors in long-term therapy is still debatable due to the risk of undesired protein and peptide absorption, disruption of nutritive protein digestion, and increased protease release (128).

Unnatural Amino Acids

The physicochemical features of peptides can be made better by substituting synthetic amino acids for natural amino acids in the following ways: D-conformation, tetra-substitution, N-methylation, amino acids, and side-chain methylation(s). As naturally occurring proteases are designed to catalyze processes relating to natural peptides, such modifications result in a peptide sequence that is more resistant to degradation by enzymes compared to the original sequence. It has been discovered that the amino acid ala2 is responsible for the breakdown of glucagon-like peptide-1. As a result, it has been demonstrated that substituting D-ala2 for ala2 improves drug stability, lengthens half-life, and increases activity. A difficulty with this technique is that the activity of the drug must be retained even if the amino acid sequence is modified, which can be difficult to achieve (129-131).

Summary and Limitations

As oral dosage forms are more convenient, oral dosage forms of insulin should be developed. Different methods are employed to get oral insulin. They have many advantages over conventional insulin forms. Biodegradable polymer-based nanoparticles have increased stability, decreased toxicity in peripheral healthy tissues, their pharmacokinetic parameters can be controlled, and drug release can be controlled and targeted (130-135). SLNs are biocompatible and can be produced on a large scale easily, peptides can be protected from being degraded, and drug release can be controlled (136-140). Liposomes provide protection against enzymatic degradation, biocompatibility and flexibility, safety and minimum toxicity, non-immunogenicity, and entire biodegradability (141-145). Enzyme inhibitors retard the peptide degradation rate by the enzyme (145-147,150), while CPPs enhance intracellular permeation (148-157). Absorption enhancer increases oral bioavailability by raising membrane permeation (147,151,152). Modifying the structure of the peptides increases peptides' oral bioavailability by minimizing the enzymatic destruction of peptides and improving the permeability across the membrane (153-156). Mucoadhesive polymers give site-specific delivery and improve membrane permeation (157). Along with all these advantages, they have some limitations. PLGA/PCL has poor drug loading and higher product cost with peptide or protein drug instability problems such as denaturation or aggregation. Chitosan has instability in the GIT due to an acidic environment (130-135). Solid nanoparticles provide low peptide entrapment efficiency, but their interaction with biological barriers is not still known (136-140). Liposomal drug delivery systems have a high manufacturing cost, poor durability against pancreatic lipase and stomach pH, low hydrophilic drug loading, and the possibility of leakage from the encapsulated drug (141-145). Enzyme inhibitor medication is a long-term treatment with serious adverse effects, including the possibility that proper digestion of nutritional proteins would be compromised (143,145-147). Using CPPs is expensive, and it has hazardous side effects as well as an immunological reaction (148,149). It has been shown that absorption enhancers can affect cell shape, produce cell damage, and have a lack of selectivity (147,151,152). Due to the fact that PEG is both immunogenic and antigenic, modifying the structure of a peptide might result in significant safety concerns (153-156). Furthermore, the conjunction process in this case may be complicated. According to (157), mucoadhesive polymers can stimulate mucus transition in absorption sites (intestine). As a result, to produce an oral dosage form of insulin, the constraints listed above should be minimized. Only extensive study in the future will be able to take advantage of the extent of another momentous event in the history of medicine.

Conclusion

Insulin is one of the most essential therapeutic options available for diabetes treatment. Developing an oral dosage form can be a milestone in insulin therapy for many reasons. Although there have been some difficulties to establish oral insulin, certain developed oral dosage forms have already been in the IIa and III phases of clinical trials. Moreover, further extensive research is necessary to incorporate oral insulin successfully in diabetes treatment.

Acknowledgments

We would like to thank the authors of the articles we cited. We also express our gratitude to the authority of the Department of Pharmaceutical Chemistry for using their computer at the Molecular Pharmacology and Herbal Drug Research Laboratory established under the HEQEP Project.

Authors' Contributions

MSA has conceived the original idea. AnS, ArS, and FY prepared the initial manuscript with referencing. JAC, AAC, TA, and SK critically reviewed the overall activities. MSA has supervised the whole activity. All authors have read the manuscript and agreed to the published version of the manuscript.

Conflict of Interests

The authors declare no conflict of interests.

Funding

This work has no funding.

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