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Non-Invasive Insulin Delivery Systems: Challenges and Needs for Improvement

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Abstract: The gastrointestinal tract (GIT) is the route of choice for the administration of most drugs, regardless of their molecular structure or weight. The manufacture of an oral dosage form does not have to meet specialized regulatory requirements relating to such issues as sterility, pyrogenicity, and particulate contamination. Insulin has an important place in drug therapies for insulin-dependent diabetes mellitus (type I) and for many patients with non-insulin-dependent diabetes mellitus (type II). However, it is still generally delivered via injections. It would be highly advantageous if insulin could be administered orally, because the oral delivery of insulin can mimic the physiological fate of insulin and may provide better glucose homeostasis. The desire to deliver protein and peptide biopharmaceuticals conveniently and effectively has led to the intense investigation of targeted delivery systems. Despite various challenges, progress toward the convenient non-invasive delivery of proteins and peptides has been made through specific routes of administration.
Keywords: pyrogenicity, sterility less-invasive, long-term dependence

Introduction

The synthesis of insulin by recombinant DNA technology represented an important scientific milestone and made large quantities of the protein available at an affordable price, a factor that led insulin to become one of the most popular proteins to be studied for non-parenteral delivery[1, 2]. Consequently, the results of research into several aspects of the delivery of the insulin are available. In recent years, there has been a great deal of interest in the exploitation of non-invasive routes for insulin delivery, and their development by the pharmaceutical industry, including oral, nasal, buccal, pulmonary, transdermal, rectal, and ocular drug delivery systems [3–5] The objective of this review is to provide an update on the most promising advances in non-invasively delivery systems for insulin that may overcome the barriers to its absorption.

Routes of insulin administration

Oral administration: This would also lessen the incidence of peripheral hyperinsulinemia, which is associated with neuropathy, retinoendopathy, and so forth. Various challenges are usually evaluated by determining the fate of insulin in the GIT. The main challenges reported involve overcoming the enzymatic degradation of insulin and the insufficient permeation of insulin through the GIT. Success in the oral delivery of therapeutic insulin would improve the quality of life of many people who must routinely receive injections of this drug. In the last few decades, various attempts have been made to overcome the limitations and drawbacks of conventional oral insulin therapy. The successful oral delivery of insulin involves overcoming the barrier of enzymatic degradation, achieving epithelial permeability, and conserving the bioactivity of the drug during formulation processing. Pharmaceutical strategies have been proposed to maximize oral insulin bioavailability in insulin delivery systems, to overcome barriers, and to develop safe and effective therapies[6-8].

Absorption enhancers: Absorption enhancers improve the absorption of drugs by increasing their paracellular and transcellular transport. They involve several different mechanisms of action, including changes in membrane fluidity, decrease in mucus

viscosity, the leakage of proteins through membranes, and the opening of tight junctions. Common examples of non-specific permeation enhancers are bile salts, fatty acids, surfactants, salicylates, chelators, and zonula occludens toxin. Bile salts in mixed micellar systems increase the permeation of insulin by accessing a paracellular pathway. A study of N-lauryl-β-D-maltopyranoside also suggested that this enhancer may open the tight junctions of the epithelium, thereby increasing the permeation of insulin via a paracellular pathway. In another interesting study, water-in-oil-in-water multiple emulsions incorporating 2% docosahexaenoic acid or eicosapentaenoic acid had dose-related pharmacological effects on insulin and may potentially become the formulations for the enteral delivery of insulin[9-11].

Mucoadhesive polymeric systems: The term 'mucoadhesion' refers to the adhesion between polymeric carriers and the mucosa and is exhibited by certain polymers, which become adhesive upon hydration[12, 13]. Thus, the goals of mucoadhesive drug delivery systems are to extend the residence time at the site of drug absorption, to intensify contact with the mucus to increase the drug concentration gradient, to ensure immediate absorption without dilution or degradation in the luminal fluid, and to localize the drug delivery system to a certain site[14, 15]. Delivery systems containing mucoadhesive polymers provide intimate contact with the mucosa, thereby reducing drug degradation between the delivery system and the absorbing membrane. They are controlled release systems that provide the simultaneous release of both drug and inhibitor, and allow the immobilization of enzyme inhibitors in the delivery systems. Novel polymers have shown excellent inhibitory activity against proteolytic enzymes and reasonable mucoadhesivity, and might therefore be a useful tool in overcoming the enzymatic barrier to oral peptide therapeutics. The binding of hydrophilic polymers, such as polyacrylates, cellulose derivatives, and chitosan derivatives, to biological surfaces is based on hydrogen bonding and ionic interactions. In the last few years, a large number of mucoadhesive systems have been developed, including super porous hydrogel-composite-based systems, lipid-based nanocarriers, thiolated polymers, and chitosan-based carriers. Some mucoadhesive polymers have also been shown to act as absorption enhancers or inhibitors of proteolytic enzymes[16-19].

Particulate carrier delivery systems: Most oral delivery strategies for insulin based on particulate carriers have been developed to circumvent the barriers to oral peptide delivery. They efficiently protect protein and peptide drugs against enzymatic degradation in the harsh environment of the GIT, provide high transfer of drugs across the epithelial mucosa, control the release rate, and target drug delivery to specific intestinal sites. Pathogens and microparticles smaller than 10 μm enter the gut-associated lymphoid tissues (GALT), which include Peyer's patches, the appendix, and small solitary lymphoid nodules. Peyer's patches are follicles of lymphoid tissue covered with a specialized epithelium containing Mcells. The potential modes of entry of submicron particles from the intestine include via M cells and enterocytes, and by paracellular routes. Histological evaluation of tissue sections has demonstrated that 100 nm particles diffuse throughout the submucosal layers, whereas larger particles (10 μm) are predominantly localized in the epithelial lining of the tissue. Similar particle size-dependent uptake was also confirmed in an experiment with Caco-2 cells[20-22]. Lysosomal degradation is normally associated with the endocytotic uptake of microparticles, but because this can interfere with the antigen-sampling role of the M cells, Peyer's patches are deficient in lysosomes. These favorable characteristics of the GALT have stimulated research into targeting Peyer's patches for peptide and protein delivery. Colloidal carrier systems that have already been studied to improve peptide delivery include microemulsions, liposomes, polymeric nano- and microparticles, and polymeric micelles. A novel oral dosage formulation of dry insulin emulsion responded to changes in the external environment, simulating gastrointestinal conditions, suggesting that this new enteric-coated dry emulsion formulation is potentially applicable to the oral delivery of peptide and protein drugs. In another related study, a new solid-in-oil-in-water emulsion for the oral administration of insulin has been developed using surfactant-coated insulin. A microemulsion of recombinant human (rh)-insulin has also demonstrated an improved efficacy of orally administered insulin. Liposomes are also a potential alternative carrier for the oral delivery of proteins. In one particular study, double liposomes containing insulin were examined in combination with apportion in. In a similar approach, Zelihagül et al. investigated the penetration properties of various liposome formulations containing insulin through a Caco-2 cell monolayer. They found that the oral administration of insulin- and sodium-taurocholate-incorporated liposomes significantly decreased blood glucose levels. Furthermore, a high in vitro/in vivo correlation was observed using the Caco-2 cell monolayer model. In one specific study, fusogenic liposomes were shown to be unique delivery vehicles capable of introducing their contents directly into the cytoplasm with the aid of the envelope glycoproteins of Sendai virus. The results indicated that fusogenic liposomes are useful carriers with which to improve the absorption of insulin via the intestinal tract[23-38].

Targeted delivery systems: The delivery of proteins and peptides to specific sites of action has been used to lower the total dose delivered and to concentrate the therapeutic dose at specific sites of pharmacological action. Absorption is not uniform throughout the GIT, and site-specific absorption occurs because of differences in the composition and thickness of the mucus layer, pH, surface area, and enzyme activity. Drug delivery to the colon, for instance, has several attractive features, including a prolonged residence time, reduced enzymatic activity, increased tissue responsiveness to absorption enhancers, and natural absorptive characteristics. Oral administration offers a potential portal to the superficial layers of the GIT (local delivery) and to the blood and lymphatic systems (systemic delivery). However, the harsh hydrolytic environment of the GIT and the epithelial barriers to absorption pose major challenges to the success of this mode of drug delivery for peptide and protein drugs. Insulin administration in a colon-targeted delivery system has been developed extensively over the past few years. The colon-targeted delivery of insulin with sodium glycocholate was more effective in increasing hypoglycemic effects after oral administration. The combination of sodiumglycocholate and poly (ethylene oxide) tended to prolong the absorption of insulin after oral administration using the colon-targeted delivery system. Tozaki et al. also reported that novel azopolymer-coated pellets may be useful carriers for the colon-targeted delivery of peptides, including insulin and (Asu1, 7) eel–calcitonin. Buccal administration in the various transmucosal routes of insulin delivery, many strategies have been used by scientists, and they are compiled in. The buccal mucosa has excellent accessibility, an expanse of smooth muscle, and a relatively immobile mucosa, and is hence suitable for the administration of retentive dosage forms. Direct access to the systemic circulation through the internal jugular vein allows drugs to bypass the hepatic first-pass metabolism, leading to high bioavailability. Other advantages include low enzymatic activity, suitability for drug excipients that mildly and reversibly damage or irritate the mucosa, painless administration, easy drug withdrawal, facility to include a permeation enhancer/enzyme inhibitor or pH modifier in the formulation, and versatility in the design of multidirectional or unidirectional release systems for local or systemic action. The mucosa lining the oral cavity represents a potentially important topical route for the delivery of proteins and therapeutic peptides. It has been shown that the buccal administration of proteins, such as insulin, interferons, and interleukins, has some advantages and reduces many related side effects. For example, the buccal route provides a constant, predictable drug concentration to the blood. Veuillez et al. have shown that peptide transport across the buccal mucosa occurs via passive diffusion and is often accompanied by varying degrees of metabolism. Various approaches have been taken to improve the buccal absorption of peptides, including the use of absorption enhancers to increase membrane permeability and/or the addition of enzyme inhibitors to increase drug stability. From this point of view, the role of absorption enhancers in the buccal transport of proteins is crucial. Many substances can function as absorption enhancers, the most popular being detergents such as bile acid salts, sodium lauryl sulfate, etc. However, many absorption enhancers have some side effects, often causing irritation of the buccal mucosa. An additional problem is the taste of buccal compositions. The most efficient absorption enhancers, bile acids salts, have a strong bitter taste, so the regular use of compounds containing bile acids is hardly acceptable for long-term administration. Pluronic F-127 (PF-127) gel containing insulin and unsaturated fatty acids, such as oleic acid (18:1), eicosapentaenoic acid (20:5), or docosahexaenoic acid (22:6), showed a continuous hypoglycaemic effect following its buccal administration in normal rats. PF-127 gels containing oleic acid showed the highest pharmacological availability 15.9%±7.9%). Comparative analyses indicated that 20% PF-127 gels containing unsaturated fatty acids are potential formulations for the buccal delivery of insulin. In particular, a good candidate for an effective absorption enhancer seems to be lysalbinic acid. Lysalbinic acid, a product of the alkaline hydrolysis of egg albumin and a mild detergent, meets those requirements. Nasal administration Nasal insulin delivery has been widely investigated as an alternative to subcutaneous injection for the treatment of diabetes and is considered to be a promising technique for the following reasons: the nose has a large surface area available for drug absorption because the epithelial surface is covered with numerous microvilli; the subepithelial layer is highly vascularized, and the venous blood from the nose passes directly into the systemic circulation, thereby avoiding the loss of drug by first-pass metabolism in the liver; it allows lower doses, more rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, fewer side effects, high total blood flow per cm3, and a porous endothelial basement membrane; it is easily accessible; and the drug is delivered directly to the brain along the olfactory nerves. The pharmacokinetic profile of intranasal insulin is similar to that achieved with intravenous injection and, in contrast to subcutaneous insulin delivery, bears a close resemblance to the ‘pulsatile’ pattern of endogenous insulin secretion during meal times. To date, attempts to implement this approach have indicated that intranasal insulin therapy has considerable potential for the control of postprandial hyperglycemia, especially in the treatment of patients with insulin-dependent diabetes mellitus. Despite the potential of

the nasal route, a number of factors limit the intranasal absorption of drugs, especially peptide and protein drugs. Mucociliary clearance, enzymatic activity, and the epithelium combined with the mucus layer constitute barriers to the nasal absorption of high-molecular-weight and hydrophilic peptides. Therefore, the use of absorption enhancers and proteolytic enzyme inhibitors, and the design of suitable dosage formulations, such as mucoadhesive and dry powder delivery systems, has been investigated to enhance the nasal bioavailability of these drugs. The effects of sodium deoxycholate (SDC) in combination with cyclodextrins (CD) as enhancers of the nasal absorption of insulin have been determined by measuring blood glucose levels. Combining SDC with beta-CD lowered the serious nasal ciliotoxicity of SDC and had a marked absorption-promoting effect, which was due not to the low concentration of SDC but to the inhibition of leucine aminopeptidase activity. The effects of a soybean-derived sterol mixture and of a steryl glucoside mixture as enhancers of the nasal absorption of insulin in rabbits have been investigated. A series of new glycosides with extended alkyl side chains (C13–16) linked to maltose or sucrose were synthesized and used effectively to enhance nasal insulin absorption in anesthetized rats. Cross comparisons of alkylmaltoses and alkanoylsucroses showed that the alkyl chain length had a greater effect than the glycoside moiety in determining the potency of potential insulin absorption-enhancing agents. When tetradecylmaltoside was applied to the nasal mucosa 15 min before insulin was applied, enhanced insulin absorption was observed. Another study reported that lipid-emulsion-based formulations were devised to enhance insulin absorption through the nasal cavity, although at lower insulin doses, but showed no statistically significant enhancement. Unfortunately, many absorption enhancers cause significant damage to the nasal mucosa or other side effects when used at very effective concentrations, particularly with long-term exposure. Most of the traditional absorption enhancers, such as surfactants and bile salts, have limited clinical use because of the irreversible damage to the nasal mucosa that accompanies their absorption enhancing effects[37-58].

Dry powder inhalation: Insulin was used as a model protein to demonstrate the feasibility of using protein crystals for the pulmonary delivery of a sustained-release protein drug formulation. The hypoglycaemic effects of the microcrystal suspension were prolonged over 7 h. These results could be attributed to the sustained release of insulin from the microcrystals, which were deposited widely throughout the entire lung. Insulin dry powder, made of insulin and other appropriate materials, was also insufflated into rat lungs from an incision in the throat. The area above the curve (AAC) for insulin (5 U/kg) administered by pulmonary delivery was very close to that of insulin given by subcutaneous administration at the same dose. Thus, the pulmonary delivery of insulin acts effectively and rapidly. The feasibility of insulinmicrocrystals as a long-acting formulation for pulmonary delivery was examined. In an in vivo experiment with rats, zinc enhanced the hypoglycaemic effects of insulin microcrystal, with minimum reductions in blood glucose of 17%. To investigate the enhancement effect of lanthanide ions (Ln3+) on the absorption of larger molecules from the pulmonary pathway, insulin (mol. wt. =5730) was chosen as the model peptide. Lanthanum is an inhibitor of calcium flux and inhibits the insulin secretion induced by glucose and acetylcholine to basal levels but does not alter the stimulatory effects of insulin. The temporal changes in the gadolinium (Gd3+) content of serum were also investigated because Gd3+ prevents the lipopolysaccharide-induced decrease in the expression of hepatic insulin-like growth factor-I (IGF) and IGF-binding protein-3. The effect of Ln3+ in promoting the bioavailability of insulin is closely related to its species, concentration, and order of delivery. The anionic form of gadolinium seems to be more effective than its cationic form. The co administration of Gd3+ with insulin was most effective in increasing insulin absorption in the lung[59-64].

Particulate carrier systems: The pulmonary delivery of peptides and proteins is complicated by the complexity of the anatomical structure of the human respiratory system and the effects on the disposition of the drug caused by the respiration process. A novel nebulizer-compatible liposomal carrier for the aerosol pulmonary delivery of insulin was developed and characterized. Experimental results showed that insulin could be efficiently encapsulated in liposomes using a method involving preformed vesicles and detergent dialysis. The optimal encapsulation efficiency was achieved when 40%ethanol was used. The particle size of the liposomal aerosols expressed from an ultrasonic nebulizer approximated 1 μm. Animal studies showed that plasma glucose levels were effectively reduced when liposomal insulin was delivered by the inhalation route using aerosolized insulin-encapsulated liposomes. By including a fluorescent probe (phosphatidylethanolamine– rhodamine) into the liposome, researchers found that the liposomal carriers were effectively and homogeneously distributed in the lung aveoli. Liposome-mediated pulmonary drug delivery promotes an increase in the drug retention time in the lungs, and more importantly, a reduction in extrapulmonary side effects, which invariably results in enhanced therapeutic efficacy. The influence of calcium phosphate (CAP) and polyethylene glycol (PEG) particles on the systemic delivery of insulin administered by the pulmonary route appears to be a crucial factor. Insulin–CAP–PEG particles in

suspension (1.2 U/kg, 110– 140 μL) were administered to the lungs of fasted rats by intratracheal instillation (INCAPEG) or spray instillation (SINCAPEG). Pharmacokinetic and pharmacodynamic analyses showed that the presence of CAP–PEG particles positively influenced the disposition of the insulin administered to the lungs of rat The great challenge for researchers remains the full optimization of the delivery system, which is the culmination of all those particle properties required for therapeutic applications: good encapsulation efficiency, the prevention of protein degradation, and the predictable release of the drug. This breakthrough in the availability of inhaled insulin is certain to have exceptions. It must be recognized that inhaled insulin may not be ideally suited to all patients. Receiving inhaled insulin had more episodes of hypoglycemia and gained more weight than did patients treated with oral agents. Mild to moderate cough was also reported in up to 25% of patients receiving inhaled insulin. Uncontrollable factors also affect pulmonary absorption, and smokers need lower and asthmatics higher doses. The pulmonary insulin dose required for a similar glycemic effect is approximately 20 times that required for a subcutaneous injection, and insulin directed antibodies are an issue. However, as a substitute for short-acting insulin, inhaled insulin appears to be safe, efficient, and satisfactory for clinical use and acceptable to patients at this early stage in its development[65-72].

Ocular administration: Numerous research groups have reported early exploratory work on systemic drug absorption via the ocular route. This efficient systemic absorption can be utilized as a non-invasive means of delivering drugs systemically. It also offers the advantages that it is much easier to administer than is an injection; the rate of systemic absorption through the ocular route is as fast as via an injection; eye tissues are much less sensitive to the development of immunological reactions than are other tissues; it bypasses first-pass gastrointestinal and liver effects, which are responsible for the low oral bioavailability of peptides and other drugs; and no tolerance and ocular side effects have been detected after long-term (3 months) daily administration of insulin eye drops. The eye presents unique opportunities and challenges when it comes to the delivery of pharmaceuticals, and it is very accessible to the application of topical medications. The potential route for insulin delivery to the anterior segment of the eye has been the conjunctival sac. More recent investigations have shown that the conjunctival route of entry plays an important role in the penetration of drugs into the anterior segment. Furthermore, topically applied drugs have been shown to have access to the sclera from the conjunctiva. Therefore, it is conceivable that such drugs could find their way to the posterior segment. It has been shown that even a high molecular-weight peptide like insulin can accumulate in the retina and optic nerve after topical application, supporting the contention that topically applied drugs can both reach the posterior segment and be therapeutic. Finally, topically applied insulin also accumulates in both the contra lateral eye and the central nervous system. After the pioneering work of Christie and Hanzal (1931), numerous investigations of the systemic delivery of insulin via the ocular route were undertaken. Bartlett et al. investigated the feasibility of using insulin eye drops in humans by studying the local toxicity and efficacy of insulin administered without surfactant to the eyes of healthy volunteers. The results of this study suggest that single-dose insulin at concentrations of up to 100 U/mL, formulated in saline, has no detectable clinical toxicity on the anterior structures of the human eye. Not surprisingly, this therapy was abandoned in humans because of its low bioavailability. Since then, the development of ophthalmic drug delivery systems has always been hindered by local irritation, rapid loss in drainage, blinking, and tearing. The results of previous studies suggest that the use of absorption enhancers should be introduced. A wide variety of absorption enhancers have been evaluated in the delivery of insulin via the ocular route. Yamamoto et al. determined the extent, pathways, and effects of absorption enhancers on the systemic absorption of insulin after the instillation of a topical solution to albino rabbit eyes. The absorption enhancers used were polyoxyethylene-9-lauryl ether (POELE), sodium glycocholate, sodium taurocholate, and sodium deoxycholate, all at a concentration of 1%. The nasal mucosa contributed about four times more than the conjunctival mucosa to the systemic absorption of ocularly applied insulin. However, the conjunctival mucosa was more discriminating in its sensitivity to the nature of the bile salts used than was the nasal mucosa. Collectively, these findings indicate that it is feasible to achieve hypoglycemia with ocularly administered insulin. Consequently, eye drops of 0.25% insulin plus 0.5% POELE or polyoxyethylene-20-stearyl ether (Brij-78) were instilled into rabbit eyes twice a day for 3 months. The efficacy of the insulin in lowering the blood glucose concentration and the uptake of insulin into the systemic circulation remained the same throughout the experimental period. No allergic responses or local side effects were detected, indicating that both insulin and the absorption enhancers (POELE and Brij- 78) are safe for instillation into the eyes over long periods. series of alkyl glycosides with various alkyl chain lengths and carbohydrate moieties were tested for their ability to enhance the systemic absorption of insulin after topical ocular delivery in anesthetized rats. All the reagents were effective only when used at concentrations above their critical micelle concentrations, and the most hydrophobic alkyl glycoside reagents were the most efficacious in

promoting systemic insulin absorption. Regular porcine insulin was administered as eyedrops, either alone or in combination with several different absorption enhancers, to eight healthy euglycemic dogs. No ocular symptoms occurred with the administration of insulin alone or together with 0.5% solutions of Brij-78, fusidic acid, POELE, dodecylmaltoside, or tetradecylmaltoside. This study demonstrated that short-acting insulin is systemically absorbed in dogs via the ocular route when applied with certain emulsants. Sucrose cocoate (SL-40) is an emulsifier used in emollients and skin-moisturizing cosmetic formulations that contains a mixture of sucrose esters of coconut fatty acids in an aqueous ethanol solution. Sucrose cocoate was examined to determine its potential usefulness and enhancing effects in nasal and ocular drug delivery.When insulin was administered ocularly in the presence of 0.5% sucrose cocoate, significant increases in plasma insulin levels and a decrease in blood glucose levels were observed. To prolong the retention time of the formulation in the precorneal area, a positively charged insulin-containing liposome was prepared. This formulation reduced the blood glucose concentrations of rabbits to 65%–70% of the initial levels for up to 5 h. Commercially available Gelfoam®, an absorbable gelatine sponge, is used in the fabrication of an ocular insert in the form of a matrix system. Both in vitro flow-through and in vivo methods of device removal were examined to determine the dissolution rate of insulin from a Gelfoam®-based eye device. Two eye drop formulations and 13 eye device formulations were evaluated. The in vivo results for devices containing 0.5 or 1.0 mg of insulin with 20 µg of Brij-78 showed substantial improvement in insulin activity and a significantly prolonged systemic delivery of insulin within the desired therapeutic levels, with no risk of hypoglycemia. The prolonged activity of the insulin was due to its gradual release from the device, which slowed tear production. Furthermore, the mean blood glucose concentration returned to a nearly normal level within 60 min of the removal of the device. Rectal administration during the past few years, considerable interest has arisen in the rectal route for insulin administration. This route is regarded as a more physiological route for the application of insulin. Rectal insulin delivery offers several advantages over some of the other enteral routes. First, the rectal route is independent of intestinal motility, gastric-emptying time, and diet. It is most likely that the presence of degrading enzymes in the gut wall decreases from the proximal end to the distal end of the small intestine and rectum. The most important advantage suggested for the rectal administration of insulin is the possibility of avoiding, to some extent, the hepatic first-pass metabolism. Hosny found that insulin suppositories containing 50 U of insulin incorporated with 50 mg of deoxycholic acid, sodium taurocholate, or both, placed in the rectum of alloxan-induced hyperglycemic rabbits, caused a large decrease in plasma glucose concentrations, and the relative hypoglycemia was calculated to be 38.0%, 34.9%, and 44.4%, respectively, compared with that observed for insulin (40 U) injected subcutaneously. The most pronounced effect was observed with the addition of polycarbophil to a suppository formulation containing a combination of deoxycholic acid and sodium taurocholate, which produced 56% relative hypoglycemia compared with that achieved with a subcutaneous injection. These suppository formulations are very promising alternatives to current insulin injections, because they are roughly half as efficacious as subcutaneous injections. Insulin suppositories were formulated usingWitepsol W35 as the base, to investigate the effects of various bile salts/acids on the plasma glucose concentrations of diabetic beagle dogs. A relative hypoglycemia of about 50% was achieved using insulin suppositories containing Witepsol W35 as the base and sodium deoxycholate (100 mg) plus sodium cholate (50 mg), sodium taurodeoxycholate (100 mg), or sodium taurocholate (100 mg) as enhancers of the rectal absorption of insulin. A desirable hypoglycemia, expressed as Cmax, and/or AUC, can be achieved by adjusting the insulin dose in the formulation according to the degree of initial hyperglycemia. Investigation of the effects of insulin suppositories on the plasma glucose concentrations of diabetic beagle dogs showed that a relative hypoglycaemic effect of about 50%–55% can be achieved using insulin suppositories containingWitepsol W35 as the base, insulin (5 U/kg), and sodiumsalicylate (50mg) or POELE (1%) as rectal absorption enhancers. Studies have recently shown that the formation of an adhesive interaction between the delivery system and the rectal mucosa can be harnessed as an absorption modifier because it increases the contact time of the coadministered drug and possibly acts as a sustained-release polymer. A thermo reversible liquid insulin suppository, which undergoes a phase transition to a bioadhesive gel at body temperature, enhances the bioavailability of insulin. The thermoreversible liquid insulin suppository (containing 100 IU/g insulin, 15% poloxamer P407, 20% poloxamer P188, 0.2%polycarbophil, and 10% sodium salicylate) could potentially be developed as a more convenient, safe, and effective rectal delivery system for insulin. Adikwu evaluated snail mucin motifs as rectal absorption enhancers for insulin. Themucins were extracted from the giant African snail Archachatina marginata by differential precipitation with acetone. Mucin administered exogenously formed disulphide linkages between its cysteine domain and those of endogenous mucin, increasing the gel network and hence the viscosity of the mucus, probably conferring

sustained- or prolonged-release properties to the dosage form. A batch with 7% mucin reduced the basal glucose level to 44% within 2 h of administration of the glycero-gelatin loaded mucin suppository. The efficacy and bioavailability of rectally delivered peptide remained low compared with those of peptide delivered by intravenous or subcutaneous injection. These delivery systems appeared to significantly increase the uptake of high-molecularweight polar drugs, such as insulin, before surfactant and other absorption enhancers were introduced. This is a concern in long-term therapy, because of the absorption of unwanted toxic molecules present in the GIT and disturbance to the permeability of the mucosal membrane. Patient compliance and the pain that accompanies rectal administration are both challenges for its widespread use as a therapeutic dosage form. Its irreproducible bioavailability and the special storage conditions required are also great obstacles to the rectal delivery of insulin[73-93].

Transdermal drug delivery systems: Transdermal drug delivery systems can be generally divided into physical, biochemical, and chemical methods. Further strategies have been investigated for the carriage of insulin using the enhancing effects of flexible lecithin vesicles containing insulin, designed for the transdermal delivery of hydrophilic proteins. The entrapment efficiencies of conventional and flexible vesicles were 35% and 81%, respectively. When flexible vesicles were non-occlusively applied to the abdominal skin of mice at a dose of 0.90 IU/cm2, an in vivo hypoglycemic study showed a percentage decrease in blood glucose of 21.42%± 10.19% at 1 h, which reached 61.48%±8.97%at 5 h and was still greater than 50% at 18 h. An advanced study evaluated the pharmacokinetic and pharmacodynamic effects of transdermally delivered insulin using novel CaCO3 nanoparticles in normal mice and those with diabetes.

Iontophoresis: More recently, one of the most advanced technologies that have been developed in the 20th century to overcome low skin permeability to insulin is iontophoresis. Iontophoresis is a technique used to enhance the transdermal delivery of compounds through the skin by the application of a small electric current. Using the processes of electro migration and electro-osmosis, iontophoresis increases the permeation of charged and neutral compounds. It offers the option of a programmed drug delivery technique that physically facilitates the transport of permeates across the skin. Transdermal iontophoresis is one such technique that shows promise and is believed to be a future option in the controlled and enhanced delivery of peptides and proteins. It allows non-invasive, continuous, pulsatile delivery, as well as pre-programmed complex dosing regimens. Combinations of iontophoresis with absorption enhancers, electroporation, and sonophoresis have been tested to increase transdermal drug permeation and decrease possible side effects further. At present, research is focused on resolving skin toxicity issues and other problems, to make the technology a commercial reality. Mao et al. reported that in the transdermal delivery of insulin by pulse-current iontophoresis, the pulse permeation rate correlates positively with the reservoir insulin concentration and with skin penetration by insulin. In another iontophoretic delivery study, Zakzewski et al. reported experimental evidence indicating a substantial increase in the penetration by insulin with the same-day application of a depilatory lotion, in conjunction with iontophoretic enhancement. Kanikkannan et al. studied the delivery of bovine insulin to streptozotocin-induced diabetic rats by iontophoresis. The iontophoresis of a monomeric human insulin analogue (B9 Asp, B27 Glu) through intact skin produced a significant decrease in plasma glucose levels in the diabetic rats. Insulin was used as the model for large peptides; to understand better the effect of peptide concentration, NaCl concentration, and Significant permeation of large peptides like insulin has been achieved using combination strategies involving absorption enhancers and transdermal iontophoresis. Recently, Choi et al. demonstrated the effectiveness and mechanism of a transdermal drug delivery system using iontophoresis plus an absorption enhancer. A combination of absorption enhancer pretreatment and iontophoresis delivered drugs more effectively than iontophoresis alone. The proposed theory is that iontophoretic drug delivery may be easiest through the dilated intercellular spaces of the stratum corneum, which have lowered electrical impedance following absorption enhancer pre-treatment[94, 95.]

Sonophoresis (ultrasound): For the past two decades, sonophoresis (ultrasound) has been used to enhance the delivery and activity of drugs. Ultrasound has an ever-increasing role in the delivery of therapeutic agents, including genetic material, proteins, and chemotherapeutic agents. There is a tremendous corpus of literature on the use of ultrasound to enhance the permeability of the skin for transdermal drug delivery. Therapeutic levels of ultrasound (1–3 MHz, 1–3 W/cm2) have been used for years to drive small hydrophobic molecules, like steroids, into or through the skin. Absorption enhancers have sometimes been used to increase skin permeability further. However, no significant transport of protein was achieved until 10 years ago, when Mitrogotri et al. showed that low-frequency ultrasound was much more effective than higher frequencies, and provided evidence of the mechanism involved. Skin permeability increased with decreasing frequency, and with increasing time of exposure and intensity (beyond a certain threshold), thus identifying collapse cavitation as a causative

mechanism. The current theory is that cavitation events open reversible channels in the lipid layers of the stratum corneum and provide less tortuous paths of transport for proteins such as insulin. Electron microscopy of skin exposed to low-frequency ultrasound revealed the removal of surface cells and the formation of pores and pockets large enough (~20 μm) to accommodate the transport of proteins and other large molecules. Tezel and Mitrogotri have formulated a model of shock-wave andmicroject cavitation events and their impact upon skin permeability. Although their model can be fitted to their data, there are many assumptions and parameters in the model, and more direct evidence is needed to identify conclusively the mechanisms of ultrasound-enhanced transdermal protein delivery[95-97].

Microneedles: A novel approach that increases transdermal transport involves the use of microneedles that pierce the skin and create micrometerscale openings. Although still extremely small on a clinical level, channels of micrometer dimensions are much larger than macromolecules and therefore should dramatically increase skin permeability to large drugmolecules. Microneedles of micrometer dimensions can create transport pathways large enough for small drugs, macromolecules, nanoparticles, and fluid flow, but small enough to avoid pain and facilitate highly localized and even intracellular targeting. The microelectronic revolution has provided tools for highly precise, reproducible, and scalable methods to fabricate structures of micrometer dimensions. This lithography-based approach can produce large arrays of microneedles that can be inserted into cells, skin, or other tissues. Arrays of micrometer-scale needles could be used to deliver drugs, proteins, and particles across skin in a minimally invasive manner. The increased importance of macromolecular therapeutics, combined with the newly acquired power of micro fabrication, has recently prompted interest in fabricating and testing microneedles for drug delivery. Practical microfabrication techniques have been developed to yield microneedle arrays of silicon, metal, and biodegradable polymers of micrometer dimensions in various geometries. Microneedle arrays having solid or hollow bores with tapered or beveled tips may provide minimally invasive method to increase skin permeability for diffusion-based transport of large molecules such as proteins. Hollow microneedles have permitted the flow of microliter quantities into the skin in vivo, including the microinjection of insulin to reduce blood glucose levels in diabetic rats. These results suggest that microneedles are a useful approach to transdermal drug delivery. Building on microneedle transdermal studies, Martanto et al. designed and fabricated arrays of solid microneedles to be inserted into the skin of diabetic hairless rats for the transdermal delivery of insulin to lower blood glucose levels. Recent marketed and developed formulations for non-invasive insulin delivery Significant progress has been reported in the recent past in the delivery of insulin using buccal and pulmonary routes, as has been achieved for many other hormonal drugs, such as calcitonin and vasopressin, which are available in intranasal sprays. The field of insulin delivery took a tremendous step forward with the approval of Exubera® from Pfizer and Nektar Therapeutics. Insulin has long been a target of drug delivery companies because of the number of patients using it and the difficulties and issues surrounding the reliance on injections. Pharmaceutical companies are confident that ease of use and the elimination of the needle will be enough to counter the added costs of alternative insulin delivery systems, and Exubera® is expected to reinforce this notion. The approval of this drug has legitimized alternative forms of drug delivery, providing patients with increased hope of eliminating or reducing their dependence on injections and instilling confidence in numerous drug delivery companies that alternative forms of insulin delivery systems can win the approval of federal authorities. Marketed developed insulin formulations and technologies for non-invasive. Researchers working in academic institutions and drug delivery companies have begun collaborative work on several novel delivery technologies to introduce a viable and feasible oral insulin dosage form that can potentially replace painful insulin therapy. The overwhelming attractiveness of oral administration is prompting numerous companies to develop technologies to overcome the challenges of oral peptide delivery. As a result, there is a high degree of innovation and competition, with multiple products already in clinical trials. One of the more unusual alternatives is from Emisphere Technology, Inc. Emisphere Technology has very recently made public that it is undertaking clinical phase II trials in type 2 diabetic patients in order to file an investigational new drug (IND) application with the U.S. Food and Drug Administration (FDA). This will be the first attempt by Emisphere Technology to test the Eligen™ oral drug delivery strategy that delivers insulin with the Emisphere® delivery agent or carrier in a capsule. Their proprietary Eligen™technology platform is based upon the use of synthetic non-acylated amino acids as carriers, which allow the peptide to enter the bloodstream through the body's natural passive transcellular transport processes in the GIT. Non-acylated amino acids, when used as carriers, do not alter the conformation of the delivered drug but do alter the physical properties at the point of transport, while not affecting the efficacy of the drug. The interim results of a U.S. phase II trial of AI-401, an oral tolerance product of AutoImmune, Inc. developed

for the treatment of diabetes, were presented at a poster session at the American Diabetes Association meeting in Chicago, in June 1998. The trial evaluated the feasibility of oral insulin therapy, delaying β-cell destruction in the pancreas, and thus preserving endogenous insulin secretion in newly diagnosed type 1 diabetic patients. This study is one of four trials undertaken using AI-401, an oral formulation of recombinant human insulin. The Eli Lilly Company is Auto Immune's worldwide partner in autoimmune (type 1) diabetes. The final data from the oral insulin arm of the NIH sponsored Diabetes Prevention Trial-Type 1 (DPT-1) showed a statistically significant benefit for patients with type 1 diabetes[98-107].

Conclusion

There has been a long history of research directed toward the development of novel routes of insulin delivery since recombinant DNA technology made insulin available at a reasonable cost in an injectable dosage form. Needle phobia and stress have encouraged scientists to investigate and exploit all promising routes of insulin delivery, ranging from oral to rectal, with a wide variety of devices and delivery systems. Many approaches have been used to study various strategies to overcome the inherent barriers to insulin uptake across the GIT and by transmucosal and transdermal routes. Each of the various routes of insulin administration has its own set of favorable and unfavorable properties. Most of the approaches described above represent long-term possibilities for insulin delivery, but difficulties in achieving adequate blood insulin concentrations are yet to be overcome. Over the last several decades, these formidable tasks have focused on oral insulin delivery. Our final achievement will be a clinically therapeutic bioavailable oral insulin that bypasses the obstacles of the GIT and overcomes the challenges inherent in the physicochemical properties of the insulin molecule. In recent years, the development of innovative oral insulin delivery carriers that improve oral insulin absorption has thrown some promising light on the new horizon of oral insulin therapy. Although extensive human clinical studies are still required, especially of long-term clinical applications, researchers in academic institutions and several drug delivery pharmaceutical companies are actively involved in the development of an oral insulin delivery system.

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