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Review Article

Non-Invasive and Less Degradative Ways of Insulin Administration

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ABSTRACT

Protein and peptide delivery systems attract great attention nowadays. They play crucial role in several diseases, but their way of administration has some disadvantages that makes patients dissatisfied. In this study, we choose insulin as a peptide that is used for type I and type II diabetic patients, but injection way of its usage is not suitable in diabetes as a chronic remedy. Although oral way is a needle-free one, but its bioavailability through that would be decreased because of degradation in gastro-intestine and consequently, further dosage should be used to get the desired hypoglycemic effect. Administration of insulin through non-parenteral and less enzymatic pathways, such as intranasal, pulmonary, transdermal, colon and vaginal routes, is new that attracts researchers' attention considerably. Although the bioavailability of insulin may be lower than the current injection way, but it may be improved by some strategies like the use of permeation enhancers. There are also some limitations in each way, but propagation of them would result in improvement of patients' quality of life and may cause some economic profits. The objective of this review was to introduce the convenient ways for long term insulin administration with few enzymatic barriers.

Introduction

Proteins and peptides are bio-macromolecules that have dramatic increase in pharmaceutical industry for medical aims. There is an intensive research in their delivery for several disease conditions and we can see suitable therapeutic effects by low dose of them. Their high specificity and potency make them to be more effective than small molecule drugs.[1,2] Most of these therapeutic peptides are delivered by parenteral route because of their low bioavailability when administered by other routes.[1] Insulin as a 51- amino acids peptide is generally delivered by invasive ways such as subcutaneous one.[3] This peptide plays the main role in therapeutic cycle of patients with type I and sometimes type II diabetes mellitus. In parenteral delivery of insulin there have been some problems that may limit use of insulin, like local hypertrophy and fat deposition at injection site, inability to handle it in certain circumstances and inject an exact daily dose of it by self-injection. Pain, cost and infections are also other restrictions that make it inconvenient to diabetic people.[4,5] Therefore, most of the patients are not pleased with this way of administration for chronic diseases such as diabetes mellitus. So, how to deliver this critical macromolecule is a challengeable issue which needs great attention.

In this respect some special delivery systems are intended to be designed through non-parenteral to make patients more convenient in insulin therapy. These non-invasive alternative routes which are proposed by scientists, are oral, nasal, pulmonary, transdermal, vaginal and colon delivery ways.[6,7] Among them, although the oral one will be more acceptable by patients, but this macromolecule may encounter a lot of barriers and its bioavailability will be decreased.[8] Because of enzymatic degradation and poor membrane permeability through this way, the half-life and bioavailability of insulin would be much lower than parenteral.[2] By improving non- invasive and less enzymatic ways, not only injection related anxiety will be decreased, but also enzymatic degradation through oral way will be bypassed.[9] Therefore, in this review we want to introduce these ways, such as intranasal, pulmonary, transdermal, colon and vaginal insulin delivery ways for diabetic patients (Figure 1.), who need this critical peptide for their chronic disease in their period of life.

Intranasal administration

Nose delivery

The nasal way is one of the non- invasive routes of insulin delivery with large, highly vascular surface (~150 cm^2) [10] that has rapid absorption and less enzymatic degradation.

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Some researchers, applied a bioadhesive delivery system including degradable starch microspheres (DSM)[11] and biological absorption enhancer lysophosphatidyl choline for nasal insulin delivery.[8] They found a rapid decrease in mean plasma glucose to about 50% of asal levels.[8]

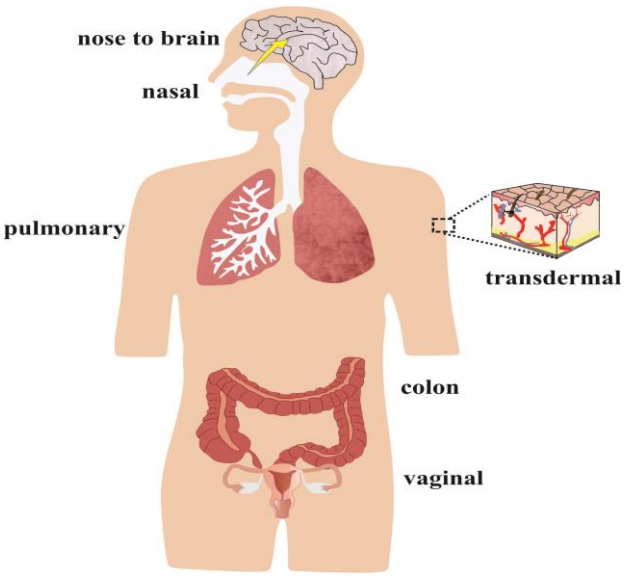

Figure 1. The ways of insulin delivery by non-invasive and less enzymatic ways.

Another research group evaluated a spray formulation that contains two bioadhesive polymer solutions (microcrystalline cellulose (MCC) or Plastoid LS0) with or without permeation enhancer (sodium taurocholate (ST), ammonium glycyrrhizinate (AG), glycyrrhetinic acid (GA)). They used white male rabbits as animal models in their experiment which were made diabetic by intravenous injection of 80 mg/kg alloxan in isotonic saline solution. Since drug absorption from nasal cavity is dependent upon some parameters such as time available and viscosity of the formulations, the presence of penetration enhancers with bioadhesives would result in an improved absorption. As a result, they found that the use of bioadhesive polymers like MCC and AG, may be introduced as a promising potential way for peptide delivery in nasal spray formulations.[12]

Chitosan as a biodegradable and biocompatible mucopolysaccharide obtained from deacetylation of chitin in crustaceans such as crabs and shrimps. Chitosan has mucoadhesive property because of its viscosity and interaction with negatively charged sites on the mucosa surface. It has shown that chitosan could be able to enhance absorption of peptides and proteins.[13,14] In a study, co-administration of hydroxyl-β-cyclodextrin (HP-β-CD) and chitosan were analyzed in male Sprogue-Dawley rats. They proved that the presence of HP-β-CD in chitosan formulation, can increase the permeability of insulin and will have maximum hypoglycemic effect.[15] In subsequent studies, chitosan derivatives co-formulated in hydrogels with polyethylene glycol (PEG) and glycerophosphate containing insulin, displays good water holding capacity and mucoadhesive potential with favorable rheological behavior. The mucoadhesive formulation based on N-trimethyl chitosan formulation demonstrate a reduction in blood glucose in diabetic rat models.[16,17] Another study also demonstrated that PEG-g-chitosan nanoparticles are promising carriers for insulin delivery through the nasal mucosa in which PEG is able to increase solubility and biocompatibility of chitosan and also resist adsorption of plasma proteins in blood by steric repulsion mechanism.[18] There is also a product in first clinical phases with "ChiSys" name and is produced by West Pharmaceutical Services. In this formulation chitosan is used as an absorption enhancer.[19]

Aminated gelatin microspheres (AGMs) have good mucoadhesive properties in nasal drug delivery in rat models. It was found that the plasma glucose level reached to its lowest at 45 min after administration of insulin-AGMs, but in administration of blank AGMs and blank insulin there was no significant lowering effect on glucose level in rats. Therefore, this positively charged gelatin derivative is investigated as a good system for the release of insulin through nasal administration.[20]

Microemulsion formation is another system for delivery of insulin via nasal administration. In this formulation, it is not necessary to use irritating absorption-enhancing adjuvants.[21] Permeability studies demonstrated that this nano-sized emulsions have the ability to improve insulin transport by opening the intracellular pathway.[22]

Thiomeric microparticles are systems that muco-adhesiveness is one of their advantages. Because of their muco-adhesive property, they can facilitate the entrance of peptides by leading a high concentration on the mucosa membrane. This polymer makes potent contact with mucosa and facilitates absorption of insulin which attaching to the polymer by disulphide bonds.[23]

Cell-penetrating peptides (CPPs) have been known as promising tools for intracellular delivery of macromolecules. It has been proven that this peptide has the ability for transcellular delivery of peptides and proteins across the nasal and intestinal epithelial membrane without local toxicity, inflammation and immunogenicity.[24,25] In one of the studies on these peptides, the researchers found that PenetraMax and L-penetrain have nasal absorption enhancing capacities without any damage to epithelial membrane of the nose. The bioavailability of insulin with novel CPPs, PenetraMax, was almost 100% relatively to subcutaneous administration of insulin.[25]

Nose to Brain delivery

Pharmacotherapy of some CNS diseases by insulin delivery is a challenging subject because of the presence of formidable obstacle, blood brain barrier (BBB). Some researchers found some ways to deliver insulin into the brain. It has been shown that insulin can cause a reliable and predictable decline in weight loss and food intake by administration into the hypothalamic area or the cerebral ventricles of the brain. The easiest way to administer this peptide into the brain, is by olfactory region of nasal cavity (Figure 2).

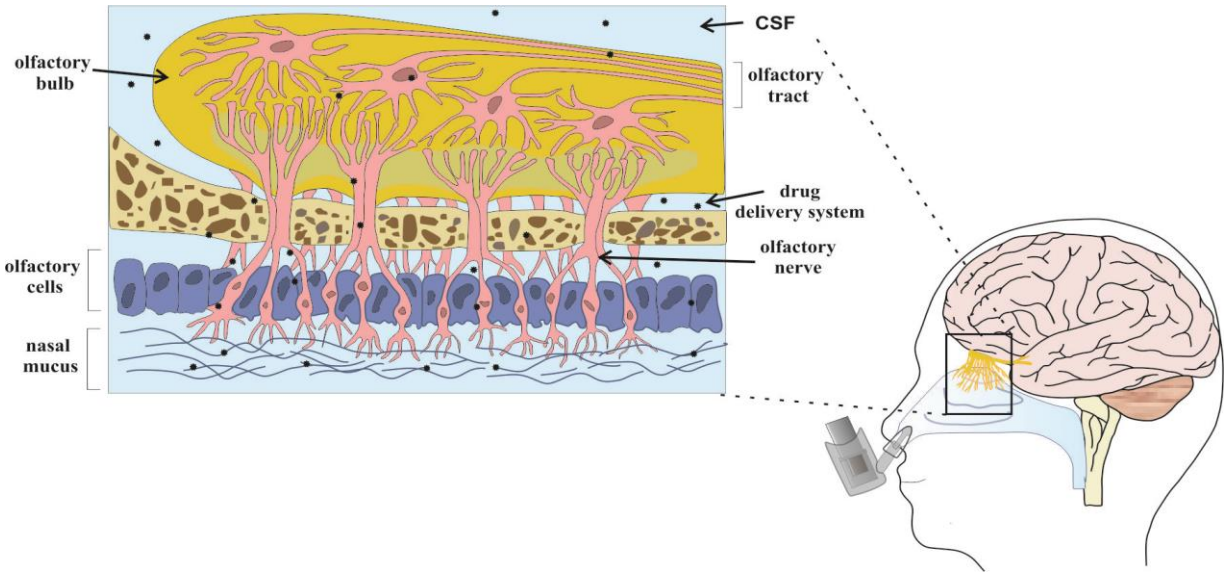

Figure 2. Nose to brain drug transport.

They propose that insulin gets access to the brain interstitial fluid through receptor mediated system and bypassing the BBB[26]. High affinity insulin receptor (Ins R)[27] in the brain endothelium and cell-penetrating peptides (CPPs) have been introduced for improving insulin transport across the brain. CPPs can enhance uptake of macromolecules like peptides and proteins via intranasal pathway to the brain. They increase the permeability of mucosal membrane by irritation or damage; although the researchers have proven its safety with local and systemic administration. This research group has been reported that co-administration of insulin with D or L penetration as CPPs is potential candidate therapy for Alzheimer's disease and can improve memory and recognition.[28] According to these studies, it is possible to deliver insulin through olfactory way into the brain efficiently and effectively by using some strategies.

Pulmonary administration

Delivery of insulin through the human lungs by inhalation is another non-invasive administration rout for systemic delivery that would receive considerable attention and high compliance.[29] The lungs have an enormous absorptive surface area (~80-120 m^2),[10] extremely thin mucosal membrane, good supply of blood, low intracellular and extracellular enzyme activity and avoidance of first pass hepatic metabolism; so these properties make lungs a good site for systemic delivery of peptide and proteins.[20,30]

Delivery of sufficient dose of drug to special target needs an efficient carrier. A good delivery system can prepare a long acting form of insulin that would improve patients' compliance. Inhaled dry powder insulin (IDPI) may be an option for delivery of insulin in diabetic patients. To date, clinical evidence suggests that IDPI improve glycemic control compared to SC or oral regimen.[31] In one study, researchers demonstrated that layer-by-layer insulin microparticles formulated by thin cationic and anionic polyelectrolytes produced sustained lowering effect of serum glucose in rats. This approach reduced the rapid clearance of this peptide from the body and decreased the time of administration.[32] Hyaluronic acid (HA), a linear polysaccharide with hygroscopic property, can make hydrogel in presence of water in low concentration and was designed as a system to prolong the pharmacokinetic of insulin delivered through the lungs. This polymer shows muco-adhesive property and protection against the lungs in inflammation. Researchers showed that pharmacokinetic of insulin was improved when administered with HA in dry powder inhalation form in conscious dogs.[33] Another experimental result declared that liposomal carrier for insulin delivery could be efficient for pulmonary delivery by increasing retention time of insulin in mice lung and protecting it in aerosol generation process.[20]

The bioavailability of peptides and proteins with large size and high hydrophilicity, like insulin, can be improved by using absorption enhancers which increasing permeability without any damage to the membrane. Sperminated pullulans (SP) and gelatin (SG) as sperminated polymers have the ability to interact with the luminal surface of mucus membrane because of their amino groups and enhance the permeability of water soluble drugs in good level. It was shown that since the SP has more amino groups than SG, it demonstrated lowering of glucose concentration of 70.5% relative to baseline glucose level in plasma of rats.[34]

Chitosan is cationic polymer that can also interact with negative surface of membrane with negligible toxicity and damage to the absorption site. Researchers incorporated insulin into chitosan nanoparticles through ionotropic gelation technique. This microencapsulated insulin-loaded chitosan nanoparticle in dry powder form, induced better hypoglycemic effect than control formulations.[35] In another work, the permeation of three kinds of microparticle vehicles for pulmonary delivery of insulin

in diabetic rats such as N-trimethyl chitosan 60 (TMC 60), TMC 20 and dextran were assessed. TMCs are chitosan derivatives but in contrast to it, they have good water solubility property without pH dependence. Both of TMC 60 and 20 show muco-adhesive property but the main difference between them is in degree of quaternization, which make TMC 60 a permeation enhancer. The bioavailability of pulmonary delivered insulin by TMC 60 was 0.95 relative to the subcutaneous way, but the others bioavailability were almost half of it. They concluded that TMC 60 insulin microparticles have capability to be introduced as an alternative carrier for chitosan in protein delivery through the lungs.[36] Incorporation of chitosan particles in phospholipid bilayer would also cause sustained delivery of insulin with low capture of them by alveolar macrophages.[37,38]

Porous nanoparticle-aggregate particles (PNAPs) are another option for insulin delivery. Yang *et al*. incorporated a model peptide, octerotide acetate (OA), in PNAPs by spray freeze drying to obtain proper aerodynamic size (~3 µm) without any high temperature in spray drying. They also improved lipophilicity of OA by electrostatic interaction with sodium dodecyl sulfate as an anionic detergent. In this way, loading entrapment of drug in PNAPs was also increased. Therefore, this way have the potential to be introduced for carrying of insulin through the lungs because of its proper aerodynamic size and maximal deposition.[39]

Transdermal administration

Transdermal drug delivery (TDD) is another non-invasive alternative way to the subcutaneous injection of insulin in diabetic patients.[40] This way is able to minimize painful administration of injection delivery[41] and may improve patient compliance.[42] This way is limited by the low permeability of stratum corneum (SC), flat and dead cells which filled with keratin fibers surrounded by lipid bilayers.[3] Researchers demonstrated some pathways to bypass the skin barrier, such as sonophoresis and iontophoresis as physical enhancers.[3,43] Theses enhancers may have some undesirable and irreversible damage to skin[44] but synergistic use of them may be safe and cost effective accomplishing by enhanced penetration of chemicals with large molecular weight. Ultrasound in sonophoresis increases diffusivity of chemicals by changing in skin structure and iontophoresis enhances electrorepulsion and water movement. When they are used in combination, electroosmosis process, plays the key role in flux of large molecular weight chemicals[45] Sintov *et al*. introduced a chemical agent that can also help for the protein/ peptide delivery through the skin. They demonstrated that application of iodine ointment before topical insulin administration elevated insulin level in plasma in comparison with control group. They related this result to inactivation of endogenous sulfhydryls such as glutathione, which can interact with disulfide bonds in hormone, by iodine.[40] "U-Strip" is a developing product in non-invasive insulin delivery that microelectronics and ultrasonic techniques are used in it.[19]

Microneedles (MNs) have been widely studied for insulin delivery.[46] A dissolving microneedle patch composed of starch and gelatin is a way of insulin delivery with less painful property than subcutaneous one. It causes rapid and efficient delivery of insulin into the skin in diabetic treatments.[47] In one study, researchers showed that lyophilized hydrogel patches could be used in combination with microneedle delivery of insulin. These lyophilized patches are easy in use, and stable form for protein. They show no leakage of drug in comparison with solution formulation and their three dimensional structure makes them preferable with microneedle systems.[42] Another delivery system, Biphasic vesicles, transdermal vesicle which is combined of liposome and emulsion in its structure and suitable for macromolecular delivery across the skin.[1] King *et al*. used transdermal patches containing Biphasix-insulin formulation. The results showed that this carrier not only decreased the blood glucose up to 43.7 % (compared with initial blood glucose level), but also made a sustained delivery of it in diabetic rats.[48]

Heat is also another combined strategy that can be used for insulin delivery through transdermal pathway by creating micropores on the skin surface and increasing blood flow.[19]

Colon administration

Colon targeted drug delivery via oral way is currently considered as a way for systemic therapeutic goals. Although large bowel has lower absorption sites in comparison with small intestine, but it has some advantages such as long transit time and low proteolytic effect, that makes it as an efficient site for systemic insulin delivery.[49] In addition, its response to permeation enhancers is also high.[50] Proximal part of the colon has been intended as a good absorption site for insulin and other peptide and protein delivery. Researchers are using microbial, pH, pressure and time-dependent strategies for systemic delivery of insulin through colon.[49] Microbiota-activated coatings as of the mentioned systems are mostly obtained from polysaccharides with natural origin and are mixed with insoluble structuring excipients that could respond to microbial flora of colon. PH-sensitive films and Pressure-sensitive coatings also show responsiveness to pH changes in the lower gut and relatively elevated pressure in large bowel, respectively. Finally, time-dependent films undergo time erosion processes by passing through the colon.[51,52]

In a study, chitosan (CS), triethylchitosan (TEC) and dimethylethyl-chitosan (DMEC) based nanoparticles were used for colon delivery of insulin. TEC and DMEC are quaternized derivatives of chitosan that make them permanently charged even in neutral and alkaline pH. Chitosan based structure of these carries[53] and the polyelectrolyte complex formation method that was used for loading insulin, make this system to release drug in a sustained manner for 5 hours.

Table 1. Advantages and disadvantages of non-invasive and less enzymatic ways of insulin administration.

Route of Administration		Advantages	Disadvantages
Intranasal administration	Nose delivery	Highly vascularized [10] Less enzymatic degradation [11] Large surface (~150 cm^2) [10]	Dependent on time of exposure and viscosity of the formulations [12] Considerable variations in bioavailability [56]
	Nose to brain delivery	The easiest way of delivering insulin into the brain [26] Bypassing the BBB [54]	Low penetration for macromolecules [54]
Pulmonary administration		Enormous absorptive surface area (~80- 120 m^2) [10] Extremely thin (0.2 µm) mucosal membrane [10] Good supply of blood [55] Relatively resistant to most peptidases [10]	Delivery of insufficient dose of drug because of patient [56] Rapid clearance by mucociliary clearance [10]
Transdermal administration		Inexpensive [56] Large surface [19]	Low permeability of the stratum corneum [3] Possibility of skin hypersensitivity [55]
Colon administration		Long transit time [19] Good response to permeation enhancers and responsive systems [19] Low level of peptidases [49]	Lower absorption sites in comparison with small intestine [49] High amount of anaerobic bacteria [58]
Vaginal administration		Wide surface area [57] High blood supply [57]	Gender specificity [59] Messiness [59] Low remaining time in site of absorption [59] Variable vaginal epithelium thickness [58]

Preparing nanoparticles under mild conditions without application of any organic solvents and surfactants is the major advantage of this method that makes it suitable for peptides. They conclude that, because of permanent charged characteristics of TEC and DMEC and sustained release effect of this formulation, they can be introduced as promising system for colon absorption via oral administration of proteins and peptides.[4]

It was demonstrated that colon targeted delivery system with an absorption enhancer (sodium glycocholate) and a gelling agent (poly ethylene oxide) could be able to show prolonged absorption of insulin and better hypoglycemic effect than a naked colon targeted system containing insulin.[60] Onuki *et al*. used water in oil in water (w/o/w) system as a protection system of insulin against proteolysis. They used also docosahexaenoic acid (a poly unsaturated fatty acid) and pluronic F127 as potential absorption enhancers and controller of hypoglycemic duration, respectively for rectal delivery of insulin in rats.[61]

Vaginal administration

To this date, there is just a few number of vaginal dosage forms because of its limitations such as gender specificity, messiness and low remaining time in site of absorption, but beside of its disadvantages, it may be a good site for peptide and protein delivery because of its wide surface area, high blood supply, efficient permeation characteristics and avoidance of hepatic first pass metabolism effect.[57,59] It is also important to say that smaller dose of insulin will be needed in comparison with rectal administration with the same formulation.[59] Kazuhiro *et al*. documented that administration of insulin suspension in polyacrylic acid gel based in 1 IU/kg of alloxan diabetic rats and rabbits through vaginal, caused remarkable hypoglycemic effect during the first 30 min. They also said that polyacrylic acid aqueous gel base can adjust the pH and viscosity over a wide range, and low viscosity of this formulation (0.1%) show better results than high viscose one (1%) but the difference isn't considerable.[7] This way of drug delivery, may have also some absorption barriers through mucosa that can be solved by absorption enhancers. Some amphiphilic compounds, such as sodium taurodihydrofusidate (STDHF), laureth-9 and lysophosphatidylcholine (LPC), dramatically increased hypoglycemic response to intravaginal administration of insulin but all of them may have effect on mucosa histology; but LPC, as an enhancer, may cause limited epithelial damage.[57]

Comparison of non-invasive and less enzymatic ways of insulin administration

Intranasal, pulmonary, transdermal, colon and vaginal delivery ways can be introduced as non-invasive and painless sites for insulin absorption with low enzymatic activity in comparison with oral and subcutaneous injection ways and are more acceptable than injection. Beside their advantages they may have some limitations that we brought them briefly in Table 1.

Conclusion

In this review we collected the ways of insulin administration that not only encounter with less enzymatic barriers in oral administration, but also avoid patients' disapprobation in comparison with current way, subcutaneous injection. The ways that were introduced, were intranasal, pulmonary, transdermal, colon and vaginal ones. All of those ways can improve quality of life in diabetic patients and reduce dose of administered drug, but all of them need more research to be used by patients readily. They need great attention and effort to be brought in clinical trials.

Conflict of interests

The authors claim that there is no conflict of interest.

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