REVIEW ARTICLE

Novel noninvasive techniques in management of diabetes

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Since the discovery, insulin remains as effective means of curing the diabetes. During the last decades, diabetes has become a life-threatening disease, because of this people initially goes for insulin injections but the inconvenience leads to the development of noninvasive techniques, which has recently replaced the insulin injection. These techniques overcome the barrier for the insulin treatment and ultimately control the glucose level of patient effectively than invasive technique. In these review, an attempt has been made to cover overall techniques available for diabetes treatment noninvasively. In an attempt of this, data has been collected, which includes insulin delivery via oral, nasal, pulmonary, rectal, buccal and ocular route. Along with these devices available in the market and under trials for diabetes treatment has also been covered. As this disease requires continuous treatment, it is advisable to go with noninvasive means of insulin delivery.

Key words: *Diabetes, insulin, iontophoresis, sonophoresis, pulmonary*

INTRODUCTION

The inconvenience, discomfort of patients, has driven rigorous pharmaceutical research to replace the existing invasive devices (injections, surgical devices etc.). Over the last several decades, the biotechnology has gained tremendous success in the development of new bioengineered products. Diseases like diabetes; cancers etc., are the life-threatening diseases, so such progress of biotechnology leads to the development of the newer drug delivery, ultimately deal with diseases. In addition to biotechnology, a tremendous increase in computer science and electronic field also leads into the development of the non-invasive devises. This also found to be beneficial in disease management. In this review, the overall novel techniques for management of diabetes have been covered.

DIABETES MELLITUS

Diabetes mellitus is a common degenerative disease among the communities with different age groups and different socioeconomic levels, characterized by elevated blood glucose levels (BGLs) and disturbance in carbohydrate, fat and protein metabolism, or is correlated with a deficiency of insulin secretion within the target cell membranes or is correlated with a deficiency of insulin secretion within the target cell membranes.[1] It is suffered by more than 180 million people and is responsible for approximately 2.9 million deaths each year.[2] This deficiency in insulin results in type 1 diabetes or insulin-dependent diabetes mellitus. Type 2 diabetes or noninsulin dependent diabetes mellitus which is a result of hyperglycemia caused by overproduction of glucose at the hepatic level.[3] Convention diabetes treatment is basically a replacement therapy, which involves administration of exogenous insulin subcutaneously to impersonate, as closely as possible, the insulin secretion of the healthy pancreas. The subcutaneous route has been a stronghold of insulin delivery until now, though it has following obstacles,

- Insulin is not delivered in a pulsatile manner
- Because of slower absorption it should be administered before 30 min of meal[4]
- Deposition of insulin in peripheral tissue may cause peripheral hyperinsulinemia
- Diabetic micro and macro-angiopathy may occur due to smooth-muscle cell proliferation, and glucose incorporation into the lipid of arterial walls.[5]

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So the quest to defeat this drawback, exploits a newer noninvasive techniques including oral, buccal, pulmonary, nasal, transdermal, ocular, and rectal drug delivery systems etc.[6-9]

ORAL INSULIN

The gastrointestinal tract (GIT) is the route of choice for the administration of most drugs, despite their molecular structure or weight.[10] The major challenges in proteins and peptide drug delivery is degradation by enzyme, keep the bioactivity of proteins during formulation processing. Proteolytic enzymes like pepsin, chymotrypsin, trypsin, and carboxypeptidase, which are positioned in the stomach and small intestinal lumen. These proteolytic enzymes are responsible for about 20% degradation of ingested proteins, while the remaining of the degradation occurs at the brush-border membrane (by various peptidases) or within the enterocytes of the intestinal tract.[11,12] Success in the oral delivery of therapeutic insulin would progress the quality of life of many people who must regularly receive injections of this drug. Different strategies have been proposed to overcome such bioavailability hampering problems.

Penetration enhancers

The intestinal epithelium represents the major obstacle to absorption of orally administered drugs and peptides into the systemic circulation. Access of molecules through the paracellular pathway is restricted by tight junctions. 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.[13] Bile salts, surfactants, fatty acids, chelators, salicylates, and zonula occludens toxin (Zot) are common permeation enhancers.

Insulin solution when administered with or without aprotinin (AP) as a protease inhibitor, or absorption enhancers such as sodium caprate, Na2EDTA or sodium glycocholate (GC), found beneficial in enhancement of insulin bioavailability more effectively in the colon than in the small intestine.[14] Cyclodextrins (CDs) have also been used to enhance the absorption of insulin from lower jejunal and upper ileal segments of rat small intestine.[15] In another interesting study inclusion of mixed micelles containing sodium GC and linoleic acid (LOA), appreciably improved insulin absorption and causes hypoglycemia in dogs. Bile salts have been reported to boost the absorption of insulin through paracellular pathway.[16] Fasano and Uzzau reported that when Zot when administered along with insulin in rabbit, shows an increase in permeability to insulin (*in vitro*), while *in vivo* studies shows absorption in both the rabbit jejunum and ileum, while no considerable changes were detected in the colon. In diabetic rats, bioavailability of oral insulin co-administered with Zot was sufficient to lower serum glucose concentrations to levels comparable to those obtained after parenteral injection of the insulin.[17] Study reveals that the insulin when formulated as enteric-coated capsules containing Witepsol W35 and sodium salicylate, significantly decreased plasma glucose levels (PGLs) in diabetic beagle dogs.[18] In another interesting study water-in-oil-water multiple emulsion containing gelatin, insulin, 2% oleic acid (OA) has been formulated according to the orthogonal experimental design for intestinal insulin delivery.[19] In similar approach potential of 2% docosahexaenoic acid or eicosapentaenoic acid for enteral delivery has also been studied.[20] Sodium 5-methoxysalicylate also enhances the absorption of insulin from the upper GIT, and gives relative bioavailability of 10% of that given intramuscularly.[21]

Protease inhibitors

Insulin is sturdily degraded by α-chymotrypsin, trypsin, and elastase. The coadministration of enzyme inhibitors provides a workable means to evade the enzymatic barrier to the delivery of peptide and protein drugs.

In an interesting study, transport studies of insulin along with a lipophilic marker ([7-3H] testosterone) and a hydrophilic marker (D-[1-14C] mannitol) across rat jejunum in the presence of chicken ovomucoid and duck ovomucoid (DkOVM) has been carried out. Result shows increase in flux values of insulin in the presence of alpha-chymotrypsin and DkOVM at the 1:2 ratio of enzyme to inhibitor, and ovomucoids has potential to modulate transcellular and paracellular permeability by inhibiting the insulin degradation. Ovomucoid (DkOVM) was further evaluated for its dissolution stability in the presence of trypsin and α-chymotrypsin. Results show that ovomucoid found beneficial in deactivation of α-chymotrypsin.[22-24]

Another report demonstrated enteric coating also beneficial approach in protecting insulin degradations, in which enteric coated tablet containing insulin formulated with polymer-inhibitor conjugate carboxymethylcellulose (CMC)-Bowman–Birk inhibitor and CMC-elastatinal, further homogenized with polycarbophil-cysteine conjugate and mannitol, shows protective effect on insulin, while in diabetic mice these conjugates, when combined with polycarbophil–cysteine, induced a 20-40% reduction in basal glucose levels for more than 80 h due to formation of inter, as well as intramolecular disulfide bonds within the polymer matrix.[25] Another study leads to the conclusion that when different protease inhibitors (soybean trypsin inhibitor, camostat mesilate, bacitracin, sodium GC and AP) Coadministered along with insulin in large and small intestinal loop of rats, showed remarkable improvement in insulin absorption from the large intestine, while no predominant effect observed from a small intestine. In addition to this bacitracin, camostat mesilate and sodium GC prevent degradation of insulin.[26]

Another interesting studies of Morishita *et al*. demonstrated that pretreatment of rat ileum by hyaluronidase improves the permeation of insulin across the mucous/glycocalyx layers.[27,28] However, its use may alter the physiology of GIT, as some proteins from food need to be degraded for their effectiveness.

Mucoadhesive polymeric systems

Mucoadhesive drug delivery systems utilize the property of certain polymers to adhere to the mucosa on hydration and held together by interfacial forces.[29] Chitosan-EDTA-protease-inhibitor (antipain, chymostatin and elastatinal) conjugates showed mucoadhesive as well as inhibitory effect towards the trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), elastase (3.4.21.36), carboxypeptidase A (EC 3.4.17.1), carboxypeptidase B (EC 3.4.17.2) and aminopeptidase N (EC 3.4.11.2).[30] Superporous hydrogel (SPH) and SPH composite has also been developed for effective delivery of peptide drugs into intestine.[31] Advancement in this field driven the development of a variety of mucoadhesive polymers. Improvement in the mucoadhesive properties of alginate by the covalent attachment of cysteine has also been carried out.[32] Thiolated polymers are bearing thiol side group emerging as newer approach for mucoadhesive delivery of proteins and peptides.[33] This innovation leads to the development of anionic thiomers poly (acrylic acid)–cysteine,[34] poly (acrylic acid)–cysteamine,[35] CMC–cysteine,[36] and alginate–cysteine[32] and the cationic thiomers chitosan–cysteine,[37] chitosan–TBA[38,39] and chitosan–thioglycolic acid,[40,41] have been formulated. Due to the formation of stronger disulphide bond which mimic natural glycoproteins, improves mucoadhesive properties up to 140 fold.[42] In another similar type of works Thiolated microspheres were formulated, cysteine was immobilized on carbopol using EDAC. These microspheres showed 75.25% ± 0.93% reduction in BGL in comparison to nonthiolated microspheres.[43] An interesting report demonstrate that Multilamellar liposomes consisting of dipalmitoylphosphatidylcholine and dicetyl phosphate coated with chitosan, facilitate enteral absorption of insulin. To protect peptide drugs from degradation in stomach and upper part of small intestine numerous work has been carried out on pH sensitive delivery. In this type of approach pH-responsive, poly (methacrylic-g-ethylene glycol) hydrogels P (MAA-g-EG) were formulated for insulin delivery which remain in unswollen in stomach, while swells or dissociate at higher pH (intestine) and releases insulin.[44-48] In Further studies effect of molecular weight and microparticle size on release of insulin from hydrogel has been carried out, The smaller-sized microparticular insulin-loaded polymer showed a rapid burst-type insulin release and higher insulin absorption compared with that achieved with larger microparticles, resulting in a greater hypoglycemic effect without detectable mucosal damage.[27,49] Yamagata *et al*. demonstrated that the reason for such kind of release lies behind $Ca2^{+}$ deprivation ability of P (MAA-g-EG).[50] 1:1 molar ratio of methacrylic acid/ethylene glycol units showed the most pronounced hypoglycemic effects.[51] In another study, hypoglycemic effect of insulin-loaded lectin-microparticle, resulted in larger glucose change (%) from the base level.[52] Another study reveals that chitosan nanoparticles (CS NPs) loaded with insulin prolong hypoglycemia over 15 h, showed bioavailability up to 14.9%,[53] while chitosan-4-thiobutylamidine (chitosan-TBA) - insulin tablets showed a controlled release of insulin over 8 h.[54] LueBen *et al*. reported that chitosan-glutamate, polycarbophil and Carbopol 934P are capable of inhibit the activity of the proteolytic enzyme trypsin at pH 6.7.[55] Recently silica based, insulin/cell penetrating peptide encapsulated mucoadhesive oral insulin formulation has been formulated, which overcomes both enzyme and mucosal barriers.[56]

As explained, these mucoadhesive polymers are serves as a promising approach for peroral delivery of proteins and peptide drugs, and their mechanism of action is probably a combination of inhibiting protease activities and modulating the intestinal epithelial permeability.

Micro and nano particulate carrier delivery systems

Major objective to formulate particulate carrier delivery systems is to circumvent the barriers to oral peptide delivery. Intestinal payer's patches are follicles of organized gut-associated lymphoid tissue, covered with a specialized epithelium containing M-cells. M-cells serves as the road for trafficking across the intestine.[57] Report of Desai *et al*. demonstrated that 100 nm particles diffuse throughout the Caco-2 cell and showed 46% uptake, whereas larger particles (10 μm) showed 6% uptake.[58] Pan *et al*. has also studied the effect of particle size of insulin-loaded bioadhesive chitosan microspheres (CS-MPs) [Figure 1].[59]

Deluca *et al*. developed acryloyl hydroxyethyl starch-PLGA microspheres for sustained release delivery of insulin.

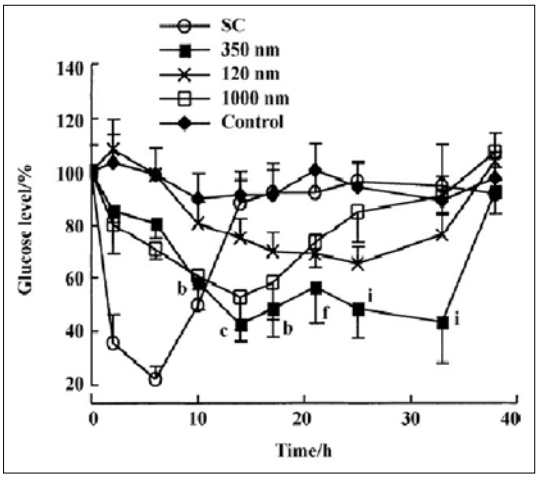

Figure 1: Hypoglycemic effect of various sizes of chitosan microspheres in alloxan-induced diabetic rats relative to insulin pharmacological saline solution (sc). $^{b}P < 0.05$, $^{c}P < 0.01$ versus 120 nm. $^{f}P < 0.01$ versus control. $^{i}P < 0.01$ versus 1000 nm. $n = 8$. Mean ± standard deviation[59]

Recently, alginate–CS-MPs were developed for insulin delivery. Result showed that under the pH conditions of the gastrointestinal environment, only 32% of insulin released during the simulated transit time of drug (2 h in the stomach and 4 h in the intestine). While, under the pH condition of blood, insulin release was stable and sustained for a long time (14 days).[60] In another study Ma *et al*. prepared hollow quaternized CS-MPs by the Shirasu Porous Glass membrane emulsification technique and glutaraldehyde cross-linking method.[61] The CH40 microspheres showed moderate hypoglycemic effect for about 20 days.[62] In another approach, polyphosphazene microspheres were formulated by three different procedures - (a) Suspension solvent evaporation; (b) double emulsion solvent evaporation; (c) suspension/double emulsion-solvent evaporation for efficient delivery of insulin. Result showed a bi-modal release behavior fast release during the first 2 h followed by a slow release. microspheres obtained with methods A and C showed~80% reduction in blood glucose, while hypoglycemic effect was prolonged up to 1000 h.[63] Based on the competitive binding of glucose and p-succinylamidoph enyl-α-D-glycopyranoside-insulin to cross-linked concanavalin A (Con A) microspheres (MSs) prepared by water-in-oil emulsion technique, Pai *et al*. developed a self-regulating insulin delivery system[64] Recently a novel chitosan phthalate microspheres containing insulin were prepared by emulsion cross-linking technique. Result showed that at pH 7.4, rapid release of insulin observed and sustained the plasma glucose at prediabetic level for at least 16 h.[65] Radwan demonstrated the use of polyisobutylcyanoacrylate (PIBCA) nanospheres as biodegradable polymeric carriers for oral delivery of insulin. Insulin associated with PIBCA nanospheres retains its biological activity up to 15 h improving insulin gastrointestinal absorption, but also in sustaining its systemic action by lowering the blood glucose to an acceptable level.[66]

In an interesting study, Kisel *et al*. formulated and evaluated the phosphatidylethanol as liposomal carrier for enteral delivery of insulin in rat. Result shows hyperinsulinemia which reduces the BGL.[67] In addition to this, the penetrating ability of liposomal insulin formulation across the Caco-2 cell monolayer has been studied. Study reveals that, the oral administration of insulin and sodium taurocholate (NaTC) incorporated liposomes significantly decreased BGLs. Moreover, high *in vitro/in vivo* correlation was observed using the Caco-2 cell monolayer model.[68] In another study on liposomes Goto *et al*. demonstrated that Fusogenic liposomes were found to be prominent approach for delivering their content directly into the cytoplasm facilitated by envelope glycoproteins of Sendai virus. Result showed significant hypoglycemic effect following the colonic and the rectal administration than ileal administration. In addition to this surface coated liposomes with poly (ethylene glycol) 2000 (PEG-Lip) or the sugar chain of mucin (Mucin-Lip) also prepared. The mean transit time of PEG-Lip was much longer than those of uncoated liposomes and Mucin-Lip.[69] Liposomes coated with silica by acid catalysis also have potential in reducing glucose levels.[70] Another interesting study demonstrated that recombinant human insulin (rhINS)-loaded sodium GC liposomes protect insulin better way from enzymatic degradation as compared to liposomes containing the bile salt counterparts of NaTC and sodium deoxycholate.[71] In another approach hybrid liposomes made up of dimyristoylphosphatidylcholine and micellar surfactant (Tween 20) showed a remarkable effect in diabetic rats.[72]

Polymeric micelles have recently gained importance as a carrier in protein and peptide drug delivery.[73] The new oral solid-in-oil-in-water dry emulsion formulations could be extensively applicable to oral delivery of pharmaceutical peptides and proteins. The release behavior of encapsulated insulin was found to be responsive to external pH.[74] Similar approach has been made using surfactant coated insulin.[75] In another study a low-shear reverse micellar approach were adopted to prepare Insulin loaded microemulsions using didodecyldimethylammonium bromide, propylene glycol (PG), triacetin (TA) and insulin solution as the surfactant, co-surfactant, the oil phase and the aqueous phase respectively. This microemulsion showed a 10-fold enhancement in bioavailability compared with plain insulin solution.[76] A microemulsion of rhINS has shown potential in improving efficacy of orally administered insulin.[77,78] Recently nanoemulsion composed of Labrafac® CC, phospholipid, Span™ 80 and Cremorphor® EL further coated with alginate/chitosan for oral insulin delivery has been formulated. The relative pharmacological bioavailability of the coated nanoemulsion with 25 and 50 IU/kg insulin were 8.42% and 5.72% in normal rats and 8.19% and 7.84% in diabetic rats, respectively.[79] Insulin loaded in microcapsules of chitosan and sodium alginate fabricated by layer-by-layer assembly has been reported. The results provide a simple method to control the loading and release of protein molecules within these polysaccharide microcapsules.[80] In similar approach insulin-loaded PLGA microcapsules has been formulated. Study reveals maintained BGLs at 100-200 mg/dL for 55 days.[81] In another study clayton prepared encapsulated islets of Langerhans in sodium alginate and poly-l-lysine to form the capsules, which has promising approach in the treatment of type 1 diabetic patient's. Over 90% of the entrapped insulin released after 3 days.[82,83] Zhou *et al*. prepared sustained microcapsules of insulin made by a novel high-voltage field method; showed drug release continued over 300 h.[84] poly (alkylcyanoacrylate) nanocapsules prepared by Interfacial polymerization of spontaneously forming water-in-oil microemulsion represents an efficient way to load proteins and peptide drugs.[85] In similar approach Graf *et al*. developed poly (alkylcyanoacrylate) nanoparticles prepared from microemulsion, showed that hypoglycemic effect over controls up to 36 h.[86] Research in this area leads to the development of CS-MPs by membrane emulsification technique and a step-wise crosslinking method, provided the

microspheres with higher chemical stability of insulin (>95%), lower burst release and steady release behavior.[87] As like enteric coated dry emulsion Eudragit S100 microspheres has the potential to show pH dependent release and protect insulin from proteolytic degradation in the GIT.[88] In another study Mundargi *et al*. also prepared pH-sensitive oral insulin delivery systems using Eudragit microspheres. The *in vivo* release studies on diabetic-induced rat models exhibited maximum inhibition of up to 86%, suggesting absorption of insulin in the intestine.[89] In a similar approach, pH sensitive polymethacrylic acid-chitosan-polyethylene glycol nanoparticles were prepared via polyelectrolyte complexation and serve as a good candidate for oral peptide delivery.[90] Recently He *et al*. designed novel biodegradable, and pH-sensitive poly (ester amide) s (PEA) microspheres based on dual amino acids. These insulin-loaded PEA microspheres after oral administration to streptozotocin-induced diabetic rats at 60 IU/kg, reduces fasting PGLs to 49.4%. The hydrophobic leucine groups on PEA seem to play an important role in the pH-dependent release mechanism and cytotoxicity of PEA microspheres.[91] Biodegradable nanoparticles loaded with insulin-phospholipid complex were prepared by a novel reverse micelle-solvent evaporation method, in which soybean phosphatidylcholine was in a job to improve the liposolubility of insulin, while biodegradable polymers acts as carrier materials to control drug release. Study reveals the hypoglycemia up to 12 h and insulin availability up to 7.7%.[92] Sarmento *et al*. developed cetyl palmitate-based solid lipid nanoparticles containing insulin, which showed biphasic way hypoglycemic effect, with an initial peak between 4 and 8 h with a decrease of 25% of the initial glucose level and later after 12 h of assay prolonged for up to 24 h. and bioavailability was found to be 5.0%.[93]

Recently Wu *et al*. developed Novel PLGA/HP55 nanoparticles for oral insulin delivery. These nanoparticles showed insulin release in pH dependent manner, and relative bioavailability was found to be 11.3% ± 1.05% compared with subcutaneous injection (5 IU/kg) in diabetic rats.[94] rhINS entrapped in multilamellar niosomes is another successful way to protect insulin against pepsin, α-chymotrypsin and trypsin.[95] These carriers may cause the toxicity.

Targeted delivery systems

The quest to deliver protein and peptide biopharmaceuticals conveniently and effectively has led to intense investigation of site-specific drug-delivery systems, utilized to lower the total delivered dose, and to concentrate a therapeutic dose at a specific site of pharmacological action.[96] The colonic region of the GI tract is the one that has been extensively investigated over the past two decades for targeted drug delivery. Targeting to the distal organ of GIT for drug as proteins and peptides are beneficial which are otherwise degraded in the stomach or small intestine.[97] Colon is emerging as an attractive site for peptide and protein drug delivery over the past few years.[98] In an interesting study, Katsuma *et al*. studied the effect of sodium GC co-administered with various absorption promoters on orally administered insulin absorption utilizing a colon-targeted delivery system. Result shows that sodium GC co-administered with poly (ethylene oxide) tended to prolong the colonic absorption of insulin and might be more effective for improvement of availability of orally administered insulin up to 5.5%.[99] Similar kind of study, demonstrated that azopolymer-coated pellets can be an efficient carrier for colon targeting of insulin and (Asu1,7) eel-calcitonin.[100] The uptake of poly (lactide-co-glycolide) particles by murine peyer's patches and their binding to payer's-free tissue was found to be influenced by surface charge and particle size.[101] In similar approach poly (lactide-co-glycolfide) microsphere were prepared for delivery of the insulinotropic hormone, glucagon-like peptide-1.[102] Transcytosis of insulin-transferrin (In-Tf) conjugate mediated by transferrin receptor (TfR) in Caco-2 cell monolayers brought forward a feasible approach for developing the oral delivery of insulin, as well as other peptide drugs. Tyrphostin-8 enhances hypoglycemic activity of In-Tf via enhancement of TfR-mediated transcytosis especially at 7 h after administration.[103,104] Though targeting drug delivery arising as a promising approach for delivery of proteins and peptides, carrier related toxicity might be arise due to continued absorption of particles via M-cells into Peyer's patches, which may perhaps induce an immune response.

Erythrocytes

Study has been carried out to check the potential of different forms of human red blood cells as oral carrier for delivery of human insulin.[105] In another approach of Al-Achi insulin solution either free or along with carrier that is, erythrocyte-ghosts (EG) or liposomes-vesicles, was administered buccally. Result showed decrease in blood glucose concentration.[106]

PULMONARY INSULIN

Lungs provide a large absorptive surface area, immense capacity for solute exchange, good vascularization, and extremely thin absorptive mucosal membrane facilitate the pulmonary delivery of protein and peptides.[107] The devices that are currently available for pulmonary drug administration, includes nebulizers, metered-dose inhalers and dry-powder inhalers (DPIs). With some adaptation, most of these devices can be used for pulmonary peptide and protein administration. Nowadays inhale insulin has immerging as a promising alternative to parenteral delivery. In an attempt of this highly dispersive insulin, dry powder formulations has been formulated for use in DPIs using high-pressure homogenization and spray-drying, showing deep lung deposition of insulin.[108] In another study insulin microcrystals along with lactose carrier were produced for pulmonary delivery. In other study insulin

microcrystals along with protease inhibitor (AP, bacitracin and soybean-trypsin inhibitor) has been formulated. Result showed enhancement in hypoglycemic effect, soybean-trypsin inhibitor (48.86% ± 3.24% at 10 mg/ml; 55.78% ± 0.71% at 5 mg/ml; 51.49% ± 5.27% at 1 mg/ml) and AP (52.57% ± 8.78% at 10 mg/ml; 51.97% ± 1.98% at 5 mg/ml; 56.90% ± 3.42% at 1 mg/ml). These conclusions suggest that the use of insulin microcrystals and protease inhibitors would be helpful to improve the hypoglycemic effect in pulmonary route. Bioavailability of insulin from microcrystal was found to be 15% which was higher than insulin solution (10%).[109] In similar approach insulin, microcrystals has been formulated using seed zone method which lowers blood glucose up to over 13 h.[110,111] Lee *et al*. demonstrated the effect of various adjuvants such as protamine, zinc, and glycerol, on the hypoglycemic effect of microcrystal suspension delivered by the pulmonary route. Result shows that at higher concentration of zinc chloride, lower percentage of minimum reduction in blood glucose and the higher D% (decrements of the BGL) observed. Furthermore, the zinc is known to enhance the stability of insulin crystal structure through the formation of zinc-containing hexamers which prolongs the collapse of insulin crystals from hexamers to monomers which can be absorbed in body.[112]

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 a model peptide. Results showed that the enhancing effect of $Ln3^+$ on the bioavailability (Fr) of insulin is closely related to its species, concentration, and delivery order. 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. Gadolinium ($Gd3^+$) hampers kupffers cells activity and prevents lipopolysaccharide-induced decrease in liver insulin-like growth factor-I and IGF-binding protein-3 gene expression. The anionic form of Gadolinium (Fr = 68.4%) seemed to be more effective compared with its cationic form (Fr = 59.5%). Coadministration of $Gd3^+$ with insulin (Fr = 80.1%) was the most effective in increasing insulin absorption from the lung.[113-115]

Zhao *et al*. demonstrated the use of phospholipid-based ultrasonic microbubbles (PUMs) to enhance the pulmonary absorption of insulin. Insulin solution containing PUMs shows 48.58% bioavailability relative to subcutaneous injection.[116] Comparative study of two lipid-based vesicles liposomes and microbubbles on the basis of *in vitro* toxicity in A549 cells showed that the minimum reductions of the blood glucose concentration produced by insulin–microbubble and insulin–liposome mixtures were 60.8% and 35.0% of the initial glucose levels, respectively, while their bioavailabilities relative to subcutaneous injection were 48.6% and 30.8%, respectively. This states the usefulness of microbubbles as a potential carrier.[117] Hyaluronic acid (HA) along with rhINS was co-spray dried to form a dry powder suitable for inhalation. Controlled release plasma pharmacokinetics were observed in HA - insulin formulation as compared to HA alone, Furthermore Addition of either HPC or $Zn2^+$ during formulation was found to trim down release rates of insulin from the HA matrix.[118] In an interesting study, demonstrated that zinc insulin follows paracellular pathway for transport across the Calu-3 cell monolayer which formed a confluent monolayer with a high transepithelial electrical resistance value of 1000 ± 150 Ω cm2.[119] In a study of Bi *et al*. Spray-freeze dried dry powder inhalation of insulin-loaded liposomes, showed successful hypoglycemic effect with low-BGL and long-term period and a relative pharmacological bioavailability as high as 38.38% in the group of 8 IU/kg dosage.[120] Lung Lavage fluid along with phospholipids shows significant enhanced absorption of insulin.[121] Todo *et al*. demonstrated the effect of various absorption enhancers on stability of insulin in dry powder form prepared by spray drying. Insulin formulated in solutions along with Bacitracin and Span 85 (potent pulmonary absorption enhancers), showed no deteriorative effect on the stability of dry insulin powder. While formulation with citric acid showed deteriorative effect. The absolute bioavailability of insulin solution and dry powder containing bacitracin or Span 85 was almost 100% and 20% of that of the insulin solution, respectively.[122,123] CD derivatives, such as tetradecyl-β-maltoside and dimethyl-β-CD (DMβCD), enhance the insulin absorption. They mainly act by either formation of the inclusion complex with insulin or solubilizing membrane component.[124] As reported Insulin can be encapsulated in liposome using detergent dialysis method. In attempts of this, novel nebulizer-compatible liposomal carrier for aerosol pulmonary drug delivery of insulin was developed. Liposomal delivery enhances the retention time of insulin and reduces side effect which invariably results in enhanced therapeutic efficacies.[125] In other study insulin calcium phosphate (CAP) and PEG particles in suspension (1.2 U/kg, 110–140 μL) were administered to lungs of fasted rats by intratracheal instillation (INCAPEG) or spray instillation (SINCAPEG). Insulin bioavailability after SINCAPEG was 1.8-fold that of insulin solution administered SC.[126] Aerosolized large porous particles deposited homogeneously throughout the lung and showed a reduction in barrier properties within the first 90 min after impingement with microparticles.[127] Kawashima *et al*. demonstrated that Insulin loaded PLGA nanospheres were found to prolong the hypoglycemia over a period of 48 h.[128] In similar approach Insulin-loaded polybutylcyanoacrylate nanoparticles were formulated by emulsion polymerization. Result showed 57.2% relative bioavailability of insulin-loaded nanoparticles by pulmonary administration over the same formulation by subcutaneous administration.[129] Chitosan/tripolyphosphate nanoparticles have also been formulated using spray drying technique that promotes peptide absorption across mucosal surfaces. Results showed 75-80% insulin release within 15 min. protein-loaded CS NPs using typical aerosol excipients, such as lactose and mannitol, producing microspheres as carriers which

have potential in pulmonary delivery of insulin.[130] Recently microencapsulated CS NPs have also been formulated for pulmonary insulin delivery. INS-loaded CS NPs exhibit a significant reduction in blood glucose ($P < 0.05$) compared to the controls. PGL was reduced by~70% as compared to basal BGL at 60 min of postadministration, and hypoglycemic effect was maintained for 4 h [Figure 2].[131]

Along with potential advantages pulmonary administration of proteins and peptides has some disadvantage. Patients receiving inhaled insulin had more episodes of hypoglycemia and gained more weight than did patients treated with oral agents.[132,133] Inhaled insulin also sensitize immune response of the body to produce antibodies for insulin.[134] Mild to moderate cough was also reported in up to 25% of patients receiving inhaled insulin.[135,136]

BUCCAL INSULIN

Difficulties associated with parenteral delivery and poor oral availability of peptides provided the momentum for exploring alternative routes for the delivery of such drugs. These include routes such as pulmonary, nasal, ocular, sublingual, vaginal, rectal, buccal, and transdermal. Among the various transmucosal routes, buccal mucosa has excellent accessibility, relatively immobile mucosa and an expanse of smooth muscle, hence suitable for administration of retentive dosage forms. Direct entry to the systemic circulation through the internal jugular vein bypasses drugs from the hepatic first-pass metabolism ultimately leads to high bioavailability. Other advantages are low enzymatic activity, painless administration, easy drug withdrawal, suitability for drugs or excipients that mildly and reversibly damages or irritates the mucosa, facility to include permeation enhancer/enzyme inhibitor or pH modifier in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic actions.[137] Various attempts have been reported to enhance the transport of peptides across the mucosa such as the use of penetration enhancers, addition of enzyme inhibitors to increase their membrane permeability and stability.[138]

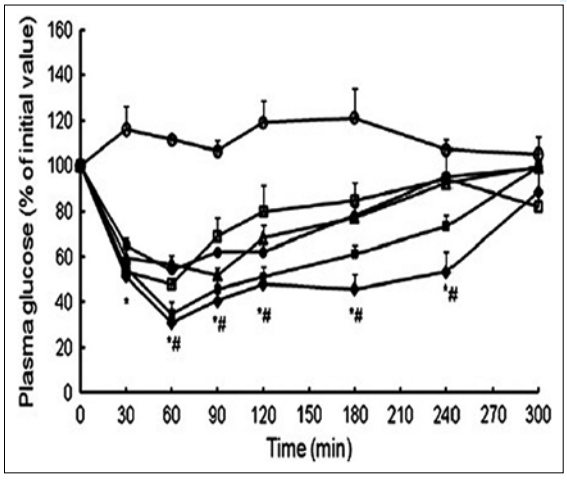

Figure 2: Hypoglycemic profiles following intratracheal administration to rats of microencapsulated insulin-loaded chitosan nanoparticles (INS-loaded CS NPs) prepared using chitosans of different MW (CS 113 and CS 213), and control formulations (mean ± SD, $n \geq 3$): (♦) Microencapsulated INS-loaded CS NPs-CS 113; (■) Microencapsulated INS loaded CS NPs-CS 213; (○) Microencapsulated blank (without insulin) CS NPs-CS 113; (□) Mannitol microspheres containing INS; (Δ) Suspension of INS-loaded CS NPs – CS 113; (●) INS solution in PBS pH 7.4; *Statistically significant differences from microencapsulated blank CS NPs ($P < 0.05$); #Statistically significant differences from INS solution ($P < 0.05$)[131]

Morishita *et al*. investigated release profiles of insulin from Pluronic F-127 (PF-127) gel containing unsaturated fatty acids such as docosahexaenoic acid, OA, and eicosapentinoic acid. Result showed remarkable hypoglycemia and decrease in insulin release while PF-127 gels containing OA showed bioavailability up to 15.9% ± 7.9%, evaluation study 20% PF-127 gels containing unsaturated fatty acids are potential formulations for the buccal delivery of insulin [Figure 3].[139]

In an interesting study Starokadomskyy demonstrated the use of lysalbinic acid, a product of the alkaline hydrolysis of egg albumin and a mild detergent, as the absorption enhancer for the buccal delivery of peptide drugs α-interferon and insulin result has shown that lysalbinic acid has no irritating or sensibilizing effects during buccal use.[140] In other approach phospholipid vesicles with or without sodium deoxycholate has been formulated by reverse phase evaporation method for buccal delivery of insulin. Comparative result showed that subcutaneous administration of insulin solution, the relative pharmacological bioavailability and the relative bioavailability in the insulin-deformable vesicles group were 15.59% and 19.78%, respectively, which were higher than in the conventional insulin vesicles ($P < 0.05$), blank deformable vesicles and insulin mixture groups ($P < 0.05$).[141] However,

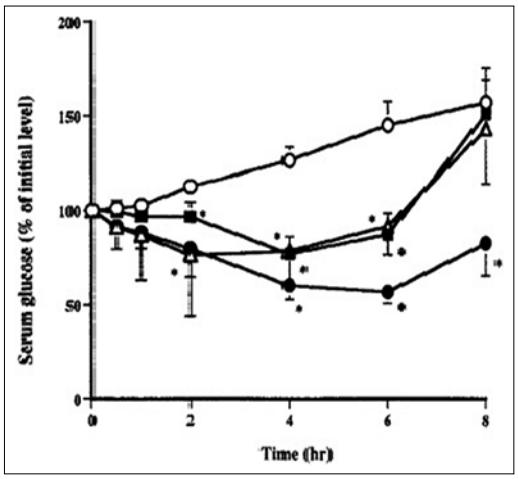

Figure 3: Effect of intra-buccal administration of insulin-loaded Pluronic F-127 (PF-127) gel formulations on serum glucose levels. PF-127 gel without fatty acid (O), containing oleic acid (●), EPA (Δ) and DHA (■). Each value represents the mean ± standard deviation ($n = 4$). Cornparisons calculated at each period PF-127 gel without fatty acid versus PF-127 gel containing oleic acid, EPA or DHA (*$P < 0.01$).[139]

the effect of salivary scavenging and accidental swallowing of delivery system; barrier property of buccal mucosa stands as the major limitations in the development of buccal adhesive drug delivery systems. To overcome this various attempt has been made on buccal adhesive delivery, adhesive dosage forms such as gels, films, tablets, and patches has been formulated that can localize the formulation and improve the contact with the mucosal surface to improve absorption of peptides and proteins. In attempts of this pelleted bioadhesive polymeric nanoparticles for buccal delivery of insulin has been formulated. A significant hypoglycemic response was observed after 7 h, without any detectable fluctuation in blood glucose profile.[142] In another related Study, insulin was formulated into mucoadhesive buccal tablets using Carbopol 934, hydroxypropyl cellulose, or hydroxypropylmethyl cellulose and different absorption promoters.[143] Xu *et al*. in their study demonstrated the effect of insulin buccal spray (IBS), a formulation with soybean lecithin and propanediol combined as the absorption enhancer for insulin on diabetic rabbits and rats. The hypoglycemic effect lasted over 5 h and 4 h in diabetic rabbits and rats, respectively, while average bioavailability of IBS by buccal delivery versus subcutaneous injection is 29.2%.[144]

NASAL INSULIN

Numerous attempts have been made to deliver a large number of peptides and proteins by this route.[145-148] The accessibility of the nasal route facilitates self-medication, thus improves patient compliance compared to parenteral route.[149,150] Nasal administration offers an alternative for achieving systemic effect. Other advantages are large surface area available for drug absorption because the epithelial surface is covered with numerous microvilli; the subepithelial layer is highly vascularized and relatively high blood flow which promotes rapid absorption thereby avoiding the loss of drug by first-pass metabolism in the liver.[151] The porous endothelial basement membrane of the nose is an easily accessible and the drug is delivered directly into the brain along the olfactory nerves.[152,153] Various attempts have been made to for increasing the residence time of drug formulations in the nasal cavity. In an attempt of these bioadhesive microspheres, liposomes and gels have been formulated.[154] The pharmacokinetic profile of intranasal insulin is analogous to that obtained by intravenous injection and in contrast to subcutaneous insulin delivery, bears a close resemblance to the "pulsatile" pattern of endogenous insulin secretion during meal-times.[150] Pringels *et al*. demonstrated that powder bulk density and the spray pattern of nasal powder do not affect the bioavailability of insulin.[155] In similar study effect of particle size on insulin-loaded adhesive, microspheres has been studied. Result showed that he relative pharmacological availability was 10.2% ± 0.5% (120 nm), 14.9% ± 1.3% (350 nm), and 7.3% ± 0.8% (1000 nm) respectively. Particles of 350 nm showed a comparatively higher availability ($P < 0.05$).[61] However, 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 has been investigated to enhance the nasal bioavailability of these drugs. In attempts of this a glycosides containing long alkyl side-chains (C13-C16) linked to maltose or sucrose were synthesized and tested for their usefulness in enhancing nasal insulin absorption in anesthetized rats. When insulin was applied to the nasal mucosa 15 min after tetradecylmaltoside was applied, improved insulin absorption was observed.[156] Potential of CS NPs as a system for improving the systemic absorption of insulin following nasal instillation has also been demonstrated.[157] Mitra *et al*. demonstrated the role of Lipid emulsions as vehicles for enhanced nasal delivery of insulin.[158] Insulin can be administered using DMβCD as an absorption enhancer in powder form, which showed 13% ± 4% absolute bioavailability compared to 1% ± 1% for both an insulin/DMβCD liquid and an insulin/lactose powder formulation.[159] As earlier mentioned to increase the residence time of drug formulations in the nasal cavity various attempt has been demonstrated to formulate gel formulation. In attempts of this carbopol-based insulin gel, spray was formulated. Study reveals the bioavailability of insulin from the nasal gel formulation was 20.6% compared with the intravenous injection in rabbit.[160] In a similar type of work bioadhesive chitosan gels has been formulated for nasal delivery of insulin which showed zero-order release kinetics from the gels and fall in the glucose level by as much as 46% of the intravenous route[161] In another study Microspheres containing 400 mg of chitosan and 70 mg ascorbyl palmitate caused a 67% reduction of blood glucose as compared to an intravenous route, and absolute bioavailability of insulin was found to be 44%. In addition, starch nanoparticles medium cross-linked with epichlorohydrin.[162] Insulin-gold nanoparticles can be delivered in diabetic Wistar rats by both oral and intranasal (transmucosal) routes. Result showed that there was a significant reduction of BGLs (postprandial hyperglycemia) when insulin was delivered using gold nanoparticles as carriers by the transmucosal route in diabetic rats.[163] Furthermore, Nasal administered chitosan–TBA–insulin microparticles led to an absolute bioavailability of 7.24 ± 0.76%.[164] In other approach Wang *et al*. investigated aminated gelatin microspheres (AGMS) as a nasal drug delivery system for peptide drugs. AGMS could significantly increase the nasal absorption of insulin in rats when administered in a dry powder formulation, but no noteworthy hypoglycemic effect was observed when given in suspensions, this may be due to increased insulin absorption was ascribed to the hydrogel nature of the microspheres that could soak up water from the nasal mucosa and thus resulted in a temporarily dehydration of the epithelium membrane and led to the gap in tight junctions. Again the electrostatic interactions between the model drugs and the microspheres were believed to

be the main aspect that controlled the release behavior.[165] In similar study Jain *et al*. prepared mucoadhesive CS-MPs for noninvasive and improved nasal delivery of insulin. It was observed that after administration of glutaraldehyde and citric acid cross-linked microspheres, maximum blood glucose reduction was found to be 66.78% ± 3.33% and 69.37% ± 3.46% of the initial BGL respectively after 7 h.[166]

Jain *et al*. formulated the novel mucoadhesive multivesicular liposomes (MVLs) coated with chitosan and carbopol as sustained release carrier for delivering insulin via nasal and ocular routes. chitosan coated MVLs showed prolonged hypoglycemic effect up to 72 h, compared to noncoated MVLs (32% at 12 h) and conventional liposomes (34% at 8 h).[167] Nasal irritation and decline in absorption of insulin after continuous administration somewhat limits nasal administration drug delivery.

TRANSDERMAL INSULIN

As skin largest organ of our body, Transdermal delivery represents an attractive substitute to oral delivery of drugs and provides an alternative to hypodermic injection also. In the recent years, a variety of topical formulations have been developed to treat local indications.[168] One of the most prominent barriers in transdermal delivery is stratum corneum, the skin's outermost layer. Various attempts has been made to overcome this barrier, includes iontophoresis, use absorption enhancers, ultrasound or microneedles.

In the attempts of this, Williams and Barry studied the effect of various permeation enhancer like sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g., laurocapram), pyrrolidones (for example 2-pyrrolidone), alcohols and alkanols (ethanol, or decanol), glycols (for e.g., PG) surfactants, fatty acid, on transdermal delivery of insulin. They act by disrupting the packing motif, or acting on desmosomal connections between corneocytes or altering metabolic activity within the skin.[169] As mentioned earlier macromolecules can't pass easily across the skin, to overcome this Karande *et al*. described particular mixtures of penetration enhancers that increase skin permeability to macromolecules, which includes sodium laureth sulfate and the phenyl piperazine (PP). Result gives > 100-fold more efficient delivery without inducing skin irritation.[170] In another interesting study short synthetic peptide, ACSSSPSKHCG, recognized by *in vivo* phage display, facilitated proficient transdermal protein drug delivery through intact skin. Result showed suppressed serum glucose levels for at least 11 h.[171]

Further enhancement of transdermal delivery it is necessary to alter physical or biomolecular structure of stratum corneum by suitable techniques or by the use of specific chemical agents or drug carriers. In attempts of this flexible lecithin vesicles were used as a carrier for transdermal delivery of insulin in mice. This vesicles containing insulin showed 50% reduction in BGL within 18 h.[172] In other approach pharmacokinetic and pharmacodynamic effects of transdermally delivered insulin was assayed using novel CaCO3-nanoparticles encapsulating insulin (nanoinsulin) in normal mice and those with diabetes. These nanoparticles were applied transdermally to the back skin of normal ddY mice and dB/dB and kkAy mice with diabetes after fasting for 1 h. Maximum serum insulin was 67.1 ± 25.9 μIU/mL at 4 h with 200 μg of transdermal nanoinsulin in ddY mice, whereas that after subcutaneous injection of 3 μg of monomer insulin was 462 ± 20.9 μIU/mL at 20 min. Transdermal nanoinsulin decreased glucose levels in a dose-dependent manner. A maximum decrease in blood glucose of 48.3% ± 3.9% (ddY), 32.5% ± 9.8% (dB/dB), and 26.2 ± 7.6% (kkAy) after 6 h was observed with 200 μg of transdermal nanoinsulin, compared with 64.1 ± 1.0% (ddY), 57.9 ± 3.4% (dB/dB), and 24.1 ± 6.7% (kkAy) after 1 h with 3 μg of subcutaneous monomer insulin.[173] In addition to this transferees can transport insulin through permeability barriers, such as the intact skin with a bio-efficiency of at least 50% of the subcutaneous dose action.[174]

Transdermal iontophoresis

Iontophoresis evolved as a transdermal enhancement technique in the 20th century, primarily for the delivery of large and charged molecules.[175] Iontophoresis provides a noninvasive means of systemic administration of a minute amount of drugs. E.g., high molecular weight electrolytes such as proteins, peptides and oligonucleotides which are normally difficult to administer except through parenteral route.[176] Iontophoresis is a technique used to enhance the transdermal delivery of compounds through the skin via the application of a small electric current. Using the processes of electromigration and electro-osmosis, iontophoresis increases the permeation of charged and neutral compounds. It is a programmed drug delivery technique that physically facilitates the transport of permeates across the skin.[177] In attempts of this Mao *et al*. employed pulse current iontophoresis for transdermal delivery of insulin. Study reveals that insulin solution at pH 3.6 below its isoelectric point (pH 5.2) showed highest permeation rate 32 3 ± 33.4 U/(cm2·h), while, at pH 4, the transdermal permeation rate of insulin declined markedly to 143.7 ± 27.3 μU/cm2·h).[178] In further study saturated fatty acids and cis-unsaturated fatty acids namely lauric acid (LA), OA, LOA and linolenic acid (LLA) were combined with iontophoresis. The flux enhancement was highest with LA, which in the presence of iontophoresis showed 20 times enhancement of insulin flux in comparison to passive flux and 9 times enhancement as compared to iontophoresis alone. Flux enhancement of unsaturated fatty acids was in the following decreasing order LOA > OA > LLA.[179] Akram *et al*. prepared Insulin emulgel using emu oil as the penetration enhancer, showed permeation flux (4.88 ± 0.09 μg/cm2/h) through excised rat skin. Emulgel in combination with iontophoresis decrease BGL to a greater extent [Figure 4].[180]

Monomeric human insulin analogue (B9 Asp, B27 Glu) has also been delivered transdermally by iontophoresis, provides a significant reduction in PGL of diabetic rats.[181] Pillai *et al*. studied the effect of NaCl and different buffer on transdermal delivery of insulin. Result showed that the permeation of insulin increased in the presence of NaCl due to ion induced convective flow. The flux enhancement of insulin in the presence of phthalate buffer was higher in comparison to citrate buffer, while the enhancement in these two buffers was the same in the presence of 0.05 M NaCl.[182] Further effect of pretreatment with commonly used vehicles such as ethanol (EtOH), PG, water and their binary combinations, dimethyl acetamide (DMA), 10% DMA in water, ethyl acetate and isopropyl myristate on insulin iontophoresis, act on the intercellular lipids has also been studied.[183] Iontophoresis followed by electroporation in the presence of 1, 2-dimyristoylphophatidylserine showed~18 fold increase in the insulin transport over electroporation alone.[184]

Gels are considered to be the most suitable delivery vehicle for iontophoresis, as they can be easily combined with the iontophoretic delivery system and can also match the contours of the skin. Insulin gel formulated along with poloxamer 407 was evaluated by ex vivo and *in vivo* skin permeation studies in rat with chemical enhancer and/or iontophoresis. 36-40% reduction in PGL was obtained by iontophoresis alone or in combination with LOA.[185] Skin irritation is a major obstacle while using chemical enhancer in combination with iontophoresis than alone.

Sonophoresis (phonophoresis)

Ultrasound is gaining a wide importance in the delivery of therapeutic agents, including genetic material, protein and chemotherapeutic agents. Cavitating gas bodies, such as microbubbles acts as a mediator and through which the energy of relatively noninteractive pressure waves

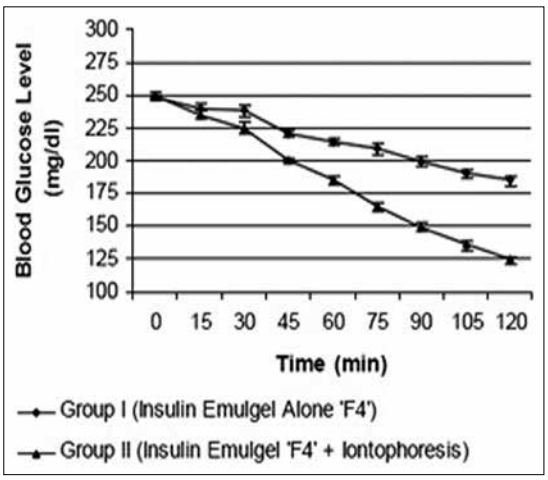

Figure 4: Change in the blood glucose level versus time period up to 120 min during the application of insulin emulgel alone and insulin emulgel + iontophoresis (mean ± standard deviation, $n = 3$).[180]

is concentrated to produce forces that permeabilise cell membrane.[186] Ultrasound exposure causes cavitations in the keratinocytes of the stratum corneum, plays a major role in transdermal drug delivery.[187] Therapeutic levels of ultrasound (1-3 MHz, 1-3 W/cm2) have been used to drive small hydrophobic molecules, through skin.[188] Tezel *et al*. studied the Synergistic effect of low-frequency (20 kHz) ultrasound and surfactants (including anionic, cationic, and nonionic with varying tail lengths [8-16-carbon atoms]) on skin permeability. In the presence of ultrasound anionic and cationic surfactant enhances skin conductivity than nonanionic surfactant. Ultrasound enhances surfactant delivery and dispersibility into skin.[189] Tezel and Mitragotri demonstrated the major mechanism of bubble-stratum corneum interactions including shock wave emission, microjet penetration into the stratum corneum, and impact of microjet on the stratum corneum were considered.[190] Lee *et al*. studied ultrasonic transdermal insulin delivery in rabbits using the light-weight cymbal array. Result showed that for the ultrasound-insulin group, the glucose level was found to decrease to − 132.6 ± −35.7 mg/dL from the initial baseline in 60 min. however, after the removal of insulin reservoir glucose level continued to decrease to − 208.1 ± 29 mg/dL from the initial baseline.[191] Similar study of Park *et al*. in pigs demonstrated that ultrasound-insulin group, the glucose level decreased to − 72 ± 5 mg/dL at 60 min ($P < 0.05$) and continued to decrease to − 91 ± 23 mg/dl in 90 min ($P < 0.05$)[192] In a similar type of interesting study transport of insulin through *in vitro* human skin were studied. Humulin®R insulin and Humalog® insulin showed seven and a fourfold increase in the ultrasound facilitated transmission respectively compared to the control.[193] Recently Al-Bataineh *et al*. studied transdermal delivery of insulin using piston shaped PZT ultrasound transducers operating in the frequency range 100-1000 kHz.[194]

Microneedles

Microneedles stab the skin and create micrometer-sized perforations. These apertures are too large to pass the macromolecules so even larger molecules can pass through it despite of that small enough to avoid pain and facilitate highly localized and even intracellular targeting. Microfabrication techniques for silicon, metal, and biodegradable polymer leads to the development of microneedle arrays having solid and hollow bores with tapered and beveled tips and feature sizes from 1 to 1,000 μm which can be used in delivering insulin across the skin.[195] Micromachined dry biopotential electrode having number of spikes etched in silicon has potentials in delivering insulin effectively.[196] Gill *et al*. demonstrated that microneedles or arrays of microneedles can be coated with proteins or DNA by GRAS coating formulation to dissolve within 20 s in porcine cadaver skin.[197] In another study arrays containing 16 microneedles measuring 500 μm in length with a 75 μm tip diameter, were used to deliver insulin transdermally in hairless rats. Result showed that BGLs dropped steadily to 47% of pretreatment

values over a 4 h. So it can be concluded that microneedles can be fabricated and used for *in vivo* insulin delivery.[198]

OCULAR INSULIN

Ocular the route seemed to be the most reasonable one because (a) It could deliver precise doses of drugs just like injections; (b) it was much easier and less expensive to administer eyedrops than an injection; (c) the rate of systemic absorption through the ocular route was as fast as an injection; (d) eye tissues were much less sensitive to the development of immunological reactions than other tissues; (e) the drug absorbed via the ocular route would first pass metabolism; and (f) no tolerance and ocular side effects could be detected after long-term (3 months) daily administration of insulin eyedrops.[199] The potential route for insulin delivery is the nasal meatus where the majority of systemic absorption of instilled drug takes place. Some absorption also take place from conjunctival sac.[200]

Yamamoto studied the effect of various absorption promoters like polyoxyethylene-9-lauryl ether, Na GC, NaTC and Na deoxycholate, on ocular administration of insulin at a concentration of 1%.Relative bioavailability was found to be The bioavailability was found to be 5.7-12.6%, 4.9-7.9%, 3.6-7.8%, 8.2-8.3% with polyoxyethylene-9-lauryl ether, GC, NaTC and Na deoxycholate respectively, as compared to 0.7-1.3% in the absence of absorption promoters.[201] Insulin can be instilled along with 0.5% BL-9 or Brij-78 which acts as absorption enhancers. Study reveals that there were no allergic responses, or local side effects could be detected, for a long period of time.[202] In another study, a series of alkylglycosides with various alkyl chain lengths and carbohydrate residues were tested for their ability to enhance systemic absorption of insulin after topical ocular delivery in anesthetized rats. This alkylglycosides found beneficial only when they used above their critical micelle concentration[203] In study of Rhea *et al*., insulin was administered along with 0.5% saponin, 0.5% and 1% BL-9, 0.5% and 1% dodecylmaltoside, and 0.5% and 1% tetradecylmaltoside Via the Ocular Route in Dogs.[204] In an interesting study, Sucrose cocoate (SL-40), an emulsifier employed in ocular delivery of protein and peptides. Absorption studies were performed in anesthetized Sprague-Dawley male rats with calcitonin and insulin, a smaller increase in plasma insulin levels, and a decrease in BGLs was observed.[205] Srinivasan *et al*. demonstrated the use of polyoxyethylene-9-lauryl ether in 0.8% w/w concentration as penetration enhancer for ocular delivery. Liposomal delivery of insulin not only promotes the ocular absorption, but also controls and prolongs the drug action.[206] In another study ocular insert fabricated in the form of a matrix system using commercially available Gelfoam absorbable gelatin sponge, USP. Study reveals that devices containing 0.5 or 1.0 mg of insulin with 20 µg of polyoxyethylene-20-stearyl ether (Brij-78) give a substantial improvement in insulin activity and a significant prolongation in its duration compared with the eyedrops, within the desired therapeutic levels without the risk of hypoglycemia.[207,208] In similar study sodium insulin and zinc insulin ocular devices consisting of Gelfoam as an insulin carrier without surfactant or absorption enhancer has been formulated. Result shows that 10% acetic acid solution-treated insulin devices produce significant blood glucose reduction.[209] In similar study effect of acid on the release of insulin has been studied, which showed that 5% or higher concentration of acetic acid solution as well as 1% HCL enhances the insulin release from the device. Device with 30% acetic acid produce no eye irritation while single device containing 0.2 mg of insulin with either water or 10% acetic acid is sufficient to control the BGLs for over 8 h.[210,211]

RECTAL INSULIN

Insulin delivery by rectal route offers some advantage over the enteral route; first, the rectal route is independent of gastric-emptying time, intestinal motility 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.

Ichikawa *et al*. studied the effect of insulin solution (100 U/kg) at various pH values when administered via rectum route of rabbits. Result showed a large decrease in blood glucose concentration except at the pH close to the iso-electric point of insulin. Anionic, cationic and amphoteric surfactants, as well as bile acids, increased the absorption. These results were similar to those after intravenous injection.[212] In another similar type of study absorption of two kinds of insulin (from porcine or bovine pancreas) from the rectum of rabbits after the administration of hollow-type suppositories containing insulin and glyceryl-1-monooctanoate (GMO) as an absorption-enhancing agent was investigated. Two types of suppositories were prepared, first one, containing insulin in an aqueous solution (approximately 25 IU/mg/100 µL citric buffer solution at pH 3.0) in the cavity of the suppository and GMO mixed with a base material (Witepsol H-15), and second one, containing insulin in a crystalline form in the same amount as in first one. Without GMO, the insulin and glucose levels in plasma were unchanged, whereas a marked increase in the plasma levels of insulin and a decrease of glucose concentrations were found following coadministration of insulin and GMO by the type I suppository. Similar enhancement of rectal absorption of insulin was obtained from porcine and bovine sources. In the case of the crystalline insulin, despite the use of the same amount of GMO, porcine insulin was more efficiently absorbed than bovine insulin by the type II suppository.

GMO enhances the absorption of insulin in an aqueous solution or a crystalline form, and the dissolution rate of insulin may be an important factor in the rectal absorption of insulin.[213] In another study effect of bile salts/acids like deoxycholic acid (DCA), sodium cholate (NaC), or sodium deoxycholate (NaDC) on Insulin suppositories formulated using Witepsol W35 as base were investigated. Study reveals that insulin suppositories containing Witepsol W35 as a base and NaDC plus NaC (100 mg plus 50 mg, respectively), NaTDC (100 mg), or NaTC (100 mg) showed relative hypoglycemia of about 50%. Use of DCA, NaC, and NaDC as rectal absorption enhancers of insulin is promising approach in rectal insulin delivery.[214]

Insulin suppositories containing sodium salicylate (50 mg) and polyoxyethylene-9-lauryl ether (1%) showed relative hypoglycemia of 49% ± 12% and 55% ± 11% relative to subcutaneous injection of insulin (4 U/kg).[215] Further research in this area leads to the development of thermo-reversible insulin liquid suppository composed of poloxamer P 407, P 188, polycarbophil and sodium salicylate, which undergoes a phase transition to bioadhesive gels at body temperature and ultimately enhances the bioavailability of insulin. These liquid suppositories have potential to deliver insulin in more convenient, safe and effective way.[216] Mucin motifs extracted from the giant African snail Archachatina marginata by differential precipitation with acetone were evaluated rectal absorption enhancer for insulin. Result showed that the mucin only at 5% and 7% gives remarkable, consistent lowering in blood glucose concentration.[217]

Rectally administered insulin gives low bioavailability as compared to subcutaneous injection. Patient compliance and pain during administration also a major obstacle in this type of drug delivery.

MARKETED FORMULATION

Oral formulations

Oral insulin research is still at the research and development stage. Numerous companies have developed, and some are working on different technologies to overcome the challenges of oral peptide delivery.

ORMD-0801

ORMD-0801 an orally ingestible insulin capsule formulated by Oramed Pharmaceuticals is now under Phase 2 clinical trial of US Food and Drug Administration (FDA).[218]

Eligen®

During December 2010, Novo Nordisk followed by agreement develops and commercialize oral formulations using Emisphere's Eligen® Technology. This technology utilizes synthetic nonacylated amino acids as carriers, which does not hamper the conformation of the drug and transport it across the physiological barrier into the bloodstream. It only changes the drug by physical means at the point of transport.[219-221]

IN-105

India's biotechnology company Biocon has entered into a partnership for its oral insulin pill IN-105, giving the US drugmaker Bristol-Myers Squibb, the option to exclusively develop and sell the product if the clinical trials prove successful. This pill possesses a polymer located at specific position in B chain to prevent insulin degradation in the stomach. It fails in the later stage trials in patients with type 2 diabetes.[222]

Macrulin®

Provalis has developed insulin based oral pill Macrulin®, which uses a water-in-oil microemulsion technique in which aqueous phase contains insulin, and the oil phase contains cholesterol, lecithin and nonesterified fatty acids.[223]

Nobex oral insulin

Biocon ties with Nobex Corporation to develop oral insulin delivery technology. This technology involves the attachment of one or more amphiphilic oligomers to peptides and proteins ehich results in stability to enzymatic degradation and improved solubility.[224,225]

9.1.6. Transgene (biotek Andhra Pradesh)

By combining different oral delivery approaches into a single-drug delivery system and utilizing biodegradable novel polymeric nanoparticles loaded with insulin, Transgene has developed an oral insulin delivery technology. Animal efficacy studies on trabioral showed impressive results for the delivery of insulin. It showed sustained glycemic level for around 10 h.[226]

AI-401 AutoImmune, Inc./Eli Lilly

Eli Lilly and Company and AutoImmune Inc., have developed AI-401 an oral form of rhINS for the treatment of autoimmune-mediated diabetes. It delays β-cell destruction in the pancreas.[227]

Cob Oral™

Access Pharmaceuticals, Inc., a biopharmaceutical company, presented poster at the 10th Annual Diabetes Technology Meeting, entitled, "Preclinical Studies with Cobalamin™ Nanoparticles for Oral Drug Delivery of Insulin." Cobalamin™ technology utilizes the body's natural vitamin B12 oral uptake mechanism to facilitate oral absorption of pharmaceuticals by a "Trojan horse" mechanism.[228,229]

NN1954

Novo nordisk, in collaboration with Merrion Pharmaceuticals, utilizes their GIPET platform for developing long-acting insulin tablet or capsule. Novo nordisk claimed that their product will become lead product after completion of early stage trials.[230]

Tamarisk technology

It involves the encapsulated insulin known as serum specific nano-encapsulate particles It travels through the cell in a similar way as fatty acid or cholesterol and company claimed that because of this body believes that it is naturally occurring molecule.[231]

BUCCAL FORMULATIONS

Oral-Lyn

Canadian company Generex Biotechnology has developed Oral-Lyn, a liquid formulation of human insulin that is sprayed into the mouth using its proprietary Rapid Mist device. It is indicated for the treatment of type 1 and 2 diabetes. In September, 2009, the US FDA approved Oral-Lyn under its treatment IND program.[232]

OCULAR

Gelfoam

Acidified Gelfoam, an absorbable gelatin sponge, USP based ocular device delivered insulin more efficiently into the bloodstream without the aid of absorption enhancer.[211]

INHALED FORMULATIONS

Exubera

The first inhaled insulin Exubera has been developed by Pfizer in collaboration with Nektar Therapeutics, approved by the US FDA in January 2006. Initially, it was predicted that Exubera would be a best-seller drug since it was the first inhaled option for people who needed to take insulin. However, Exubera's high price and bulky inhaler, as well as concerns about its effects on lung function, led to much lower sales than had been expected. So it was removed from the market in 2007.[233-235]

Afrezza

Afrezza (human insulin [recombinant DNA origin]; (Mankind Corporation, Valencia, California, USA) is a drug-device combination consisting of Technosphere insulin (TI) inhalation powder and a product-specific breath-activated inhaler. The formulation is designed for use with first generation palm-sized MedTone™ DPI, a capsule-based high-impedance inhaler and thumb-sized second-generation DreamBoat inhalers that uses a passive powder deagglomeration mechanism. The US FDA is reviewing it for use as inhaled mealtime insulin for managing hyperglycemia in patients with type 1 and type 2 diabetes mellitus.[236,237]

Technosphere insulin

Technosphere insulin is an inhaled form of regular human insulin consisting of fumaryl diketopeperazine microspheres (2-3 μm) loaded with Regular human insulin. It has a rapid onset of action (~15min) that is being considered for approval for the treatment of type 1 and type 2 diabetes mellitus. It also reduces the level of glycosylated hemoglobin (A1c) in a dose-dependent manner.[238,239]

AERx iDMS

AERx (Aradigm Corp., Hayward, CA, USA) insulin diabetes management system (iDMS) (Novo Nordisk A/S, Bagsvaerd, Denmark), first electronic inhaled-insulin-system, uses unique liquid human insulin strip to deliver an aerosol of insulin to the lungs. The bioavailability was found to be 13-17% followed by use of AERx iDMS.[240,241]

Aerodose

The Aerodose insulin inhaler is uniquely designed by Aerogen Inc., to provide patients with a small, discreet and easy-to-use inhaler. The highest bioavailability of insulin was achieved using the smaller of two aerosol particle sizes and four seconds of aerosolization time. Dose-response relationships study showed no significance difference between inhaled insulin delivered via the Aerodose insulin inhaler and subcutaneously injected insulin with respect to relative bioavailability or relative biopotency in type 2 diabetic patients.[242,243]

AIR

Alkermes Inc. and Eli Lilly and Company has developed AIR Inhaled Insulin for type 1 diabetes and Byetta (foremerly exenatide) LAR for type 2 diabetes. That arrangement ended with Lilly's withdrawal from the inhaled insulin program.[244]

Spiros

Dura and Eli Lilly has developed inhaled insulin (r DNA origine) based upon Dura's Spiros technology for proteins and peptides. It is a small handled, breath-actuated battery operated powder inhaler. The inhaler has an impellar which gets actuated when patient inhales, leads to dispersion of powder for inhalation.[245]

ProMaxx®

PROMAXX, a microsphere technology, was developed by Epic Therapeutics. It is a water based delivery technology for proteins, peptides etc.[246,247] Different devices meant for inhaled delivery of insulin are shown in Figure 5.

NASAL

Nasulin™

Nasulin, an intranasal spray, consisting of regular short-acting human recombinant insulin dissolved in water patented by CPEX Pharmaceuticals, Inc., which consists of CPE-215 as a permeation facilitator. CPE-215 is obtained from *Angelica archangelica*. Repeated dosing of Nasulin™ in the same nostril enhances the insulin absorption.[248,249]

QDose

MicroDose, through its collaboration with Vectura Group plc, QDose Limited, has developed a highly efficient, speedy

Figure 5: Different devices meant for inhaled delivery of insulin (a) Exubera (b) Spiros (c) Technosphere (d) Alkermes (e) Aerodose (f) AERx

acting, insulin inhaler, based upon MicroDose's proprietary electronic inhaler technology and Vectura's dry powder insulin formulation. Glucose Clamp Study showed that relative bioavailability of inhaled insulin was ~18%.[250]

ChiSys™

It is a chitosan-based drug delivery system.[251]

TRANSDERMAL

Macroflux®

This consists of drug-coated titanium microprojection array affixed to a polymeric adhesive back. These microprojections penetrate through the skin's barrier layer into the epidermis.[252]

PassPort™

Altea Therapeutics has developed the basal insulin skin patch system, uses extremely short bursts of focused thermal energy to create hundreds of tiny channels, or micropores, in the surface of the skin. These pores allow the permeation of proteins into the body without pain.[253]

U-Strip™

U-Strip Insulin System, a programmable transdermal drug delivery patch, uses ultrasonic energy for delivery of insulin. This leads to pore dilation and deposition of large drugs molecule drugs into the dermis.[254]

NONINVASIVE DIAGNOSTIC DEVICES FOR DIABETES

Zhang *et al*. monitored the tear glucose level by using contact lens based sensor. This involves the encapsulation of fluorescence resonance energy transfer pair-labeled protein within mesoporous nanoparticles which acts as analyte (tear glucose) collector.[255]

Noninvasive glucometer

Recently, Grove Instruments™ has made-up a new noninvasive devise [Figure 6] based on utilization of near-infrared spectroscopy to determine the BGL. This device takes reading from the blood, not interstitial fluid due to "optical bridge".[256]

CONCLUSION

Since many years diabetes is emerging as life threatening disease. Management of diabetes requires continuous insulin treatment. Hence, to avoid patient discomfort and enhance patient compliance, noninvasive insulin delivery is the best approach. In the future, many of the inhaled devices emerge as a potential tool to reduce the risk of diabetes.

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Figure 6: Groves noninvasive glucometer

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