

Advanced Approaches in Insulin Delivery

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Abstract: Diabetes is a syndrome of disordered metabolism and inappropriate hyperglycemia resulting from a deficiency of insulin secretion or insulin resistance. Insulin, a pancreatic hormone, helps to lower the blood sugar levels. The structural features of insulin and insulin receptors are summarized. Diabetic patients use insulin in the form of injections, which involves lots of pain, and a need for non-invasive, alternative mode of insulin administration is desired. These challenges have led to attempts in insulin therapy using oral, nasal, pulmonary, rectal, transdermal, buccal, gene therapy, islet cell transplantation and diabetes vaccine. Among all the approaches pulmonary administration has achieved some clinical significance. Future approaches that can be exploited for insulin therapy in Insulin Dependent Diabetes Mellitus [IDDM] have been summarized. Insulin inhalers or tablets for IDDM are interesting alternatives.

Key Words: Diabetes, insulin delivery, alternative approaches, gene therapy, islet cell transplantation, diabetes vaccine.

INTRODUCTION

Diabetes is a syndrome of disordered metabolism and inappropriate hyperglycemia resulting from a deficiency of insulin secretion or resistance. A normal blood sugar level in human ranges between 60 to 120 mg/dl. The hormones, insulin and glucagons, keep the blood glucose level within the normal limits. New diagnostic criteria incorporate 3 ways to diagnose diabetes.

- Symptoms of diabetes plus a casual plasma glucose concentration ≥ 200 mg/dL. Casual is defined as anytime of day without regard to the last meal. Symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
- FPG (Fasting Plasma Glucose) ≥ 126 mg/dL. Fasting is defined as no caloric intake for at least 8 hours.
- 2-h PG (Plasma Glucose) ≥ 200 mg/dL during an oral glucose tolerance test (OGTT). The OGTT should be performed as described by WHO [World Health Organization], using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. In clinical practice, this test is rarely used anymore [1].

In 2004 according to WHO more than 150 million people suffer from diabetes worldwide. In US 16 million people suffer from diabetes, the sixth leading cause of death by disease in the nation, reaching nearly 200, 000 deaths each year [2-4].

CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus is of two types- *type 1* and *type 2* diabetes mellitus. Type 1 diabetes mellitus is a syndrome of absolute insulin deficiency. It is characterized by pre-disposition to recurrent Ketoacidosis in the absence of insulin therapy, and most cases are due to autoimmune destruction

of pancreatic β -cells. Most type 1 diabetics are diagnosed before the age of 35; however, the incidence of type 2 diabetes at younger ages has increased. The requirement for insulin may also confuse the proper classification of diabetes mellitus, since many type 2 diabetics require insulin therapy for optimal blood glucose control. Type 1 diabetic patients require insulin therapy to prevent recurrent ketoacidosis. A variant of type 2 diabetes termed maturity onset diabetes of the young (MODY) can present with acute hyperglycemia and ketoacidosis in teenagers, but may be managed by diet and oral agents for many years without recurrent acidosis. The occurrence of impaired glucose tolerance during pregnancy in a woman without a previous history of diabetes is termed gestational diabetes mellitus (GDM). GDM is seen in approximately 4% of all pregnancies in the United States [5] and is associated with an increased risk of fetal abnormalities, adverse outcomes, and increased life long maternal risk of chronic diabetes. Insulin resistance is normal in pregnancy and results, in part, from high progesterone concentrations. Glucose tolerance is most impaired during the third trimester, and oral glucose tolerance testing is recommended between weeks 24 and 28 of gestation. The treatment of GDM focuses on tight control of blood glucose to improve perinatal outcomes, as well as behavioral interventions to minimize the risk of type 2 diabetes later in life [5]. The treatment for type 1 diabetes is insulin injections under the skin. Type 2 diabetes is generally treated with medications such as with a single medication of Alpha-glucosidase inhibitors [e.g. Acarbose] Thiazolidinediones [e.g. Pioglitazone], sulfonylurea [e.g. Tolbutamide] or biguanides [e.g. Metformin] [6-10]. If the diabetes is not controlled with this approach, combination therapy that is drugs with two different classes may be used, combination of metformin and sulfonylureas agents has been used successfully [11]. The next step would be oral therapy plus insulin, then insulin only [6].

INSULIN

Insulin was 1st discovered by Banting and Best in the year 1921 [12]. Insulin is a polypeptide hormone that travels

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around the blood stream; insulin has molecular weight of (pork): 5777.66 [13], (beef): 5733.61 [13], Human (semi synthetic, biosynthetic): 5807.69 [13], consisting of two amino acid chains A & B (Fig. 1), the A chain has 21 amino acids and B chain has 30 amino acids. The two chains are connected by two disulphide bridges, bonds formed between the sulphur atoms in the amino acid cystine. The A chain also has a third internal disulphide bridge. The disulphide bridges hold the molecule together. Although the amino acid sequence of insulin varies among species (Table 1), certain segments of the molecule are highly conserved, including the positions of the three disulphide bonds, both ends of the A chain and the C-terminal residues of the B chain [14]. The disulfide bridges and Amino acid sequences are essential for insulin's biological activity [15]. The helical structure of A12-18 is essential for biological activities of insulin. A8-10 is not much concerned with biological activities, but is much more important antigenically in binding to its antibodies [15].

MECHANISM OF ACTION/EFFECT

Insulin is a polypeptide hormone that controls the storage and metabolism of carbohydrates, proteins, and fats. This activity occurs primarily in the liver, in muscle, and in adipose tissues after binding of the insulin molecules to receptor sites on cellular plasma membranes. Although the mechanisms of insulin's molecular actions in the cellular area are still being explored, it is known that cell membrane transport characteristics, cellular growth, enzyme activation and inhibition, and alterations in protein and fat metabolism are all influenced by insulin [16]. More specifically, insulin promotes uptake of carbohydrates, proteins, and fats in most tissues. Also, insulin influences carbohydrate, protein, and fat metabolism by stimulating protein and free fatty acid synthesis, and by inhibiting release of free fatty acid from adipose cells [16, 17]. Insulin increases active glucose transport through muscle and adipose cellular membranes, and promotes conversion of intracellular glucose and free fatty

acid to the appropriate storage forms (glycogen and triglyceride, respectively). Although the liver does not require active glucose transport, insulin increases hepatic glucose conversion to glycogen and suppresses hepatic glucose output. Even though the actions of exogenous insulin are identical to those of endogenous insulin, the ability to negatively affect hepatic glucose output differs because a smaller quantity of exogenous insulin reaches the portal vein [18].

Table 1. Amino Acid Sequence of Human, Porcine and Bovine Insulin [14]

Insulin	A8	A10	B30
Human	Theonine	Isoleucine	Threonine
Porcine	Theonine	Isoleucine	Alanine
Bovine	Theonine	Valine	Alanine

METABOLISM OF INSULIN IN THE BODY

Insulin has a half-life of about 5 to 6 minutes in normal subjects and patients with uncomplicated diabetes [19]. The half-life of proinsulin is longer than that of insulin (about 17 minutes), and this protein accounts for about 10% of the immunoreactive insulin in plasma [20]. C-peptide is secreted in equimolar amounts with insulin; however its molar concentration in plasma is higher because of its considerably longer half life (about 30 minutes) [20]. Degradation of insulin occurs primarily in liver, kidney, and muscle [21]. About 50% of the insulin that reaches the liver *via* the portal vein is destroyed and never reaches the general circulation. Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules, which also degrades it. Severe impairment of renal function appears to affect the rate of disappearance of circulating insulin to a greater than does hepatic disease [22]. He-

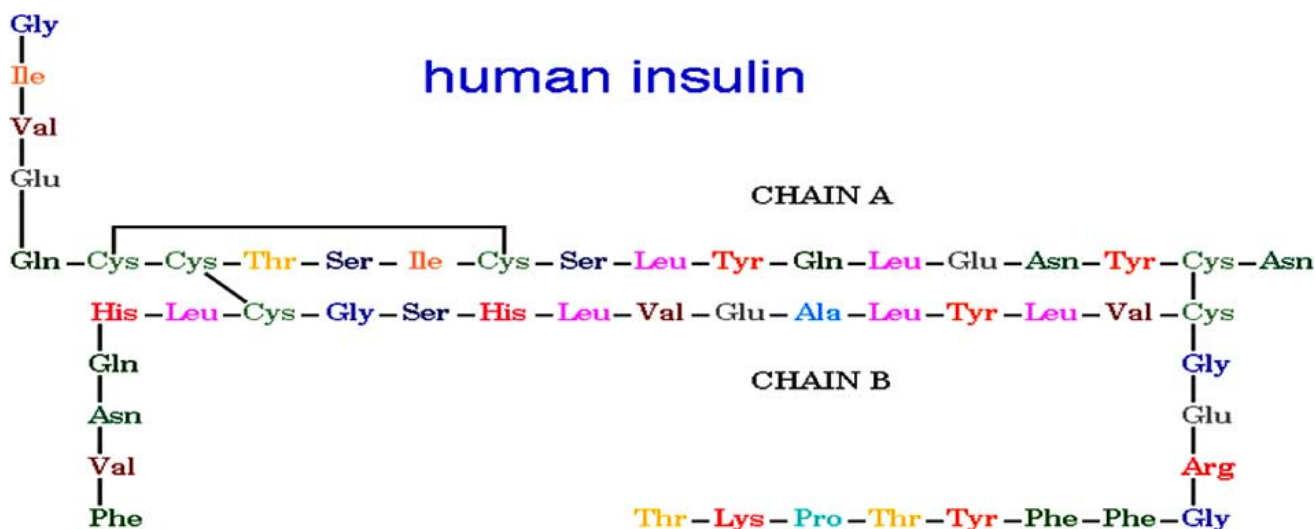


Fig. (1). Human Insulin structure consisting of chain A and B.

patic degradation of insulin operates near its maximal capacity and cannot compensate for diminishing renal breakdown of the hormone. The oral administration of glucose appears to reduce hepatic extraction of insulin [23]. Peripheral tissues such as fat also inactivate insulin but this is of less significance quantitatively. Proteolytic degradation of insulin in the liver occurs primarily after internalization of the hormone and its receptor and to a lesser extent, at the cell surface [24]. The primary pathway for internalization is receptor-mediated endocytosis. The complex of insulin and its receptor is internalized in to small vesicles termed endosomes, where degradation is initiated [21]. The extent to which internalized insulin is degraded by the cell varies considerably with cell type. In hepatocytes, over 50% of the internalized insulin is degraded; where as most internalized insulin is released intact from endothelial cells. In the latter case, this finding appears to be related to the role of these cells in transcytosis of the insulin molecules from the intravascular to the extravascular space. Transcytosis has an important role in the delivery of insulin to its target cells in tissues where endothelial cells form tight junctions, including skeletal muscle and adipose tissues [25].

METHODS OF PREPARATION OF HUMAN INSULIN

'Human' insulin is so called because structurally and chromatographically it is identical to the insulin produced by the human body. Human insulin produced by recombinant DNA technology is less allergenic. Three methods of synthesis of human insulin have been developed to the stage of commercial production and they are as follows

- Biosynthetic human insulin- Biosynthesis is a process in which a synthetic gene sequence of DNA coded for a desired protein is inserted into a plasmid. This plasmid is then inserted into E.coli and cultured. For the A and B chains of insulin, fermentation is carried out separately. The DNA sequence coding for A and B chains are synthesized chemically. The A-chain gene is linked to a large protein tryptophane synthetase gene by a methionine codon. This synthesized plasmid is inserted in E.coli culture. The B-chain gene is also prepared in a similar manner and inserted in a separate E.coli culture. The cells are harvested and insulin chains are cleaved by treatment with cyanogens bromide. A and B chains are then combined by air oxidation to produce human insulin. This insulin is described as chain recombinant bacterial (crb) [26-30].
- Semi synthetic Human Insulin- Basically, porcine and human insulin's differ only by a single amino acid, i.e., alanine in B-30 position. Semi synthesis of human insulin was carried out using porcine insulin. Enzymatic transpeptidation of porcine insulin with L-threonine tert-butyl ester gives Human insulin ester. This Human insulin ester is then purified by HPLC or ion exchange chromatography by treating with a mixture of trifluoroacetic acid and anisol to produce fully biologically active human insulin. This insulin is designated as enzymatically modified porcine (emp) insulin chains [31, 32].
- Proinsulin Route: In this method the entire proinsulin gene is inserted into E.coli cultures and harvested to produce more proinsulin chains. Proinsulin is later cleaved

by carboxypeptidase's-B to yield human insulin and C-peptide, which is then further purified. This type of insulin is called proinsulin recombinant bacterial (prb) [33-34].

NEW INSULIN ANALOGUES

The discovery of insulin in 1921 was one of the milestones in medical history that revolutionized the treatment of diabetes. However, it is almost impossible to use traditional human insulin to achieve sustained normoglycemia without hypoglycemic events. Modern recombinant DNA technology stands behind the market success of insulin analogues with its improved pharmacokinetic and therapeutic efficacy The newer rapid-acting insulin has given more flexibility to pre-meal dosing while decreasing the risk of early postprandial plasma hyperglycemia and later hypoglycemia (Table 2) [35-40].

Table 2. Commercially Available Insulin Preparations and their Activities [35-40]

	Onset (hrs)	Peak (hrs)	Duration (hrs)
Aspart	0.1 - 0.3	1 - 3	3 - 5
Lispro	0.1 - 0.5	0.5 - 2.5	2 - 4
Regular	0.5	2 - 4	5 - 8
NPH	1 - 3	5 - 10	13 - 18
Lente	1 - 3	5 - 10	13 - 18
Glargine	1 - 2	No peak	20 - 24

INSULIN LISPRO

It is the first clinically available insulin analogue. Introduced in 1996, lispro differs from human insulin by a switch at lysine B28 and proline B29. In the vial, the lispro exists as a hexameric formulation, but when it is injected, it dissociates into a monomeric formulation, leading to a more rapid absorption and shorter duration of action. Patients using lispro are able to use less insulin and experience fewer hypoglycemic events. Lispro has been beneficial in patients with end-stage renal disease patients and in pregnant patients. It can be absorbed more promptly than regular human insulin, with rapid onset of action and a short duration of action. Therefore it can be administered immediately prior to meal, compared with 30-45 minutes in advance of eating with regular insulin. This makes it a regimen that can greatly enhance patient compliance [35, 36].

INSULIN ASPART

Aspart is the newest rapid-acting insulin analog; it differs from human insulin through substitution of aspartic acid at B28. The time to maximum concentration is 52 minutes vs. 145 minutes with regular insulin. Aspart has shown better PPG [Postprandial Glucose] control and less nocturnal hypoglycemia compared with regular insulin [37]. A small study comparing aspart and lispro demonstrated them to be

very similar; however, lispro peaked approximately 10 minutes earlier [38].

INSULIN GLARGINE

Glargine differs from human insulin by replacing asparagine at A21 with glycine and adding 2 arginines to the C-terminus of the B-chain. Glargine was designed to mimic normal basal insulin, with a slow absorption and long half-life. Glargine is stable at a pH of 4 and will precipitate at physiologic pH, thereby causing the slow absorption. Glargine was shown to cause less nocturnal hypoglycemia in patients with type 1 diabetes [39]. In another study, involving type 2 diabetic patients, insulin glargine decreased the incidence of nocturnal hypoglycemia from 28% to 12.6% compared with once-daily NPH. Also, the pre- and post-prandial blood glucose levels were lower in the glargine group vs. NPH [40].

NEWER ROUTES FOR INSULIN DELIVERY

The most unpleasant thing about insulin is that it needs to be given through an injection repeatedly which involves lots of pain and hence patient compliance is not there. Therefore there is a search for a non-injectable preparation of insulin, which could be administered by oral, nasal, pulmonary and other non-invasive methods. Following are the approaches tried to develop alternative non-invasive mode of insulin administration.

A. Oral

This is the most preferred route & most of the drugs available today are in the form of tablets or capsule. But insulin's tablet is still a dream. The basic problem with insulin administration *via* oral route is its inherent instability in the harsh condition of the gastrointestinal tract (pH; Enzymes; adsorption to solids) and also absorption through GIT [Gastro Intestinal Tract] is not guaranteed [41]. Therefore for improved oral absorption, it's important to protect the formulation from degradation and increase its absorption through GIT [Gastro Intestinal Tract]. Following are the approaches that are tried for oral insulin administration.

- a) Micro spheres as oral delivery system for insulin: Gowthamarajan *et al.* [42] investigated the feasibility of delivering insulin systemically by oral route using micro spheres prepared with Eudragit L-100, sodium glycocholate and aprotinin. Eudragit L-100 was used as carrier for micro spheres to give a site-specific release of insulin in the upper intestine; sodium glycocholate was used as penetration enhancer and aprotinin as a protease inhibitor. The in-vivo hypoglycemic effect study carried out using alloxan induced diabetic rats showed that the micro spheres prepared with Eudragit L-100, 1% aprotinin and 1% sodium glycocholate showed prolonged hypoglycemic effect for 4 hours which was not even observed with subcutaneous bovine insulin injection [42].
- b) Chitosan capsules for insulin delivery: Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides such as in-

sulin. Tozaki *et al.* (43) developed colon-specific insulin delivery with chitosan capsules. *In vitro* drug release experiments from chitosan capsules containing 5(6)-carboxyfluorescein (CF) were carried out by rotating basket method with slight modifications. The intestinal absorption of insulin was evaluated by measuring the plasma insulin levels and its hypoglycemic effects after oral administration of the chitosan capsules containing insulin and additives. Little release of CF (5(6)-carboxyfluorescein) from the capsules was observed in an artificial gastric juice (pH= 1), or in an artificial intestinal juice (pH= 7). However, the release of CF was markedly increased in the presence of rat caecal contents [43].

- c) Water-in-oil type of emulsion: Human insulin was incorporated into a w/o emulsion by high-pressure homogenization. A fine stable dispersion of the aqueous phase was achieved and the emulsion was able to protect insulin against gastric degradation *in vitro* without further encapsulation [44].
- d) Biocarrier insulin: In this system insulin was first entrapped in Liposome's. The preparation was developed using ghost erythrocytes as bio carriers for intraduodenal administration of insulin because proteolytic enzymes in duodenal region break erythrocytes. From such a system insulin was absorbed and showed its glucose lowering effects [45, 46]
- e) Nanocapsules for insulin delivery: Damge *et al.* [47] developed nanocapsules using biodegradable Polymer Poly (isobutyl / Cyanoacrylate) [mean size 220nm]. When administered orally by force-feeding to diabetic rats, insulin nanocapsules (12.5, 25, and 50 U/kg) decreased fasted glycemia 50-60% by day 2. This effect was maintained for 6 or 20 days with 12.5 or 50 U/kg, respectively. Only the dose of 100 U/kg decreased fed glycemia by 25% in diabetic rats. In normal rats, hyperglycemia induced by an oral glucose load was reduced by 50% with the same dose of oral insulin nanocapsules [47]. Sharma *et al.* [48] have loaded insulin in ceramic nanoparticles prepared from hydroxyapatite and encapsulated these particles in sodium alginate. *In vitro* release profile of insulin was carried out in simulated gastric (pH 1.2) and intestinal fluids (pH 7.4). 100 mg of insulin loaded nano particle was introduced into 10 ml of respective medium. 0.1 ml of sample was withdrawn at various time intervals and evaluated for insulin using Lowry's method for protein estimation. Present investigation show that insulin loaded HA (hydroxyapatite) nanoparticle encapsulated in sodium alginate can effectively release almost 100 % of the drug in SIF [Simulated Intestinal Fluid] during a period of 2 hours. However, during the same period only 24-28 % of insulin was released in SGF [Simulated Gastric Fluid] [48].
- f) Entrapment in Liposome's: Stefanov *et al.* [49] investigated the feasibility of delivering insulin systemically by oral route using liposomes prepared from phosphatidyl choline and cholesterol (1:9). No change in blood glucose levels was noted in normal animals but a significant reduction was obtained with diabetic rats with the maximum effect observed within 3 hrs.

B. Nasal

Clinicians have attempted to administer insulin through the nasal mucosa. However, the bioavailability of nasally administered insulin was <10 % [50-52], a value that could only be achieved with the help of "absorption enhancers" [50-55]. Such agents increase the absorption rate of relatively large peptide such as insulin through the epithelial barrier. Only few of the many agents studied, increased the absorption rate without irritating the nasal mucosa or having other side effects [55, 56]. Absorption enhancers reported for transmucosal insulin delivery include saponins; sodium caprylate; sodium laurate; polyacrylic acid; fusidic acid and bile salts [57]. Recently, chitosan, a polysaccharide, has been investigated to increase insulin absorption with no evidence of toxic manifestation [58]. Both liquid and powder systems have been evaluated in humans. Chitosan is a bioadhesive material and therefore slows mucociliary clearance allowing more time for the therapeutic material to be absorbed. Chitosan also causes a transient and reversible effect whereby the tight junctions of the nasal mucosal cells open to allow the passage of insulin [59]. Increasing the time of contact with the nasal mucosa can increase the nasal absorption of insulin. The clearance half-life can be increased from 15 min with nasal solution to 240 min using starch micro spheres (SMS) [60]. Insulin administered in combination with SMS resulted in 497% increase in AUC for plasma insulin as compared to insulin solution. The AUC increased by 1657% compared to insulin solution when an enhancer lysophosphatidyl choline was used with insulin and SMS [60]. When a surfactant such as saponin, sodium glycocholate or BL-9 was added to the preparation, the absorption of insulin from the nasal mucosa was enhanced independent of pH. Gordon *et al.* [61] studied the effects of different bile salts at various concentrations on intranasal insulin absorption in man. Maximum increase in serum insulin was obtained using deoxycholate followed by *chenodeoxy-*cholate and cholate at 1% w/v. Blood glucose concentration reduced by approximately 50% with deoxycholate and peak serum insulin concentration was reached in 10 min after administration of the spray. Similar results were also obtained by Moses *et al.* [62]. Absorption enhancing effect of different cyclo-dextrins on intranasally administered insulin in rats and rabbits were determined. Dimethyl-beta-cyclodextrin was found to be most potent [63, 64]. Nasal absorption is also promoted by medium chain fatty acid salt [65] glycyrrhetic acid derivatives in rat [66] sodium tauro-24, 25-dihydrofusidate in sheep [67]. Etr *et al.* [68] demonstrated that the blood glucose remained low for 3 h after lunch when insulin was used intranasally with 1% deoxycholate in type1 diabetics.

C. Pulmonary

Delivery of medication to the respiratory tract for the localized therapy of respiratory diseases has been practiced for several decades. The lungs are an attractive site for the systemic delivery of insulin because lung has some inherent advantages for insulin administration. These includes,

- a vast (in humans 50-140 m², ~500 millions of alveoli) and well-per fused absorptive surface (~5 l blood/min, pulmonary capillary blood volume ~0.25 l),

- the absence of certain peptidases that are present in the gastrointestinal tract,
- no immediate degradation of the absorbed insulin by the liver ("first pass metabolism"),
- a thin alveolar-capillary barrier, and
- marginal variance in the amount of mucus production [69].

More than 95% of the lung surface is composed of the ultra thin (0.1 μm) alveolar epithelium, i.e. the gas exchange surface is much larger than that of the branching system. These conditions allow a fast absorption of peptides into the bloodstream and a rapid onset of action after inhalation, i.e., the lung represents a highly permeable "port of entry" into the blood for macromolecules [70]. Particle size is also essential for pulmonary delivery of insulin; the optimal particle size for pulmonary insulin administration appears to be in the range of 2-5 μm [71]. Since the volume of particles increases by power of three if the radius doubles, a compromise must be found for the particle size to allow a good penetration into the fine bronchial branches and alveoli on one hand (which means small particles) and a sufficient amount of insulin on the other hand (which means larger particles with slow absorption) [71]. The kinetics of insulin absorption across the respiratory mucosa tends to mimic the bolus insulin needs and peak levels are achieved in 15 to 20 minutes with return to the base line in 40 to 60 min's [72, 73].

The pharmacodynamic effects of insulin formulations administered *via* the lung are comparable to or even faster than, those of subcutaneously injected regular insulin or rapid acting insulin analogues [74]. The relative biopotency of inhaled insulin in most cases is approximately 10% i.e. the dose of insulin administered must be ten fold higher than with subcutaneous application [74]. Most experience with inhaled insulin has been obtained using either dry powder formulation in the Nektar Pulmonary Inhaler/Exubera device (Nektar Therapeutics Inc., San Carlos, CA, Aventis, Bridgewater, NJ, Pfizer, NY) or a liquid aerosol formulation in the AERx InsulinDiabeticsManagementSystem (AradigmCorp. Hayward, CA, NovoNordiskA/S, Copenhagen, Denmark). The products of Aradigm/Novo Nordisk are in phase- II and that of Nektar/Pfizer/Aventis are in phase-III of clinical trials [74]. The published results of clinical trials thus far indicate that metabolic control is comparable to that of subcutaneous insulin therapy and as of to date no serious side effects have been reported from these human trials [75].

D. Transdermal Route

The transdermal delivery of polypeptides such as insulin has been evaluated by many different groups but the results so far not encouraging [76]. The skin of a man provides a good barrier. Novel approaches to "driving" Insulin across the skin, such as Iontophoresis and ultrasound; have been explored but clinical trials have been disappointing [77, 78]. Insulin is an interesting case where physiological pH is a problem when considering Iontophoresis. The charge on the molecule changes with pH- gradient across the skin. Under electrophoresis; the molecule can be forced into skin; but, as the pH changes as one moves from the skin surface to tissue,

the charge on the molecule reverses and the molecule is forced back out [77, 78]. A possibility is studied of the transdermal delivery of insulin by using a mixture of synthetic analogues of phosphoglycerides (SAP); as a potential activator of insulin diffusion through the skin. Experimentally *in vitro*; it was proven that the diffusion of insulin through the skin of two types of transdermal therapeutic form (TTF)-matrix-type and matrix- hydrogel-type is possible only in presence of activator SAP-M-99 [79]. The detected optimal composition of insulin matrix TTF with the area of 40 sq cm collagenous sponges enabled to increase the insulin diffusion upto 0.54 units/hour [79]. Transferosomes are ultra flexible liposomes with low pore resistance (Cevc) [80]. Insulin incorporated in this system transported therapeutic amounts of peptide across intact mammalian skin [80]. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. Preliminary studies with plasmids and insulin revealed that the ethosomal carrier might be used for enhanced delivery of these agents [81].

E. Buccal Route

Efforts have been made to develop insulin patch from which insulin would be absorbed through patch through the lining of the mouth cavity. This would mimic the basal insulin secretion. By buccal delivery drugs are absorbed rapidly into the reticulated veins, which lie under the oral mucosa and enter the systemic circulation directly, by passing the liver [82]. A mucosal adhesive delivery system was developed for the buccal delivery of insulin. It was found that the systemic delivery of insulin through the buccal mucosa was significantly affected by the formulation composition used [82]. Insulin could not be effectively absorbed by a simple disk-shaped dosage form prepared by direct compression of insulin in a mixture of hydroxypropyl-cellulose [HPC] and carbopol-934 [82]. Buccal absorption was achieved using a dome shaped, two phased mucosal adhesive device prepared by dispersing insulin crystals with sodium glycocholate, an absorption promoter, in an oleaginous core and then overlapping the medicated core with an adhesive dome [83]. Modi *et al.* [84] reported on the buccal administration of insulin using a spray device (MDI), which are both surprising and potentially exciting. Reported clinical studies in diabetic subjects have demonstrated good efficacy with bioavailabilities in the range 5-10% [84].

F. Rectal

Despite its poor social acceptability, the rectal mucosa has also been investigated as a potential route of insulin administered. Insulin can be absorbed through this route in the presence of absorption enhancers, the co administration of absorption enhancers such as sodium glycocholate; has been reported to enhance the rectal absorption of insulin [85]. The effectiveness of insulin administration by rectal suppository was examined in normal and non-insulin-dependent non-obese diabetic subjects. A 100-U insulin suppository (mean 1.8 U/kg) given to the diabetic subjects caused four times as great a fall in plasma glucose compared with the normal subjects given the same dose (mean 1.6 U/kg). Diabetic subjects given a 100-U insulin suppository (mean 1.7 U/kg) 15 min after meals three times daily showed a significant (P

less than 0.05) improvement in postprandial hyperglycemia accompanied by a reduction of urinary glucose from 26+/-5.9 to 2.0+/-1.0g/day [86].

G. Bioresponsive Insulin Delivery System

Horbett *et al.* [87] developed bioresponsive insulin delivery that consists of artificial beta cells of a glucose-sensitive hydro gel membrane for the feed back – controlled delivery of insulin. The glucose sensitive membrane is fabricated by entrapping glucose oxidase enzymes in a polyorthoester coat [87]. Because the conventional polyorthoesters do not have the required pH sensitivity, their structure was modified by incorporating tertiary amine groups in a polymer backbone [89]. As glucose diffuses into polymer; glucose oxidase catalyzes its conversion to gluconic acid (pKa= 3) thereby lowering the micro environmental pH in the membrane [88]. The reduced pH results in increased ionization of the tertiary amine groups. Electrostatic repulsion between the ionized amine groups increases the swelling and thus the permeability of the hydro gel membrane to insulin contained in the reservoir. Thus the membrane permeability to insulin is a function of the glucose concentration surrounding the membrane, and the release of insulin is accelerated by the increase in glucose levels [89].

RECENT ADVANCES IN TREATMENT OF DIABETES

Cell-Replacement and Islet Cell Transplantation

Implantation of surrogate β -cells involves the creation or expansion of insulin-producing cells *in vitro* [90]. This is followed by their implantation (or reimplantation if from the same individual) in the patient. The cells of β -cell origin are allowed for unlimited expansion in culture to produce insulin. Alternatively these cells can be obtained from stem cells (adult or embryonal) [90]. These cells have been induced to differentiate into β -cells (or selected to this end) *in vitro*. The poor outcome of the numerous attempts to transplant human islets during the past quarter of a century is interpreted to be caused by a host of factors; one is allojection [90]. Despite long-term immunosuppression, insulin production in type 1 diabetic patients with transplanted islets has historically been transient at best [90]. State-of-the-art of immunosuppression that prevents allojection of kidney, heart, or liver seems to fail with human islets. Transduction of cells from the patient per se alleviates this problem. Indeed, the best results of islet transplantation are seen with auto transplantation of islets obtained from the patient's own pancreas after resection for chronic pancreatitis [91]. The complexity of dealing with three different immune attacks, i.e., acute rejection, chronic rejection, and recurrence of the disease, has drastically hampered clinical islet transplantation. In addition, there is a wealth of *in vitro* data suggesting that β -cell function is affected by cyclosporine, prednisone, or azathioprine that are widely used immunosuppressants [92, 93]. Despite immunosuppression and matching for HLA, several studies suggest the loss of function of transplanted islets was caused by recurrence of disease, as was amply demonstrated in pancreas transplantation between monozygotic twins [94]. Failed islet transplants have been reported to be associated with reappearance of GAD65 autoantibodies, a major serological

marker for type 1 diabetes [95]. This is prevented by enveloping the cells in thin coats of gelatin-like material (microencapsulation). Marc Garfinkel *et al.* [96] have been working on a new method of microencapsulation, which involves creating a layer between two liquids, like oil and water, and applying suction above the layer to generate a thin spout of the lower liquid. This spout then breaks into small beads, surrounding particles (or islets) contained in the water layer. These beads are then exposed to a process that causes them to gel. These researchers propose experiments to show whether islets microencapsulated by this method will reverse diabetes without rejection-preventing medications. Also islet cells microencapsulation with special materials such as alginate-polyL-lysine also showed good result [97-99]. Finally, it is self-evident that new and more plentiful sources of insulin-secreting cells will be needed to treat type 1 diabetes. The most recent developments in stem cell research are promising in this regard, with islets or islet cells having been derived from embryonal murine [100, 101] and embryonal human [102], as well as adult human pancreatic ductal stem cells [103].

GENE THERAPY

The human insulin gene has been isolated. This gene is located on the short arm of chromosome 11 [104]. Insulin gene therapy involves the introduction of a foreign gene into any cell type in the body. The gene(s) introduced could be the insulin gene itself, perhaps under control of a tissue-specific promoter, allowing for expression in a select non- β -cell type, or a gene encoding a factor that in turn activates the insulin gene, thereby allowing for ectopic insulin production [90]. Insulin gene therapy should comprise several important components: (1) an effective insulin gene transfer system; (2) a regulatory system that results in the expression and release of insulin in response to glucose; (3) the biochemical machinery for the appropriate processing of proinsulin into mature, active insulin in the transfected cells; and (4) an appropriate target cell that has biochemical characteristics similar to β cells but is not a target for autoimmune attack [105]. Insulin gene transfer can be accomplished by either Non Viral gene transfer system or viral gene Transfer system [105]. Nonviral methods for transferring genetic material include the direct injection of DNA, either naked or enclosed in a liposome, electroporation and the gene gun method [105]. Riu *et al.* [106] have indicated that engineered muscle cells can continuously secrete basal insulin to enhance glycemic control in conjunction with insulin therapy. When fed, transgenic mice were able to have a normal level of blood glucose and insulin. When treated with streptozosin, they had mild hyperglycemia with an elevated level of blood insulin. Injection of a small dose of insulin was able to correct their hyperglycemia. However, Streptozocin-treated control mice remained hyperglycemic even with insulin treatment. Furthermore, streptozosin-treated transgenic mice presented normalization of both skeletal muscle and liver glucose metabolism [106]. Basal hepatic insulin production can be used as an auxiliary treatment to conventional insulin therapy. Sustained gene expression of basal hepatic insulin has been achieved by Dong *et al.* [107]. Widely used viral methods of gene transfer include vectors based on retrovirus, adenovirus and adenoassociated virus (AAV) [108,

109]. Adenovirus-mediated preproinsulin gene transfer into adipocytes can ameliorate hyperglycemia in diabetic mice [110]. Genetically obese type 2 diabetic mice were treated with a single subcutaneous injection of recombinant adenovirus Adex1CA-human preproinsulin into epididymal fat pads. Mature insulin was produced in adipose tissue. Three days after virus injection, these mice showed a marked decrease in blood glucose levels with an improved glucose tolerance [110]. The normalized glucose levels in diabetic rats were maintained for at least two weeks after the virus injection [110]. Recently, a new generation of adenovirus vectors has been developed that are completely devoid of all viral protein coding sequences and are therefore less immunogenic [111]. Although these 'gutless' viruses require the presence of a helper virus for replication, contamination by the helper virus can be avoided by genetically engineering a conditional defect in the packaging domain of the helper virus [111]. Moreover, the gutless vectors are known to have a prolonged expression of the transgene [111]. It has been difficult to develop therapies that target those T cells initiating and mediating the pathogenesis of autoimmune disease. Indeed, most current treatments indiscriminately affect both the auto reactive T cells and the "good" T cells, putting the patient at risk of compromised immune function. A new approach by Tian *et al.* [112] raises the possibility of *targeted* therapy for autoimmunity. Tian *et al.* [112] describe a remarkable way to reestablish central tolerance in NOD mice through reconstitution with hematopoietic stem cells (HSCs) retrovirally transduced to express a protective form of the MHC class II chain. Here the investigators achieve coexpression of protective chains (I-A^d or A^k) with the endogenous disease-prone chain (I-A^{g7}) in about 15 % of bone marrow derived HSCs [hematopoietic stem cells] however this is sufficient to block disease progression in all irradiated/reconstituted NOD recipients. Mice transplanted with forms MHC class II that are protective against disease exhibit no detectable leukocyte infiltration in to pancreatic islets and no cytokine [IFN- γ , IL-2 and IL-4] production by splenocytes in response to GAD65 and insulin, two putative auto antigens of Type I diabetes mellitus [112]. Among the current strategies investigated for the treatment of Type I diabetes mellitus, the gene therapy approach proposed by Tian *et al.* could be one of the most difficult to apply to humans but if this is possible, it might ensure long lasting protection against the development of Type I diabetes mellitus.

ANTIDIABETIC VACCINE

The understanding of the immunopathogenesis of diabetes has led to the development of a diabetic vaccine [113]. Instead of using immunosuppressive agents to suppress an already dysfunctional immune system, antigen specific vaccines or non antigen-specific immunostimulants present a unique opportunity to boost regulatory function. Many types of vaccines have been developed to prevent diabetes including immunosuppressants, lymphocytes, mycobacterium and insulin [114-116]. Sirolimus is an immuno suppressant that inhibits IL-2-induced T-Cell proliferation (but not T-cell apoptosis) [116]. Rabinovitch *et al.* [116] discovered a combination of sirolimus and IL-2 to show a synergistic effect in preventing female non-obese diabetic [NOD] mice from diabetes development [117]. Disease prevention continued

for 13 weeks after the mixture was discontinued. The combination of sirolimus and IL-2 protects islet B-cells from autoimmune destruction by the deletion of auto reactive Th1 cells, making a shift from Th1 to Th2 and Th3 producing cells [117]. The vaccine development principles involve the balance of Th1 and Th2 immune cells. These two types of immune cells are unbalanced in patients with type-1 diabetes (excessive Th1 cells). Two plasma DNA vaccines were created and tested in NOD mice. One encoded a fusion protein consisting of a fragment of glutamic acid decarboxylase 65(GAD-65) Linked to Ig GFc, which activates immune cells that recognizes friendly islet cells. Another encoded IL-4 assists these immune cells to become Th2 cells [118]. The insulin B-chain bear's major type -1 diabetes associated epitopes in humane and NOD mice. Bot *et al.* [119] in a recent investigation have developed a DNA vaccine. The vaccine encoding insulin B-chain results in substantial protection of female NOD mice against diabetes. Co-administration of DNA vaccine with IL-4 expressing plasmid (or extension of vaccination schedule) corrected unfavorable response of male NOD mice. Some studies have shown that orally or nasally administered whole insulin, B-chain insulin or GAD can suppress the development of diabetes in NOD mice [120, 121]. The mechanism is increase in regulatory Th2 cell response.

CONCLUSION

Work has been done on each of above-mentioned routes but of these entire routes pulmonary route is leading the race. Future approaches such as gene therapy, islet cell transplantation, and diabetes vaccine are still in their initial stages but in future they have a vital role to play in treatment of IDDM. However up to now we have only Insulin injections lets hope in the near future we may have Insulin inhalers or Insulin tablets for IDDM Diabetic patients.

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