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Mini Review

Glucose - Responsive Smart Insulin

Aishwarya Krishnamurthy, Ravinder Goswami
Department of Endocrinology and Metabolism
All India Institute of Medical Sciences, New Delhi.

ABSTRACT

The discovery of insulin in 1920s revolutionized the management of Type 1 Diabetes Mellitus. The evolution of insulin over the last nine decades has seen three phases, namely discovery and understanding of the molecule, advances in synthesis of the molecule and advancements in the optimization of insulin structure and delivery. These advances aim to minimize hypoglycemia while simultaneously improving anti-hyperglycemic efficacy. Glucose-responsive insulin (GRI) systems were first conceptualized in 1979. These are novel insulin formulations that provide anti hyperglycemic activity appropriate to circulating glucose levels but with a mechanism to avoid hypoglycemia, that often accompanies stringent glycemic control. GRIs are of three major types- algorithm-based mechanical, polymer-based, molecular GRI analog systems. They differ in the mechanisms of achieving glucose responsiveness. Algorithm-based systems are closed loop insulin pumps that adjust insulin delivery commensurate with blood glucose levels on the basis of predetermined algorithms, that terminate or increase insulin delivery according to blood glucose trends. The second category of GRI includes glucose-responsive polymer-based matrices that house insulin, releasing insulin as needed based on ambient glucose levels. The third approach is to incorporate glucose sensitive motifs in the insulin molecule itself that would decrease or increase insulin availability based on blood glucose levels. The mechanisms behind these novel approaches to insulin delivery and action will be the focus of this review article.

Introduction

The prognosis for patients with youth-onset diabetes mellitus was poor till the discovery of insulin in the early 1920s. This was preceded by several other complementary discoveries. Paul Langerhans identified islet cells in the pancreas while pursuing doctoral studies in 1869 (1). Twenty years later, in 1889, German researchers Oskar Minkowski and Joseph von Mering observed that removal of pancreas in dogs led to them developing symptoms suggestive of diabetes mellitus (2). In 1921, Frederick Grant Banting, an orthopedic surgeon and Charles Herbert Best, a medical student were able to extract insulin from dog's pancreas. They received the Nobel Prize for Medicine /Physiology in 1923 (3) for the discovery.

Mayer *et al* have described the chemical aspects of insulin's history in three distinct phases (4). Initial phase of 30 years culminated in protein sequencing of the insulin molecule by Sanger *et al* in 1954 (5). In the second phase, developments were related to advances in insulin synthesis in the laboratory by chemical synthesis, semi-synthesis and rDNA technology in 1970s (6). This provided unlimited amounts of pure insulin as an alternative to animal insulin. The third phase of insulin development

Correspondence: Dr. Aishwarya Krishnamurthy, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi-110029. Email: aishwaryakr1410@gmail.com.

pertains to optimization of insulin structure and delivery to minimize its current therapeutic limitations while simultaneously improving anti-hyperglycemic efficacy. Efforts in this direction include production of ultra-rapid and longer acting basal insulin analogues, single chain insulin analogues, hepato-selective analogues, insulin receptor isoform selective analogues, oral and pulmonary insulin delivery (7, 8).

Glucose-responsive insulin systems are another innovative approach that is being developed to reduce the hypoglycemic potential of insulin while retaining anti-hyperglycemic efficacy.

Glucose - Responsive Insulin Systems

The concept of glucose-responsive insulin (GRI) was first proposed in 1979 (8). GRI represent new insulin formulations that provide insulin activity appropriate to the circulating glucose levels so as to have the good control of hyperglycemia but no serious hypoglycemia. There are three categories of insulin/delivery systems fitting the theme of glucose regulated delivery :

(1) Algorithm-based mechanical GRI systems - closed loop continuous glucose monitors (CGMs) coupled insulin pumps (10).

(2) Polymer-based systems- in which insulin is housed in a glucose-responsive polymeric matrix-based vesicle or hydrogel insulin (11).

(3) Molecular GRI analog systems- which incorporate glucose-responsive motifs in the insulin molecule by way of altering bioavailability or activity (12).

Algorithm-based Mechanical GRI Systems

Closed loop pumps include CGMs, an insulin pump capable of receiving data from the CGM and a computer encoded algorithm which predicts dose of insulin. The limitations, of these systems, are delay in onset of action of insulin after subcutaneous injection, imprecision in estimation of algorithm-based insulin dose due to lag time between plasma and interstitial glucose. Besides, insulin action might persist once a bolus dose is delivered. Therefore, the current algorithms need fine tuning to provide insulin dosages appropriate for prevailing blood glucose in relation to the interstitial blood glucose (13-16). Modern pumps can provide glucagon hormone to counter hypoglycemia. These pumps are equipped with dual hormone algorithms that can predict hypoglycemia and stop insulin supply. These pumps reduce glycemic excursions and hypoglycemias better than conventional closed loop pumps (17, 18).

Intraperitoneal insulin delivery with an implantable insulin pump has the added advantage of providing first-pass metabolism similar to native insulin secretion (19, 20). Currently, these devices have not received regulatory approval because of susceptibility to catheter occlusion possibly due to pro-inflammatory amyloid formation from the peritoneal side and fibrillation/aggregation of insulin molecule within the pump.

Polymer-based Systems

In this system, glucose sensing mechanisms are coupled to a polymer-based matrix. Insulin is sequestered within this polymer matrix. The usual material for construction of the matrix are poly-N-vinyl-pyrrolidone, polyethyleneglycol (PEG), succinyl- amidophenyl-glucopyranoside (21-23) and modified peptides or lipids (24, 25). This matrix is injectable and is designed to function as a “smart” subcutaneous insulin depot. Structural changes occur in this matrix with increasing ambient glucose levels leading to increased permeability of encapsulated insulin or detachment of insulin from its structural attachment to the polymer matrix. The insulin release is similarly decreased with a fall in ambient glucose (Fig. 1). Molecular

glucose sensing/responsive motifs are incorporated into these polymer scaffolds that serve as on-off switches for effecting the structural change following alteration in glucose level. These glucose-responsive motifs are of three kinds.

Glucose-binding proteins

Concanavalin A (Con A), a lectin was used by Brownlee and Cerami (25) in their initial model of glucose-responsive insulin. Glucose responsiveness in this system can be achieved through two mechanisms. First is through immobilization of a sugar-modified insulin on Con A, which is released from the injected subcutaneous deposit upon displacement by ambient glucose. The second is by incorporating Con A lectin with its tetrameric structure and four sugar-binding sites as a cross-linker in a polymer backbone. Competitive binding of glucose with Con A will disrupt the structural integrity of the polymer and release the insulin contained within it (Fig. 2). The clinical utility of Con A is limited by its non- physiological glucose affinity (competition for glucose binding at ambient glucose levels that are higher than typical diabetic range), immunogenicity and mitogenic potential (26-28).

Glucose oxidase

This enzyme catalyses glucose oxidation. This reaction results in the formation of gluconic acid, H_2O_2 and drop in pH. The resulting change in pH leads to structural changes in polymers that are built to respond to pH by virtue of their acidic/basic functional groups. A polymer with predominantly acidic group would shrink and one with basic groups would swell. Some polymers are built to degrade in an acidic environment thus releasing the contained insulin. (Fig. 1, 3). Glucose oxidase (GoD) based polymersomes incorporated on cross-linked hyaluronic acid microneedle arrays are being developed for painless transcutaneous delivery (29). Experimental data suggests these glucose sensing system can lower blood glucose within one hour followed by maintenance up to five hours without any hypoglycemia in mice pre- treated with insulin.

Phenylboronic acid

Glucose responsiveness property of Phenylboronic acid (PBA) is achieved by its formation of reversible esters with cis- diol molecules. The ambient glucose acts as a competitive inhibitor of diol molecules bound to PBA. In PBA based system, diols are integrated with insulin and immobilized on a PBA based polymer scaffold. With hyperglycemia, diol-insulin ester bond with PBA is dissociated and insulin is released in proportion to glucose levels. PBA has also been used, like Con A lectin as a component maintaining the structural integrity of the polymer backbone, competitive binding to glucose would cause degradation of a polymer leading to insulin secretion (Fig. 4). The advantage of PBA over Con A is due to its affinity for glucose which is in the physiological range (11). However, it has a tendency for spontaneous degradation (11) and the interaction, which is mediated by the diol group, is not specific to glucose, thereby limiting its clinical translational value.

Currently, polymer based GRI systems have several limitations. These challenges include limited particle stability, non-physiological range of action and too slow or too rapid response. Strategies being considered to overcome these hurdles include: use of multiple glucose sensing mechanisms like GoD and PBA in polymer matrix, variations in particle size, permeability of surface area and use of multilayered polymer matrices. Another interesting innovation is the integration of glucose-responsive system with externally positioned β-cell capsules. Ye *et al* (30) fashioned beta cells embedded in an alginate based microgel capsule, fitted with a microneedle array made of hyaluronic acid. The principle is that the interstitial glucose permeates through the microneedle patch and reaches the β-cells stimulating insulin release.

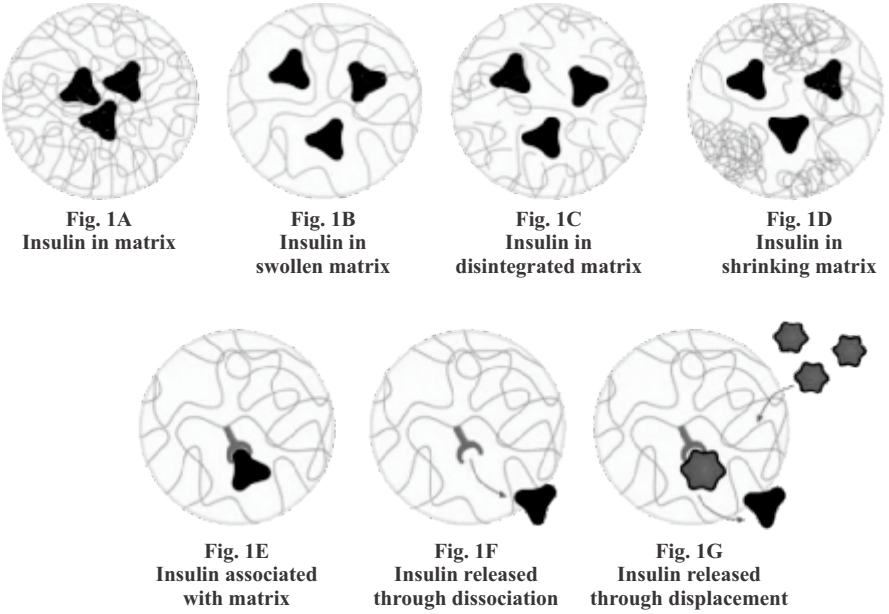

Fig. 1 (A-G) : Different types of changes in injectable polymer matrices leading to release of encapsulated insulin.

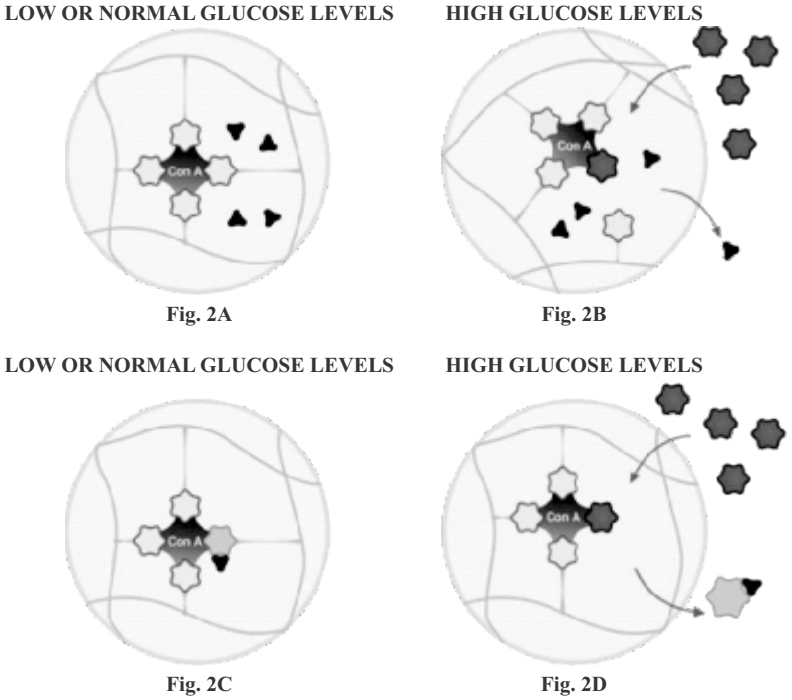

Fig. 2 (A&B) : Concanavalin A forms an important structural component of the matrix that entraps insulin molecules. When it interacts with the ambient glucose molecules the interaction is interrupted leading to disintegration of the matrix and release of insulin from the polymer matrix.

Fig. 2 (C&D) : Concanavalin A forms an integral part of the polymer matrix along with glucose conjugated insulin molecule. Ambient glucose displaces the insulin glucose conjugate by competitive binding to concanavalin A, thereby effecting the release of insulin from the polymer.

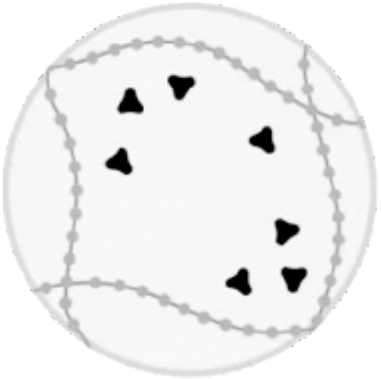

Fig. 3A

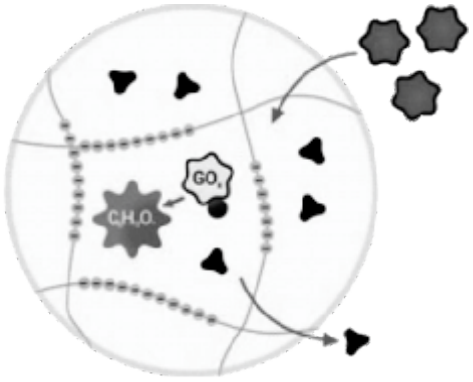

Fig. 3B

Fig. 3 (A): shows a vesicle (polymer matrix) with the encapsulated insulin.
Fig. 3 (B): shows production of gluconic acid by interaction of glucose oxidase enzyme contained within the vesicle with the ambient glucose molecule (resulting in an acidic environment). The vesicle being acidic shrinks in an acidic environment and the pores increase in size resulting in escape of insulin.

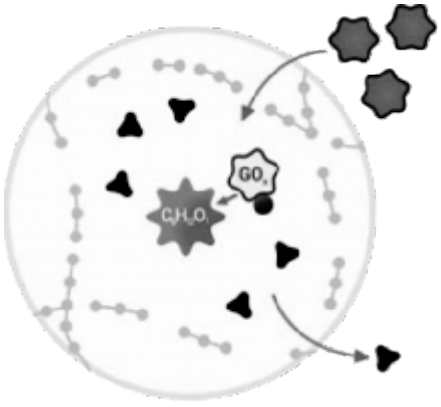

Fig. 3C

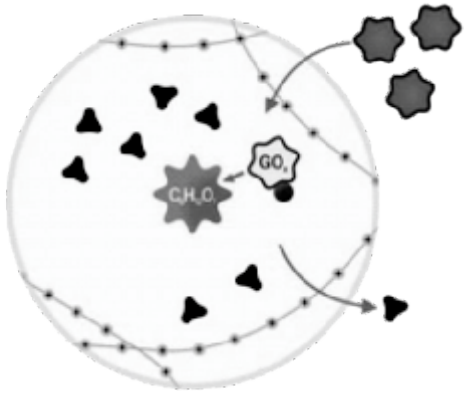

Fig. 3D

Fig. 3 (C): shows the same reaction resulting in production of gluconic acid from glucose oxidase contained within the vesicle and ambient glucose (pink) and the disintegration of the vesicle matrix with release of insulin in an acidic environment.
Fig. 3 (D): shows the same reaction with a basic vesicle matrix demonstrating swelling and release of contained insulin in response to an acidic environment due to production of gluconic acid by glucose oxidase enzyme acting on ambient glucose.

However, it was found that the diffusion of glucose through the microneedle patch is inadequate, resulting in an insignificant insulin release. To overcome this, the microneedles, are embedded with α-amylose as well as self-assembling polymers containing GoD, α-amylase, and glucoamylase enzymes. The GoD in these microneedles converts interstitial glucose into gluconic acid leading to pH mediated disintegration of the polymers on the microneedle arrays. Release of α-amylase from within the polymers hydrolyses α-amylose embedded on the microneedle into disaccharides and tri-saccharides. Glucoamylase converts

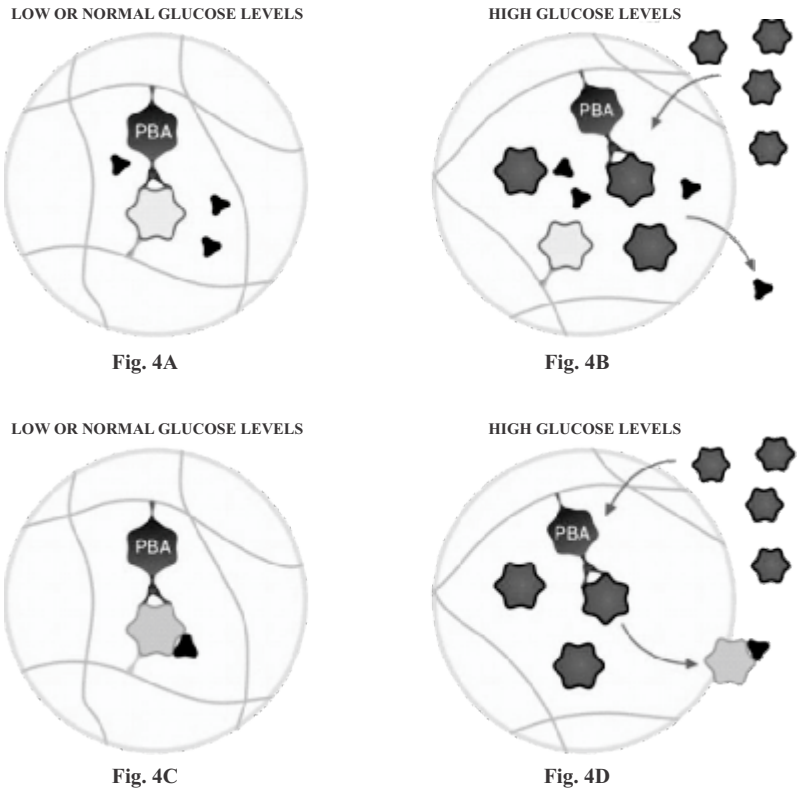

Fig. 4A Fig. 4B

Fig. 4C Fig. 4D

Fig. 4 (A&B): Phenylboronic acid (PBA) is an integral part of the polymer structure that houses insulin. The interaction in the polymer is mediated through another monosaccharide unit which gets competitively displaced by ambient glucose causing disruption of the structural attachments of PBA and disintegration of the matrix with release of insulin.
Fig. 4 (C&D): PBA and glucose conjugated insulin form an important component of the polymer structure. The ambient glucose displaces the insulin glucose conjugate from the PBA molecule by competitive inhibition thereby freeing the glucose conjugated insulin from the matrix.

these saccharides to glucose. Thus effectively the glucose concentration in the vicinity of the microneedles/beta cell capsule structure increases, providing stimulus to beta cells for insulin secretion. This strategy of amplifying glucose signal overcomes the disadvantage of beta cell capsules, i.e. poor response at physiological glucose levels (Fig. 5). A single patch of this type of GRS was effective in Type 1 diabetic mice for up to 10 hours (30).

In brief, all the three technologies discussed above were based on sequestering insulin in a glucose-responsive matrix followed by appropriate release of insulin as per the ambient glucose.

Molecular GRI Analog Systems

Molecular GRI are different class of system wherein the glucose responsiveness is inbuilt in the insulin molecule itself. The strategy involves altering the structure of insulin to modulate its pharmacokinetics.

GoD based approach uses GoD-insulin compound wherein a cysteine based linkage is disrupted by the enzymatic glucose oxidation to affect insulin release. GoD-based acidification at local injection site can also increase the bioavailability of glargine due to its enhanced solubility at acidic pH (31). Both the approaches have not resulted in any clinically significant

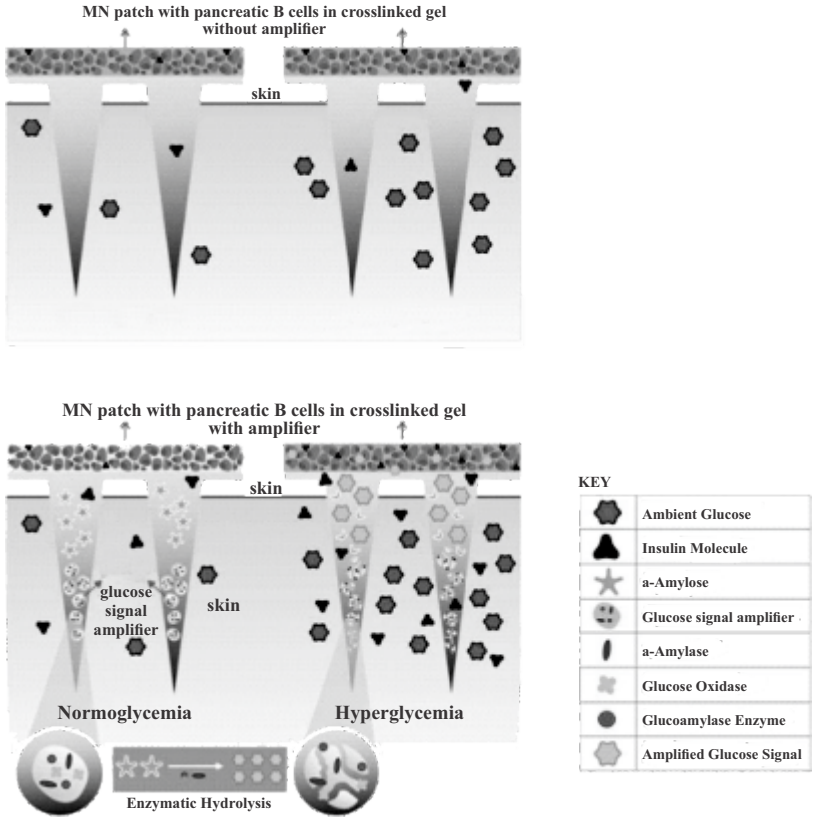

Fig. 5: β cell capsule with an α-amylase and polymers (encapsulating glucose oxidase, α-amylase, glucoamylase enzyme) embedded micro needle patch. This system helps to amplify the glucose concentration in the vicinity of the β cells so as to elicit a more robust insulin secretory response.

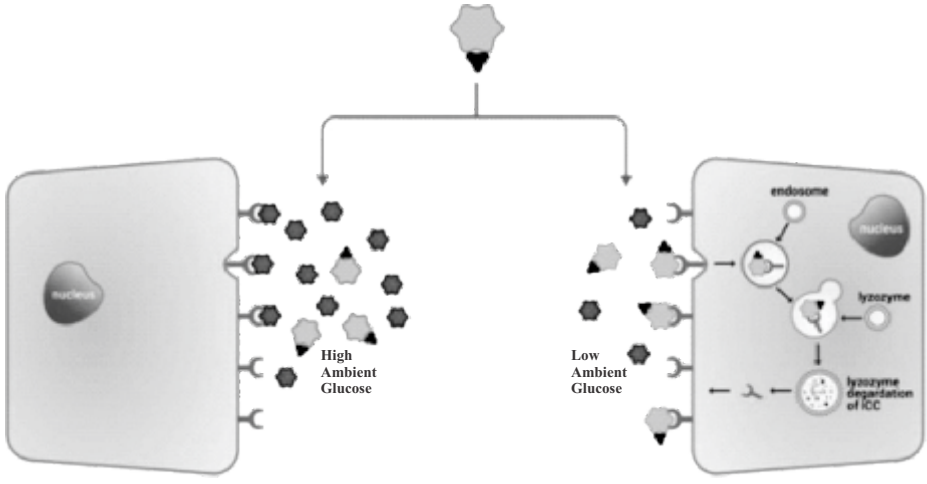

Fig. 6(A) Fig. 6(B)

Fig. 6: Insulin carbohydrate conjugates (ICC) compete with ambient glucose for binding to the mannose receptors (MR) on hepatocyte cell surface. At low ambient glucose levels (Fig. 6B), more molecules of ICC bind to the MR and are subjected to lysosomal degradation, thereby clearing excess insulin and preventing hypoglycemia. At high ambient glucose levels (Fig. 6A) there is little attachment to the MR and therefore minimal degradation.

effects due to adverse effects. The important drawback is the low Km of GoD for glucose leads to excessive insulin release at even low ambient glucose levels and results in hypoglycemia. Besides, tissue damage can occur at the local injection sites due to liberation of H_2O_2 (32). The *in-vitro* efficacy of GoD-based glargine, in reality, did not translate to improved glycemic control in pilot animal studies (33).

PBA based approaches to generate molecular GRI analog have been attempted. Insulin conjugated to a diol or sugar molecule is anchored to PBA molecule through a reversible ester bond. The interstitial glucose, competitively binds to PBA molecule thus freeing the diol labelled insulin for action. A second approach is through addition of a PBA tag to the insulin analogue detemir. Detemir has long half-life consequent to its myristic acid chain mediated binding to albumin and slow release from albumin. This release remains unrelated to the glucose concentration in blood. Addition of a PBA molecule to detemir in such a way that the interaction of detemir with albumin becomes glucose-responsive has been attempted. However, both the above approaches were unable to achieve the desired result in-vitro (34).

Zion and Lancaster proposed another novel alternative strategy for a GRI, based on endogenous lectin–based clearance (35). In this approach, addition of saccharides to the native insulin molecule results in an analog that can bind to insulin receptor as well as the mannose binding receptor. The mannose receptor (MR) normally binds and transports proteins and pathogens tagged for intracellular destruction and degradation through lysosomes without eliciting any immune response. Glucose is a competitive inhibitor of MR binding. At high ambient glucose level, insulin binding to MR and destruction is decreased. As a result more insulin is available for normal action through insulin receptor. In hypoglycemia there is a reverse sequence of events, i.e. higher fraction of circulating modified insulin is destroyed via uninhibited binding to MR (Fig. 6). Kaarsholm *et al* (36) were able to demonstrate *in vitro* glucose responsiveness of this approach, with the molecule MK 2640. However, the insulin analogue though safe and well tolerated, had poor *in vitro* potency for clinical use. Further modifications in this approach are underway, to address the potency of these formulations.

Conclusion

The journey of insulin over the last century from the initial crude alcoholic extracts of canine pancreatic tissue to a sophisticated molecule is inspiring. Recombinant DNA technology has resulted in ease of availability of human insulin molecules. Modification of insulin amino-acid sequence and addition of side chain has given insulin of different duration of action to facilitate better glycemic control. Currently efforts are on to make smart insulin molecules which are released in a controlled manner as per the ambient glucose in order to prevent hypoglycemia while maintaining normal glycemic condition for prolonged duration. On the other hand parallel efforts are on to design smart insulin, which is degraded in proportion to the ambient glucose so as to reach the same objective, i.e. good glycemic control without any risk of hypoglycemia.

Declaration

The authors have nothing to declare and there is no conflict of interest.

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