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Engineering Synthetically Modified Insulin for Glucose-Responsive Diabetes Therapy

Matthew J. Webber\textsuperscript{1,2}, Daniel G. Anderson\textsuperscript{1,2,3,4,5}, and Robert Langer\textsuperscript{1,2,3,4,5,*}

\textsuperscript{1}David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge MA 02139, USA

\textsuperscript{2}Department of Anesthesiology, Boston Children’s Hospital, Boston, MA 02115, USA

\textsuperscript{3}Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge MA 02139, USA

\textsuperscript{4}Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge MA 02139, USA

\textsuperscript{5}Harvard-MIT Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Summary

Though a suite of different insulin variants have been used clinically to provide greater control over pharmacokinetics, no clinically used insulin can tune its potency and/or bioavailability in a glucose-dependent manner. In order to improve therapy for diabetic patients, a vision has been the development of autonomous closed-loop approaches. Toward this goal, insulin has been synthetically modified with glucose-sensing groups or groups that can compete with free glucose for binding to glucose-binding proteins and evaluated in pre-clinical models. Specifically, it was demonstrated that site-specific modification of insulin with phenylboronic acid can result in glucose-responsive activity, leading to faster recovery in diabetic mice following a glucose challenge but with less observed hypoglycemia in healthy mice. This strategy, along with several others being pursued, holds promise to improve the fidelity in glycemic control with routine insulin therapy.

Keywords

diabetes; phenylboronic acid; lectin; Concanavalin A; fully synthetic pancreas; bioartificial pancreas; bionic pancreas; drug delivery

*Person to whom correspondence should be addressed: Prof. Robert Langer, The Koch Institute at MIT, Building 76 Room 661, 500 Main Street, Cambridge, MA 02139, rlanger@mit.edu, Phone: 617-253-3107, Fax: 617-258-8827.

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Current State of Insulin Therapy

Diabetes, a class of diseases characterized by high fasting blood glucose levels, is increasing in prevalence globally.\[1–3\] The endocrine function of insulin, a small 51 amino acid signaling hormone produced in the pancreas, is central both to disease manifestation as well as therapeutic management. In insulin-dependent patients, exogenous insulin is most commonly self-administered subcutaneously via syringe injection or pump infusion. Best therapeutic outcomes are observed when a strict insulin administration schedule is followed. \[4\] While routine insulin therapy enables sufficient glycemic control for some patients, many experience complications that arise from poor adherence to therapy, inaccurate dosing, or fluctuations in blood glucose levels.\[5\] Acute complications, generally occurring from insulin overdose-induced hypoglycemia, can result in coma or death,\[6\] while chronic fluctuations in blood glucose levels increase the long-term risk of developing cardiovascular disease, stroke, non-healing wounds, blindness, cancer, and many other co-morbidities.\[7\]

In an effort to improve glycemic control for insulin-dependent patients, extensive efforts have been taken to develop and commercialize insulin variants with tunable pharmacokinetics.\[8, 9\] These efforts have led to fast-acting, intermediate-acting, and long-acting insulin variants; use of various types in combination may afford improvements in patient-specific therapy. Native unmodified insulin, traditionally considered to be intermediate-acting, has an onset time of 30–60 minutes, a peak window of action from 2–3 hours, and a duration of action up to 8 hours.\[4\] In comparison, fast-acting variants such as Insulin lispro, designed to inhibit dimer formation and accelerate uptake through switching the B29 lysine residue and the B28 proline residue, have an onset time of just 5–15 minutes, with peak action at 30–60 minutes and duration of action up to 5 hours.\[4, 10\] Long acting variants such as Insulin detemir, with a fourteen-carbon saturated alkyl chain covalently attached to the ε-amine of B29 lysine to enable insulin to bind to and be sequestered by circulating serum albumin, have an onset of action of 1–2 hours, with peak action at 3–9 hours and duration lasting 24 hours or more.\[4\] As a component in diabetes management, long-acting insulin is particularly useful as a daily injection to provide basal insulin and limit spikes in blood glucose levels throughout the day.\[11\] Though combinations of different insulin variants may afford better control over the bioavailability of insulin, there remains a need to improve the fidelity of glycemic control and thus avoid both acute and long-term complications that arise in managing diabetes.

Toward Closed-Loop Insulin Therapy

An abiotic approach has been envisioned that could sense elevations in blood glucose and respond with a metered dose of insulin and/or glucagon for closed-loop therapy, in order to recapitulate the natural dynamics of glycemic control with both peaks and troughs.\[12, 13\] One of the routes envisioned, termed the “Fully Synthetic Pancreas”, would make use of synthetic materials or formulations that are sensitive to elevated blood glucose levels, prompting a structural or conformation changes within the material. An ideal strategy would respond quickly to elevation in blood glucose and shut off insulin release during normoglycemia. It would also be compatible to serial self-administration over a lifetime of use, which would necessitate ease of delivery and minimize adverse acute or chronic
reaction at the injection site. Many different approaches to develop a synthetic system have been evaluated in pre-clinical studies, including polymeric hydrogels, nanoparticle formulations, and swelling or degradable membrane-sealed reservoirs.[12, 14–16] These materials traditionally rely on a glucose-sensing mechanism to facilitate insulin release. Enzymatic methods to sense glucose have primarily used glucose oxidase (GOx), an enzyme that catalyzes the conversion of glucose into hydrogen peroxide and gluconic acid, with the concomitant drop in pH used to induce structural or conformational changes in a material. [17–24] Another strategy has incorporated glucose-binding lectin proteins, such as tetravalent concanavalin A (ConA), into materials to cross-link glycosylated polymers, with these cross-links subsequently ruptured to release encapsulated insulin in response to elevated free glucose levels.[25–28] Instead of using enzymes or proteins, a third approach has used phenylboronic acids (PBAs) to bind glucose reversibly to prepare glucose-responsive cross-links in materials or glucose-inducible material swelling.[29–33] Synthetic materials using each type of glucose-sensing strategy have been evaluated in pre-clinical studies.[13] A number of studies using GOx-based glucose sensing have demonstrated particular promise, with a report of acid-sensitive nanoparticle networks that can reverse diabetes in a mouse model for up to 10 days following a single administration.[23]

An alternative approach for closed-loop insulin therapy has focused on the development of automated external control processes using a combination of digital pumps and glucose sensors, an approach termed the “Bionic Pancreas” in reference to the electromechanical hardware required for its use.[34, 35] One promising approach that has been used in a number of clinical trials has leveraged process control algorithms to prepare a fully automated system in which glucose readings produced from a wearable continuous glucose monitor are processed and then translated into an appropriate dose of insulin or glucagon provided by a wearable dual-hormone pump.[36–39] Fast-acting Insulin Lispro is typically used in these pumps, and its pharmacokinetic properties combined with information from real-time blood glucose readings provide the basis for the algorithms used. One key benefit demonstrated by this system is a reduction in the number of hypoglycemic episodes.[36] Additionally, this system is amenable to continuous patient-specific adaptation for enhanced therapeutic efficacy over time,[37] and can be achieved through the combination of wireless continuous glucose monitors and pumps with common hand-held consumer electronics (i.e. an iPhone) to minimize device-imposed restriction.[36] Though this type of device has demonstrated unmatched glycemic control with an engineered approach, the substantial amount of hardware and devices required, as well as known issues including discomfort and/or infection at sites of implantation for continuous glucose monitors and pumps,[40, 41] presents opportunities to improve upon this approach.

One additional approach to closed-loop insulin therapy has focused on transplantation of some type of glucose-sensing and insulin-secreting cell population. Termed the “Bioartificial Pancreas”, this approach relies on the natural biological sensing and feedback mechanisms that lead to glycemic control in a healthy patient with normal β-cell function. The success of some patients treated with the Edmonton Protocol, using intraportal transplantation of cadaveric islets along with immunosuppression, points to the clinical utility of this approach. [42] In this first study using this protocol, all seven patients treated remained insulin-independent one year following treatment. However, practical limitations including the
availability of sufficient numbers of viable islets as well as complications that may arise from life-long immune suppression, necessitate new approaches.[43] Cells derived from different sources, including xenogeneic islets or de novo stem cell-derived populations, might offer a sufficient source of tissue for transplantation.[44–46] Unless from an autologous source (i.e. induced pluripotent stem cells), this approach would still necessitate circumventing the host immune system in order to prevent destruction of the donor tissue. Toward this goal, biomaterial constructs that provide drug-free immunoisolation of donor cells have been explored.[47, 48] Though hurdles remain, including further development of the cell source and encapsulation materials, it is possible that an optimized “Bioartificial Pancreas” approach could deliver on a “cure” for diabetes that is not possible using the abiotic approaches described.

**Synthetically Modified Insulin for Glucose-Responsive Therapy**

An alternate abiotic approach to enable glucose-responsive insulin activity is based on synthetic modification of the insulin molecule to impart glucose-sensing properties. As previously discussed, modification of insulin has allowed some control over pharmacokinetics and bioavailability. Though this has improved insulin therapy, none of the presently available insulin molecules has activity that can be modulated as a function of blood glucose levels. Endowing insulin with glucose-responsive character could therefore offer considerable benefits in reducing complications associated with poor fidelity in glycemic control. In addition, it would circumvent the need for the various accessories (i.e. polymers, electronics, etc.) required in other abiotic approaches for closed-loop therapy and might also represent a less distant horizon than envisioned in the bioartificial pancreas approach. Moreover, glucose-responsive modified insulin could be administered using the same pumps or injector technologies that are used for traditional insulin therapy, and thus is compatible with standard patient administration techniques.

Efforts to evaluate glucose-responsive therapy with synthetically modified insulin were reported as early as 1979.[49] In this work, insulin was synthetically modified via glycosylation and used in combination with ConA for glucose-mediated lectin binding. The insulin-ConA complex exhibited insulin release that was controllable by soluble glucose concentration, and the activity of the insulin in reducing blood glucose levels in dogs was not compromised by the modification. Variations on this approach were subsequently explored in which polymers or microcapsules with immobilized ConA were used for glucose-mediated release of synthetically glycosylated insulin.[50–54] Synthetic glycosylation of insulin preserves protein bioactivity, and when combined with ConA, makes a stable but reversible complex. The glycosylated insulin competes in equilibrium with free glucose for binding to ConA; therefore an increase in local glucose concentration promotes freely soluble, functional, synthetically modified insulin.[55] SmartCells, Inc., which was acquired by Merck in 2010, developed synthetically modified insulin with pendant sugar units that, similarly, could be coupled with glucose-binding proteins for glucose-regulated insulin therapy.[56–58] Some manifestation of this approach is, at present, being evaluated by Merck in Phase I clinical trials in the United States (Clinical Trial #MK-2640).
Phenylboronic acid (PBA) has also been conjugated to insulin to enable glucose sensing. [59–61] PBAs are Lewis acids that bind reversibly to cis-1,2 or cis-1,3 diols, such as glucose, which stabilizes a negative charge on the boronic acid. [62] This binding of PBA to diol affords two separate mechanisms by which molecular properties can be varied; the stabilized negative charge can act as an electrostatic driving force for improved solubility of the conjugate, or alternatively free glucose in blood could disrupt an interaction between PBA-conjugated insulin and an immobilized diol. An example of this used insulin conjugated with small molecule PBA groups in combination with glucamine-derived polyethylene glycol polyacrylamide to act as an immobilized diol. [60] In this work, glucose-induced release of PBA-modified insulin from diol-containing polymers was demonstrated, with the slope dependent on whether the modified insulin was in the monomeric form or used as zinc-formulated hexamers. Taking this a step further, modified insulin-only self-assembly was achieved with conjugates that contained both PBA groups and glucose-like diols. [59] In this work, reversible insulin self-assembly was achieved that was controlled by soluble glucose concentration. In addition to published reports, information available in patents indicate that Novo Nordisk A/S has investigated PBA-modified insulin toward glucose-responsive therapy. [59, 60, 63, 64]

Recently, we reported on glucose-responsive behavior observed for a PBA-modified insulin in a mouse model of diabetes. [61] In this work, aliphatic conjugates were synthetically prepared by fusing a saturated alkyl chain with a PBA group. Through controlling reaction conditions and stoichiometry, site-specific modification of insulin with these conjugates yielded insulin specifically modified at the ε-amine of the B29 lysine residue with the PBA-containing groups. This enabled four different PBA-containing modified insulin derivatives to by synthesized and evaluated. The use of the aliphatic domain was inspired by the design of long-acting Insulin detemir, as in this clinically used insulin an aliphatic group affords binding to serum albumin which leads to prolonged circulation half-life. [65, 66] Meanwhile, in our design the PBA group was intended to afford a change in properties for the conjugate upon glucose binding to PBA. A variety of PBA chemistries were evaluated in order to modify the pKa of the PBA group to alter its binding to glucose. An example structure of one of our best-performing insulin molecules from our published report (Ins-PBA-F) is shown in Figure 1A, alongside the structure for insulin detemir (Ins-LA-C14) which served as inspiration for our design. When administered subcutaneously into an STZ-induced diabetic mouse, all four PBA-conjugated insulins resulted in reversal of blood glucose, and some including Ins-PBA-F responded to repeated intraperitoneal glucose challenges (i.e. simulated meals) over a thirteen-hour period. When compared head-to-head with long-lasting Insulin detemir in diabetic mice, Ins-PBA-F responded more quickly to an intraperitoneal glucose challenge than did identical doses of Insulin detemir (Figure 1B). However, in healthy mice Ins-PBA-F induced less hypoglycemia. Taken together, these findings were supportive of glucose-mediated activity for the aliphatic PBA-modified insulin derivatives described. [61] These PBA-modified insulins appear to exhibit differential potency in diabetic mice compared to healthy mice. However, the precise threshold blood glucose concentration at which this shift in potency is observed remains unclear. In an additional measure of the utility of these PBA-modified insulins, their response to glucose challenge was compared to that for a healthy mouse with full pancreatic function. In this...
case, Ins-PBA-F exhibited responsiveness in a diabetic mouse that was nearly identical to that observed for a healthy pancreas, a benchmark that serves as the true “gold standard” for restoring glycemic control in the diabetic state.

Expert Commentary and Five-Year View

The isolation and use of exogenous insulin dramatically changed the lives of patients with diabetes. Some patients respond well to disease management with standard of care use of insulin therapy, including a cohort who have lived for 50 or more years as insulin-dependent diabetics.[67] However, many patients experience complications that arise from both acute and chronic instability in blood glucose levels, leading to a myriad number of health complications. A major research effort has been to restore glycemic control in a manner that could be more autonomous, and has envisioned using glucose-sensing materials, combinations of sensors and pumps, or insulin producing cells in order to achieve closed-loop control of blood glucose. Some such closed-loop strategies, including autonomous control with bi-hormonal pumps and transplantation of donor islet tissue, have been evaluated clinically. An alternative approach has been envisioned that would leverage experience in preparing modified insulin analogues or derivatives with modified pharmacokinetics, and add a glucose-responsive element to afford glucose-mediated changes in insulin potency. Toward this aim, strategies to control the availability of insulin by modification with either glucose-like groups or PBA groups intended to bind to glucose have been demonstrated in pre-clinical studies, and show promise for future insulin therapy.

Based on early demonstrations of success in pre-clinical studies, it is envisioned that a glucose-responsive insulin variant will see clinical use in the coming years. Presently, Merck is evaluating a glucose-responsive “smart” insulin, and has the requisite infrastructure and experience to bring an effective new insulin variant to market. The clinical translation of a glucose-responsive insulin is still rife with challenges; both efficacy and safety. Many of the strategies described to-date lack a definitive “off” switch, and though insulin availability may be reduced as a function of glucose levels, insulin may remain available and signaling at low glucose, resulting in hypoglycemia. Additionally, it is possible that modification of insulin could induce a humoral response or elicit an allergic reaction, both of which are side effects that could end development of a compound requiring routine administration over a prolonged time. Should safety be established, and early pre-clinical efficacy be reproduced in providing enhanced glycemic control to patients, it is foreseeable that a glucose-responsive insulin variant could have a dramatic impact on the landscape of insulin therapy and offer more autonomous glycemic control with a significant impact on improving the health of diabetic patients. Successful realization of this approach would provide a route to improved disease management, but the quest for a “cure” for diabetes remains still remains on the horizon.

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* = of interest

** = of considerable interest

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Key Issues

- A number of clinically used insulin variants have demonstrated controllable pharmacokinetics, though none have activity that is tunable based on blood glucose levels.

- More closely matching natural blood glucose dynamics may reduce both the acute and chronic complications associated with diabetes.

- Multifarious efforts toward autonomous closed-loop insulin therapy have been evaluated in preclinical and clinical settings.

- Synthetic modification of the insulin protein to afford glucose-responsive insulin activity is one approach to affording glucose-responsive therapy.

- Insulin with glucose-mediated potency could be useful in both improving response to a glucose insult (i.e. meal) as well as limiting hypoglycemia.

- Significant efforts remain in clinical translation of glucose-responsive modified insulin to ensure safety and efficacy and to determine strategies for predictable and reliable therapy.

- There is great promise for the vision of glucose-responsive modified insulin in improving disease management, with subsequent benefits in improving quality of life and reducing co-morbidity associated with diabetes.
Figure 1.
(A) The structure of insulin detemir (Ins-LA-C14) compared to the structure of our modified insulin (Ins-PBA-F) with a terminal phenylboronic acid conjugated to a saturated alkyl segment. (B) Results from in vivo evaluation of PBA-containing Ins-PBA-F compared to long-lasting Ins-LA-C14 and native insulin. A dose of insulin, in this case 3 IU/kg, was administered subcutaneously at the beginning of the study. Serial blood glucose measurements were collected over the next 6 hours. Three hours following insulin administration, an intraperitoneal glucose tolerance test (IPGTT) was performed in order to quantify the responsiveness of insulin to glucose challenge. Figure reproduced from Chou et. al. (Reference 61), Copyright 2015, National Academy of Science, USA.