Bioconjugate Chemistry

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Zwitterionic Polymer Conjugated Glucagon-like Peptide-1 for Prolonged Glycemic Control

Caroline Tsao, Peng Zhang, Zhefan Yuan, Dianyu Dong, Kan Wu, Liqian Niu, Patrick McMullen, Sijin Luozhong, Hsiang-Chieh Hung, Yu-Hong Cheng, and Shaoyi Jiang*

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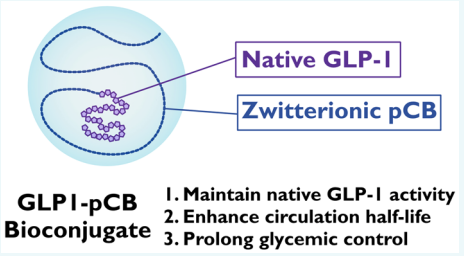
ABSTRACT: Glucagon-like peptide-1 (GLP-1) is of particular interest for treating type 2 diabetes mellitus (T2DM), as it induces insulin secretion in a glucose-dependent fashion and has the potential to facilitate weight control. However, native GLP-1 is a short incretin peptide that is susceptible to fast proteolytic inactivation and rapid clearance from the circulation. Various GLP-1 analogs and bioconjugation of GLP-1 analogs have been developed to counter these issues, but these modifications are frequently accompanied by the sacrifice of potency and the induction of immunogenicity. Here, we demonstrated that with the conjugation of a zwitterionic polymer, poly(carboxybetaine) (pCB), the pharmacokinetic properties of native GLP-1 were greatly enhanced without serious negative effects on its potency and secondary structure. The pCB conjugated GLP-1 further provided glycemic control for up to 6 days in a mouse study. These results illustrate that the conjugation of pCB could realize the potential of using native GLP-1 for prolonged glycemic control in treating T2DM.



INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a long-term progressive metabolic disease characterized by high blood glucose (hyperglycemia) due to the dysfunction of pancreatic β-cells and insulin resistance.[1,2] The incidence of diabetes is expected to increase as it has affected 463 million adults (20−79 years old) worldwide in 2019, in which T2DM is accounting for ∼90% of all cases.[3] The number of people affected by diabetes is expected to reach 700 million by 2045, estimated by the International Diabetes Federation.[3] Native glucagon-like peptide-1 (GLP-1(7−36), abbreviated to GLP-1) is an incretin peptide secreted from intestinal L-cells in response to meal intake, in order to induce insulin secretion from pancreatic β-cells and suppress glucagon release in a glucose-dependent fashion.[4−6] While overweight and obesity are now considered as the major risk factor of non-insulin-dependent diabetes mellitus, continuous subcutaneous infusion of GLP-1 has resulted in a sustained weight loss over a period of at least 6 weeks in a human study.[7] Therefore, GLP-1 shows great potential use in the treatment of T2DM and obesity. However, native GLP-1 has limitations for clinical use due to the rapid proteolytic inactivation and degradation by dipeptidyl peptidase IV (DPP-IV) enzyme.[8−10] The peptide bond in Ala^8-Glu^9 is cleaved by DDP-IV which results in a metabolite of GLP-1(9−36) that has a 100-fold lower binding affinity compared to the native intact peptide.[11,12] To overcome this shortcoming, two possible approaches have been considered: (1) utilize natural analogs or synthetic analogs by modifying the residues vulnerable to DPP-IV, and (2) "shield" the native peptide with synthetic materials to protect it from proteolytic inactivation.

A number of GLP-1 analogs, mainly GLP-1 receptor agonists (GLP-1 RAs), have been developed based on the first approach, including exenatide, a lizard-derived analog; tapsoglutide, an analog with α-aminoisobutyric acid (Aib) substitutions; and liraglutide, an acylated derivative.[13−15] Unfortunately, these GLP-1 analogs have raised undesired immune responses: antidrug antibodies (ADA) toward GLP-1 RAs due to the foreignness introduced upon residue modifications. The incidences of antibody formation in clinical use were 45.4% of the patients treated with exenatide, 49% with tapsoglutide, 69.8% with lixisenatide, and 8.6% with liraglutide.[16−18] The generation of ADA could lead to a compromise of therapeutic efficacy of GLP-1 RAs and an increase in hypersensitivity reactions after administration.[19−22] Thus, the delivery of native GLP-1 for treating T2DM is still an attractive idea, as there is low risk of ADA generation, which is desirable for long-term treatments. On the other hand, limited studies have been done using the second approach to prevent rapid proteolytic inactivation. Therefore, we aim to further explore the possibility

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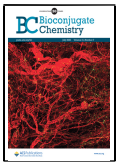

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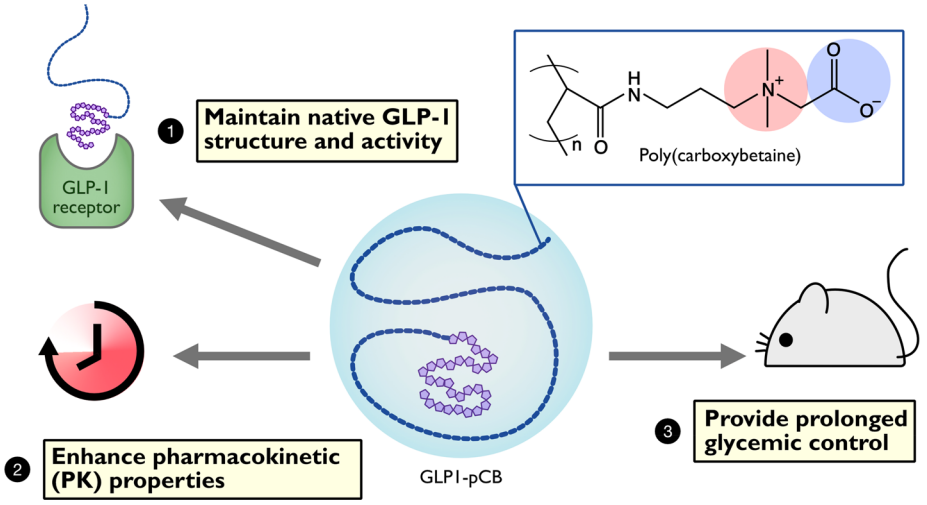
Scheme 1. Through the Conjugation of Zwitterionic Poly(carboxybatine) Polymer onto Native Glucagon-like Peptide-1, the Bioconjugate GLP1-pCB Has Three Potential Advantages: It (1) Maintains the Secondary Structure and Activity of GLP-1, (2) Enhances the Pharmacokinetic Properties *In Vivo*, and (3) Provides Prolong Glycemic Control in a Mouse Study



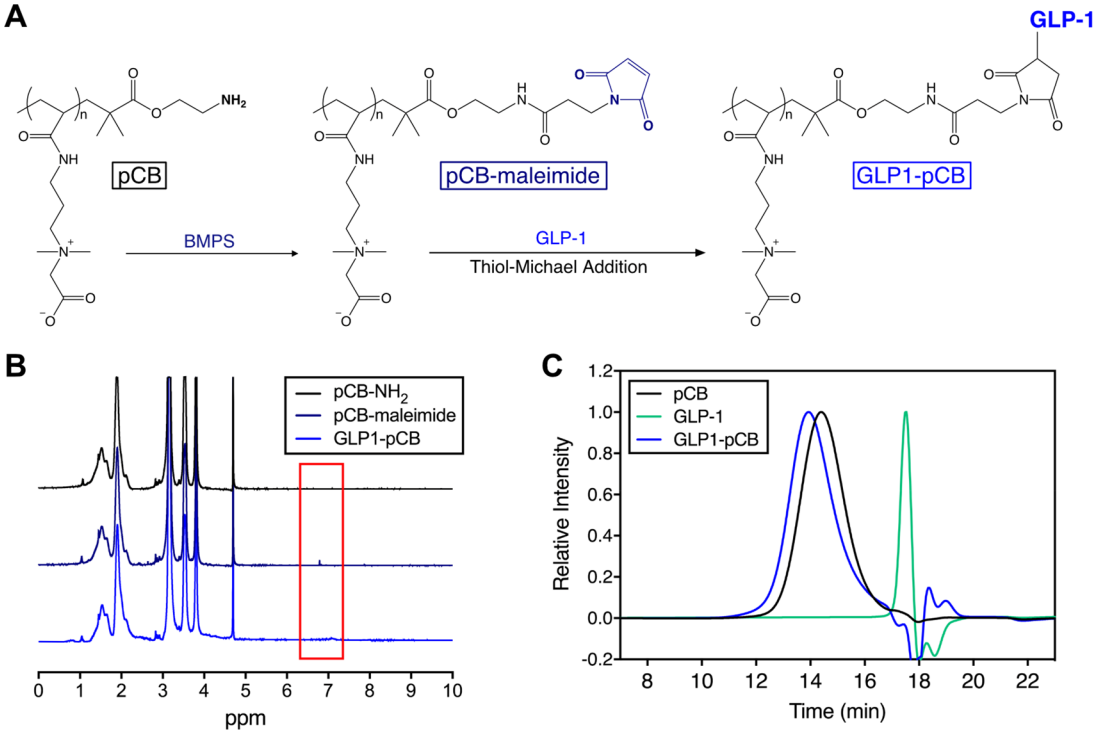


Figure 1. A. Synthetic scheme for pCB conjugation onto GLP-1. B. Nuclear magnetic resonance spectroscopy (^{1}H NMR, 300.10 MHz, D_2O) profile of each conjugation step. The appearance of the small peak indicates the successful addition of the maleimide, while the disappearance indicates the successful conjugation onto GLP-1. C. Gel permeation chromatography (GPC) traces of GLP1-pCB. The left-shift of GLP1-pCB peak of suggests the success of conjugation as GLP1-pCB is larger than pCB alone.

of using synthetic materials to improve the therapeutic effects of native GLP-1 in this study.

One common problem that GLP-1 and GLP-1 analogs face is the short circulation half-life from rapid renal clearance due to their low molecular weights. The conjugation of natural or synthetic materials has been shown to improve the pharmacokinetic (PK) properties of GLP-1 analogs. Conjugation of different lengths of poly(ethylene glycol) (PEG) or polysaccharides has increased the half-life from 1.5−5 min to 12.1−30.3 h when injected subcutaneously in rodents.[23,24] Integration of human serum albumin or fragment crystallizable region (Fc) has improved the half-life to 8.5−38.2 h in rodents when injected subcutaneously.[25−27] Lipidation of the GLP-1 analogs also extended the half-life to 12−46.1 h when delivered intravenously in rodents.[28−30] Yet, the addition of materials could sacrifice the activities of the GLP-1 and GLP-1 analogs. The conjugation of polymers (such as PEG) or human serum albumin has been reported to dramatically reduce the potencies of GLP-1 analogs due to unwanted interactions between the bulk conjugate and the peptide, and steric hindrances of the bulk conjugate.[23−25,31] Furthermore, some synthetic materials, such as PEG, have been demonstrated to be immunogenic and

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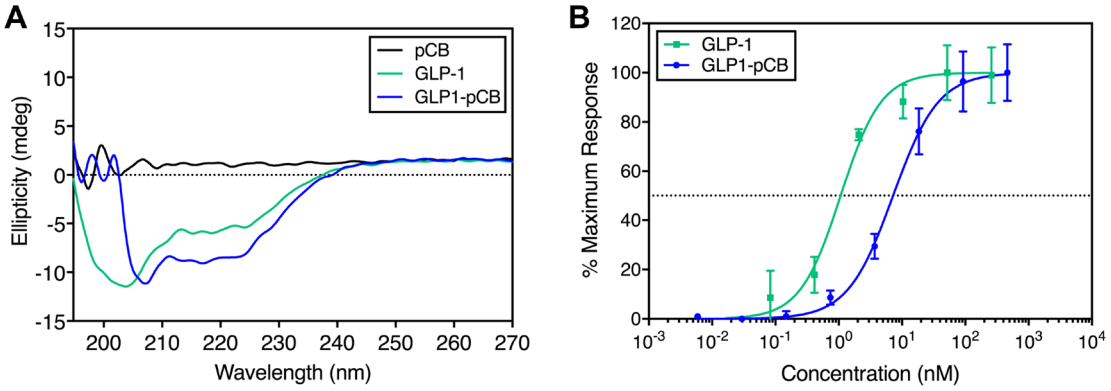

Figure 2. A. Circular dichroism (CD) spectroscopy of pCB, GLP-1, and GLP1-pCB. GLP1-pCB maintains the secondary structure of GLP-1. B. *In vitro* insulinotropic activity of GLP-1 and GLP1-pCB. Properties of both are summarized in Table 1. All studies were done in triplicate.

vulnerable to pre-existing anti-PEG antibodies.[32,33] Therefore, it is ideal to use synthetic alternatives that would not affect peptide activity and have low immunogenicity for peptide conjugation.

Zwitterionic polymer poly(carboxybetaine) (pCB) has been demonstrated to be efficacious in protecting biologics from undesired interactions in the biological environment and in improving the PK properties of the biologics.[34−36] Due to their superhydrophilic nature, the conjugation of pCB polymers has been demonstrated to retain protein stability and bioactivity.[37−39] Moreover, studies have shown that pCB polymers induce little to no anti-pCB antibodies even when conjugated to highly immunogenic proteins.[35,36,40,41] In this study, we develop a bioconjugate of native GLP-1 with a superhydrophilic zwitterionic polymer pCB. We first investigate the secondary structure and activity of the native GLP-1 after the conjugation of pCB. The PK properties of the bioconjugate, and its ability to provide glycemic control, are next evaluated. We aim to demonstrate the potential of using pCB-conjugated native GLP-1 for prolonged glycemic control (Scheme 1).

RESULTS AND DISCUSSION

The structure−activity relationship of GLP-1 has been well investigated, and several amino acid residues on the N-terminus have been identified as critical for receptor binding and activation.[42] Previous studies on the site-specific conjugation of PEG onto GLP-1 have demonstrated that the N-terminus of GLP-1 is essential for receptor activation.[23] Though the conjugation of PEG close to the N-terminus can mitigate the proteolytic inactivation of GLP-1 by DDP-IV, the potency of GLP-1 was greatly reduced.[23,31] Hence, in this study, a zwitterionic polymer pCB was conjugated via thiol-maleimide "click chemistry" to the C-terminus of GLP-1 modified with a cysteine residue to ensure a site-specific conjugation (Figure 1A). Considering that the renal filtration cutoff size for globular proteins is 50−70 kDa,[43,44] we synthesized a 66 kDa pCB polymer for the conjugation. We hypothesized that the increased hydrodynamic volume from the conjugation of 66 kDa pCB could prevent the filtration of GLP-1 in the kidney. A two-step conjugation process was performed, and the success of each step was evaluated through nuclear magnetic resonance spectroscopy (^{1}H NMR). During the first step of adding the *N*-*β*-maleimidopropyl-oxysuccinimide ester linker (BMPS) onto pCB, the spectrum of the product (labeled as pCB-maleimide) would show the peak specific for maleimide. After the conjugation of GLP-1, the peak for maleimide was no longer present, indicating the success of the process (Figure 1B). The final product of conjugating pCB onto GLP-1 was further evaluated through gel permeation chromatography (GPC). The left shift of the peak compared to pCB alone indicated the success of producing pCB-conjugated GLP-1 (denoted as GLP1-pCB) (Figure 1C). Additionally, GLP-1 was poorly dissolved in phosphate-buffered saline before the conjugation of pCB; GLP1-pCB experienced no issues dissolving back into any water-based solution.

When attaching a high-molecular-weight polymer onto a fairly small peptide, it is of particular concern whether the secondary structure of the peptide would be affected, which can affect the activity of the peptide. Although PEG has been considered to be inert and is widely used in many different biotherapeutics applications, recent studies have revealed that PEG interacts with peptides or proteins, which could cause the loss in activity or stability.[45,46] It is important to identify polymeric materials that would not interfere with the structure of the peptide. We evaluated the secondary structures of GLP-1 and GLP1-pCB using circular dichroism spectroscopy (Figure 2A). GLP-1 is a peptide consisted of two *α*-helix structures.[11] While pCB showed no structure at all, GLP1-pCB exhibited *α*-helix structures similar to that of GLP-1 with a slight shift at the far-UV region, potentially resulting from pCB conjugation. This result has further suggested that pCB can be considered a good candidate for peptide conjugation due to the minimum interferences of the peptide from the polymer.

We next investigated the activity GLP1-pCB through *in vitro* insulin secretion assay as GLP-1 augments insulin release with a glucose-dependent manner. The activity of GLP1-pCB was evaluated through analyzing its potency and efficacy. Potency denotes the amount of GLP-1 or GLP1-pCB needed to produce a half-maximal response (EC_{50}), which is inversely proportional to the binding affinity of the peptide to its receptor; while the relative efficacy (E_{max}) is the maximal insulin release that GLP1-pCB produces compared to GLP-1 irrespective of concentration, and is related to the activation of receptors.[47] Rat pancreatic/islet RIN-m5F cells were incubated with different concentrations of GLP1-pCB or GLP-1 in the presence of glucose to determine the concentration of a peptide that gives the EC_{50}. GLP-1 showed an EC_{50} value of 1.08 nM, consistent with what was previously reported,[11,24,31,42] whereas GLP1-pCB showed an EC_{50} value of 7.24 nM (Figure 2B and Table 1). The decrease in the potency of the GLP1-pCB was expected, as large 66 kDa pCB inevitably brings steric hindrance due to the flexibility of the polymer chain and distinctive hydration layer.[48−50] In comparison, the site-specific mono-PEGylated

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Table 1. *In Vitro* Potency and Relative Efficacy of GLP-1 and GLP1-pCB

	Potency EC_{50} (nM)	Relative E_{max}[a] (%)
GLP-1	1.08 ± 1.17	100%
GLP1-pCB	7.24 ± 1.12	98.8%

[a]Relative E_{max}: Maximum insulin release relative to GLP-1 as 100%.

GLP-1 with a similar molecular weight of 50 kDa only retained an EC_{50} of 1870 nM, corresponding to 0.03% of the unconjugated peptide.[23] Similarly, when GLP-1 was conjugated to a human serum albumin (66 kDa) through a short PEG linker, the potency of GLP-1 was reduced by 3−4 orders of magnitude.[25] Even though there was a decrease in potency, E_{max} of GLP1-pCB was 98.8% with respect to GLP-1 set as 100%, suggesting that conjugation of pCB would not affect the activation of GLP-1 receptors, but only the binding affinity. We hypothesize that pCB draws water away from the relatively hydrophobic peptide, allowing the peptide and the receptor to interact by shifting the equilibrium, which maintains the potency and efficacy of GLP-1. Furthermore, as suggested before, it is hypothesized that the minimized nonspecific interactions between pCB and the GLP-1 receptor due to the superhydrophilic nature of the zwitterionic polymer can further facilitate the interaction between the peptide and its receptors.[39]

The short circulation half-lives of GLP-1 and GLP-1 analogs due to their small size is a major challenge for clinical use. The conjugation of natural or synthetic materials has improved the PK profiles of GLP-1. PEG polymers with different lengths or polysaccharides have increased the half-lives of GLP-1 analogs to 12.1−30.3 h from 1.5−5 min with a single subcutaneous injection;[23,24] the integration of human serum albumin or Fc has also increased the half-life to 8.5−38.2 h in rodents.[25−27,51] With pCB having a molecular weight similar to that of human serum albumin, we next investigated the PK properties of GLP1-pCB. Fluorescent-labeled GLP1-pCB was given subcutaneously with a dosage of 300 nmol/kg (equivalent to 1 mg/kg of GLP-1). Concentrations of GLP1-pCB are expressed as the amount of fluorescent-labeled GLP-1 detected in the serum. Concentrations of GLP1-pCB at different time points were fitted into a two-compartment model to analyze the PK profile (Figure 3 and Table 2). GLP1-pCB had a much longer circulation half-life ($T_{1/2\beta}$ = 43.0 h) than that of GLP-1, and with a maximum serum concentration observed (C_{max}) of 1427.1 ng/mL at T_{max} = 13.4 h (time of maximum concentration observed). The $T_{1/2\beta}$ was comparable to other modifications of GLP-1 reported previously

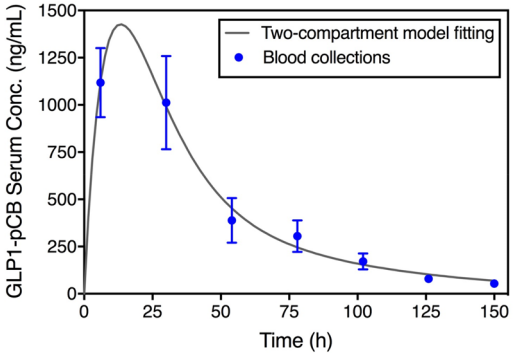

Figure 3. Circulation profile of a single subcutaneous injection of GLP1-pCB. The concentration of GLP1-pCB is expressed as the amount of fluorescent-labeled GLP-1 detected in the serum.

Table 2. Pharmacokinetics Profile of GLP1-pCB

$T_{1/2\beta}$ (h)	T_{max} (h)	C_{max} (ng/mL[a])	$AUC_{0-\infty}$ (ng/mL h)
43.0	13.4	1427.1	72931.1

[a]ng/mL: the concentration of GLP1-pCB is expressed as the amount of GLP-1 detected in the serum. 10 ng/mL of GLP-1 detected is equivalent to 3 nmol/mL of GLP1-pCB in the serum.

($T_{1/2\beta}$ = 12.1−38.2 h), while C_{max} was slightly better than other modifications of GLP-1 reported previously (C_{max} = 684−1022 ng/mL).[23,24] These results indicated that more GLP1-pCB was able to enter the systemic bloodstream and maintain a prolonged circulation profile. It has been reported that protein therapeutics and nanoparticles with a molecular weight more than 16 kDa administered subcutaneously exhibit limited direct transportation into the blood capillaries upon administration and enter the systemic circulation via an indirect route, through the lymphatic absorption.[52−54] It is therefore suggested that the conjugation of pCB could effectively facilitate GLP-1 entering the systemic circulation by protecting GLP-1 from degradation and clearance when passing through the lymphatic system. This could possibly be due to the prevention of nonspecific interaction with the environment owing to the superhydrophilicity of pCB.

As GLP1-pCB showed good activity *in vitro* and improved circulation half-life *in vivo*, we last evaluated its pharmacodynamic (PD) profile through the intraperitoneal glucose-tolerance test (IPGTT) (Figure 4). C57BL/6 mice were subcutaneously injected with a single dose of 300 nmol/kg (equivalent to 1 mg/kg of GLP-1) of GLP-1, GLP1-pCB, or saline (negative control) at time 0. IPGTTs were performed once on each mouse at different time points: 6, 30, 54, 78, 102, 126, and 150 h after sample injection. Mice were fasted for 6 h before the IPGTT, when the mice were challenged with an intraperitoneal injection of glucose at 2 g/kg. Blood glucose levels were monitored for 120 min after the glucose challenge (Figure 4A). For the group of mice that only received saline as the control, there was an elevation of blood glucose level to ∼350 mg/dL, 20 min after the challenge. The glucose level eventually returned to the baseline after 2 h. The group that received a single dose of GLP-1 was able to maintain the glucose level close to baseline on the same day challenge, but failed to maintain the glucose level during the second IPGTT challenge (at the 30 h time point). GLP1-pCB, on the other hand, was able to maintain the blood glucose level below 300 mg/dL when challenged up to 126 h after a single injection (Figure 4B−H). The loss of activity after 150 h could due to the low concentration of GLP1-pCB remaining in the bloodstream. The area under the curve (AUC_{0-120}) further illustrated the differences in the accumulative blood glucose level for up to 7 days (Figure 4I). These results showed the GLP1-pCB exerted activity for up to 6 days, suggesting that pCB could provide protection for GLP-1 from proteolytic cleavages in the bloodstream and provide prolonged glycemic control.

CONCLUSIONS

In this study, we developed a bioconjugate GLP1-pCB consisting of the native GLP-1 peptide with a zwitterionic polymer pCB. We first demonstrated that the conjugation of pCB onto the peptide did not alter the secondary structure of GLP-1. Even though the GLP1-pCB conjugate had a modest loss of potency due to steric hindrance as expected for polymer−protein conjugates, it still possessed very good efficacy *in vitro*.

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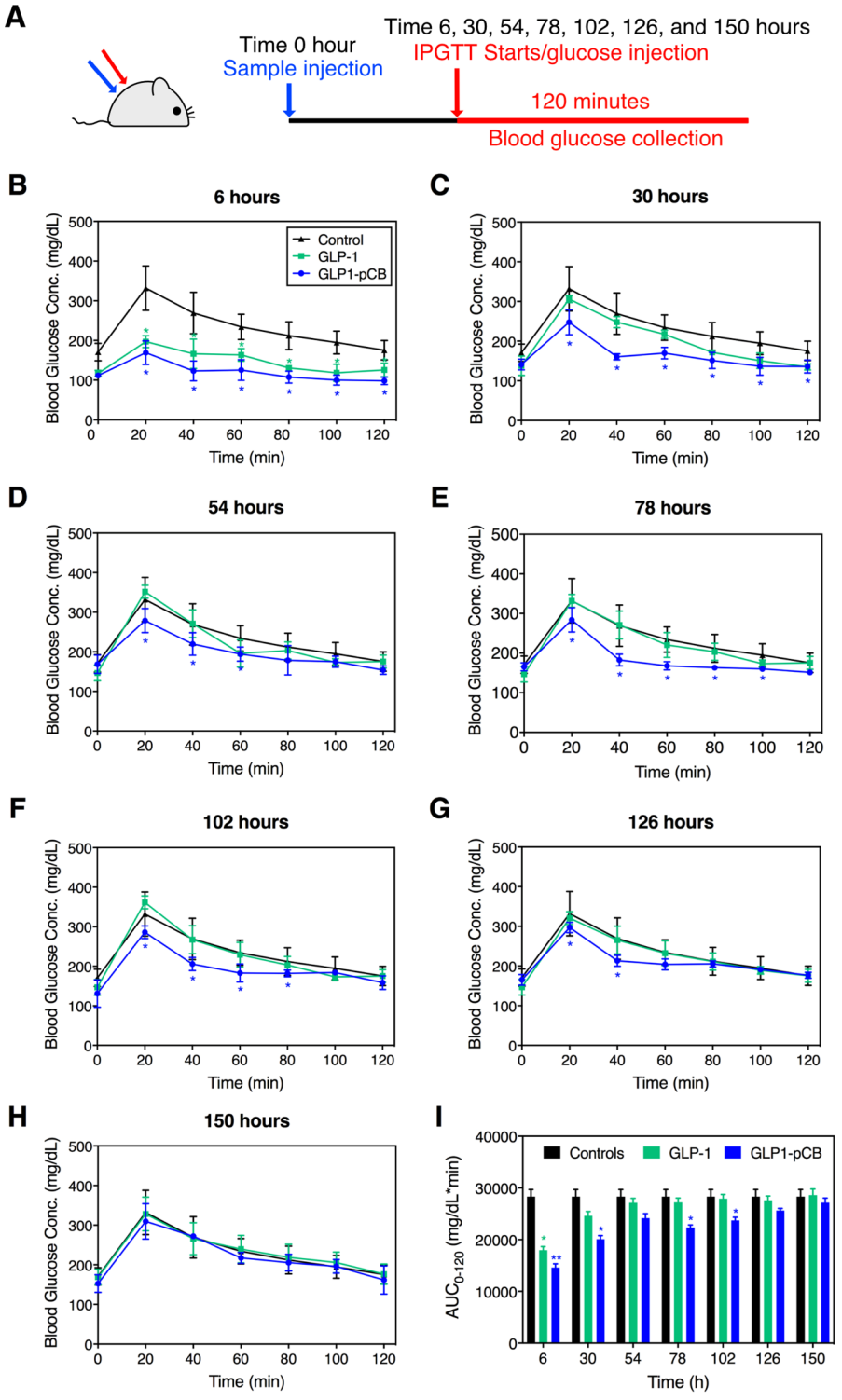

Figure 4. Intraperitoneal glucose tolerance test (IPGTT) of GLP-1 and GLP1-pCB. A. Experimental design for the IPGTT. Mice were first subcutaneously injected with a single dose of 300 nmol/kg GLP-1, GLP1-pCB, or saline (labeled as Control) at time 0 h. IPGTTs were then conducted on animals with an intraperitoneal injection of glucose (2 g/kg) at different time points: 6, 30, 54, 78, 102, 126, and 150 h post-sample injection. Six animals were challenged with IPGTT for each time point. Blood glucose levels of each animals were then monitored for 120 min during each IPGTT. B.−H. Blood glucose profiles of IPGTT at different time points after sample injection. I. Accumulative blood glucose AUC_{0-120} (area under the curve) profiles of IPGTT. The AUC was calculated based on the blood glucose profiles from B. to H. Results are reported as mean ± SD $^{*}p < 0.05$, $^{**}p < 0.01$.

GLP1-pCB had a prolonged circulation half-life and exerted activity up to 6 days after a single subcutaneous injection in a mouse study. While we present proof-of-concept experiments, more studies regarding the long-term immunogenicity and the optimization of the GLP1-pCB construct should be done in the future. The conjugation of pCB provides a potential solution for the fast clearance and rapid inactivation of GLP-1, which shows promise for providing long-term glycemic control for treating T2DM.

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EXPERIMENTAL PROCEDURES

General Experimental Details. Glucagon-like Peptide-1 (GLP-SH, HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR-SH) was purchased from GenScript (Piscataway, USA). Poly(carboxybetaine) (pCB) was synthesized as reported previously.[55] In brief, 3-acrylamido-*N*-(2-(*tert*-butoxy)-2-oxoethyl)-*N*,*N*-dimethylpropan-1-aminium (t-Butyl CBAAm) and chain transfer agent (CTA) were synthesized. pCB polymer was next prepared by a combination of reversible addition−fragmentation chain transfer (RAFT) polymerization, aminolysis, acid deprotection, and amine-to-thiol conversion steps utilizing the t-Butyl CBAAm and CTA prepared earlier. *N*-*β*-Maleimidopropyl-oxysuccinimide ester (BMPS) was from TCI American. Amicon Ultra centrifugal filter units were purchased from EMD Millipore (Billerica, MA). Pierce 660 nm Protein Assay was purchased from Thermo Fisher Scientific (Waltham, MA). Fetal bovine serum (FBS), Gibco RPMI 1640 (ATCC modification) medium, and RPMI 1640 medium (none ATCC modification) were all purchased from Thermo Fisher Scientific (Waltham, MA). RIN-m5F cells were purchased from ATCC (Manassas, VA). Alexa Fluor 555 NHS Ester (succinimidyl ester) was purchased from Thermo Fisher Scientific (Waltham, MA).

Preparation of pCB-Conjugated GLP-1 (GLP1-pCB). pCB (72 mg) were first dissolved in phosphate-buffered saline (PBS) pH 7.6 at 36 mg/mL, slowly mixed with 7.23 μM BMPS/DMSO (100 μL of 20 mg/mL), and reacted for 40 min at room temperature. The reaction mixture was then purified through centrifugal filters with a molecular cutoff at 10 kDa through multiple washes using PBS pH 7.35. GLP-1 (5 mg) was first dissolved in deionized water at 12.5 mg/mL (400 μL). The GLP-1 solution was then mixed with maleimide-modified pCB at 1.725 mg/mL (400 μL + 2500 μL) in PBS pH 7.35 by slowly dripping droplets while stirring, and reacted for 2 h at room temperature. Reaction mixture was then transferred to 4 °C fridge and reacted overnight. Reaction mixture was then purified and collected through the ENrich Size Exclusion Chromatography 650 column (10 mm × 300 mm), using NGC 10 Quest Chromatography System (Biorad). The concentration of GLP1-pCB was determined through Pierce 660 nm Protein Assay. Products from each step of the conjugation procedure were determined using ^{1}H NMR (300.10 MHz, D_2O) and the Agilent Technologies 1260 Infinity binary high performance liquid chromatography (HPLC) system with Waters Ultrahydrogel 1000 column (7.8 mm × 300 mm).

Structural Analysis of GLP1-pCB. GLP1-pCB and GLP-1 were characterized by circular dichroism. A Jasco J-720 spectropolarimeter was used to measure the far-UV spectra of the proteins diluted to a concentration range of 20−200 μM in 20 mM sodium phosphate buffer, pH 7. The mean residue ellipticity was measured from 190 to 250 nm in a 0.1 cm path length quartz cuvette at 25 °C. All spectra were accumulated with standard sensitivity. Studies were done in triplicate to ensure reproducibility.

***In Vitro* Insulinotropic Activity Assay.** The insulinotropic activity of GLP-1 and GLP1-pCB was evaluated by static incubation of RIN-m5F cells. Cells were seeded in 10% FBS/Gibco RPMI 1640 (ATCC modification) medium in Corning Costar Flat Bottom cell culture plate, 96 well, at a density of 1.5 × 10^5 cells/well, and grown overnight at 37 °C, under 5% CO_2. Acute tests for insulin release upon contact with samples were preceded by 2 h preincubation at 37 °C, under 5% CO_2 in glucose-free/serum-free RPMI 1640 medium (no ATCC modification). Test incubation was performed in the presence of 2 mM glucose (Thermo Fisher Scientific) and samples in serum-free RPMI 1640 medium at the final concentration from 10^{-12} to 10^{-6} M. After 30 min of incubation, the supernatant of each well was collected and centrifuged at 1000 × *g* for 5 min at 10 °C. The insulin content was analyzed forthwith by using rat insulin ELISA kit (Thermo Fisher Scientific). Concentration−response curves were analyzed by using a nonlinear curve fitting computer program (GraphPad Prism, San Diego, CA, USA), which yielded EC_{50} (concentration producing half-maximal response) and E_{max} (maximal effect) values.

Pharmacokinetic Studies. All animal experiments in this study adhered to federal guidelines and were approved by the University of Washington Institutional Animal Care and Use Committee. GLP1-pCB was first tagged with Alexa Fluor 555 with NHS ester targeting the lysines (K) of the construct. GLP1-pCB was dissolved in 1 mL of PBS pH 7.6 at 2 mg/mL; Alexa Fluor 555 was dissolved in 100 μL DMSO at 20 mg/mL. The Alexa Fluor 555/DMSO mixture was then slowly mixed with the GLP1-pCB solution by dripping droplets while stirring and reacted for 2 h at room temperature. The reaction mixture was then transferred to 4 °C refrigeration and reacted overnight. Reaction mixture was then purified and collected through multiple buffer exchanges (PBS pH 7.4) using 30 kDa MWCO Amicon Ultra centrifugal filter units.

Male C57BL/6 mice (5−8 weeks old, n = 12) received subcutaneous injections of 1 mg/kg Alexa Fluor 555-labeled GLP1-pCB conjugate at time 0 h. Blood samples were collected from tail vein at 6, 30, 54, 78, 102, 126, and 150 h post-administration. Serum samples were prepared from the blood samples by centrifuging at 11,000 rmp for 10 min. The concentrations of GLP1-pCB were determined by the fluorescence intensity (excitation at 552 nm, emission at 576 nm) measured with a microplate reader (BioTek, Cytation 5). Pharmacokinetic parameters were determined through PKSolver.[56]

Pharmacodynamic Studies with Intraperitoneal Glucose-Tolerance Test (IPGTT). Male C57BL/6 mice (5−8 weeks old, n = 144) received subcutaneous injections of pharmaceutical-grade saline, 1 mg/kg of GLP-1, or 1 mg/kg of GLP1-pCB conjugate at time 0 h. At different time points, including 6, 30, 54, 78, 102,126, and 150 h, 6 animals were challenged with IPGTT. Each animal has only been challenged once throughout the whole experiment. For each IPGTT challenge, animals were first fasted for 6 h. After the fast, they were given an intraperitoneal injection of sterilized pharmaceutical grade glucose at 2 g/kg. Blood glucose levels of the 6 animals at each IPGTT time point were measured at time 0, 20, 40, 60, 80, 100, and 120 min after the glucose challenge with a self-monitoring blood glucose meter (Advocate, Redi-Code$^+$, 2−5 μL of blood samples). The blood glucose levels were plotted over time, and the AUC_{0-120} was obtained using GraphPad Prism7. No animals were excluded due to complications.

Statistical Analysis. Results are reported as mean ± SD. Two-tailed Student's *t*-test was used to compare two small sets of quantitative data.

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AUTHOR INFORMATION

Corresponding Author

Shaoyi Jiang − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0001-9863-6899; Email: sjiang@uw.edu

Authors

Caroline Tsao − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0001-5815-4035

Peng Zhang − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Zhefan Yuan − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0002-4724-6214

Dianyu Dong − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States; School of Chemical Engineering and Technology and Key Laboratory of Systems Bioengineering of Ministry of Education, Tianjin University, Tianjin 300350, China;* orcid.org/0000-0002-2293-7959

Kan Wu − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0002-8900-5574

Liqian Niu − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Patrick McMullen − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Sijin Luozhong − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Hsiang-Chieh Hung − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Yu-Hong Cheng − *Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.bioconjchem.0c00286

Notes

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ABBREVIATIONS

GLP-1, glucagon-like peptide-1; T2DM, type 2 diabetes mellitus; pCB, poly(carboxybetaine); DPP-IV, dipeptidyl peptidase IV; GLP-RAs, GLP-1 receptor agonists; Aib, α-aminoisobutyric acid; ADA, antidrug antibodies; PK, pharmacokinetics; PEG, poly(ethylene glycol); Fc, fragment crystallizable region; ^{1}H NMR, nuclear magnetic resonance spectroscopy; E_{max}, maximum response (insulin release); EC_{50}, half-maximal response; BMPS, *N*-β-maleimidopropyl-oxysuccinimide ester linker; GPC, gel permeation chromatography; GLP1-pCB, poly(carboxybetaine) polymer conjugated glucagon-like peptide-1; $T_{1/2\beta}$, circulation half-life; C_{max}, maximum serum concentration observed; T_{max}, time of maximum concentration observed; PD, pharmacodynamic; IPGTT, intraperitoneal glucose-tolerance test; AUC_{0-120}, area under the curve (from 0 to 120 min); HPLC, high performance liquid chromatography.

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