Bioconjugate Chemistry

Article

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Hydrogel Drug Delivery System Using Self-Cleaving Covalent Linkers for Once-a-Week Administration of Exenatide

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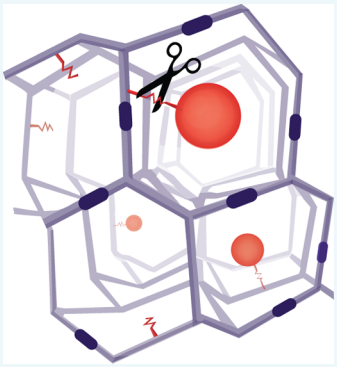
Supporting Information

ABSTRACT: We have developed a unique long-acting drug-delivery system for the GLP-1 agonist exenatide. The peptide was covalently attached to Tetra-PEG hydrogel microspheres by a cleavable β-eliminative linker; upon s.c. injection, the exenatide is slowly released at a rate dictated by the linker. A second β-eliminative linker with a slower cleavage rate was incorporated in polymer cross-links to trigger gel degradation after drug release. The uniform 40 μm microspheres were fabricated using a flow-focusing microfluidic device and in situ polymerization within droplets. The exenatide-laden microspheres were injected subcutaneously into the rat, and serum exenatide measured over a one-month period. Pharmacokinetic analysis showed a $t_{1/2,\beta}$ of released exenatide of about 7 days which represents over a 300-fold half-life extension in the rat and exceeds the half-life of any currently approved long-acting GLP-1 agonist. Hydrogel−exenatide conjugates gave an excellent Level A in vitro−in vivo correlation of release rates of the peptide from the gel, and indicated that exenatide release was 3-fold faster in vivo than in vitro. Pharmacokinetic simulations indicate that the hydrogel−exenatide microspheres should support weekly or biweekly subcutaneous dosing in humans. The rare ability to modify in vivo pharmacokinetics by the chemical nature of the linker indicates that an even longer acting exenatide is feasible.

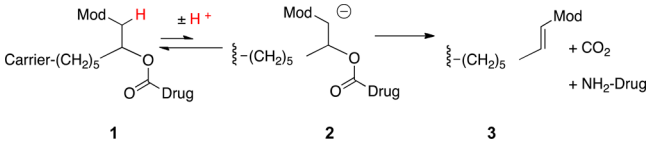


INTRODUCTION

Exenatide, a 39-amino-acid peptide from the Gila monster, is a potent agonist of the glucagon like-1 (GLP-1) receptor, and has glucoregulatory actions similar to GLP-1. In the human, exenatide has a significantly longer plasma lifetime than GLP-1, but nevertheless has a $t_{1/2,\beta}$ of only ∼2.5 h. It is marketed for type 2 diabetes as Byetta, and is administered subcutaneously (s.c.) twice a day. There is both demand for and benefit in long-acting forms of GLP-1 agonists,[1−3] and agonists have been developed that are administered s.c. once a day (Victozia, Lyxumia) or once a week (QWK) (Bydureon, Tanzeum, and Trulicity are approved and Semaglutide is in late-stage trials). There is also an implantable osmotic pump, ITCA 650, in late clinical trials that delivers exenatide over 6 to 12 months;[4] however, the level of patient-acceptance of an implantable device vs simple s.c. drug injections remains uncertain.

We have recently developed a chemical approach for half-life extension of drugs; here, a drug is covalently attached to a long-lived macromolecule through a β-eliminative linker that is slowly cleaved to release the native drug.[5,6] The carrier may be a circulating macromolecule such as polyethylene glycol (PEG) for half-life extensions of up to one or two weeks,[5] or noncirculating hydrogel depots, for half-life extension of weeks to months.[6] In this approach, a macromolecular carrier is attached to a linker that is attached to a drug via a carbamate group (**1**; Scheme 1); the β-carbon has an acidic carbon−hydrogen bond (C−H) and also contains an electron-withdrawing pK_a "modulator" (Mod) that controls the acidity of that C−H. Upon hydroxide ion-catalyzed proton removal to give **2**, a rapid β-elimination occurs to cleave the linker-carbamate bond and release the free drug and a substituted alkene **3**. The rate of drug release is proportional to the acidity of the proton which is controlled by the chemical nature of the modulator; thus, the drug release rate is controlled by the modulator. When using noncirculating hydrogel carriers, we also incorporate slower-cleaving β-eliminative linkers in the cross-links so polymer degradation can be triggered after drug release.[6]

Scheme 1

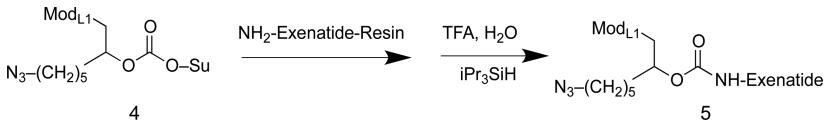


We previously reported a releasable PEGylated exenatide that had an estimated $t_{1/2,\beta}$ of ∼50 h in the human.[5] However, as with any circulating conjugate, the renal elimination rate of the conjugate limits the potential half-life extension. Thus, a noncirculating hydrogel−exenatide conjugate was prepared that showed a $t_{1/2,\beta}$ of ∼80 h. The problems of this conjugate were that the $t_{1/2,\beta}$ was not sufficiently long for QWK administration, s.c injection required a large 18 gauge needle, and the hydrogel was not biodegradable. Recently, we reported a drug delivery

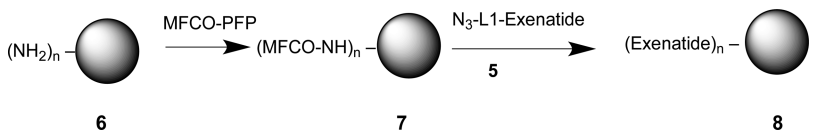
Received: December 23, 2015
Revised: January 20, 2016
Published: March 1, 2016

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210−1215

Scheme 2



Scheme 3



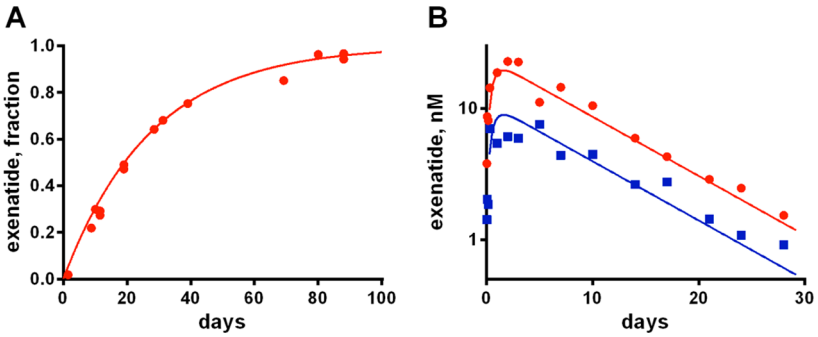


Figure 1. Release of exenatide from hydrogel microsphere conjugates. (A) In vitro release determined under accelerated cleavage conditions of pH 8.4, 37 °C, and shown after extrapolation to pH 7.4 by $k_{pH\ 7.4} = k_{pH} \times 10^{(pH-7.4)}$.[5,6] (B) Serum exenatide levels after s.c. injection of rats with hydrogel-exenatide microspheres. Gels containing 1.2 μmol (blue ■) and 2.7 μmol (red ●) exenatide/kg showed C_{max} values of 8.9 and 20 nM, respectively. Lines are the best fit to data using simplex optimization to models giving a $t_{1/2,\beta}$ of 160 ± 13 (SE) for the hydrogel–exenatide conjugates.

system (DDS) based on a Tetra-PEG hydrogel[7] carrier that is injectable, biocompatible, and biodegradable.[6]

In the present work, we have attached exenatide via a β-eliminative linker to degradable 40 μm Tetra-PEG hydrogel microspheres and demonstrated a $t_{1/2,\beta}$ for released drug of about 7 days in the rat. Simulations of the pharmacokinetics (PK) in humans suggest that this noncirculating hydrogel–exenatide conjugate should maintain therapeutic levels of the drug with weekly or biweekly s.c. administration.

■ RESULTS

The general approach used for preparing the hydrogel–exenatide conjugate DDS was to prepare an N-terminal azido-linker (L1)-exenatide,[5] and then couple it by strain-promoted azide–alkyne cycloaddition (SPAAC) to monofluorocyclooctyne (MFCO)-derivatized hydrogel microspheres containing cleavable cross-links (L2).[8]

Chemistry. In a modification of a reported method,[5] exenatide was synthesized by SPPS using Fmoc/tBu chemistry and reacted on-resin at the α-amine group with 7-azido-1-(methylsulfonyl)-2-heptyl succinimidyl carbonate (**4**; N$_3$-L1-[MeSO$_2$-]-HSC)[5] in DMF containing *N*-methyl morpholine (Scheme 2); here, scrupulous removal of amines from DMF and use of a weak base were important to avoid β-elimination of the linker. In early experiments, we observed that when the peptide-resin and blocking groups were cleaved with 95:5 TFA:H$_2$O, 5% DTE, the azide moiety of the linker was reduced. Subsequently, we found that reaction of the peptide-resin with 95:5 TFA:H$_2$O containing 5% triisopropylsilane instead of DTE provided good yields (~60%) of the N$_3$-L1[MeSO$_2$-]-exenatide **5**.

The chemistry used for amine-derivatized Tetra-PEG hydrogel production is a slight modification of that previously reported.[8] Here, we created a prepolymer in which PEG$_{20\ kDa}$-(NH$_2$)$_4$ was first acylated with an N$_3$-L2-[(MeOCH$_2$CH$_2$)$_2$NSO$_2$-]-Lys(Boc)-HSE, followed by removal of the Boc group. The azide groups of this prepolymer were then reacted with PEG$_{20\ kDa}$-(MFCO)$_4$ by SPAAC to provide the amine-derivatized hydrogel microspheres containing cleavable cross-links.

Microsphere Production. We developed a method to prepare the hydrogel as 40 μm amine-derivatized microspheres **6** in a flow-focusing microfluidic device (Figure S1). The two prepolymer solutions are introduced through a Y-junction to mix in the device; the resulting aqueous stream is sheared with an immiscible continuous phase to form droplets that undergo spontaneous polymerization into microgels. When operated continuously, the single 7-channel device used could produce almost 200 mL of swollen amino-microspheres per day, which is scalable by running multiple chips in parallel. These microspheres (a) can easily be chemically modified by acylation of the amine groups, (b) have cleavable β-eliminative cross-links to allow biodegradation or base-catalyzed degelation, and (c) can readily be injected through a 27-gauge needle. Initially, we produced these microspheres using fluorinated solvents and surfactants commonly employed in microfluidic droplet production.[9] Subsequently, the amine-derivatized microspheres were produced by a method that replaced these materials of unproven safety and high cost. We were able to substitute the fluorinated solvent, HFE-7500, as the continuous phase and wash solvent with decane. In place of the perfluorinated PEG-Krytox block copolymer surfactant, we used Abil-EM90 and polyglycerol polyresorcinoleate[10] which are extensively used in cosmetics and foods, respectively. Substitution of the

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210–1215

fluorinated specialty reagents by these commodity chemicals also reduced the cost of materials by well over 100-fold.

The tendency of the hydrogel microspheres to adhere to vessel surfaces made them difficult to transfer, and differential solvent- and modification-dependent swelling made their quantitation problematic. Inspired by SPPS methodology, we constructed a syringe-based reaction vessel (Figure S2) that allowed aseptic introduction and removal of reagents, as well as chemical modification and washing without transfer or loss of microspheres; the vessel also permitted quantitative syringe-to-syringe transfer of the microsphere slurries. Using this assembly, TFA-sterilized[11] amino-microspheres **6** were acylated with MFCO-pentafluoro phenyl ester,[12] and the MFCO-microspheres **7** then coupled with the azido-linker-exenatide by SPAAC to give the exenatide-loaded microspheres **8** (Scheme 3).

Under accelerated base-catalyzed linker cleavage conditions[5,6] the Tetra-PEG hydrogel-exenatide microspheres showed an extrapolated in vitro release $t_{1/2}$ of 460 h for exenatide (Figure 1A) and a reverse gelation time (t_{RG}) of 200 days at pH 7.4, 37 °C. Previously, we empirically observed that in vitro rates of linker cleavage at pH 7.4, 37 °C, are ~2- to 3-fold slower than in vivo rates.[5] The total exenatide released corresponded to a loading of ~2 μmol peptide/mL packed microspheres.

PK in the Rat. Figure 1B shows the serum exenatide C vs t plots over 28 days after s.c. injection of microspheres containing 1.2 μmol and 2.7 μmol exenatide/kg in the rat.

The PK were modeled as three consecutive first-order reactions occurring in the subcutaneous (SC) or central (CC) compartments (Scheme 4).

Scheme 4

$$\text{Gel-Drug}_{SC} \xrightarrow{k_1} \text{Drug}_{SC} \xrightarrow{k_a} \text{Drug}_{CC} \xrightarrow{k_2} \text{elimination}$$

Here, k_1 and k_2 are the linker cleavage and exenatide elimination rate constants, respectively, and k_a is the rate constant for absorption of the released free exenatide into the central compartment. The model was first expanded to a triexponential integrated rate equation.[13] The fast elimination of free exenatide in the rat[14] relative to release from the hydrogel (k_1) and absorption from the s.c. compartment (k_a) allows the data to be described by the simpler two-exponential eq 1

$$[\text{Drug}]_{CC,t} = \text{Dose}\cdot Q\cdot(e^{-k_1 t} - e^{-k_a t}) \quad (1)$$

and when absorption is also fast relative to release

$$Q = \frac{F}{V_{SS}\cdot k_2}\cdot k_1 = \frac{F}{CL}\cdot k_1 \quad (2)$$

The PK analysis provides values for k_a and k_1, but not for bioavailability, F, volume of distribution, V_{ss}, or drug elimination rate, k_2, as these parameters are fit to data as the aggregate scaling factor Q.

The two data sets of Figure 1B, differing only in the initial dose, were simultaneously fit to eq 1 using a Nelder–Mead downhill simplex[15] with k_1, k_a, and Q as variables and the sum of residuals squared as error function. This analysis showed dose-proportional drug levels and provided estimates of $t_{1/2,a}$ = 8.7 h, $t_{1/2,1} = t_{1/2,\beta}$ = 160 h, and Q = 0.0092. Using eq 2, CL/F is estimated as 7.8 mL/kg-min, in good agreement with the reported value of 8.6 mL/kg-min.[16] The $t_{1/2,\beta}$ represents a 343-fold half-life extension over bolus exenatide in the rat.

Figure 2A shows excellent agreement of the cumulative in vitro release and in vivo absorption profiles for the Tetra-PEG hydrogel–exenatide conjugate with a $MeSO_2$-modulator, as well as the previously PEGA hydrogel–exenatide conjugate having a faster cleaving linker;[5] although only the higher dose of Figure 1 is shown, the lower dose matched as well. To obtain the in vivo–in vitro fits, the in vivo $t_{1/2,\beta}$ values are scaled to be 2.9-fold lower than the $t_{1/2}$ of in vitro release. Figure 2B shows the Level A in vitro–in vivo correlation (IVIVC) plots[17,18] of in vivo exenatide absorption vs in vitro release of the Tetra-PEG-exenatide conjugate (R^2 = 0.995) as well as PEGA hydrogel conjugate (R^2 = 0.993) from Figure 2A.[5] The data show that in vivo exenatide release from the hydrogels is well predicted by in vitro release studies, and that in vivo drug release is about 3-fold faster than in vitro release.

PK Simulations in the Human. We have described approaches for simulations of the PK of conjugates containing β-eliminative linkers in humans.[5] First, we simulate parameters for a single dose of the drug conjugate that would maintain a specified minimal plasma drug concentration, C_{min}, for the time interval desired in multiple dosing; then, steady state values are estimated by nonparametric superposition of sequential single dose simulations. Simulations of interspecies PK with

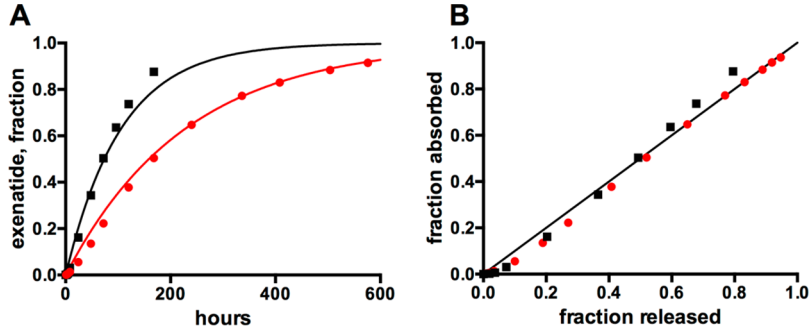


Figure 2. IVIVC of releasable hydrogel-exenatide conjugates. (A) Cumulative in vitro (lines, black, red) and in vivo (red ●, black ■) release from hydrogels with $MeSO_2$- (red, ●) and $ClPhSO_2$- (black, ■) modulators; in vitro release rates are scaled by an increase of 2.9-fold. In vivo absorption was calculated from data in Figure 1B and from Figure 3B in ref 5 using the FDA-recommended[18] deconvolution method of Nelson-Wagner.[19] (B) Level A correlation between in vitro release and in vivo absorption from hydrogels using $MeSO_2$- (red ●) and $ClPhSO_2$- (black ■) modulators for release. The line depicts a perfect correlation of absorbed and released exenatide.

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210–1215

Table 1. Reported and Simulated PK Parameters of Long-Acting Exenatide in Humans at Steady State

drug (modulator)	dosing interval	Exenatide					
		dose, mg	$t_{1/2,\beta}$, h	C_{min}, nM	C_{max}, nM	C_{max}/C_{min}	AUC nM-h/wk
Bydureon[a]	QWK	2	NA	0.07	0.120	1.7	12
Simulations							
PEG-exenatide ($PhSO_2^-$)	QWK	1.3[b]	53[c]	0.07	0.67	9.6	49
Hydrogel-exenatide ($pClPHSO_2^-$)	QWK	0.9[b]	78[c]	0.07	0.28	4.0	27
Hydrogel-exenatide ($MeSO_2^-$)	QWK	0.6[b]	160	0.07	0.14	2.0	17
Hydrogel-exenatide ($MeSO_2^-$)	Q2WK	1.9[b]	160	0.07	0.29	4.1	54

[a]Bydureon PK parameters from refs 23,24. [b]Dose values for simulations of exenatide conjugates assume 100% bioavailability of exenatide. [c]$t_{1/2,b}$ of released exenatide taken from ref 5.

circulating PEG−drug conjugates have been reported,[6] but analogous approaches for simulations of noncirculating releasable conjugates have, to our knowledge, not been previously described.

For noncirculating carriers, the simulation requires knowledge of the in vivo linker cleavage rate, k_1, in one species—such as the rat—the k_1 in the human, and the parameters $t_{1/2,\beta}$ and V_{ss} of the released free drug in the human. Although k_1 values for these linkers have not yet been determined in humans, since linker cleavage rates are chemically controlled and are the same in the mouse, rat, and monkey,[5,20] it is reasonable to assume they are species independent. Estimation of the steady state dosing of a noncirculating carrier−drug conjugate needed to provide a given C_{min} of the drug at the end of a designated dose interval is performed as follows.

In cases of eq 1 where absorption of drug is fast relative to release from the hydrogel, i.e., $k_a \gg k_1$ in Scheme 4, after a short initial absorption phase eq 1 reduces to the single exponential eq 3.

$$[\text{Drug}]_{CC,t} = \frac{\text{Dose}\cdot F}{V_{SS}}\cdot\frac{k_1}{k_2}\cdot e^{-k_1 t} \tag{3}$$

Since $[\text{Drug}]_{CC,t} = C_{min}$ when $t = t_{min}$

$$\text{Dose}_{total} = C_{min}\cdot\frac{V_{SS}\cdot k_2}{F\cdot k_1}\cdot e^{-k_1 t_{min}} = C_{min}\cdot\frac{CL}{F\cdot k_1}\cdot e^{-k_1 t_{min}} \tag{4}$$

Here, Dose_{total} is the amount of drug on the carrier at initial dosing that is needed to achieve the desired steady state C_{min} at dose-interval time t_{min}. The drug remaining on the carrier at time t_{min}, $\text{Dose}_{remnant}$, is given by

$$\text{Dose}_{remnant} = \text{Dose}_{total}\cdot e^{-k_1 t_{min}} \tag{5}$$

The amount of drug required for steady-state dosing, Dose_{SS}, is the amount to be added to the remnant to reach Dose_{total}.

$$\text{Dose}_{ss} = \text{Dose}_{total} - \text{Dose}_{remnant} = \text{Dose}_{total}\cdot(1 - e^{-k_1 t_{min}}) \tag{6}$$

which by substituting eq 4 for Dose_{total} becomes

$$\text{Dose}_{ss} = C_{min}\cdot\frac{V_{ss}\cdot k_2}{F\cdot k_1}\cdot(e^{k_1 t_{min}} - 1) = C_{min}\cdot\frac{CL}{F\cdot k_1}\cdot(e^{k_1 t_{min}} - 1) \tag{7}$$

Since $V_{ss}\cdot k_2 = CL$, estimates may be made using either parameter in eqs 4 and 7. The number of doses to reach steady state, N, with a noncirculating carrier-linked drug can be estimated by eq 8, where f_{SS} is the fraction of the steady state C_{min} reached after the Nth dose. For example, if the dosing interval t_{min} is 168 h, and $t_{1/2,1}$ is 160 h, ∼95% steady state[21] C_{min} is reached after four doses.

$$N = -\frac{\ln(1 - f_{SS})}{\ln 2}\cdot\frac{t_{1/2,2}}{t_{min}} \tag{8}$$

For steady-state dosage estimations of hydrogel−exenatide conjugates in humans, we used $t_{1/2,\beta}$ and V_{ss} of the released exenatide of 2.4 h and 0.4 L/kg, respectively, which are values for bolus s.c. injected exenatide,[22] and targeted a C_{min} of ∼70 pM which is the exenatide C_{min} achieved with 2 mg weekly dosed Bydureon.[23,24] Steady-state values of exenatide released from weekly dosed Bydureon and simulated PK parameters of previously and presently reported exenatide conjugates are compared in Table 1.

We previously reported a circulating PEG−exenatide conjugate that released exenatide with an in vivo cleavage $t_{1/2}$ of 78 h.[5] Simulations indicated that in the human the $t_{1/2,\beta}$ of released exenatide should be 53 h, and that the conjugate could maintain 70 pM drug with once weekly administration of an exenatide dose similar to Bydureon. However, because of the relatively short in vivo $t_{1/2}$ for linker cleavage and concurrent elimination of ∼30−40% the PEG−peptide conjugate with $t_{1/2}$ ∼ 5 to 6 days,[25] utilization of the drug would be inefficient and C_{max} would be high. We therefore examined a noncirculating s.c.-administered hydrogel−exenatide conjugate that coincidentally also had $t_{1/2,\beta}$ of ∼80 h for exenatide but was not lost by elimination. As expected, the estimated dose required to maintain a 70 pM C_{min} was lower than those of either the PEG-conjugate or Bydureon, but C_{max} was 2.3-fold higher that of exenatide released from Bydureon.

By using the Tetra-PEG hydrogel-exenatide DDS described here with a linker having a longer cleavage $t_{1/2}$ of 160 h, exenatide concentrations of ≥70 pM should be maintained with weekly dosing of a hydrogel−exenatide conjugate containing only ∼0.6 mg exenatide, with a C_{max}, C_{max}/C_{min}, and AUC similar to Bydureon. This would require an injection of ∼0.1 mL of the microsphere−exenatide conjugate per 70 kg person. Simulations indicate that the same hydrogel-conjugate might also serve for biweekly dosing with quite acceptable dose and C_{max}.

DISCUSSION

In the present work, we describe the preparation and properties of a DDS in which exenatide is covalently attached to Tetra-PEG hydrogel microspheres by a releasable β-eliminative linker; upon s.c. injection, the exenatide is slowly released by a rate dictated by the linker. A second β-eliminative linker with a slower cleavage rate was incorporated in polymer cross-links to trigger gel degradation after the drug had been released.

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210−1215

For synthesis, the azide-terminated linker was attached to the N-terminus of exenatide as the final coupling step in SPPS, followed by cleavage and purification of the azido-linker-exenatide. Separately, degradable Tetra-PEG amino-microspheres of 40 μm diameter were prepared in a microfluidic device, and derivatized with a cyclooctyne. Then, the azido-linker-exenatide was coupled to the cyclooctyne-microspheres by SPAAC to give the microsphere-exenatide DDS.

The exenatide-laden Tetra-PEG microspheres were injected s.c. into the rat at two doses, and serum exenatide was measured over a one month period. PK analysis showed high bioavailability and a $t_{1/2,\beta}$ of ~7 days, a ~340-fold half-life extension over bolus exenatide. This conjugate and another having a faster release rate both gave an excellent Level A IVIVC in the rat. The results showed that in vitro release serves as a surrogate for in vivo release, and indicates that the in vivo cleavage is about 3-fold faster than in vitro. Although the reason for the faster in vivo release rates is unknown, it cannot be due to foreign body responses to the s.c. microspheres—as suggested for PLGA-encapsulated exenatide[26]—since circulating PEG-conjugates containing these linkers show similar in vivo rate enhancements.[5]

Since the β-eliminative linker cleavage rate is species independent,[5,20] using known PK parameters of exenatide in the human, we could simulate the PK of the hydrogel DDS in the human. Results indicate that weekly s.c. dosing of only ~0.6 mg exenatide attached to the microspheres should maintain therapeutic levels of the drug with a PK profile similar to Bydureon, and biweekly administration of about 1.9 mg should maintain therapeutic levels of the peptide with acceptable C_{max} and peak-over-trough values. Although these doses were calculated assuming 100% bioavailability of the released exenatide, the assumption is justified by the high bioavailability of s.c. exenatide in the human.[27]

There are three current formats for half-life extension in marketed GLP-1 agonists—controlled release, peptide agonist fusions, and albumin binding; the benefits and harms of these have recently been reviewed.[28] Our releasable hydrogel-exenatide is most analogous to the QWK PLGA-controlled release formulation of exenatide, Bydureon, and derives substantial benefit from the large information-base derived from this DDS. Notably, extensive correlations of the PK of exenatide with its PD and toxicity provide a high degree of confidence that if we achieve the desired PK, we can attain the desired therapeutic outcome. Further, the strong PK–PD relationship of exenatide allows pathways that can significantly shorten the time to regulatory approval (i.e., the FDA 505b2 pathway).

The hydrogel–exenatide DDS has several benefits over Bydureon. First, the in vivo release profile of exenatide from the hydrogel DDS is much simpler and predictable than from the PLGA encapsulated formulation. Each dose of the former is followed by a simple first-order in vivo release of exenatide that allows easy estimation of drug levels upon multidosing. In contrast, a single dose of Bydureon shows an initial burst, a lag period, and then a period ~4 to 8 weeks after administration during which most of the exenatide is released;[29] achieving a desired steady-state drug level requires harmonized superpositioning of the irregular periods of drug release upon sequential doses of the polymer. Second, because the drug in the current DDS is covalently bound to the carrier, there is no burst effect due to loosely adhering or rapidly escaping drug. Third, whereas Bydureon must be stored dry and reconstituted shortly before injection through a 23 gauge needle, the Tetra-PEG hydrogels and linkers are stable at reduced pH as an aqueous suspension[5,6] and the 40 μm microspheres can be easily injected through a small-bore 27 gauge needle. Finally, unlike PLGA formulations that can significantly glycolate and lactoylate amines of an encapsulated peptide,[26,30] the current DDS releases the peptide in the form that is attached to the carrier.

The other approved once-weekly GLP-1 agonists consist of a fusion of the peptide with a long-lived circulating macromolecule (Abglutide, Dulaglutide), or a peptide agonist modified such that it binds to serum albumin (Semaglutide). With a $t_{1/2,\beta}$ of nearly 7 days, the released exenatide from our DDS has a lifetime comparable to or exceeding the approved long-acting GLP-1 agonists. Further, two unique features of the current DDS distinguish it from all others. First, a second peptide could be connected to the hydrogel–exenatide conjugate by the same β-eliminative linker to synchronize their in vivo half-lives. This should provide a simple method to create a dual-effector DDS functionally analogous to single peptides developed to have GLP1R-GCGR[31] or GLP1R-GIPR[25] co-agonism. Second, a unique feature of the current DDS is the ability to modify the half-life of the released drug by modifying the β-elimination rate of the linker. Thus, it should be feasible to create a DDS that delivers its cargo over a period of one month or longer. The preparation of such dual-effectors using the current hydrogel-exenatide DDS and ultra-long-acting hydrogel–exenatide conjugates are in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00690.

The source of specialized materials is provided along with their use. Detailed synthetic and conjugation procedures are described as well as analytical procedures. Methods of preparing and conjugating hydrogel microspheres are also furnished. In vitro kinetic procedures are provided as are in vivo PK methods and analyses. (PDF)

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Notes

The authors declare the following competing financial interest(s): All authors are employees of and have equity in Prolynx.

■ ACKNOWLEDGMENTS

This work was supported in part by NSF grant 1429972.

■ REFERENCES

(1) McDonald, A. I., and Isaacson, J. (2012) Bydureon Survey, *LifeSci Advisors*; http://www.lifesciadvisors.com/clientinfo/marketresearch/marketresearch__Bydureon%20Survey_2-19-2012__clientinfo.pdf.

(2) Madsbad, S., Kielgast, U., Asmar, M., Deacon, C. F., Torekov, S. S., and Holst, J. J. (2011) An overview of once-weekly glucagon-like peptide-1 receptor agonists–available efficacy and safety data and perspectives for the future. *Diabetes, Obes. Metab. 13*, 394–407.

(3) Tibble, C. A., Cavaiola, T. S., and Henry, R. R. (2013) Longer Acting GLP-1 Receptor Agonists and the Potential for Improved Cardiovascular Outcomes. *Expert Rev. Endocrinol. Metab. 8*, 247–259.

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210–1215

(4) Henry, R. R., Rosenstock, J., Logan, D., Alessi, T., Luskey, K., and Baron, M. A. (2014) Continuous subcutaneous delivery of exenatide via ITCA 650 leads to sustained glycemic control and weight loss for 48 weeks in metformin-treated subjects with type 2 diabetes. *J. Diabetes Complications 28*, 393−8.
(5) Santi, D. V., Schneider, E. L., Reid, R., Robinson, L., and Ashley, G. W. (2012) Predictable and tunable half-life extension of therapeutic agents by controlled chemical release from macromolecular conjugates. *Proc. Natl. Acad. Sci. U. S. A. 109*, 6211−6.
(6) Ashley, G. W., Henise, J., Reid, R., and Santi, D. V. (2013) Hydrogel drug delivery system with predictable and tunable drug release and degradation rates. *Proc. Natl. Acad. Sci. U. S. A. 110*, 2318−23.
(7) Sakai, T., Matsunaga, T., Yamamoto, Y., Ito, C., Yoshida, R., Suzuki, S., Sasaki, N., Shibayama, M., and Chung, U.-i. (2008) Design and Fabrication of a High-Strength Hydrogel with Ideally Homogeneous Network Structure from Tetrahedron-like Macromonomers. *Macromolecules 41*, 5379−5384.
(8) Henise, J., Hearn, B. R., Ashley, G. W., and Santi, D. V. (2015) Biodegradable tetra-PEG hydrogels as carriers for a releasable drug delivery system. *Bioconjugate Chem. 26*, 270−8.
(9) Holtze, C., Rowat, A. C., Agresti, J. J., Hutchison, J. B., Angile, F. E., Schmitz, C. H., Koster, S., Duan, H., Humphry, K. J., Scanga, R. A., et al. (2008) Biocompatible surfactants for water-in-fluorocarbon emulsions. *Lab Chip 8*, 1632−9.
(10) Steinhilber, D., Rossow, T., Wedepohl, S., Paulus, F., Seiffert, S., and Haag, R. (2013) A microgel construction kit for bioorthogonal encapsulation and pH-controlled release of living cells. *Angew. Chem., Int. Ed. 52*, 13538−43.
(11) Lasch, P., Nattermann, H., Erhard, M., Stammler, M., Grunow, R., Bannert, N., Appel, B., and Naumann, D. (2008) MALDI-TOF mass spectrometry compatible inactivation method for highly pathogenic microbial cells and spores. *Anal. Chem. 80*, 2026−34.
(12) Baumhover, N. J., Martin, M. E., Parameswarappa, S. G., Kloepping, K. C., O'Dorisio, M. S., Pigge, F. C., and Schultz, M. K. (2011) Improved synthesis and biological evaluation of chelator-modified alpha-MSH analogs prepared by copper-free click chemistry. *Bioorg. Med. Chem. Lett. 21*, 5757−61.
(13) Westman, A. E. R., and DeLury, D. B. (1956) The differential equations of consecutive reactions. *Can. J. Chem. 34*, 1134−1138.
(14) Parkes, D., Jodka, C., Smith, P., Sonali Nayak, S., Rinehart, L., Gingerich, R., K, C., and Young, A. (2001) Pharmacokinetic Actions of Exendin-4 in the Rat: Comparison With Glucagon-Like Peptide-1. *Drug Dev. Res. 53*, 260−267.
(15) Nelder, J. A., and Mead, R. (1965) A simplex method for function minimization. *Computer Journal 7*, 308−313.
(16) Gao, W., and Jusko, W. J. (2011) Pharmacokinetic and pharmacodynamic modeling of exendin-4 in type 2 diabetic Goto-Kakizaki rats. *J. Pharmacol. Exp. Ther. 336*, 881−90.
(17) Sakore, S., and Chakraborty, B. (2011) In Vitro−In Vivo Correlation (IVIVC): A Strategic Tool in Drug Development. *J. Bioequiv Availab S3*, 1−12.
(18) FDA Guidance for Industry Extended Release Oral Dosage Forms: Development, E., and Application of In Vitro/In Vivo Correlations. (1997).
(19) Wagner, J. G., and Nelson, E. (1963) Per cent absorbed time plots derived from blood level and/or urinary excretion data. *J. Pharm. Sci. 52*, 610.
(20) Santi, D. V., Schneider, E. L., and Ashley, G. W. (2014) Macromolecular prodrug that provides the irinotecan (CPT-11) active-metabolite SN-38 with ultralong half-life, low C(max), and low glucuronide formation. *J. Med. Chem. 57*, 2303−14.
(21) Hauck, W. W., Tozer, T. N., Anderson, S., and Bois, F. Y. (1998) Considerations in the attainment of steady state: aggregate vs. individual assessment. *Pharm. Res. 15*, 1796−8.
(22) Byetta, P. i. Prescribing information.
(23) Drucker, D. J., Buse, J. B., Taylor, K., Kendall, D. M., Trautmann, M., Zhuang, D., and Porter, L. (2008) Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Lancet 372*, 1240−50.
(24) Kim, D., MacConell, L., Zhuang, D., Kothare, P. A., Trautmann, M., Fineman, M., and Taylor, K. (2007) Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care 30*, 1487−93.
(25) Finan, B., Ma, T., Ottaway, N., Muller, T. D., Habegger, K. M., Heppner, K. M., Kirchner, H., Holland, J., Hembree, J., Raver, C., et al. (2013) Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci. Transl. Med. 5*, 209ra151.
(26) Li, X., Zhao, Z., Li, L., Zhou, T., and Lu, W. (2015) Pharmacokinetics, in vitro and in vivo correlation, and efficacy of exenatide microspheres in diabetic rats. *Drug Delivery 22*, 86−93.
(27) European Medicines Agency; Byetta: EPAR - Scientific Discussion 12/14/2006; http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000698/human_med_000682.jsp&mid=WC0b01ac058001d124.
(28) Zaccardi, F., Htike, Z. Z., Webb, D. R., Khunti, K., and Davies, M. J. (2016) Benefits and Harms of Once-Weekly Glucagon-like Peptide-1 Receptor Agonist Treatments: A Systematic Review and Network Meta-analysis. *Ann. Intern. Med. 164*, 1−12.
(29) Fineman, M., Flanagan, S., Taylor, K., Aisporna, M., Shen, L. Z., Mace, K. F., Walsh, B., Diamant, M., Cirincione, B., Kothare, P., et al. (2011) Pharmacokinetics and pharmacodynamics of exenatide extended-release after single and multiple dosing. *Clin. Pharmacokinet. 50*, 65−74.
(30) Houchin, M. L., and Topp, E. M. (2008) Chemical degradation of peptides and proteins in PLGA: a review of reactions and mechanisms. *J. Pharm. Sci. 97*, 2395−404.
(31) Day, J. W., Ottaway, N., Patterson, J. T., Gelfanov, V., Smiley, D., Gidda, J., Findeisen, H., Bruemmer, D., Drucker, D. J., Chaudhary, N., et al. (2009) A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat. Chem. Biol. 5*, 749−57.

DOI: 10.1021/acs.bioconjchem.5b00690
Bioconjugate Chem. 2016, 27, 1210−1215