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Sustained release subcutaneous delivery of BMS-686117, a GLP-1 receptor peptide agonist, via a zinc adduct

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ABSTRACT

BMS-686117 is an 11-mer GLP-1 receptor agonist with a short intrinsic pharmacokinetic half-life ($t_{1/2}$) of ~2 h. In order to develop an extended release formulation for once-daily (QD) subcutaneous administration, a non-covalently bonded Zn/BMS-686117 adduct with very low aqueous solubility was prepared through mixing zinc acetate and BMS-686117 solutions, followed by filtration or spray drying. At pH 6.8, free BMS-686117 concentration decreased continuously with the increase of Zn:BMS-686117 ratio. Furthermore, free BMS-686117 concentration increases in the presence of ethylenediaminetetraacetic acid (EDTA), indicating the reversibility of the zinc–peptide association. As solids, the glass transition temperature of Zn/BMS-686117 adduct increases with the increase of Zn:BMS-686117 ratio. A Zn/BMS-686117 adduct suspension, with a molar ratio of zinc:BMS-686117 of 3:1, was dosed subcutaneously to dogs along with two other solution formulations. The Zn/BMS-686117 adduct showed a prolonged BMS-686117 terminal $t_{1/2}$ of 8.5 h, a mean residence time (MRT) of 16 h, and a C_{max} value 6–8 times lower than the solution formulations. Additionally, the Zn/BMS-686117 was encapsulated into poly(lactide-*co*-glycolide) (PLGA) microspheres. The Zn/BMS-686117 microspheres showed an almost zero-order release profile *in vitro* for at least 18 days, with minimal initial burst, indicating the potential of using this approach for long-term sustained release.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is secreted from gut endocrine cells in response to nutrient ingestion and plays multiple roles in metabolic homeostasis following nutrient absorption. GLP-1 administration by continuous infusion or subcutaneous injection acutely normalizes elevated plasma glucose in the fasting and the fed state with low risk of hypoglycemia, lowers glucagon concentrations, delays gastric emptying of solid and liquid meals, reduces caloric intake, and causes weight loss in type II diabetic patients. GLP-1 treatment has been shown to improve beta cell function in aged and diabetic rats, suggesting that this mechanism may also improve glucose tolerance and potentially delay or prevent the progression of diabetes in humans with chronic treatment (Drucker, 2001; Kjems et al., 2003; Nourparvar et al., 2004; Holst and Orskov, 2004).

BMS-686117 is a potent 11-mer GLP-1 receptor agonist (Fig. 1). Following subcutaneous administration in dogs, BMS-686117 has a bioavailability of 93%, a short $t_{1/2}$ of ~2 h and mean residence time (MRT) of ~4 h. While this peptide clearly does not possess the adequate pharmacokinetic properties to be a QD subcutaneous product, clinical study has indicated that GLP-1 peptide blood level must be maintained to obtain the optimal therapeutic effect (Larsen et al., 2001). As a chronic use product for diabetic patients, the drug product is intended for self-administration and patient compliance is of great importance. Therefore, a QD formulation that delivers ~1 mg/ml dose, combined with a fine syringe needle (27 gauge or smaller) for subcutaneous injection, are highly desirable for clinical and marketing success.

Various drug delivery technologies have been developed for sustained release of peptides (Putney and Burke, 1998; Maa and Prestrelski, 2000; Shi and Li, 2005; Sadrzadeh et al., 2007). Common approaches include PEGylation (Schmidt et al., 2007; Nande et al., 2008; Yu et al., 2008), polymers depots (biodegradable microspheres, hydrogels) (Okada and Toguchi, 1995; Tamber et al., 2005; Mok and Park, 2008; Garbayo et al., 2008; Jin et al., 2008; Yu et al., 2008), polymeric micelles (Giddi et al., 2007; Wang et al., 2008; Xiong et al., 2008), complexation (Ye et al., 2006), or combination of the above. However, many of the above technologies are not suitable for the formulation requirement of BMS-686117. For a rela-

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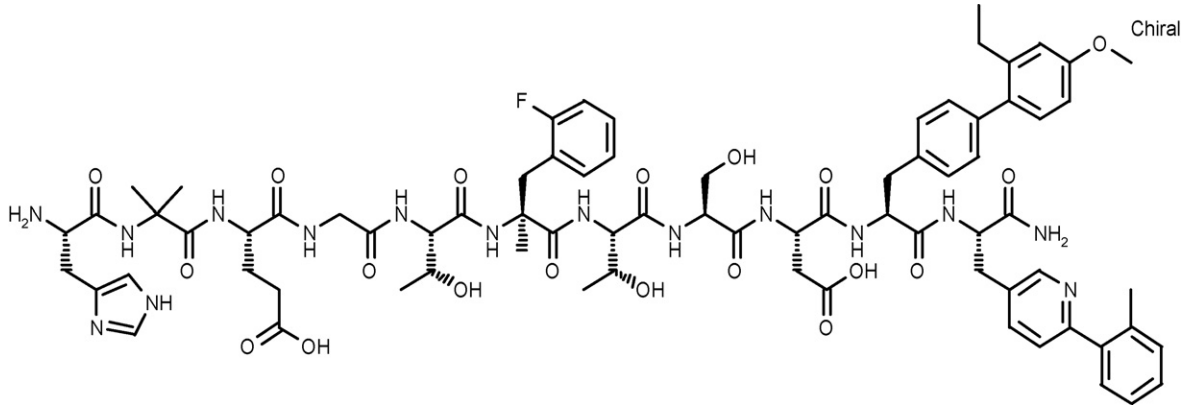


Fig. 1. Structure of BMS-686117, an 11-mer GLP-1 receptor agonist (MW: 1528.7).

tively small peptide like BMS-686117 (MW 1528.7), PEGylation with a long PEG chain (usually > 5 K (Hadziyannis and Papatheodoridis, 2003; Cooksley, 2005; Ye et al., 2006)) is very likely to shield the peptide from its binding site. Biodegradable polymeric systems, such as PLGA, are typically used for longer term sustained release (once monthly or longer) instead of QD; also the typical low drug loading (~10%) will increase the daily injection mass significantly and make the injection through fine needle much more difficult. In this paper, investigation of a zinc–peptide adduct formulation as a potential sustained release delivery system for BMS-686117 is reported. The adduct suspension is suspendable for months in aqueous medium, easy to inject through a 27 G needle and demonstrated sustained release profile *in vivo* in dogs. When the Zn/BMS-686117 adduct is encapsulated in a biodegradable polymer, a long-term sustained release formulation with a much decreased initial burst and an almost zero-order release profile can be developed.

2. Materials and methods

2.1. Materials

BMS-686117 was synthesized in house by the discovery chemistry division of Bristol-Myers Squibb Company. The peptide is a white, amorphous powder and no crystalline form has been identified. Zinc acetate and ethylenediaminetetraacetic acid (EDTA), were purchased from Sigma–Aldrich (St. Louis, MO). Poly(lactide-*co*-glycolide) (PLGA) polymer (Medisob 5050DL2A, uncapped, MW: 14K) was purchased from Alkermes (Cambridge, MA). HPLC grade solvents were purchased from EMD Chemicals (Bibbsons Town, NJ).

2.2. Apparent solubility of BMS-686117 in presence of zinc

Excess amount of BMS-686117 was suspended in pH 6.8, 50 mM phosphate buffer and placed on an orbital shaker (AROS 160, 100 rpm) at ambient temperature for 12 h. The suspension was then centrifuged and the supernatant was collected into 10 ml centrifuge tubes. Into the saturated BMS-686117 solution, zinc acetate solution (50 mM, pH 6.8 phosphate buffer) was added and a precipitate was formed. Concentration of free BMS-686117 in solution was determined by analyzing the clear supernatant using a validated HPLC method after centrifugation of the suspension. A plot of apparent solubility of BMS-686117 vs. Zn:BMS-686117 molar ratio is shown as Fig. 2A.

In order to investigate reversibility of Zn-peptide binding, excess amount of EDTA, a strong chelating agent for zinc, was added into the Zn/BMS-686117 suspension in pH 6.8, 50 mM phosphate buffer. The Zn/BMS-686117 suspension with and without EDTA was centrifuged, the supernatant was analyzed by HPLC.

The HPLC analysis utilized a Waters HPLC system (Waters 2690 Separations Module and Waters 996 Photodiode Array Detector, YMC ODS-AQ C18 column). A gradient method was used where solvent A is 0.05% trifluoroacetic acid (TFA, Sigma) in water, and solvent B is 0.05% TFA in acetonitrile. Wavelength of 220 nm was used to detect the absorption of BMS-686117.

2.3. Zn/BMS-686117 adduct formation and isolation

BMS-686117 and zinc acetate were dissolved separately in ethanol at concentration of ~30 mg/ml. The two solutions were

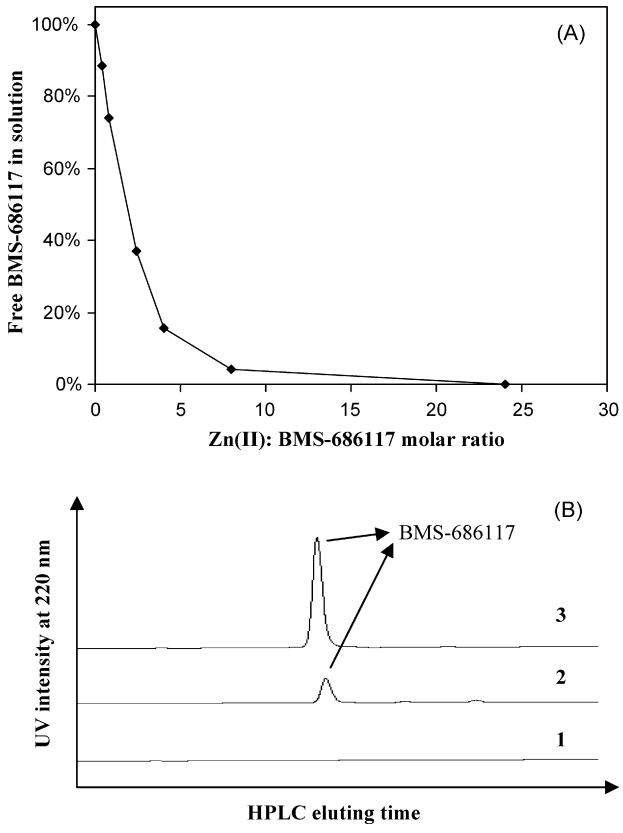


Fig. 2. (A) Free BMS-686117 in presence of Zn(II) at different molar ratios. (B) HPLC chromatographs from bottom to top: 1, Zn/BMS-686117 saturated solution; 2, free BMS-686117 standard solution (4.3μg/ml); 3, slurry of Zn/BMS-686117 and EDTA (buffer used: pH 6.8, 50 mM phosphate buffer, 25 °C).

mixed at pre-determined Zn:BMS-686117 molar ratio, and a white precipitate of Zn/BMS-686117 adduct was formed. To isolate the solid for different purposes, two methods were utilized. Method 1: the Zn/BMS-686117 ethanol suspension was poured into large volume of de-ionized water. The suspension was stirred for 2 h, then filtered and washed with large amount of water. The obtained solid Zn/BMS-686117 adduct was vacuum dried at room temperature for 48 h. Method 2: to produce Zn/BMS-686117 with controlled particle size, the ethanol suspension of Zn/BMS-686116 was spray dried using a Buchi B-191 mini-spray dryer (Brinkmann Instruments, Westbury, NY). Spray drying parameters were: inlet temperature 60 °C, solution pumping rate 3 ml/min, atomizing N_2 flow rate 500 Nl/h, aspirator 100%. The outlet temperature was maintained at 35 °C.

2.4. Physical characterization of Zn/BMS-686117 adduct

2.4.1. Atomic absorption study of Zn content in Zn/BMS-686117 adduct

Approximately 20–40 mg of Zn/BMS-686117 adduct was weighed and dissolved in 10 ml of DMSO. The samples were assayed using a PerkinElmer Optima 4300 ICP-AES instrument (Shelton, CT), with calibration standards of 1 and 5 ppm Zn, which were also prepared in DMSO. Results from two Zn wavelengths, 206.200 nm and 213.857 nm, have good agreement between them and the mean value was used.

2.4.2. Modulated differential scanning calorimetry (MDSC)

The glass transition temperature (T_g) of BMS-686117 and Zn/BMS-686117 adduct was determined by a TA DSC Q1000 Differential Scanning Calorimeter (New Castle, DE). Approximately 3 mg of sample was placed into an aluminum pan with crimpled lid; all samples were heated from 0 to 250 °C. The ramp rate was 2 °C/min, and modulates 0.32 °C every 60 s.

2.4.3. Scanning electron microscope (SEM)

A scanning electron microscope (Philips XL 30ESEM, FEI Philips, Hillsboro, OR) was used to study the morphology of the spray dried Zn/BMS-686117 powder. Spray dried powder was mounted on the aluminum stubs by double-sided tape and sputter coated with Pd (Pelco SC-7 Auto Sputter Coater). The SEM analysis was carried out at an accelerating voltage of 15–20 kV.

2.4.4. Particle size distribution and zeta potential measurement

Spray dried Zn/BMS-686117 particles were suspended in water, stirred by gentle hand shaking, and the particle size distribution was analyzed by a Horiba LA-910 laser scattering particle size distribution analyzer (Irvine, CA). Zeta potential was measured by a Zetasizer Nano ZS particle size/zeta potential analyzer (Malvern Instrument, Worcestershire, UK).

2.5. Pharmacokinetic study in dogs

This animal study was performed by Covance (Kalamazoo, MI) according to Standard Operating Procedures (SOPs). All procedures in the protocol and study specific procedures are in compliance with the Animal Welfare Act Regulations (9 CFR 3), and adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985). Briefly, the test formulations were administered to four purebred beagles male dogs subcutaneously through 21 gauge needle. Blood (approximately 2–3 ml) samples were collected from each animal pre-dose and at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h post-dosing. It's worth noting that the Zn/BMS-686117 suspension is easily injectable thorough a 27 G or finer needle. However, 21 G needles were required for some control formulations with high viscosity. To keep the dosing procedure consistent, 21 G needles were used for all formulation legs in this study.

BMS-686117 plasma concentrations were determined by liquid chromatography tandem mass spectrometry (LC/MS/MS) using a validated method. Briefly, the LC/MS/MS system included binary Shimadzu LC-10AD pump (Shimadzu Corp., Columbia, MD), a CTC PAL autosampler (Leap Technologies, Switzerland), and a Sciex API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). A Phenomenex Synergi C18 column, Fusion-RP, 2.0 mm × 50 mm, 4 μm (Phenomenex, Torrance, CA) was used for separation at a flow rate of 0.3 ml/min with a rapid gradient elution. Mobile phases consisted of 10 mM ammonium formate and 0.1% formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). ESI mass spectra were acquired in the positive ion mode with multiple reaction monitoring (MRM), multiple charged at ion pair of 765.1 → 195.2 and 740.7 → 210.0 for BMS-686117 and internal standard, BMS-501143, respectively. The plasma levels of BMS-686117 were calculated against a standard curve concentration, which were prepared in the same matrix. Peak area ratio was used for calculation and the calibration curve was fitted to a weighed $1/x^2$ linear regression model using Sciex softwareAnalyst version 1.2.

Pharmacokinetic parameters were determined using Kinetica™ software (Thermo Electron Corporation, Philadelphia, PA).

2.6. Preparation of PLGA microspheres by spray drying

BMS-686117 was dissolved together with poly(D,L-lactide-*co*-glycolide) (PLGA) with or without zinc acetate in DCM/EtOH (4/1, v/v). Concentration of total solid is 5%, w/v. The solution was spray dried with a Buchi B-191 spray dryer (Brinkmann Instruments, Westbury, NY) to generate microspheres. Spray drying parameters were: inlet temperature 50 °C, solution pumping rate 7 ml/min, atomization N_2 flow rate 500 Nl/h, aspirator 100%, outlet temperature 28 °C. Potency of the PLGA microspheres was determined by dissolving three samples in DMSO and analyzing by HPLC. The BMS-686117 encapsulation efficiency was 100%.

2.7. In vitro dissolution study of PLGA microspheres

For either PLGA formulation (with or without zinc acetate), approximately 10 mg of PLGA microspheres ($n = 3$) were suspended in 10 ml of pH 6.8, 50 mM phosphate buffer, and the suspensions were placed on an orbital shaker (90 rpm) under ambient conditions. At 1, 4 h and daily afterwards, the whole suspension was centrifuged and the supernatant was analyzed by HPLC. The microspheres were then re-suspended in 10 ml of fresh buffer to continue the release study until 18 days. The remaining PLGA microspheres were dissolved in DMSO completely and the residual BMS-686117 was analyzed by HPLC.

3. Results and discussion

3.1. Solubility of BMS-686117 in presence of Zn(II)

BMS-686117 is an amorphous powder with an apparent solubility of 17 μg/ml (pH 6.8, 50 mM phosphate buffer). With the addition of increasing amount of Zn(II) into BMS-686117 saturated solution, the free BMS-686117 concentration decreased continuously (Fig. 2A).

Solid Zn/BMS-686117 adduct made by precipitation and washing (Method 1) is an amorphous solid and is free of non-bound zinc salt. The maximal Zn:BMS-686117 ratio in the adduct was determined to be ~1.5:1 according to the atomic absorption study, regardless of the molar ratio of the starting materials.

The precipitated Zn/BMS-686117 (1.5:1) adduct showed an undetectable level of BMS-686117 in pH 6.8 phosphate buffer.

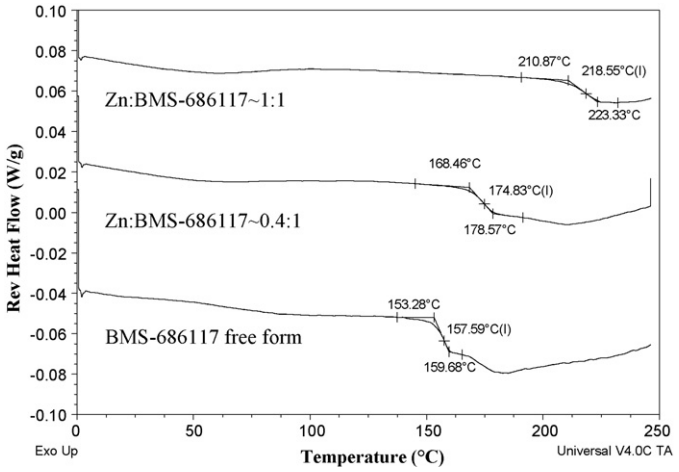


Fig. 3. Modulated temperature DSC results of Zn/BMS-686117 adducts with different Zn:BMS-686117 molar ratio (revising signals only).

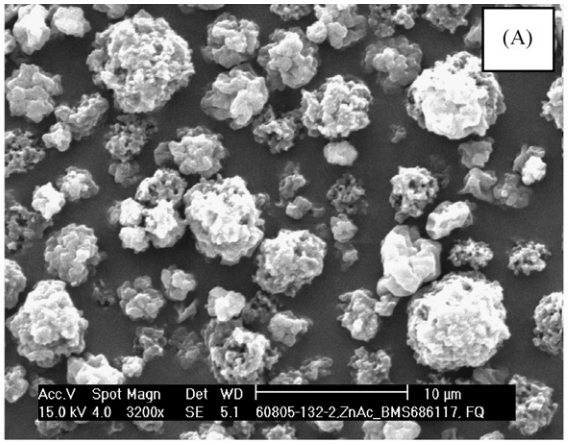
However, after excess amount of EDTA, which chelates strongly with Zn(II) (Nyborg and Peersen, 2004; Blanusa et al., 2005), was added into the Zn/BMS-686117 suspension, BMS-686117 was released and the solution concentration of BMS-686117 reached saturation level of 17 μg/ml (Fig. 2B). This result demonstrated that the binding between Zn(II) and BMS-686117 is reversible, which is a prerequisite for the Zn/BMS-686117 adduct to be able to release the free BMS-686117 and exert its therapeutic effect.

3.2. Molecular mobility of BMS-686117 in presence of Zn(II)

The Zn/BMS-686117 is an amorphous solid and its molecular mobility is qualitatively reflected by its glass transition temperature (T_g). As shown in Fig. 3, with the increase of Zn in the Zn/BMS-686117 adduct, the glass transition temperature (T_g) of the Zn/BMS-686117 increases. Free BMS-686117 has a T_g of 158 °C, while the adducts with 0.4:1 and 1:1 Zn:BMS-686117 ratio have elevated single T_g of 175 °C and 219 °C, respectively. No T_g of the free BMS-686117 was detected in the adducts, indicating that the Zn/BMS-686117 adducts were not merely physical blends of zinc salt and free BMS-686118, but a single phase entity. Since higher T_g is a result of lower molecular mobility, Zn(II) in the adducts appears to link and hold the BMS-686117 molecule together. This lower molecular mobility of Zn/BMS-686117 adduct is also consistent with its low solubility.

For an amorphous material like the Zn/BMS-686117 adduct, it is difficult to conclusively determine its solid state structure even by tools like solid state NMR. Except for the T_g measurement as discussed, we also performed solution NMR study to investigate the nature of zinc–peptide binding in solution (data not shown). Once Zn(II) was added to the BMS-686117 solution in deuterated DMSO/water (3/1, v/v), the observed proton shifts indicated that Zn is bound at the N-terminal region (Histidine) of BMS-686117. The coordinate complexation between Zn(II) and Histidine is well know and has been widely reported in structure biology, biochemistry, and protein formulation (i.e., zinc insulin) literatures (Christianson, 1991; Loomans et al., 1998; Miller and O'Dowd, 2000; Bonaccio et al., 2005; Enyedy et al., 2006). We hypothesize that similar complexation occurs between zinc and the Histidine of BMS-686117, although the exact solid state structure is elusive due to the amorphous nature of Zn/BMS-686117.

Furthermore, it was observed in the solution NMR study that the maximum chemical shift changes and NMR line broadening occur upon addition of 0.5 molar equivalent of Zn(II); and the NMR resonance line narrowing was observed on going from 0.5:1 to 1.5:1 zinc:BMS-686117 ratio. No further NMR resonance line narrowing was observed when zinc:BMS-686117 ratio increased from 1.5:1 to 2:1. These observation suggested that the Zn/BMS-686117 could be a dynamic complex, and there were more than one affinity sites on the peptide for Zn(II). The relationship between NMR proton shift/line broadening/narrowing and zinc–peptide ratio was also consistent with the earlier observation that the maximal binding ratio between zinc and BMS-686117 is about 1.5:1 (see "*Solubility of BMS-686117 in presence of Zn(II)*").



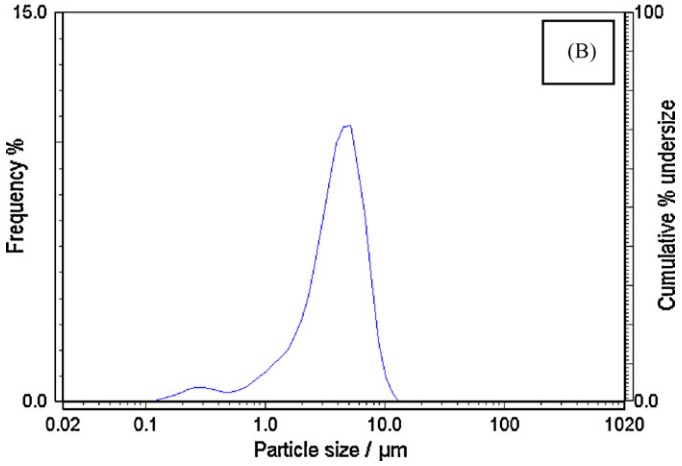


Fig. 4. (A) SEM picture of spray dried Zn/BMS-686117 adduct. (B) Particle size distribution of spray dried Zn/BMS-686117 adduct. D50 = 3.8 μm, D90 = 6.5 μm.

3.3. Morphology, zeta potential, and suspendability of spray dried Zn/BMS-686117

Spray dried Zn/BMS-686117 adduct from ethanol suspension are spherical agglomerates of fine powders in the sub-micron range (Fig. 4A). Adduct particles under SEM range from ~1 to 10 μm, consistent with the particle size in suspension measured by laser light scattering (D50 = 3.8 μm, D90 = 6.5 μm, Fig. 4B). The similar particle size distribution between solid and suspension states indicates that there was no further aggregation of the spray dried particles after being suspended in an aqueous medium. The aqueous suspension of the spray dried Zn/BMS-686117 is very homogenous and can be re-suspended even months after sedimentation. This could be partially due to the negative surface charge of the particles, i.e., −34.4 mV by zeta potential measurement. Particles in micron size range settle in water due to their gravity, and van der Waals attractive force could bind the particles together strongly (i.e., cannot re-suspend) when the particles are close enough to each other. However, accord-

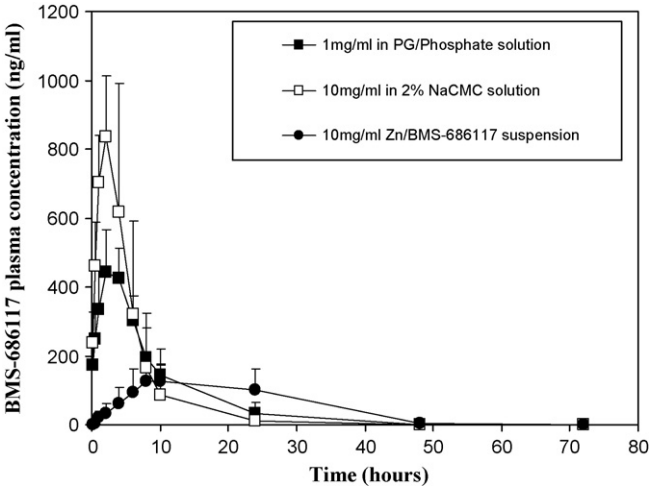


Fig. 5. BMS-686117 pharmacokinetic profiles following subcutaneous administration of different formulations. Doses are 0.1 mg/kg for PG/phosphate solution and 0.5 mg/kg for all the other formulations. The PK profile of PG/phosphate solution was normalized linearly to 0.5 mg/kg for comparison ($n = 4$ for all formulations).

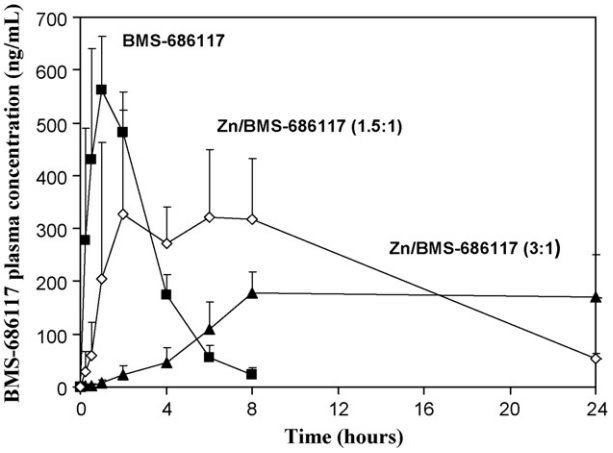


Fig. 6. BMS-686117 pharmacokinetic profiles following subcutaneous administration of BMS-686117 solution, Zn/BMS-686117 (1.5:1) suspension and Zn/BMS-686117 (3:1) suspension. Doses are 0.5 mg/kg for all formulations ($n = 4$ for all formulations).

ing to the DLVO theory (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948), a re-suspendable, flocculated suspension, such as the Zn/BMS-686117 suspension, could occur when the free energy of the system is held in the secondary minimum by the electrostatic (or steric) repulsive force. Targeting a self-administrated subcutaneous drug product, the small particle size, narrow size distribution, as well as the good suspendability, collectively allow fine syringe needles (27 gauge or smaller) to be used for the injection of the Zn/BMS-686117 suspension, which presents a significant advantage in patient compliance.

3.4. Dog PK profile following subcutaneous injection of Zn/BMS-686117

Three formulations were dosed in dogs subcutaneously in a cross-over fashion: formulation 1, BMS-686117 solution in 50% propylene glycol (PG) phosphate buffer (20 mM, pH 7.4). Formulation 2, high viscosity BMS-686117 solution in Tris buffer (100 mM, pH 8) containing 2% Sodium Carboxyl Methylcellulose (NaCMC); and formulation 3, suspension of spray dried Zn/BMS-686117 particles (3:1) in 0.2% methylcellulose (MC) aqueous solution (pH ~6). The *in vivo* PK profiles of the three formulations are shown in Fig. 5.

Systemic exposure of dogs to BMS-686117 was observed in all three formulations tested. Absorption of BMS-686117 following a single subcutaneous dose of BMS-686117 solution, i.e., formulations 1 and 2, was rapid with a higher C_{max} value of 461 ± 94 and 867 ± 223 ng/ml that were reached at approximately 2.5 h post-dosing, respectively. The mean residence time (MRT) of formulations 1 and 2 were 6.7 ± 2.7 and 3.9 ± 1.2 h; and terminal half-lives ($t_{1/2}$) were 3.9 ± 1.7 and 2.2 ± 0.7, respectively, indicating that these solution formulations are not suitable for QD administration and a high solution viscosity (formulation 2) did not help prolonging the release of the peptide. It has been reported that high C_{max} level of GLP-1 agonist drugs is linked with high incidence of nausea (Drucker, 2001; Elbrond et al., 2002) and is highly undesirable.

Unlike the solution formulations, Zn/BMS-686117 suspension (formulation 3) showed a significantly lower C_{max} of 140 ± 53 ng/ml, and a prolonged T_{max} of about 16 h. The MRT and terminal $t_{1/2}$ were also extended to 16.2 ± 0.5 h and 8.5 ± 0.6 h, respectively. The concentration at 48 h post-dose above the lower limit of quantitation (LLOQ, 2 ng/ml) was only observed after dosing the zinc adduct, again indicating the extended release of this formulation. Despite the slower release of BMS-686117, the *in vivo* exposure of this formulation is not compromised: as compared to both BMS-686117 solution formulations, the relative bioavailability of BMS-686117 after administering the zinc adduct reached about 80–90%, indicating a nearly complete absorption.

As verified by atomic absorption, the maximal Zn:BMS-686117 binding ratio is about 1.5:1, regardless of how much Zn(II) is in excess during the manufacturing. The excess Zn(II) is unbound and is readily soluble in water. Although the unbound Zn(II) should not decrease the molar mobility of BMS-686117, more Zn(II) in an aqueous environment could push the dissociation equilibrium of Zn/BMS-686117 to the opposite direction, thus further extending the release of free BMS-686117. The spray dried Zn/BMS-686117 particles used for the dog PK study in this work (Fig. 4) has excess amount of Zn(II), with Zn:BMS-686117 molar ratio at ~3:1. In a separate dog PK study (Fig. 6), it was observed that the PK profile can be continuously adjusted by varying the Zn:BMS-686117 molar ratio. A Zn/BMS-686117 with 1.5:1 ratio showed a PK profile in between that of the free BMS-686117 and the 3:1 Zn/BMS-686117 suspension. The flexibility of adjusting the PK profile with such simple manipulation is another advantage of this formulation approach.

3.5. In vitro dissolution of zinc containing PLGA microspheres

Two BMS-686117 containing (10%) PLGA microsphere formulations were prepared by spray drying, one with 5% zinc acetate while the other without. The two formulations showed comparable particle size of about 3–5 μm under SEM (Fig. 7). The surface of the zinc containing microspheres appears to be rough, presumably due to the existence of the zinc salt particles. As confirmed by HPLC analysis, both PLGA microspheres have 100% drug loading efficiency during the spray drying process.

In vitro release of both PLGA microspheres was followed for 18 days (Fig. 8). Both microsphere formulations showed sustained release profiles over this time range. The zinc containing PLGA microspheres showed a minimal initial burst (i.e., release in the first hour) of 3%, while the microsphere without zinc showed a greater initial burst of about 16%. In the remaining time, the zinc containing PLGA microspheres demonstrated an almost zero-order sustained release over the 18 days, while the control of release rate from the microspheres without zinc is obviously less consistent. After 18 days, the remaining PLGA microspheres were analyzed for the remaining BMS-686117 amount. A mass balance calculation showed that for both microspheres, a majority of ~90% of BMS-

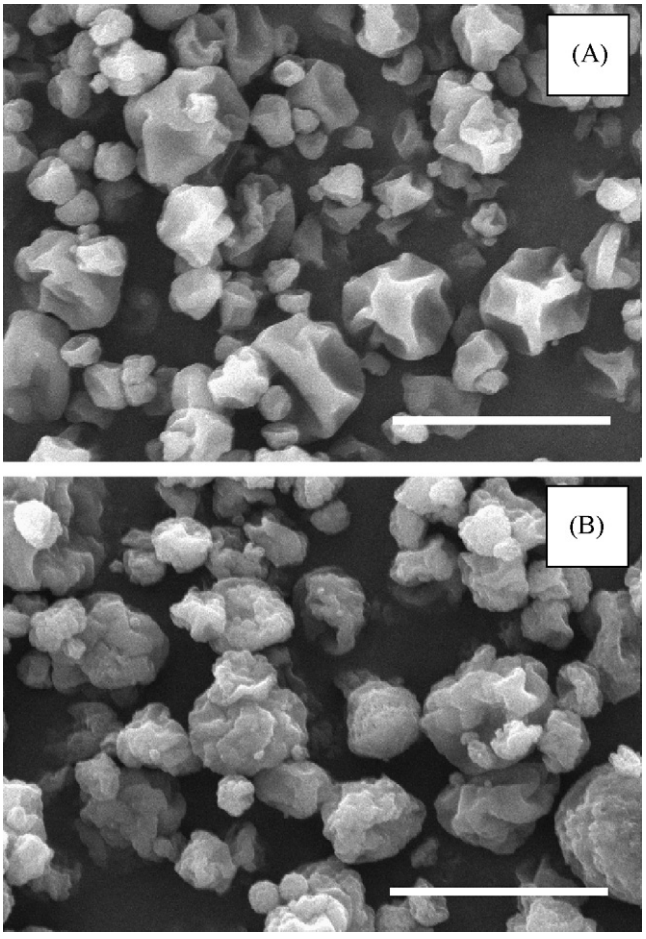


Fig. 7. SEM images of spray dried PLGA microspheres without (A) and with (B) zinc acetate (scale bar: 10 μm).

686117 is accounted for (released or remaining) after the release study.

This study demonstrated that by combining approaches of zinc adduct formation and PLGA encapsulation, it is possible to develop a long-term (once-a-month or longer) sustained release formulation with optimized release profile.

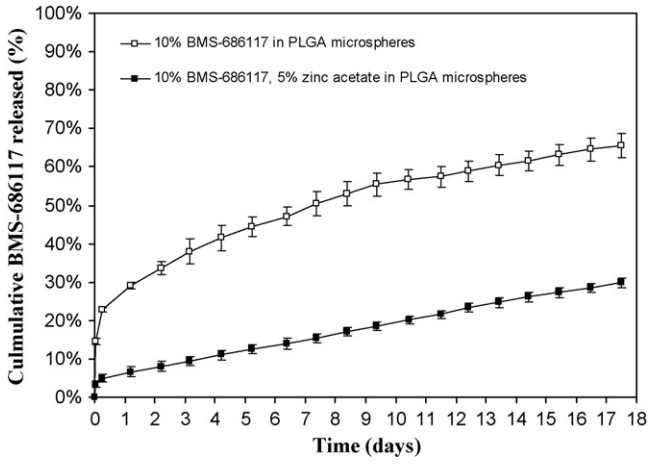


Fig. 8. BMS-686117 *in vitro* release from PLGA microspheres ($n = 3$) containing either BMS-686117 alone or Zn/BMS-686117. Dissolution medium: 10 ml, pH 6.8, 50 mM phosphate buffer.

4. Conclusion

Zn(II) forms a poorly water soluble adduct with the BMS-686117 peptide. The binding between Zn(II) and BMS-686117 is reversible, and the release of BMS-686117 can be tuned by varying the Zn:BMS-686117 ratio. Spray dried Zn/BMS-686117 (Zn:BMS-686117 = 3:1) particles form uniform and re-suspendable suspension, and demonstrated a PK profile *in vivo* in dogs that is adequate for once-daily dosing. A long-term sustained release formulation with minimal initial burst and constant release rate can be achieved by incorporating Zn(II) into PLGA microspheres. This work demonstrated a simple, effective and scalable formulation approach, which could potentially convert a peptide with short pharmacokinetic $t_{1/2}$ into a feasible drug product.

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