Preparation of PEGylated Exendin-4-loaded PLGA Microspheres for Enhanced Stability and Anti-diabetic Activity

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ABSTRACT SUMMARY
Exendin 4 (Ex4, subsequently renamed exenatide) has a potent efficacy for the treatment of type 2 diabetes under the trade-name of Byetta®, because of its short half-lifes twice-daily injections are still required. Then, in order to develop an improved Ex4 delivery system for the safe and effective delivery of bioactive Ex4 using extended release dosage forms without peptide acylation and immunogenicity, PEGylated Ex4 was encapsulated into PLGA microspheres by w/o/w double emulsion solvent evaporation method. The results obtained showed that the stability of Ex4 was greatly improved by PEGylation.

INTRODUCTION
Ex4 is a 39 amino acid peptide found in venom from the Gila monster Heloderma suspectum, and is considered to be a potent therapy for type 2 diabetes patients. However, Ex4 has short half-lifes and it required twice-daily injection. Several experiments developed the encapsulation of some synthetic peptides into PLGA microspheres with the aim of sustained peptide delivery and reduced dosage frequency. However, PLGA is reduced activity by acylation with by-products and peptides owing to degradation of PLGA. We recently proposed to develop an improved Ex4 delivery system for the safe and effective delivery of bioactive Ex4 using extended release dosage forms without peptide acylation and immunogenicity.

EXPERIMENTAL METHODS
Chemical conjugations to lysine residues (Lys27) of Ex4 by mPEG-SPA (Mw: 5, 20 kDa) were carried out using previously published method.1 The reaction was then stopped and the positional isomers of Lys27-PEG5k-Ex4 and Lys27-PEG20k-Ex4 (PEG-Ex4 analogues). To confirm stability of PEGylation against various environmental factors, proteolytic stabilities of PEG-Ex4 analogues were performed in the rat plasma. Receptor-binding affinities were found to be closely related to the conjugated PEG Mw.

RESULTS AND DISCUSSION
The purified PEG5k-Ex4 and PEG20k-Ex4 was confirmed by MALDI-TOF MS. For the stability of Ex4, the t1/2 of PEG5k-Ex4 and PEG20k-Ex4 was 3.8- and 6.4- fold greater than that of Ex4, respectively, in the rat plasma. Receptor-binding affinities were found to be closely related to the conjugated PEG Mw.

The PLGA microspheres were observed by FE-SEM. From these micrographs, it was seen that they were spherical with a smooth surface.

As expected, depending on the materials, we obtained a peptide encapsulation efficiency of 57.4–94.7% and the particle size of 10.7–14.6 μm (Table 1). Both type of PEGylated Ex4 loaded PLGA microspheres, PEG5k-Ex4 had an encapsulation efficiency of 94.7% and a uniform particle size of 11.2 μm ± 1.9 μm, were selected for subsequent in vitro and in vivo experiments.

Table 1. PLGA microspheres formulation and physico-chemical characterization (n=3).
The results obtained from the in vitro release studies indicate that both types of microspheres were significant differences. The sustained-release profile of PEG5K-Ex4 was achieved with a minimal burst of about 10.8% of the total loadings released at day 1 and above 93.7% of loadings released up to 18 days (Figure 1).

Figure 1. Ex4 and PEG5K-Ex4 in vitro cumulative release profiles from the PLGA microspheres (n=3).

After the PLGA polymers had been incubated with PEG5K-Ex4, these results became subject to dramatic changes. Results demonstrates that PEGylation can stabilize Ex4 against the acylation occurred in PLGA polymer degradations. The rate of adsorbed peptide occurred during PLGA polymer degradation was investigated to determine if such PEG5k-Ex4 could inhibit adsorption more effectively than Ex4. These results indicated that PEGylation might prevent the acylation of free amino group by steric hindrance of the PEG chains attached to lysine residues of Ex4.

Figure 2. RP-HPLC chromatogram of native and acylated products from PLGA polymer degradations (A,B); Relative amounts of ionic interacted peptide and acylated products after incubation with PLGA polymers (C,D).

The PEGylation of Ex4 reduced its immunogenicity by almost 40%, suggesting that PEGylation represents a suitable simple strategy to eliminate the immunogenicity of these Ex4.

Figure 3. Anti-Ex4 specific titers obtained by ELISA. Columns, SD for 4 animals were used for each group. **, p < 0.001, *, p < 0.05.

CONCLUSION
This study demonstrated that site-specific PEGylated Ex4 had enhanced proteolytic stability. Its enhanced stability, biological potency and eliminated acylation highlight its potential as a source for a PLGA microsphere delivery system for weekly dose of Ex4 to treat type 2 diabetes mellitus.

REFERENCES