Potential of Site-specific PEGylated Exendin-4 Modified with a High-molecular Weight Trimeric Polyethylene Glycol for Effective Type 2 Anti-diabetic Therapy

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ABSTRACT SUMMARY
Exendin-4 (Ex4) is viewed as a potent therapeutic peptide for type 2 diabetics, but its short circulating life (4-6 h) means that high doses must be administered frequently. PEGylation is playing an increasingly important role in the production of enhanced peptide and protein drugs delivery systems. This study was to optimize an Exendin-4 (Ex4) site-specific PEGylation method with a high-molecular weight trimeric PEG.

INTRODUCTION
PEGylation of peptides and proteins increases molecular sizes, shields their proteolytic sites, and masks their immunogenic sites. This process can prolong in vivo pharmacokinetics, and diminish the immunogenicities of peptides and proteins, which in turn, enhances therapeutic efficacy and reduce undesirable effects versus their non-PEGylated counterparts.

Site-specific PEGylation is an attractive approach for maximizing the therapeutic value of drugs, because this process generates only PEGylated isomers with optimized properties. In recent years, pharmaceutical technology of protein site-specific PEGylation has greatly improved with genetic engineering and various functional PEG analogues. Accordingly, site-specific PEGylation has become a generally adopted procedure. The major benefit of site-specificity is its increased production yields and, therefore, significantly reduced production costs.

Exendin-4 (Ex4-Cys) is viewed as a potent therapeutic peptide for type 2 diabetics, but commercial exendin-4 (Exenatide, Byetta) must be injected by diabetic patients at least twice a day because of its short in vivo lifetime. This has led to main drawbacks of less than optimal clinical compliance and poorer quality of life. Reduction of the required frequency of s.c. injections is one way that would significantly enhance compliance. It has been reported that continuous exenatide therapy reached compliance of 100%. Exendin-4 is 39 amino acids long, unlike GLP-1, which has 30 amino acids. Because of its nonmammalian (lizard) origin and unique C-terminal sequence, exendin-4 may induce immunogenic response in humans. In the present study, we prepared C-terminal specific PEGylated Ex4-Cys using trimeric PEG (C40-tPEG-Ex4-Cys) and then explored its physico-chemical and biological characteristics. Furthermore, the pharmacokinetic and anti-diabetic characteristics of C40-tPEG-Ex4-Cys were investigated and compared using a diabetic mouse model. Ex4-Cys acts as a GLP-1 receptor agonist for treatment of type 2 diabetes mellitus. We hypothesize that the site-specific PEGylation can increase the therapeutic potential of this diabetes drug.

EXPERIMENTAL METHODS
We prepared C-terminal specific PEGylated Ex4 using trimeric PEG and then explored its physicochemical and biological characteristics.
We describe the preparation of C-terminal specific PEGylated Ex4 (C40-tPEG-Ex4), which was performed using cysteine and amine residue specific coupling reactions using Ex4-Cys and activated trimeric PEG. The biological activities of PEG-Ex4 analogs were determined using competitive receptor-binding assays in the steady state using RIN-m5F cells. The pharmacokinetic and anti-diabetic characteristics of C40-tPEG-Ex4 were investigated and compared using a diabetic mouse model.

RESULTS AND DISCUSSION

The C40-PEG-Ex4 obtained at high yields (~ 83 %) and characterized by MALDI-TOF mass spectrometry. The receptor binding affinity of C40-PEG5K-Ex4 was 3.5-fold higher than that of N-terminal PEGylated Ex4 (Nter-PEG5K-Ex4), and receptor binding by the trimeric PEG (tPEG; 23, 50 kDa) adduct was much higher than that of branched PEG (20 kDa). Furthermore, C40-tPEG50K-Ex4 was found to have greater blood circulating t1/2 and AUCinf values than native Ex4 by 7.53 and 45.61-fold, respectively. Accordingly, its hypoglycemic duration was much greater than that of native Ex4 at 59.2 h, at a dose of 25 nM/kg (native Ex4 7.3 h). The results of this study show that C-terminal specific PEGylation using trimeric PEG is effective when applied to Ex4, and suggest that C40-tPEG50K-Ex4 has considerable potential as a type 2 anti-diabetic agent.

CONCLUSION

We prepared C-terminal specific PEGylated Ex4-Cys, which was performed using Ex4-Cys and activated trimeric PEG (C40-tPEG-Ex4-Cys). C-terminal of Ex4-Cys is an optimal site for GLP-1 receptor binding. High-molecular-weight trimeric PEG (tPEG23K,50K) shows reduced steric hindrance and increasing type 2 antidiabetic therapeutic effects of Ex4-Cys. Importantly, the longevity of C40-tPEG50K-Ex4-Cys in vivo, investigated by pharmacokinetics and pharmacodynamics, suggests that it has therapeutic potential as an effective long acting GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus.

REFERENCES