Smart PEGylation: Enabling Extended Half-life without Loss of Bioactivity

Kang Choon Lee
School of Pharmacy, SungKyunKwan University, Suwon, 440-746, Korea
kclee@skku.edu

ABSTRACT SUMMARY
PEGylation is a commonly utilized technique to improve drug solubility and stability, prolong blood circulation time, reduce immunogenicity, and decrease dosing frequency. As with any form of molecular modification, the active site is affected and can drastically decrease the bioactivity of the therapeutic agent, especially when the modification is performed on a small molecular weight molecule like a peptide. Therefore, it is generally accepted that a balance must be struck between the molecular weight of the PEG and the activity of the therapeutic molecule to reach sufficient drug efficacy. The smart PEGylation technique introduced here offers tremendous benefits over branched and non-PEGylated forms of peptides and proteins. Specifically, this abstract focuses on the smart PEGylation of potent therapeutic peptides for type 2 diabetes, GLP-1 and exenatide.

INTRODUCTION
PEGylation is the covalent binding of poly(ethylene glycol) (PEG) to molecules or materials. It is a commonly utilized technique for bioactive and therapeutic molecules (proteins, peptides, enzymes, antibody fragments, oligonucleotides, small synthetic drugs, etc.) that are limited by poor physiochemical and pharmacokinetic properties. After covalent attachment of PEG, bioactive molecules can have prolonged circulation half-lives, improved drug solubility and stability, reduced immunogenicity and as a result decreased dosing frequency. PEG provides the biomolecule with protection from proteolysis and autoimmune responses. These positive advantages have made PEGylation a common method in the pharmaceutical industry. Over 10 PEGylated drugs have been approved by the FDA to date. However, steric hindrance from high molecular weight PEG can lead to a dramatic loss in the biological and pharmacological activity of the molecules. The higher the molecular weight, the lower the bioactivity. With smart PEGylation a uniquely shaped PEG is used during PEGylation that offers tremendous benefits over traditional high molecular weight linear or branched PEG. The novel PEGylation platform incorporates PEG shape as an equally important factor in PEGylation as the PEG molecular weight and the specific binding site.

GLP-1 receptor (GLP-1R) agonists such as GLP-1 or exenatide (Ex4) are viewed as potent therapeutic peptides for type 2 diabetes. Yet due to the short circulating half-life, high doses of the drug must be administered frequently which gives rise to concentration-dependent adverse effects. By applying smart PEGylation to GLP-1R agonists, not only is a long half-life achieved, but also the glucose-lowering efficacy is extended. In turn, this can lead to weekly dosing frequency with low adverse effects and overall provide higher patient compliance.

In this abstract, the smart PEGylation of Ex4 is compared with traditionally branched PEGylated Ex4. The pharmacokinetics in rats and the pharmacodynamics in diabetic mouse models are compared.

EXPERIMENTAL METHODS
C-terminal specific PEGylated Ex4 using smart PEGylation is synthesized and compared based on its in pharmacokinetic and pharmacodynamics profiles. We describe the preparation of C-terminal specific PEGylated Ex4, Olaedin, which was performed using cysteine 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 Olaedin were investigated and compared with dimer PEGylated Ex4 using rat and diabetic mouse models.

RESULTS AND DISCUSSION
The Olaedin obtained at high yields (> 80 %) and characterized by MALDI-TOF mass spectrometry. The receptor binding by the trimeric PEG (tPEG; 23, 50 kDa) adduct was higher than that of linear and branched PEG (20, 40 kDa). Furthermore, Olaedin was found to have greater blood circulating t1/2 and AUCinf values than native Ex4 by 7.5 and 45.6-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 Olaedin has considerable potential as a type 2 anti-diabetic agent.

CONCLUSION
Smart PEGylated Ex4-Cys, which was performed using Ex4-Cys and activated trimeric PEG showed significantly improved activity over traditionally PEGylated Ex4-Cys both in the pharmacokinetic and pharmacodynamics profiles. C-terminal of Ex4-Cys is an optimal site for GLP-1R binding. High-molecular-weight trimeric PEG shows reduced steric hindrance and increasing type 2 antidiabetic therapeutic efficacy. Overall, smart PEGylated Ex-4 has therapeutic potential as an effective long-acting GLP-1R agonist for the treatment of type 2 diabetes. Smart PEGylation can be a platform technology to extend the half-life while preserving the biological activity of therapeutics.

REFERENCES