Analysis of Absorption and Excretion Route of Efpeglenatide Using Radiolabeled [125I]-CA-Ex4 Efpeglenatide, [125I]-IgG4 Fc Efpeglenatide and [14C-PEG] Efpeglenatide

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ABSTRACT
Efpeglenatide is composed of CA-Ex4 conjugated to recombinant human insulinomab G4 Fc fragment through a non-peptidyl linker. Efpeglenatide has an extended pharmacokinetic (PK) profile with enhanced pharmacodynamic (PD) properties through unique LAPSOVERY conjugation and through CA-Ex4’s hyperglycemic superagonistic property on the GLP-1 receptor. The absorption and the excretion profiles of Efpeglenatide were evaluated in Rats and following intravenous (IV) or subcutaneous (SC) administration of radiolabeled efpeglenatide at the dose of 24 nmol/kg. The radioactivity was labeled on three different positions: [125I]radiolabeling on CA-Ex4 and IgG4 Fc fragment and [14C]radiolabeling on the non-peptide linker PEG. The level of radioactivity in serum and excreta was determined based on liquid scintillation counting or gamma counting methods. The bioavailability (BA) was in the range from 94% to 98% and long terminal elimination half-life was 14.7 ± 2.4 hr. The radioactivity profile of HF-PEG demonstrated the radiolabeled Efpeglenatide. Following both IV and SC administration of Efpeglenatide labeled with [125I] or [14C], the excretion profiles were qualitatively similar with a fast initial elimination and a slower subsequent process until the end of study. The major route of excretion was via the kidneys with a total recovery of radioactivity at Day 21 of 64.9%. The amount of dose eliminated via feces after [125I] Efpeglenatide administration was slightly higher than [14C] Efpeglenatide (10%, 7%, 10%). In conclusion, radioactivity of [125I] and [14C] Efpeglenatide exhibited similar excretion profiles with BA (80%-85%) and showed long terminal half-lives, and the urinary excretion was the major route of elimination.

BACKGROUND
Efpeglenatide is a long-acting glucagon-like peptide-1 receptor agonist under development for the treatment of type 2 diabetes. Efpeglenatide is the site-specific conjugate for [125I]-CA-Ex4 and the constant region of human insulinomab G4 fragment linked via a non-peptidyl 3.4 Da PEG linker which is based on a novel strategy LAPS/OVERY.

STUDY OBJECTIVE
To evaluate the pharmacokinetic and excretion profiles of differently radiolabeled Efpeglenatide in vivo.

METHODS
Materials
Three differently labeled Efpeglenatide molecules were used in this study. The labeled sites were as follows:
- [125I]radiolabelling on Incapulinomab G4 (IgG4 fragment) and [14C]radiolabelling on the non-peptide linker PEG (FH-PEG).
- The level of radioactivity in serum and excreta was determined based on liquid scintillation counting or gamma counting methods.
- [125I]radiolabelling on the constant region of human insulinomab G4 fragment (Fc) and [14C]radiolabelling on the non-peptide linker PEG (FH-PEG).

Study Design
For PK study, rats were assigned to total of 6 groups (n=3). Each group received [125I] CA-Ex4, [125I]-IgG4 Fc and [14C]-PEG Efpeglenatide via IV or SC administration. For excretion balance study, rats were divided into 6 groups (n=3). Each group received [125I] CA-Ex4, [125I]-IgG4 Fc and [14C]-PEG Efpeglenatide via IV or SC administration.

Dosing
Efpeglenatide was intravenously (IV) and subcutaneously (SC) administered at the dose of 24 nmol/kg. The radioactivity dose of [125I] labeled Efpeglenatide was approximately 238 MBq; the radioactivity dose of [14C] labeled Efpeglenatide was 353 kBq/kg.

Sample collection
Serum/feces samples of about 0.5 mL were collected from each animal at 0.5, 1, 2, 6, 12, 24 and 48 hr intervals up to 504 hr post dose for [125I] and [14C] labeled Efpeglenatide. Urine and feces samples were collected from each animal with 24 hours intervals up to 504 hr post dose for labeled Efpeglenatide and up to 648 hr post dose for [14C] labeled Efpeglenatide. Concentration depending on each group were collected 504 hr for [125I]-labeled Efpeglenatide at 648 hr for [14C]-labeled Efpeglenatide.

Quantification of [125I]radiolucency in samples, was measured by gamma counting and [125I]radioactivity was measured by Liquid Scintillation Counting (LSC) using Packard analyzers. The data were processed as mean ± standard deviation (SD).

RESULTS
Pharmacokinetic profile of Efpeglenatide
- "High bioavailability, a long half-life and high stability in the system were observed"
Underlying Superagonistic Mechanisms of Efpeglenatide in Glycaemic Control and Weight Loss Potency

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ABSTRACT
Efpeglenatide is a long-acting GLP-1 receptor (GLP-1R) agonist developed for the treatment of type 2 diabetes mellitus (T2DM). It consists of an engineered long-acting GLP-1R agonist and human Fpg covalently coupled as a prodrug moiety. At previously reported efficacies, efpeglenatide demonstrates superior glycemic control and weight loss than dulaglutide and liraglutide. The present study aimed to elucidate the underlying mechanisms of efpeglenatide in the regulation of glycemic control and weight loss.

METHODS
Non-linear mixed-effects modeling was employed to assess the pharmacodynamic responses to efpeglenatide and other GLP-1RAs across various dose levels in mice. The pharmacodynamic models were fitted to the concentration-response data. The pharmacodynamic interactions among efpeglenatide and other GLP-1RAs were assessed using the group-mean effects model.

RESULTS
Efpeglenatide showed significant lower glycemic and weight loss compared to other long-acting GLP-1RAs.

CONCLUSIONS
Efpeglenatide shows superior glycemic control, weight loss and lower glycemic response compared to other long-acting GLP-1RAs. These results suggest that the superagonistic effect of efpeglenatide in reducing glycemia and weight loss is due to multiple mechanisms, including improved receptor binding affinity, higher receptor internalization, and enhanced receptor signaling.

REFERENCES

FURTHER INFORMATION
This study was supported by a grant of the Korea Drug Development Fund R&D Project (KDOF201304-05).

Figure 1. In vitro activity of CA-Exendin-4
(a) CA-Exendin-4 weight loss
(b) CA-Exendin-4 glycemic control

Figure 2. Receptor kinetics for GLP-1 receptor

Figure 3. GLP-1 receptor internalization by GLP-1RAs

Figure 4. Glucose lowering and body weight loss efficacy in a human weekly microergic condition in diabetic and obese animal models

Figure 6. Benefits of efpeglenatide on β-cells in a late T2DM model mice

Figure 7. β-cell preservation

Figure 8. GLP-1R agonists internalization compared to long-acting GLP-1RAs

Superganostic activity

CA-Exendin-4 possesses superagonist property derived from fast dissociation for GLP-1 receptor.

Efpeglenatide (CA-Exendin-4 analogue) Fast dissociation Compared to other GLP-1RAs

Continuous activation by less dissociation

In vivo efficacy of efpeglenatide vs. other GLP-1RAs

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