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

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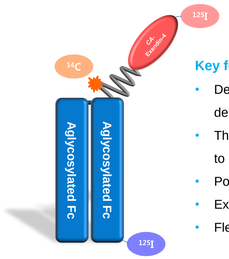
ABSTRACT

Efpeglenatide is composed of *CA*-Exendin-4 chemically conjugated to recombinant human immunoglobulin G4 Fc fragment through a non-peptidyl linker. Efpeglenatide has an extended pharmacokinetic (PK) profile with prolonged pharmacodynamic (PD) action through its unique LAPSCOVERY conjugation and through *CA* Exendin-4's hypothesized super-agonistic pharmacologic properties on the GLP-1 receptor. The absorption and the excretion profiles of Efpeglenatide were evaluated in a rat model following intravenous (IV) or subcutaneous (SC) administration of radiolabeled Efpeglenatide at the dose of 24 nmoles/kg. Efpeglenatide was labeled on three different positions: [^{125}I]-radiolabeling on *CA*-Exendin-4 and IgG4 Fc fragment, and [^{14}C]-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 81 to 88% and long terminal elimination half-lives (47-56h) were the most pronounced PK characteristics of radiolabeled Efpeglenatide. Following both IV and SC administration of Efpeglenatide labeled as [^{125}I-*CA*-Exendin-4] and as [^{125}I-IgG4 Fc], the excretion profiles were qualitatively similar with a fast initial elimination up to 7 to 9 days and a subsequent slower process until the end of study. The major route of elimination was via the kidneys (78-84%) with a total recovery of radioactivity at Day 21 of 96-99%. The amount of dose eliminated via feces after [^{14}C]-Efpeglenatide administration was slightly higher than [^{125}I]-Efpeglenatide (18% vs. 7-10%). In conclusion, radioactivity of [^{125}I]-and [^{14}C]-Efpeglenatide exhibited similar absorption profiles with BA (81-88%) 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 conjugated form of *CA* Exendin-4 and the constant region of human immunoglobulin G4 fragment linked via a non-peptidyl 3.4 kDa PEG linker which is based on a novel strategy LAPSCOVERY.



Key features

- Decrease food intake and increase glucose-dependent insulin secretion
- The long half-life and slow absorption lead to low peak-trough ratio
- Potent HbA1c reduction & body weight loss
- Excellent Gastrointestinal Tolerability
- Flexible Dosing Strategy (Weekly to Monthly)

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 following:
[^{125}I]-radiolabeling on *CA*-Exendin-4
[^{125}I]-radiolabeling on immunoglobulin G4 (IgG4) Fc fragment
[^{14}C]-radiolabeling on the non-peptide linker PEG

- **Study Design**

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

- **Dosing**

Efpeglenatide was intravenously (IV) and subcutaneously (SC) administered at the dose level of 24 nmoles/kg. The radioactivity doses of [^{125}I]-labeled Efpeglenatide were approximately 200 kBq/kg; the radioactivity dose of [^{14}C]- labeled Efpeglenatide was 263 kBq/kg.

- **Sample collection**

· Serial blood samples of about 0.5 mL were collected from each animal at 0.5, 1, 3, 6, 12, 24 and 24 hr intervals up to 504 hr post dose for [^{125}I]- and [^{14}C]- labeled Efpeglenatide.
· Urine and feces samples were collected from each animal with 24 hr intervals up to 504 hours post dose for [^{125}I]-labeled Efpeglenatide and up to 648 hour post dose for [^{14}C]-labeled Efpeglenatide.
· Carcass samples were collected at 504 hour for [^{125}I]-labeled Efpeglenatide and at 648 hour for [^{14}C]-labeled Efpeglenatide.

- **Quantification of Efpeglenatide**

[^{125}I]-radioactivity in samples was measured by gamma counting and [^{14}C]-radioactivity was measured by Liquid Scintillation Counting (LSC) using Packard analyzers. The data were presented as mean ± standard deviation (SD).

RESULTS

Pharmacokinetic profile of Efpeglenatide

" High bioavailability, a long half-life and high stability in the system were observed "

Figure 1. Pharmacokinetic profiles following IV and SC administration at a dose of 24 nmol/kg in rats (*n=3*)

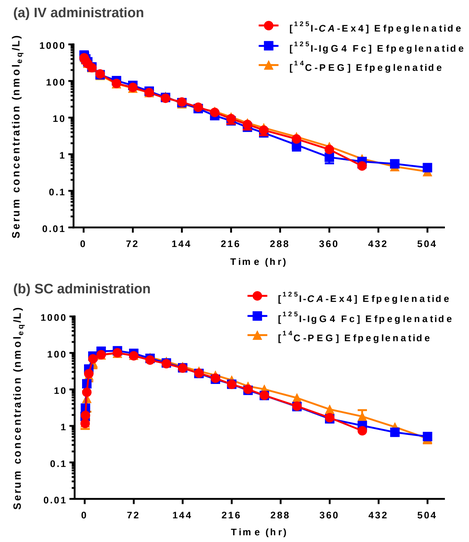


Table 1. Pharmacokinetic parameters following IV and SC administration at a dose of 24 nmol/kg in rats (*n=3*)

Radiolabeled Efpeglenatide	Routes	AUC_{inf} (hr*nmol$_{eq}$/L)	C_0 or C_{max} (nmol$_{eq}$/L)	T_{max} (hr)	$t_{½}$ (hr)	BA (%)
[^{125}I-*CA*-Ex4]	IV	15900 ± 1190	452 ± 39.9	-	49.1 ± 4.1	-
[^{125}I-IgG4 Fc]	IV	16700 ± 1100	559 ± 17.8	-	47.7 ± 7.3	-
[^{14}C-PEG]	IV	15900 ± 733	562 ± 38.9	-	58.2 ± 1.7	-
[^{125}I-*CA*-Ex4]	SC	12800 ± 565	101 ± 6.7	48.0 ± 0.0	47.1 ± 1.8	81
[^{125}I-IgG4 Fc]	SC	14300 ± 1140	116 ± 11.7	40.0 ± 13.9	53.3 ± 4.2	86
[^{14}C-PEG]	SC	14000 ± 1320	99.2 ± 0.8	56.0 ± 13.9	55.8 ± 3.6	88

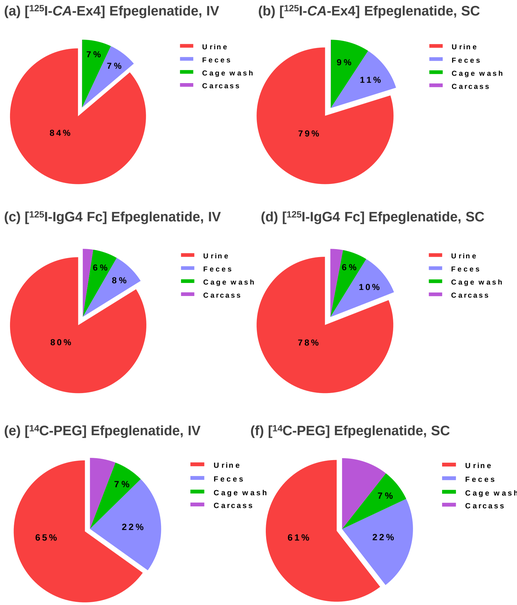
p> 0.05; in AUC and C_{max} of Radiolabeled Efpe. analyzed by one-way ANOVA, Mean ± SD

- The slow absorption with long half-life (47.1-55.8 hr) and high BA (81-88%) of [^{125}I]- and [^{14}C]-labeled Efpeglenatide after SC administration
- The pharmacokinetic profiles of the three differently labeled Efpeglenatide were similar.

Excretion profile of Efpeglenatide

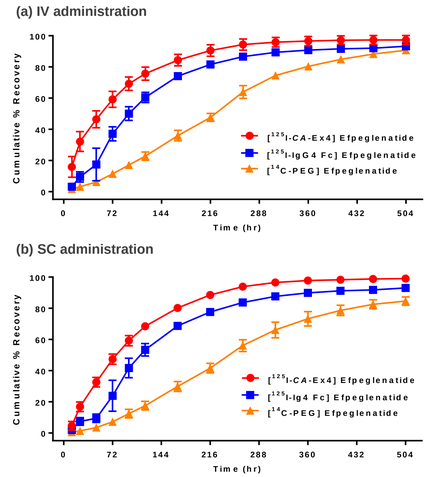
"Fragments of Efpeglenatide were mainly excreted in urine"

Figure 2. Summary of excretion in rats (*n=3*)



- Fragments of the [^{125}I-*CA*-Ex4] Efpeglenatide and [^{125}I-IgG4 Fc] Efpeglenatide were mainly excreted by urine (83-91%) and the range of total recovery of radioactive doses was 96-99%.
- In terms of fragments of [^{14}C-PEG] Efpeglenatide, the amounts of radioactivity excreted were 61-65% in urine and 22% in feces. The total recovery was 91-93% of the administered doses.

Figure 3. Excretion of radioactivity in excreta and cage wash (24 nmol/kg, *n=3*)



CONCLUSION

- **When differently labeled on three comprising moieties, the absorption and elimination profiles were well characterized and comparable indicating that the compound is stable in the body.**
- **Fragments of Efpeglenatide were cleared from the system via urine and the urinary excretion was the major route of elimination.**
- **In conclusion, this study demonstrated that Efpeglenatide has favorable pharmacokinetic properties with a high bioavailability and a long half-life ensuring the prolonged PD effect and a satisfactory elimination profile.**

REFERENCES

1. David Parkes et al. *Drug Development Research*, 2001, 53:260-267
2. L.L. Nielsen and A.D. Baron. *Current Opinion in Investigational Drugs*, 2003, 4:401-405