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Bioanalysis and Pharmacokinetic Profile of FE 203799: A Novel Glucagon-Like Peptide-2 (GLP-2) Long Acting Peptide Agonist

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FERRING PHARMACEUTICALS

OBJECTIVES

- GLP-2 is a 33-amino acid peptide that is released from intestinal L-cells following nutrient-ingestion
  - It is known to stimulate intestinal growth, nutrient absorption and mesenteric blood-flow
- Native GLP-2 has a short elimination half-life (6.4 and 7.2 min in rats and humans, respectively) and high clearance (24.6 and 6.8 ml/min/kg in rats and humans, respectively)
  - In part due to susceptibility of GLP-2 for the dipeptidyl peptidase-IV (DPP4) enzyme
- A DPP4 resistant analog of GLP-2, teduglutide, has marginally lower clearance (9.9 and 2 ml/min/kg in rats and humans, respectively) than GLP-2
- Herein, we describe The pharmacokinetics (PK), protein binding, and in vivo efficacy of a novel GLP-2 agonist, FE 203799, which retains potency and selectivity at the hGLP-2 receptor but has a superior PK/pharmacological profile.

METHODOLOGY

Peptide Synthesis: Peptides were synthesized by solid phase peptide synthesis and purified by RP-HPLC.

Rat, Monkey and Minipigs Pharmacokinetics (PK)

The jugular vein was used for compound administration (IV PK) and the carotid artery was used for blood sampling (IV and SC PK). Dosing solutions prepared in 25 mM phosphate buffer, pH 7.4 with no NaCl. PK parameters were determined by non-compartmental analysis using PK solutions and WinNonLin software.

Rat: Dosing solutions were administered subcutaneously (SC) or intravenously (IV) by bolus injection to catheterized male Sprague Dawley rats (~0.3 kg). Blood was collected at multiple time points up to 5 h post-injection (IV) and 103 h post-injection (SC).

Monkey: Dosing solutions were given SC/ IV by bolus injection to male cynomologous monkeys (~5.4 kg). Blood was collected at multiple time points up to 6 h post-injection (IV) and 80 h post-injection (SC).

Minipig: Dosing solutions were administered SC or IV by bolus injection to catheterized male Yucatan pigs (~67 kg). Blood was collected at multiple time points up to 10 h post-injection (IV) and 169 h post-injection (SC).

Plasma Protein Binding (PPB)

PPB was measured using TRANSIL® 96 wells plates containing immobilized human albumin and alpha-1-acid glycoprotein beads (at physiological ratio of 24:1). The final concentration and volume for incubation (RT, 10 min) in the wells were 2.5 uM and 450 ul (max DMSO 5%), after which samples are transferred to new plates containing the internal standard. This was followed by LC/MS/MS analysis.

Bioanalysis

The blood samples collected were processed on ice to isolate plasma, then flash frozen and stored at -20ºC. Bioanalysis was conducted using protein precipitation followed by electrospray ionization LC/MS/MS methods (AB Sciex API4000 MS, Shimadzu Prominence HPLC (CBM-20A) and SIL-20ACHT Autosampler). The gradient HPLC method involved reverse-phase column (Phenomenex Jupiter 00B-4053-B0, 50x2.0mm, 5um, 300A, C_{18}) and mobile phase (A: 0.01% TFA and 1.0% Formic acid in water, B: 0.01% TFA and 1.0% Formic acid in 70% ACN) at a flow rate of 0.5 ml/min.

Rat Pharmacodynamics (Small Intestine Growth)

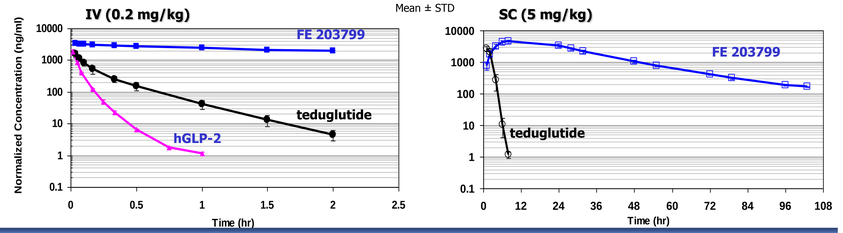
Compounds or vehicle were administered (dose-range 0.3 -1000 nmol/kg) by SC injection to male Sprague Dawley rats (body weight 235-275 g). Compounds were dosed daily for five days, the rats euthanized, and small intestines were carefully dissected, cleaned, and weighed.

BIOANALYSIS: Method Precision and Accuracy

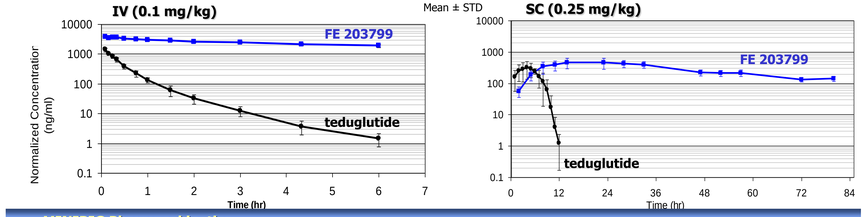
FE 203799 intra-run data	QC L (ng FB/mL)	QC ML (ng FB/mL)	QC MH (ng FB/mL)	QC H (ng FB/mL)
Run 1	4.47	43.0	838	3460
Run 2	4.46	42.0	843	3740
Run 3	4.62	36.7	804	3460
Run 4	3.99	38.2	842	3450
Run 5	3.92	40.7	802	3730
Run 6	3.55	39.3	826	3420
Nominal (ng FB/mL)	4	40	800	3600
Observed Mean (ng FB/mL)	4.17	40.0	826	3543
Standard Deviation	0.1	0.0	18.3	40.1
Precision (%CV)	2.9	0.0	2.2	1.1
Accuracy (%Error)	4.2	0.0	3.2	-1.6

RESULTS

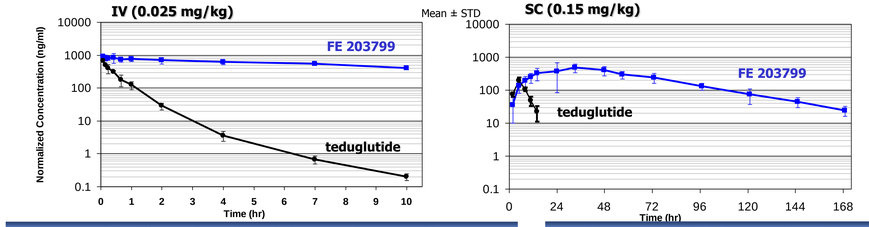
RAT Pharmacokinetics



MONKEY Pharmacokinetics



MINIPIG Pharmacokinetics



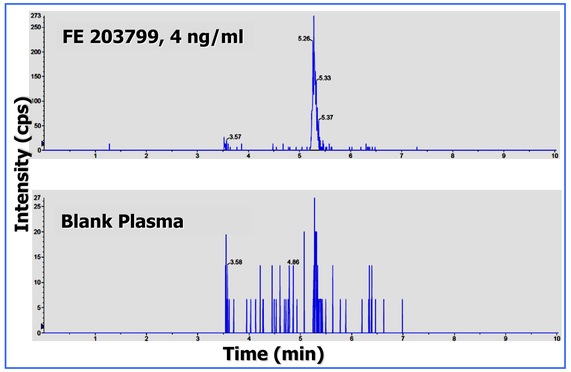
Summary of Key PK Parameters in Different Species

Peptide	Rat PK				Monkey PK				Minipig PK			
	IV Dose		SC Dose		IV Dose		SC Dose		IV Dose		SC Dose	
	Elimination half-life (min)	CL (ml/kg/min)	Terminal half-life (min)	Bioavailability (%)	Elimination half-life (min)	CL (ml/kg/min)	Terminal half-life (min)	Bioavailability (%)	Elimination half-life (min)	CL (ml/kg/min)	Terminal half-life (min)	Bioavailability (%)
Dose (mg/kg)	0.2	0.2	5	5	0.1	0.1	0.25	0.25	0.025	0.025	0.15	0.15
FE 203799	159	0.27	1349	54	474	0.046	1941	32	782	0.032	1808	43
teduglutide	18.7	9.9	31.3	74	43	2.4	56	77	88*	0.99*	164	53
hGLP-2	6.4*	24.6*	21.4*	27.5*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

* hGLP-2 dose: IV 1 mg/kg, SC 2 mg/kg

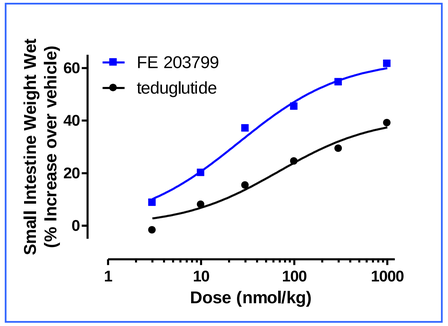
* tedulgutide IV dose: 0.1 mg/kg

BIOANALYSIS: LC/MS/MS Chromatogram of Plasma Extracts

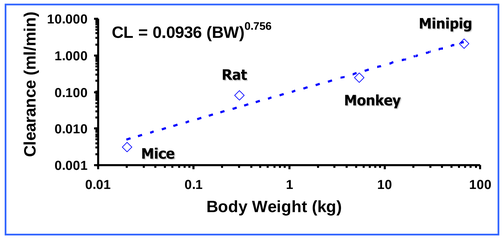


Superior Rat PK Translates to Greater In Vivo Efficacy

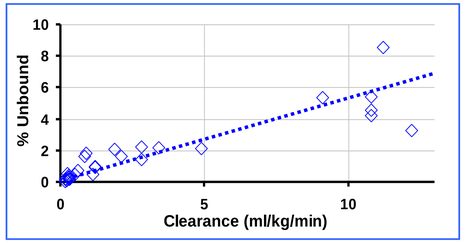
Rat PD (Small Intestinal Growth). Dose Response of FE 203799 and teduglutide, 24 h Dosing Interval.



Interspecies Allometric Scaling of Clearance (FE 203799)



PPB versus Rat IV Clearance Correlation



CONCLUSIONS

- **Methodology:**
  - The bioanalysis methods were precise, accurate and reliable
  - The PPB in vitro method was reproducible and the % unbound drug showed good correlation with the observed clearance
  - Multispecies experiments showed that FE203799 possesses a favorable PK profile
- **Properties of FE 203799:**
  - A novel peptide analog of hGLP-2 with potency/selectivity at the hGLP-2 receptor comparable to native hGLP-2
  - Unique PK profile with a very low systemic clearance and long elimination half-life
  - Unprecedented prolonged SC half-life in multiple species
    - Flip-flop pharmacokinetics observed, possibly due to solubility limiting SC absorption
  - Greater in vivo potency for stimulation of intestinal growth in rats compared to tedulglutide
- **The above properties of FE 203799 may confer it a superior therapeutic profile for the treatment of GI diseases**