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Pharmacological characterization of FE 203799, a novel long acting peptide analog of glucagon-like peptide-2 (GLP-2)

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FERRING PHARMACEUTICALS

1. Introduction

Glucagon-Like-Peptide-2 (GLP-2) is a 33 amino acid peptide derived from posttranslational processing of proglucagon in intestinal L-cells. GLP-2 is released into the circulation following nutrient ingestion and acts at distinct G protein coupled GLP-2 receptors that are primarily expressed in the small intestine and colon in a localized manner. GLP-2 has pronounced biological actions in the intestine resulting in potent stimulation of intestinal growth and beneficial effects to promote healing and maintain intestinal epithelial integrity under a variety of conditions. Hence, GLP-2 agonists have therapeutic potential to treat gastrointestinal disorders. [1-3]

Native GLP-2 has rapid clearance and a short half-life, limiting its development as a therapeutic agent. A dipeptidyl peptidase IV resistant analog of GLP-2 (teduglutide, h[Gly^2]GLP-2 (1-33)) with somewhat lower clearance than GLP-2 is in clinical trials. [4,7]

Through chemistry SAR, we identified GLP-2 analogs with low clearance resulting in longer half-life and greater duration of action. Herein we report on the characterization of FE 203799, a novel peptide analog of GLP-2 that was identified through these efforts. FE 203799 was directly compared to hGLP-2 and teduglutide.

2. Experimental Methods

Peptide Synthesis

Peptides were synthesized by solid phase peptide synthesis and purified by reverse phase HPLC.

In Vitro Receptor Assays

Activity of peptides at the GLP-2 receptor and selectivity vs. GLP-1 and glucagon (GCGR) receptors were determined using cell-based functional assays. HEK-293 cells transiently or stably co-transfected with human GLP-2, GLP-1 or GCGR receptor and a reporter plasmid containing a luciferase gene under the control of cAMP responsive elements were incubated with compounds for 5 hours, followed by lysis and determination of luciferase activity.

The respective native ligand was run as a control in each assay. Efficacies, expressed as maximum possible effect (MPE%) of native ligand and EC_{50} were determined by non-linear regression.

Rat Pharmacokinetics (PK)

Dosing solutions were administered subcutaneously (SC) or intravenously (IV) by bolus injection to catheterized male Sprague Dawley rats (carotid artery for blood collection and the jugular vein for compound administration in IV PK studies). Blood was collected at multiple time points up to 5 hours post-injection (IV PK) and 103 hours post-injection (SC PK).

Plasma samples were analyzed for test peptide concentration using standard extraction and LC/MS methods. PK parameters were determined by non-compartmental analysis.

Rat Pharmacodynamics (Small Intestine Growth)

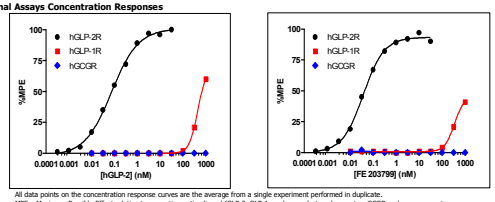
GLP-2 agonists are known to have pronounced intestinotrophic activity.[1-3] Intestinal growth in rats was measured as a primary pharmacodynamic endpoint. Compounds or vehicle were administered by SC injection to male Sprague Dawley rats (body weight 235-275 g). Compounds were tested at dose intervals of 24 h (once daily for 5 days) and 48 h (two injections at times 0 and 48 h) and the rats euthanized 96 h after the first dose. The compounds were also tested after a single injection with the rats euthanized 72 h post dose (72 h interval) and after 96 h (96 h interval). After sacrifice, the gastrointestinal tract was excised and the small intestines were carefully dissected, cleaned, and weighed. Intestinal wet weight was normalized to body weight and is reported as % increase over corresponding vehicle group run in the same study.

In the once daily dosing studies, rats received a range of doses (0.3 -1000 nmol/kg) to generate a complete dose response curve. ED_{50} and efficacies (fitted maximum response, %Max) were determined using a modified three-parameter Hill equation.

3. Results

In Vitro Pharmacological Profile

Cell-Based Functional Assays Concentration Responses



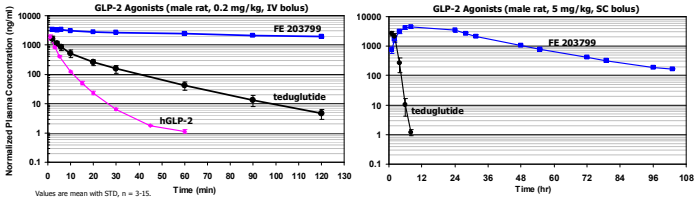
All data points on the concentration response curves are the average from a single experiment performed in duplicate.
MPE – Maximum Possible Effect relative to respective native ligand (GLP-2, GLP-1 or glucagon) at each receptor; GCGR – glucagon receptor

hGLP-2R Cell Based Functional Assay Potency and Efficacy					
Peptide	MeanEC_{50} (nM)	EC_{50} (95% CI)	Efficacy (%MPE)	Selectivity Ratio vs. GLP-1^	N
FE 203799	0.03	(0.03-0.04)	95	>28,000	3
teduglutide	0.09	(0.07-0.11)	98	>11,000	7
hGLP-2	0.07	(0.06-0.10)	100	7,059	7

^Selectivity ratio was calculated as the mean EC_{50} at the hGLP-1R divided by the mean EC_{50} at the hGLP-2R
MPE – Maximum Possible Effect relative to GLP-2; CI – Confidence Interval

Rat Pharmacokinetics

Plasma Concentration Time Course Plots



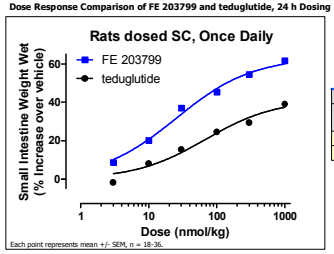
Values are mean with STD, n = 3-15.

Rat PK Parameters					
Peptide	Elimination half-life (minutes), IV	Terminal half-life (minutes), SC		SC bioavailability (F_{SC}%)	
	0.2 mg/kg	1 mg/kg	5 mg/kg	1 mg/kg	5 mg/kg
FE 203799	159	701	1349	74	54
teduglutide	18.7	30.9	31.3	63	74
hGLP-2	6.4	Not tested			

Values are mean, n = 3-15.

Rat Pharmacodynamics (Small Intestine Growth)

Dose Response Comparison of FE 203799 and teduglutide, 24 h Dosing Interval

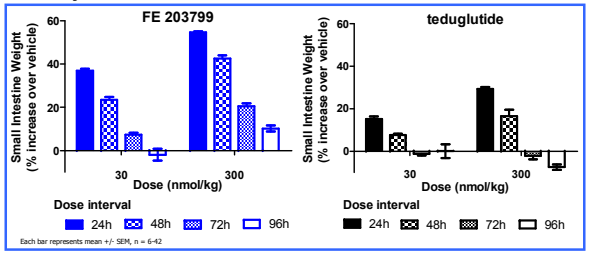


Each point represents mean +/- SEM, n = 18-36.

Intestinal Growth Potency and Efficacy			
Peptide	ED_{50} (nmol/kg)	ED_{50} (95% CI)	Efficacy (%Max)^
FE 203799	25.4	(20.6-30.4)	63%
teduglutide	68.2	(39.4-97.0)	41%

^% increase intestinal wet weight over vehicle treated

Variable Dosing Interval



Each bar represents mean +/- SEM, n = 6-42

4. Summary and Conclusions

- FE 203799
  - Novel peptide analog of GLP-2.
  - Potency and selectivity at the hGLP-2 receptor comparable to the native ligand (hGLP-2).
  - Unique PK profile of very low clearance contributing to an unprecedented prolonged half-life.
  - Greater in vivo potency and sustained duration of action to achieve pharmacodynamic efficacy for stimulation of intestinal growth in rats compared to teduglutide.
- These properties of FE 203799 may confer a superior therapeutic profile for the treatment of gastrointestinal diseases.

5. References

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