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Discovery of Novel and Long Acting GLP-1 Analogs

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Introduction

Glucagon Like Peptide-1 (GLP-1) is a 30 amino acid gut hormone produced by intestinal L cells and pancreatic α cells. GLP-1 induces post prandial glucose-dependent insulin secretion and inhibits gastric secretion and motility, thus reducing circulating glucose levels and increasing satiety. These physiological effects make GLP-1 an interesting target for both diabetes and obesity therapy. However, GLP-1 lacks the therapeutic potential due to its very short half-life. Described are our efforts using ortholog screening and fragment substitution to discover novel and long acting GLP-1 analogues with modifications in the GLP-1 sequence,

HGEGTFTSDLTEYLEEEAVR**E**FIEWLKNGGPKKIRYS-NH_2.

These analogs have desired potency and efficacy over endogenous GLP-1 with a 15h duration of action in an acute food intake reduction mouse model.

Results and Discussion

The World Health Organization estimates that as of 2008, 347 million people have diabetes mellitus [1] and by 2030, type II diabetes mellitus (T2DM) is projected be one of the leading non-communicable causes of death [2]. Comorbidities of T2DM include heart disease, fatty liver disease, hypertension and, importantly, obesity. While the physiological effects make GLP-1 therapy an interesting target for both diabetes and obesity, infusion studies in healthy subjects show rapid degradation of GLP-1 by the enzyme DPP-4 making the endogenous peptide unsuitable as a therapy. Exenatide (Byetta) and Liraglutide (Victoza) are stabilized versions of native GLP-1 and have shown utility in T2DM. Sitagliptin (Januvia) and Linagliptin (Tradjenta), inhibitors of DPP-4, are also marketed agents for T2DM. In addition Liraglutide is being investigated for obesity. Herein we describe our efforts in the discovery of novel, stable GLP-1 mimetics for the treatment of T2DM and obesity.

Infusion studies in healthy subjects demonstrate GLP-1(7-36) t½ to be around 2 minutes [3], due to rapid degradation by the enzyme DPP-4. DPP-4 cleaves dipeptide segments from the *N*-terminus of polypeptides containing a proline or alanine in the 2 position. Known GLP-1 cleavage products of DPP-4 and NEP (Neutral Endopeptidase) [5] are reasonable starting points for modification to enhance the stability of GLP-1 analogs.

Exendin-4, a uniquely stable peptide with a considerably longer half-life in human plasma [4] is a good example of the benefits of the ability to withstand DPP-4 degradation as it contains a glycine in the 2 position of the *N*-terminus. While one could attempt to stabilize hGLP-1 through analogs to address the DPP-IV and NEP cleavage sites, our search for a stabilized GLP-1 mimetic began with the selection of a series of GLP-1 orthologs from various species. The thought being that nature has preselected acceptable modifications. Selected Orthologs (Table 1) were prepared and screened in a melanophore assay using hGLP-1 as a standard. Orthologs with confirmed potency were then screened in an overnight acute food intake reduction mouse model (6h and 15h time points) using Exendin-4 as a control. The overnight food intake reduction model was employed as a crude measure of stability, making the assumption that a longer duration of action would require greater stability in general.

It has been suggested that the *C*-terminal tail portion of Exendin-4 is partially responsible for its plasma stability. To investigate the importance of the tail region, analogs of G-2 modified human 3-36 GLP-1 were synthesized with the Exendin-4 tail (PSSGAPPPS-NH_2), Cane Toad tail (*Bufo Marinus*, PKKQRLS-NH_2), and African Clawed Frog tails (*X. Laevis* 1A and 1B, PSKEIIS-NH_2, PKKIRYS-NH_2 respectively). The analogs prepared with both the toad and frog tails outperformed the analog with the Exendin-4 tail at the 15h time point in the overnight food intake assay, suggesting enhanced peptide stability (Table 2).

A group of 5 GLP-1 orthologs with either desired potency or potential stability was chosen as the basis of a strategy in which each peptide was divided into 5 amino acid segments. The *C*-terminal tail portion was selected from *X. Laevis* 1b GLP-1 (PKKIRYS-NH_2). A set of 23 novel analogs were then constructed by combining a mixture of the 5 amino acid segments. The first 5 amino acids were maintained as HGEGT as protection from DPP-4. The peptides were screened in both the melanophore assay and overnight food intake assay. Chimeric peptides 12 and 21 were then selected and used in a two-fold strategy: 1) Improve potency of peptide 12 and maintain stability and 2) Improve stability of peptide 21 and maintain potency via single point mutations in the peptide sequence. Chimeric peptides 25, 26, and 27 were then scaled and run in a 14 day DIO rat model to determine the ED_{50} of the optimized GLP-1 analogs.

While endogenous GLP-1 lacks the stability to be used as an effective therapy, the introduction of modifications to the peptide sequence gleaned from natural GLP-1 orthologs is a highly effective way to generate analogs of GLP-1 which have a superior stability profile. The incorporation of two 5 amino acid sections (11-15 and 21-25), the KKIRYS-NH_2 tail from *X. Laevis* and a single E21 modification yielded a GLP-1 peptide analog with apparent protection from NEP and DPP-4. The novel peptide 27 (Chimera-12, E21) demonstrated a pEC_{50} of 10.5 and a duration of action greater than 15h in an acute DIO mouse model (Table 3). The improvements translate to a 0.21 mg/kg/day and a calculated weight loss at the ED50 of 13.5% compared to the 0.08 mg/kg/day and 8.4% calculated weight loss demonstrated by Exendin-4 in the 14 day DIO rat model (Figure 1).

Table 1. Selected orthologs.

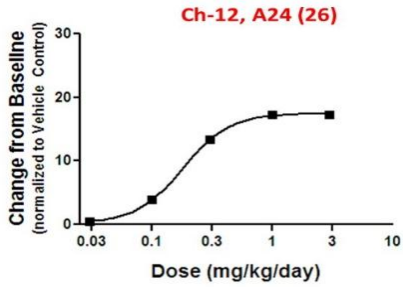
Sequence							Species
HAEGT	FTSDV	SSYLE	GQAAK	EFIAW	LVKGR		Human
HGEGT	FTSDL	SKQME	EEAVR	LFIEW	LKNGG	PSSGAPPPS-NH2	Exendin
HSEGT	FTNDV	TRLLE	EKATS	EFIAW	LLKGL		Platypus
HGEGT	YTSDI	SSYLQ	DQAAQ	NFVAW	LKSGQ		F.Minnow
HGEGT	YTNDV	TEYLE	EKATK	AFIEW	LIKGK		X.Laevis 1b

Table 2. Tail scanning.

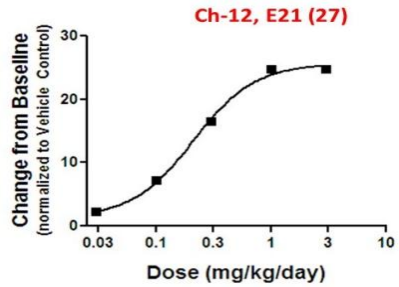
Sequence	pEC50	6h	15h
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR**PKKIRYS-NH2**	9.9	-44	-20
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR**PSKEIIS-NH2**	10.4	-48	-21
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR**PKKQRLS-NH2**	10.3	-53	-22
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR**PSSGAPPPS-NH2**	10.7	-60	-11

Table 3. Chimeras.

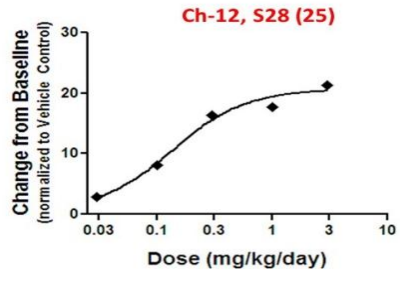
ID		Single point changes in Ch12	pEC_{50}	6h	15h
12	Ch-12	HGEGT FTSDL TEYLE EEAVR AFIEW LKNGG PKKIRYS-NH2	9.8	-97	-83
21	Ch-21	HGEGT YTNDV SKQLE EEAVR EFIAW LKSGQ PKKIRYS-NH2	11.4	-56	-30
25	**Ch-12, S28**	HGEGT FTSDL TEYLE EEAVR AFIEW LK**S**GG PKKIRYS-NH2	**10.4**	**-74**	**-67**
26	**Ch-12, A24**	HGEGT FTSDL TEYLE EEAVR AFI**A**W LKNGG PKKIRYS-NH2	**10.0**	**-96**	**-79**
27	**Ch-12, E21**	HGEGT FTSDL TEYLE EEAVR **E**FIEW LKNGG PKKIRYS-NH2	**10.5**	**-94**	**-79**



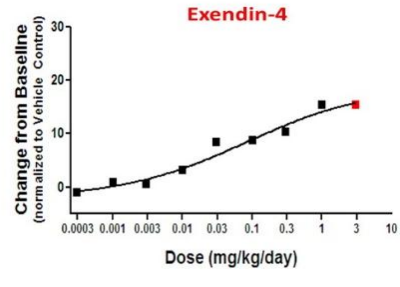
ED 50 Value (mg/kg/day)	Calculated Weight Loss at ED50 (% ΔΔ)
0.179	8.84



ED 50 Value (mg/kg/day)	Calculated Weight Loss at ED50 (% ΔΔ)
0.211	13.52



ED 50 Value (mg/kg/day)	Calculated Weight Loss at ED50 (% ΔΔ)
0.137	10.64



ED 50 Value (mg/kg/day)	Calculated Weight Loss at ED50 (% ΔΔ)
0.082	8.42

Fig. 1. In vivo results body weight / ED_{50} data (DIO rat).

References

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