Controlled Delivery Depots of Liraglutide, A GLP-1 Analogue, via Subcutaneous Injection

Michael Sekar, William Van Osdol, **Su IL Yum**, Felix Theeuwes, Keith Branham*,Whitney B Moro* and John Gibson*

Pharmaceutical Systems Research and Development, DURECT Corporation, Cupertino, CA, and *Lactel Absorbable Polymers, Birmingham, AL

Abstract

Durect reports the controlled delivery of liraglutide, a biologically active GLP-1 analogue, over durations up to 14 days using an aqueous formulation for subcutaneous injection. Pre-formulation studies of solubility and stability showed that the liraglutide, could be combined with custom-synthesized, biodegradable polymers of varying composition, and molecular weight. These peptide-polymer mixtures were lyophilized to dry powders, and subsequently suspended in a purely aqueous vehicle, (DURECT Depot formulations). Such suspension formulations were evaluated for physical and chemical stability, and for controlled release *in vitro*. In addition, a PK study was conducted in Sprague-Dawley rats, utilizing a bioanalytical method based on ELISA. Aqueous suspensions performed at a controlled rate with C_{Max}/C_{14d} < 4. Bioavailability of liraglutide in the suspension formulations was nearly 100% relative to a subcutaneous bolus of the peptide in aqueous solution. Aqueous suspension of this peptide-polymer complex can deliver active liraglutide, up to 14 days, without the need of additional additives that comprises an *in situ* forming, rate controlling depot. These results can be extended to larger non-clinical models and to man, and show promising delivery performance for peptide and protein therapeutics.

Introduction

- Liraglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist used clinically to treat type 2 diabetes mellitus
- It is composed of 32 amino acids and palmitic acid, and has a M_W of 3751 Da
- Liraglutide is currently marketed as a once-daily, subcutaneously administered solution, with each mL containing 6 mg of active drug, and daily maintenance doses of 1.2 mg or 1.8 mg
- The development of an injectable depot formulation of liraglutide with a dosing interval extended to 7 or 14 days could provide several benefits
- Rapid injectability through 23 G needle (or finer); injection volume < 500 µL
- Possible benefits might include:
  - Fewer injections
  - Lower likelihood of missed doses
  - Less fluctuation in plasma concentrations, possibly resulting in more stable plasma glucose levels

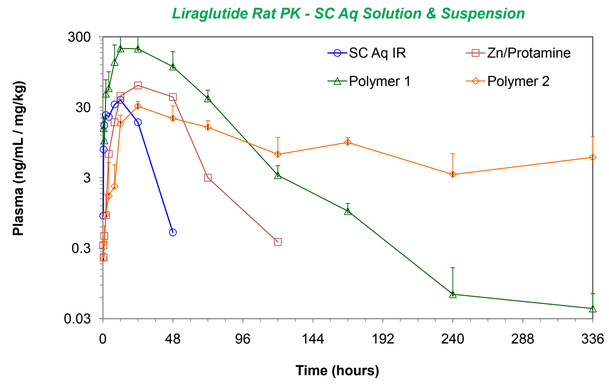
		His 7	*Ala*	*Glu 9*	*Gly*	*Thr*	*Phe*	*Thr*	*Ser*	*Asp*
										Val
		Ala	*Ala*	*Gln*	*Gly*	*Glu*	*Leu*	*Tyr*	*Ser*	*Ser*
Palmitoyl	*Glu*	*N^6 Lys*								
		Glu	*Phe*	*Ile*	*Ala*	*Trp*	*Leu*	*Val*	*Arg*	*Gly*
										Arg
										Gly 37

Rat PK Study Design

- Male Sprague Dawley rats, 5 animals / treatment group
- Each treatment group administered one of the following as a single subcutaneous injection:
  - Aqueous solution of liraglutide (20 mg/mL)
  - Aqueous suspension of liraglutide-polymer complex with target liraglutide concentration of 20 mg/mL, incorporating one of 3 polymers:
    - Zn/Protamine
    - Polymer-1
    - Polymer-2
- Injection volume ~100 µL; target dose was 5 mg/kg
- Bioanalytical method:
  - An ELISA / EIA protocol
  - GLP-1 (residues 1-37) (human, bovine, guinea pig, mouse, rat)
  - Working range for plasma [liraglutide] ≤1250 ng/mL

Study Results

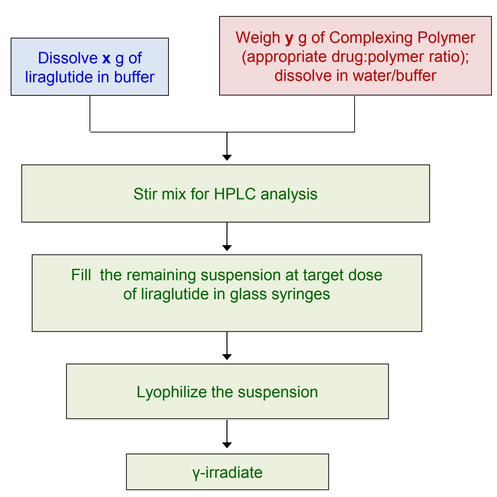
Liraglutide Rat PK - SC Aq Solution & Suspension



- A clear progression emerges in the control of liraglutide release into the *subcutis* with subsequent absorption into the systemic circulation: Polymer-2 > Polymer-1 > Zn/Protamine from aqueous formulations
- Only Polymer-2 achieved a sufficiently flat and extended plasma profile to be considered for weekly (C_{Max}/C_{7d} ~ 3.5 ± 1.1) or bi-weekly (C_{Max}/C_{14d} ~ 4.4 ± 1.3) dosing
- Polymer-2 also produced plasma levels at 28 days that were well above the 0.5 ng/mL quantitation limit (not shown in the graph)

Manufacturing Process: Liraglutide-Polymer Complex

- DURECT depot technology has an option of selecting the vehicle either with aqueous or non-aqueous media as suspension formulations
- In the present case, a complexing polymer was used to form liraglutide-polymer complex from an aqueous solution, which was isolated and lyophilized
- Just prior to administration, the formulated liraglutide powder was suspended in a purely aqueous medium (note: step for generation of API powder not shown in flow chart)
- Thus, these depots relied on dissolution rate to control API release



Study Results

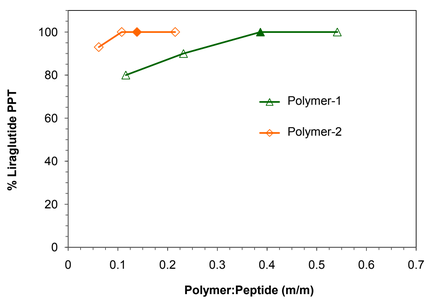
MRT for individual animals in each treatment group

Animal	*Group 1*	*Group 2*	*Group 3*	*Group 4*
1	11.6	40.6	35.7	115
2	8.50	38.0	34.7	145
3	20.7	33.2	64.9	114
4	16.9	27.4	27.6	116
5		18.7	30.2	166
Mean	14.4	31.6	38.6	131
95% CI	5.33	7.68	13.2	20.5
$MRT_{PK + Abs}$	14.4			
$\Delta\ MRT_{Complex}$		17.1	24.2	117

- By comparing mean residence times (***MRT***) between treatment group 1 (SC IR bolus, MRT_{PK+Abs}) and groups 2-4 one can define the contribution of each polymer to extended delivery of liraglutide ($MRT_{Complex}$). Groups 2, 3 and 4 are, respectively, Zn/Protamine, Polymer-1, and Polymer-2
- Zn/Protamine and polymer-1 extended release only ~2-fold beyond the SC IR bolus, but Polymer-2 extended release ~8-fold
- Absolute bioavailability from these formulations was 5%-15%

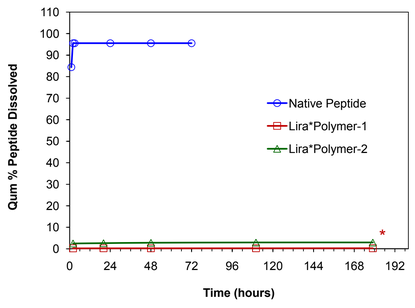
In Vitro Testing: Dissolution and Stability

Precipitation Efficiency of Liraglutide-Polymer Complex



- We prepared precipitates of liraglutide with complexing polymers (a native peptide, Polymer-1, and Polymer-2, both synthetic)
- We identified the minimum amount of each polymer that would saturate binding sites on the peptide resulting in precipitation (*solid symbol*)
- To assess the extent of dissolution rate control, dissolution of the lyophilized complexes was measured in PBS at 37°C

Liraglutide Dissolution from Peptide-Polymer complex



*After the last time point, mass balance was calculated, and total recovery of native peptide was >95%

γ-Radiation Stability of Polymer Complexes

API	*Complexing Polymer*	*γ Radiation Dose (kGy)*	*% Purity*
Liraglutide	None	15	85
"	Polymer-1	"	95
"	Polymer-2	"	99

Summary / Conclusions

- We achieved 5 days to 2 weeks duration of liraglutide delivery from aqueous suspensions of peptide-polymer complexes injected subcutaneously
- The best-performing formulation had a peak-trough ratio of ~4
- The polymer complexes extended liraglutide *MRT* from 2- to almost 10-fold relative to an SC IR aqueous solution of peptide
- Initial results suggest that liraglutide-polymer complex is radiation stable against a sterilizing dose of γ-radiation and hence terminal sterilization of liraglutide polymer complexes by γ-irradiation is possible
- Reaction of the dermis to injection of the formulations (data not presented here), as assessed by histopathology was quite mild
- Preparation of the peptide powder used in the suspension involved a short sequence of simple operations
- The simplicity of the complexation process, and its dependence on general and common characteristics of bio-therapeutics suggest a broad range of applicability for this technique for producing for 1- and 2-week subcutaneously injectable depots of protein and peptide-based drugs

BD Contact
jim.matriano@durect.com