Chapter 7

General Discussion & Summary

It is well accepted that the glucagon-like peptide-1 (GLP-1) receptor system offers unique promise for the treatment of type 2 diabetes due to its properties to regulate glycemia in a glucose-dependent manner and to reduce energy intake. As such, several therapeutics are available or are under development that target the GLP-1 axis. Exenatide was one of the first GLP-1 receptor (GLP-1R) agonists to be identified and to progress through clinical development. This thesis manuscript focuses on specific components of that clinical development plan that helped to make exenatide-based therapies available to patients.

Dose and Dose-Frequency Selection

Given that GLP-1 is a postprandial glucoregulatory hormone with a short half-life, the original exenatide clinical studies were designed to assess the dose-response of exenatide on postprandial plasma glucose. Details of the dose-response studies are described elsewhere[1-3], but a brief summary is provided here for context.

In studies that administered doses of 0.01-0.40 µg/kg exenatide prior to a standardized liquid meal challenge, 0.1 µg/kg was determined to be maximally effective at lowering postprandial glucose.[2] A dose of 0.02 µg/kg appeared to be minimally effective at lowering postprandial plasma glucose. In those studies, plasma glucagon was only measured at the higher doses (0.1-0.4 µg/kg) and maximal suppression occurred at the lowest one (0.1 µg/kg). In contrast, gastric emptying was assessed at doses of 0.02-0.1 µg/kg and dose-dependent decreases were observed throughout the dose range. Thus, it is likely that doses above 0.1 µg/kg could slow gastric emptying further, although there does not appear to be an advantage of that from a glucose lowering perspective.

The dose-response of exenatide on insulin secretion is difficult to interpret in the prandial state because the effect on insulin secretion is glucose-dependent and the plasma glucose exposure is dose-dependently lowered due to reductions in gastric emptying rate and glucagon secretion. It is clear, however, that exenatide has effects on insulin secretion in the prandial state, as the insulin to glucose ratio is higher with exenatide administration than with placebo.[2, 4] In the fasted state, increases in insulin secretion were dose-proportional with doses of 0.05-0.2 µg/kg.[1] Fasting glucagon was maximally suppressed at 0.05 µg/kg.

Following subcutaneous injection, native exenatide is rapidly absorbed and achieves peak plasma concentrations in approximately 2 hours.[2, 5-7] The mean terminal half-life is 2.4 hours providing measureable exposure for 6-7 hours. Although exenatide could improve both fasting and postprandial plasma glucose if present in the circulation, a simple formulation of exenatide would require four times a day dosing to ensure 24-hour coverage. As four times a day administration is impractical, twice a day and three times a day dosing were explored. Chapter 2 of this thesis describes a 28-day study with exenatide (0.08 µg/kg) in 116 patients with type 2 diabetes. Twice-daily (BID) injections at breakfast and dinner (bd) or breakfast and bedtime (bs) were explored as well as three times a day (TID) injections of exenatide at breakfast, dinner, and bedtime.[8] Lunch injections were not explored as mid-day administration of chronic therapies typically has poor compliance. All three regimens produced statistically significant reductions in hemoglobin A1c (A1C) compared to placebo [1.1%, 0.7%, and 1.0% for BID (bd), BID (bs), and TID, respectively] with the greatest reductions observed when exenatide was administered with meals (bd and TID). The third injection at bedtime did not offer a significant advantage and thus BID dosing was selected for further clinical development.

Weight-Normalized vs. Fixed-Dose Regimens

In early phase 2 exenatide studies, patients were dosed on a weight-normalized basis (μg/kg). This type of regimen adds complexity to administration and thus increases the odds of dosing errors. To determine if μg/kg dosing was necessary to manage pharmacokinetic (PK) and/or pharmacodynamic (PD) variability, a PK/PD model was developed to quantify the effects of bodyweight on the PK and glucose lowering ability of exenatide.[9] Baseline bodyweight did not have an effect on the glucose lowering potential of exenatide. There was a significant effect of bodyweight on exenatide PK, however the magnitude of effect was very small indicating that switching to fixed dose administration would have a minimal effect. In order to select doses for further clinical study, the same PK/PD model was used to simulate PK and postprandial glucose profiles with fixed doses of 5-12 μg. In 10 clinical trial simulations of 100 patients each (bodyweights ranging from 50-100 kg), a dose of 10 μg most often resulted in a maximum glucose lowering effect while maintaining plasma exenatide below values typically associated with side effects. The 5 μg dose resulted in a lower glycemic effect on average, but would be expected to have good tolerability in almost all patients. After the completion of phase 3, the PK/PD models were updated with additional PK and PD data and the simulations were repeated. That analysis confirmed the dose selection of 5 and 10 μg.[10]

Induction of Tolerance to Dose-Limiting Side Effects

Consistent with GLP-1 and other GLP-1R agonists, nausea and vomiting are the most common adverse events associated with exenatide treatment. These gastrointestinal side effects are dose-dependent and typically peak at the time of maximum exenatide concentration in the plasma. With acute administration, nausea was reported in 30%-60% of subjects receiving doses above 0.2 μg/kg.[2] In the 28-day study described above, 31% of subjects receiving a dose of 0.08 μg/kg reported nausea. Interestingly, the nausea was most pronounced in the first few days of treatment and dissipated thereafter. By Day 28, only 13% of subjects were still reporting nausea. The reduction in gastrointestinal adverse events observed in this study could not be explained by study dropouts as only 3.7% of subjects withdrew due to nausea. These observations suggested that tolerance to the dose-limiting side effects could be induced with chronic therapy.

As described in Chapter 3, in order to definitely determine if tolerance to gastrointestinal adverse events could be induced, we designed a blinded, randomized, controlled clinical trial in patients with type 2 diabetes that compared dose-titration to receiving a high dose of exenatide without titration.[11, 12] In the titration arm, patients received increasing doses of exenatide 0.02 μg/kg TID every 3 days until they reached a dose of 0.24 μg/kg on day 35. The dose of 0.24 μg/kg was chosen as it was known to cause significant gastrointestinal side effects if administered acutely. In the control arm, patients received placebo TID with increasing volume every 3 days to preserve the blind. On Day 35, control patients received exenatide 0.24 μg/kg TID as their first exenatide exposure. At the end of 35 days, 55.7% of patients in the control arm experienced severe nausea, nausea leading to withdrawal, or vomiting. With dose-titration, however, the incidence was reduced to 27.4%. On the last day of dosing, the Kaplan-Meier estimate was 0.68 for the control arm and 0.28 for the titration arm. The difference was highly statistically significant ($p \leq 0.001$). Similar results were observed when all nausea (mild, moderate, or severe) was compared ($p = 0.0047$). Based on these results, exenatide therapy is now initiated at a 5 μg dose for at least 1 month prior to titrating up to 10 μg.

This same titration strategy is employed by another GLP-1R agonist. In order to reduce gastrointestinal symptoms, it is recommended that liraglutide be initiated at 0.6 mg per day for one week and titrated to 1.2 mg and 1.8 mg in weekly increments.[13] Additionally, in an 8-week tolerability study with taspoglutide, the incidence of nausea was highest after the first weekly dose of 20 mg and did not increase when patients were titrated to 30 or 40 mg after 4 weeks.[14] The authors concluded that dose-titration improved tolerability. Taspoglutide is a once weekly GLP-1R agonist that was in development until 2010 when immunogenicity concerns were identified. Regardless of those issues, the above mentioned study is relevant to the question of induction of tolerance. Although these other examples suggest that dose titration induces gastrointestinal tolerance for those drugs, their study designs do not allow for definitive conclusions as high dose treatment arms without titration were not included. Our study is the only published blinded and controlled clinical trial that can definitely conclude the positive effects of dose titration. Gastrointestinal adverse event data from the extended-release formulation of exenatide also supports the notion that tolerability can be induced with slow titration (see Exenatide Extended-Release section below)

Exenatide Extended-Release

Although the plasma half-life of exenatide BID is a significant improvement over native GLP-1, twice a day administration remains a barrier for some patients. In addition, exenatide BID (also referred to as the immediate-release formulation in this thesis) predominantly exerts its effects during the postprandial period after injection at the morning and evening meals. Effects at the midday meal and in the fasting state are modest. When exenatide was infused continuously for 24 hours, both fasting and postprandial glucose were reduced throughout the day.[3] Thus, to achieve the full potential of exenatide, continuous exposure is needed. For this reason, an extended-release formulation was developed that can provide continuous exenatide exposure with once weekly administration (Chapter 4).[15] Following a single subcutaneous injection, native exenatide is slowly released into the subcutaneous space over approximately 10 weeks. Due to the complex degradation process of the microspheres in the formulation, the single dose pharmacokinetic profile has high peak-to-trough variability with three distinct peaks on day 1, week 2, and between weeks 4-8. To smooth out the PK profile, smaller doses were administered weekly allowing for additive accumulation over 10 doses. With weekly injections of 2 mg, concentrations of plasma exenatide slowly rise, achieving steady state concentrations within 6-7 weeks with minimal peak-to-trough fluctuation. The weekly dose of 2 mg was selected using superpositioning of the single dose data and was designed to achieve steady state plasma concentrations similar to the C_{max} of a 10 µg dose of exenatide BID (200-300 pg/mL). Within 2 weeks of therapy with 2 mg exenatide once weekly, plasma exenatide concentrations exceeded the minimally effective concentration of 50 pg/mL determined with a continuous subcutaneous infusion of native exenatide.[3] Maximum glucose lowering was achieved within 4-6 weeks. In the 15-week multi-dose study, doses of 0.8 mg and 2.0 mg achieved similar reductions in fasting plasma glucose, but a dose-response for postprandial glucose and A1C was observed.[15, 16] This supports the hypothesis by Holst and others[17, 18] that a greater concentration of GLP-1 or GLP-1 agonist is needed to effect postprandial glucose mechanisms (gastric emptying) compared to fasting glucose mechanisms (enhancement of insulin secretion and suppression of glucagon secretion). In addition, bodyweight was reduced with 2 mg but was not different than placebo for the 0.8 mg dose. This would suggest that effects on food intake may require even higher concentrations of GLP-1 than are needed to affect gastric emptying.

Consistent with the induction of tolerance experiment described in Chapter 3, the slow rise in exenatide concentrations inherent to the exenatide once weekly formulation appears to improve tolerance. In two long-term studies comparing exenatide BID to exenatide once weekly, the incidence of nausea was approximately 25-50% lower with exenatide once weekly despite having higher peak plasma concentrations of exenatide. This is not surprising as the PK of exenatide once weekly provides a much more gradual rise in plasma exenatide than can be achieved with the 2-step titration of exenatide BID. It also more closely mimics the PK profile achieved with the slow titration utilized in the induction of tolerance study (increases of 0.02 µg/kg every 3 days). The incidence of nausea was also less with liraglutide compared to exenatide BID[19] supporting the notion that continuous GLP-1 agonism with a faster rise to steady state (~ 3 weeks instead of 6-7 weeks) can also induce improved tolerance.

As outlined in Chapter 5, there appears to be tachyphylaxis of the gastric emptying effect with continuous GLP-1[20] and GLP-1R agonists[21, 22] that reduces their effects on postprandial glucose. In the case of GLP-1, the tachyphylaxis was evident within 4 hours suggesting that the downregulation occurs at the level of the vagal nerve rather than a consequence of GLP-1 receptor downregulation or desensitization.[20] The fact that GLP-1R agonist effects on insulin secretion, glucagon secretion, and bodyweight remain durable with both intermittent and continuous exposure supports the notion that systemic downregulation of GLP-1 receptors is unlikely. Interestingly, tachyphylaxis of both nausea and gastric emptying are evident with continuous GLP-1 exposure, suggesting that the two effects share a common pathway. Results with exenatide BID, however, are in conflict with that hypothesis as nausea is transient[23-25] but the gastric emptying and postprandial glucose lowering effects are durable. The effects of GLP-1R agonists on food-intake also appear to be independent of nausea since with exenatide BID, nausea is transient and weight loss is progressive for at least 3 years.[26] Further mechanistic work is necessary to deconvolute these observations.

Immunogenicity

Antibodies to peptide therapeutics can completely neutralize or partially attenuate the efficacy of the drug and, more importantly, may be associated with deleterious effects on safety and tolerability. Severe safety consequences can include IgE-mediated hypersensitivity reactions, anaphylaxis and immune complex disease. In addition, an antibody (Ab) response can neutralize an important endogenous system if the Ab cross-reacts with endogenous peptides. This could have lasting effects even after the therapy is discontinued. Thus, it is important to fully characterize the immune potential of a therapeutic and the consequences of the antibodies observed. Chapter 6 provides a comprehensive characterization of the immune response to both exenatide BID and exenatide once weekly.

As exenatide is a non-mammalian peptide with only 53% sequence identity to GLP-1, it is not surprising that almost 37% of exenatide BID patients and 57% of exenatide once weekly patients develop anti-exenatide antibodies. Importantly, Ab titers peaked within 22 weeks and declined thereafter allowing for a full characterization when the immune response was at its highest.

In contrast to exenatide, only 8% of liraglutide-treated patients develop antibodies consistent with an almost complete peptide sequence overlap between liraglutide and GLP-1 (97%).[27] As plasma concentrations of liraglutide interfere with the anti-liraglutide Ab measurement, assays are only conducted at study termination following a 5 day washout. Thus, it is not possible to ascertain the timecourse of Ab response in an individual patient. Comparison of

anti-liraglutide Ab responses in 26 vs. 52 week studies, however, suggests that peak responses occur prior to Week 26.[27]

Little has been reported on the immune potential of albiglutide, a dimer of a GLP-1analog fused to human albumin. Albiglutide appears to have a low immune potential (2.5%),[28] but a critical evaluation of the methods and data is not available.

Comparisons of Ab incidence across compounds must be done with caution, however, because different assay methodologies were used (ELISA for exenatide vs. RIA for liraglutide) that likely have different sensitivity and specificity characteristics. Even identical assay formats are difficult to compare across compounds as each assay will have its own test equilibrium based on individual peptide characteristics (e.g. albumin binding) and there are no standards by which to normalize responses. The ELISA format was chosen for the detection of anti-exenatide antibodies as they tend to detect lower affinity antibodies and are less sensitive to circulating drug concentrations.[29] Notably, the ELISA format was able to detect antibodies across a range of titers, and was able to discriminate between antibodies of no clinical relevance and antibodies that may be associated with an attenuated response.
Importantly, there does not appear to be a safety consequence of antibodies to exenatide or liraglutide.[27] This is in contrast to taspoglutide where rare but serious hypersensitivity reactions were reported and appeared to be associated with Ab formation.[30]

The effects of anti-exenatide Ab formation on efficacy was well characterized. While the overall incidence of Ab formation is high with exenatide treatment, the vast majority of responses (95% of BID Ab positive subjects and 88% of exenatide once weekly Ab positive subjects) are low titer and do not have an efficacy consequence. In the small subset of subjects with the highest Ab titers (5% exenatide BID; 12% exenatide EQW), there is a more variable A1C response resulting in a smaller mean reduction in A1C. Importantly, approximately half of the higher titer subjects continued to have a clinically relevant reduction in A1C. Thus, only ~3% and ~6% of exenatide BID and exenatide once weekly patients, respectively, may be poor responders due to Ab response.

Despite the use of a different assay formats, our findings are consistent with those from the LEAD-6 trial comparing exenatide BID with liraglutide. For exenatide-treated patients, mean A1C reductions at 26 weeks were attenuated in the small percentage of patients with higher Ab responses (greater % bound) to exenatide (0.5%), compared with low response subjects (1.0%).[27, 31] After completing 26 weeks of exenatide therapy, patients were crossed over to liraglutide treatment. The 6.7% of subjects with the highest anti-exenatide Ab response (those with the lowest mean A1C response on exenatide treatment) had the greatest improvements in A1C with liraglutide treatment. This suggests that the anti-exenatide antibodies did not meaningfully cross-react with liraglutide and thus liraglutide could have clinical utility in subjects that were not responding to exenatide due, at least in part, to a higher titer Ab response.

The effects of anti-liraglutide antibodies on efficacy are less clear, in part because there are fewer patients to characterize. For liraglutide-treated patients in LEAD-6,[27, 31] mean A1C reductions were attenuated in the small number of patients that were positive for antibodies to liraglutide (-0.5%), compared to the A1C reduction reported for all liraglutide patients (1.3%) at week 40. In other 26-week trials, mean reductions in A1C were similar between Ab positive and Ab negative subjects.[27]

Treatment emergent antibodies to exenatide did not cross-react with human GLP-1 or glucagon reducing the probability of lasting consequences if exenatide treatment is discontinued. These results are consistent with epitope mapping experiments that suggest

that antibodies to exenatide are likely conformational, and are targeted to the amino acid sequence regions that differ from GLP-1. In contrast to our data, ~4% of the anti-exenatide Ab positive samples in LEAD-6 were cross-reactive to GLP-1. These differences are likely a result of differences in assay format or reflect GLP-1 Ab positivity at baseline in the LEAD-6 study (baseline samples were not reported).[27] Consistent with its greater GLP-1 sequence similarity, the majority of anti-liraglutide antibodies (56%-100%) do cross-react with GLP-1.[27, 31] The clinical consequences of this reactivity are not known. No data on cross-reactivity of liraglutide antibodies to glucagon or to the GLP-1 metabolite are publically available at this time.

Differential Effects on Fasting and Postprandial Glucose

The positive attributes of the GLP-1 receptor system have spawned the development of several new GLP-1 based therapeutics that can be divided into three unique drug classes: DPP-4 inhibitors, short-acting GLP-1R agonists, and long-acting GLP-1R agonists. Short and long-acting GLP-1 receptor agonists are not typically differentiated classes, but as described in Chapter 5, differences in exposure (intermittent vs. continuous) result in differential effects on gastric emptying and ultimately on postprandial plasma glucose.

DPP-4 inhibitors have the advantage of simple oral administration, a low risk of hypoglycemia and clinically relevant reductions in fasting plasma glucose. They have modest effects on postprandial glucose and are weight-neutral. Short-acting GLP-1R agonists have the disadvantage of BID injectable dosing but they have a profound effect on postprandial glucose, a low risk of hypoglycemia, and positive weight loss that is progressive for at least three years.[26] Effects on fasting glucose are more modest. Finally, long-acting GLP-1R agonists are injectable, but have the advantage of less frequent administration (once-daily or once-weekly), robust effects on fasting glucose, a low risk of hypoglycemia, and sustained weight loss. It is important to note that the low risk of hypoglycemia is inherent to all 3 classes of GLP-1 mediated therapies, but if used in combination, can increase the risk of insulin or sulfonylurea induced hypoglycemia.

The three classes of GLP-1 mediated therapies represent significant advancements in the treatment of diabetes and the choice of which one to use, should depend on the specific needs of the patient. Tolerability, administration constraints (injectable vs. oral, frequency), degree of hyperglycaemia, and type of dysglycemia (fasting and/or postprandial) should all be considered. This is analogous to the current use of modern insulins as short, intermediate, and long-acting versions are all used to optimize the 24-hour plasma glucose profile as needed. Given that GLP-1 mediated therapies have advantages over insulins in terms of hypoglycemia risk and weight gain, optimized use of these compounds could represent a significant paradigm shift for the treatment of type 2 diabetes. Unlike the insulins, however, short and long-acting GLP-1R agonists are not currently approved for use in combination with each other, nor are DPP-4 inhibitors approved for use with GLP-1R agonists. Such combinations may be useful, but further exploration is needed to explore their combined safety and efficacy.

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List of Abbreviations

A1C	hemoglobin A1c
AACE	American Association of Clinical Endocrinologists
Ab	antibody
ACE	American College of Endocrinology
ADA	American Diabetes Association
BID	Latin: “bis in die”meaning two times per day
bd	breakfast and dinner
bs	breakfast and bedtime
AUC	area under the curve
BMI	body mass index
cAMP	cyclic AMP
C_{max}	maximum concentration
DPP-4	dipeptidyl-peptidase 4
EASD	European Association for the Study of Diabetes
Emax	maximum effect
EQW	exenatide once-weekly (extended release formulation)
FDA	United States Food and Drug Administration
FPG	fasting plasma glucose
GLP-1	glucagon-like peptide-1
GLP-1R	glucagon-like peptide 1 receptor
HbA1c	hemoglobin A1c
IDF	International Diabetes Federation
IIV	interindividual variability
IR	immediate-release
LAR	Long-acting release
NICE	National Institute for Health and Clinical Excellence
OFV	objective function value
PD	pharmacodynamic
PK	pharmacokinetic
PPG	postprandial plasma glucose
PYY	peptide YY
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SFU	sulfonylurea
$T_{1/2}$	half life
TID	Latin: “ter in die” meaning three times per day
T_{max}	time to maximum concentration
TZD	thiazolidinedione
US	United States
WHO	World Health Organization

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Curriculum Vitae

Mark Fineman has worked in the pharmaceutical industry since 1986 and has held various positions in bioanalytical chemistry, clinical pharmacology, and medical development. He was at Amylin Pharmaceuticals, Inc. from 1989-2010, where he focused on the clinical pharmacology of pramlintide and exenatide, two peptide hormone therapeutics for the treatment of type 1 and/or type 2 diabetes. While at Amylin, Mark also was responsible for Clinical Affairs at Psylin Neurosciences, Inc., a virtual biotech company spun out of Amylin and PsychoGenics Inc., to evaluate peptide hormones for the treatment of psychiatric disorders. In January 2011, Mark joined Elcelyx Therapeutics, Inc. as the Vice President of Development. Elcelyx is a start-up phase biotechnology company developing novel treatments for diabetes and obesity. Mark has authored over 40 original publications and is an inventor on several patents.

Mark graduated from California Polytechnic State University, San Luis Obispo with a B.S. in Microbiology (Medical Technology emphasis) in 1986. While employed at Amylin, he received Masters of Advanced Studies and Masters of Science degrees in Clinical Research (2006) and Molecular Pathology (2009) from the University of California, San Diego. Mark and his wife Kathy live in San Diego, California, USA with their three children, two dogs, three birds, and two guinea pigs.