Chapter 1

General Introduction
and Outline of the Thesis
Obesity and Diabetes

Obesity and type 2 diabetes are highly interrelated diseases, often dubbed “diabesity.” Both have a strong genetic basis with heritability scores of over 70%. Many predisposing genes have been identified but they account for a small minority of cases, as both conditions are heterogeneous and polygenic. Lifestyle, nutritional and environmental factors strongly influence the phenotypic expression of the disease. In 2008, 1.5 billion people worldwide were overweight and 500 million people were obese. Obese individuals are predisposed to cardiovascular disease, diabetes, osteoarthritis, cancer, and biliary diseases and obesity is the fifth leading cause of global death. In the United States (US) alone, societal costs of obesity and its related conditions are in excess of $100 billion. Over 80% of patients with type 2 diabetes are obese.

An estimated 26 million people in the US have type 2 diabetes and one new case is diagnosed every 37 seconds. Another 79 million have pre-diabetes (conversion to diabetes is approximately 6% per annum). Worldwide there are over 280 million people with diabetes and incidence rates are accelerating in developing countries, with a projected prevalence of 480 million by 2030. In the US, total societal direct and indirect costs are $174 billion making it the nation’s most costly disease. Estimated global expenditures on diabetes were estimated to be at least $418 billion in 2010, and are projected to be at least $561 billion by 2030. Diabetes is the seventh leading cause of death in the US and the most common cause of non-traumatic lower limb amputation. It causes blindness and kidney failure, and is a major risk factor for coronary artery disease, the number one killer in the US.

Over 90% of people with diabetes have type 2 diabetes. In its simplest form, type 2 diabetes is characterized by insulin resistance (reduced response of tissues to insulin) and a failure of the pancreatic β-cells to secrete sufficient insulin to overcome the resistance. Insulin resistance is aggravated by obesity and weight loss can successfully improve it. Beta cell failure appears to be progressive and leads to increased use of medications over time, culminating with insulin replacement therapy. Large prospective intervention trials have unequivocally demonstrated that glucose control prevents microvascular complications.

Diagnosis of diabetes is mainly based on overnight fasting plasma glucose concentration (>126 mg/dL), although elevations of postprandial glucose may be the earliest sign of dysglycemia. Long-term glucose control in patients with diabetes is assessed by measuring hemoglobin A1c (A1C), which reflects glucose exposure over the previous two to three months. The American Diabetes Association recommends treatment to safely achieve A1C at or below 7%, while normal A1C is <6.0%. The International Diabetes Federation, the American association of Clinical Endocrinologists, and the American College of Endocrinology all recommend a treatment goal of ≤6.5% A1C. Fasting hyperglycemia is the major contributor to A1C in poorly controlled subjects, while postprandial hyperglycemia is the major contributor to A1C in subjects nearing target goals. Thus, to achieve the recommended target A1C, control of both fasting and postprandial glucose must be achieved.
The first line intervention for prevention of type 2 diabetes is diet and exercise (lifestyle intervention). While effective in many cases (particularly those with preserved beta cell function), lifestyle intervention has been met with disappointing long-term results secondary to poor adherence and the progressive nature of the underlying disease. Lifestyle intervention and metformin are recommended as first line therapy for the management of hyperglycemia. If A1C goals are not achieved with metformin and lifestyle intervention, various oral and injectable therapies are often utilized. As diabetes is a progressive disease, intensive insulin therapy (basal and mealtime) may be ultimately necessary to maintain glycemic control. Most of the above treatments are associated with adverse effects including hypoglycemia, weight gain, edema and/or nausea and vomiting. These adverse effects often limit treatment effectiveness and, in the case of weight gain, may add to the progression of the disease and to obesity-related comorbidities. Consistent with these treatment limitations, >60% of patients with diabetes are currently not achieving recommended therapy goals. Additionally, recent treatment algorithms suggest that diabetes therapy should address the multiple pathophysiologies that may be present in patients with type 2 diabetes (e.g. insulin resistance, β-cell dysfunction, obesity) early in the disease progression. Thus, effective and safe novel therapies for diabetes that cause weight loss could have a significant impact on long-term health outcomes.

**Glucagon-Like Peptide-1**

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from gastrointestinal L cells in response to nutrients. Following food ingestion, GLP-1 appears in the plasma within minutes and due to its rapid degradation by the ubiquitous glycopeptidase, dipeptidyl peptidase-4 (DPP-4), is undetectable by three hours. Given that enteroendocrine L cells are predominantly located in the ileum, colon, and rectum, it is proposed that rapid appearance of GLP-1 in the plasma following a meal is likely a result of indirect hormonal and/or neural signaling as opposed to direct nutrient contact with GLP-1 secreting cells in the proximal gut lumen.

The human GLP-1 receptor (GLP-1R) is a 463 amino acid G-protein coupled receptor expressed on pancreatic islet α and β cells and many other tissues including the gastrointestinal tract, heart, kidney, lung and the peripheral and central nervous systems. Receptor activation is linked to the cyclic AMP (cAMP) second messenger pathway. GLP-1 lowers blood glucose by several mechanisms including enhancement of glucose stimulated insulin secretion, suppression of glucagon secretion, and slowing of gastric emptying. Importantly, the effects of GLP-1 are glucose dependent, thus limiting the probability of GLP-1 induced hypoglycemia. GLP-1 also may have a role in weight maintenance by increasing satiety. All of these effects work in concert to regulate energy intake and glucose flux during the prandial period. When GLP-1 was infused in patients with type 2 diabetes, near-normalization of both fasting and postprandial plasma glucose was observed. A comprehensive review of GLP-1 biology is provided in Chapter 5 of this manuscript.
Glucagon-Like Peptide-1 Based Therapies

To circumvent the short half-life constraints of GLP-1, two novel classes of glucose-lowering therapeutics have emerged: DPP-4 inhibitors and GLP-1R agonists. DPP-4 inhibitors increase the concentration of endogenous GLP-1 by inhibiting the enzyme that inactivates it (GLP-1 enhancers). GLP-1R agonists mimic the actions of GLP-1 but have DPP-4 resistant properties by virtue of their amino acid sequence and/or through chemical modification (GLP-1 mimetics). Representatives from both classes of drugs are clinically available in the US and Europe and have demonstrated effective utility for the treatment of type 2 diabetes.19, 32-34

DPP-4 Inhibitors

DPP-4 inhibition leads to a doubling of plasma concentrations of GLP-135 which in turn enhances glucose-dependent insulin secretion and suppress inappropriately elevated glucagon secretion.36 DPP-4 inhibitors do not slow gastric emptying and have minimal effects on food intake.36, 37 With long-term treatment, they lower A1C by approximately 0.75%38 predominantly through a reduction in fasting plasma glucose with minimal effects on postprandial plasma glucose. DPP-4 inhibitors have a low risk of both hypoglycemia and gastrointestinal side effects and they are typically weight neutral.

GLP-1R agonists

Currently, there are two GLP-1R agonist molecules available for clinical use in the US and Europe: exenatide and liraglutide.

Exenatide

The first GLP-1R agonist to be approved was exenatide. Exenatide is a synthetic version of exendin-4, a 39 amino acid peptide isolated from the salivary secretions of the gila monster (Heloderma suspectum). It shares 53% sequence identity with GLP-1 and is equipotent at the GLP-1 receptor.39 Exenatide has an extended half-life relative to GLP-1 (2.4 hours)40 and is detectable for approximately six to seven hours following a subcutaneous injection in the abdomen, arm, or thigh.41 Like GLP-1, exenatide slows gastric emptying42, suppresses glucagon43, 44, and enhances first-phase and second-phase insulin secretion.45, 46 Importantly, the actions of exenatide on insulin and glucagon secretion occur when blood glucose is in the euglycemia to hyperglycemia range, but not during hypoglycemia.46 The glucose-dependence of GLP-1R agonists provides an important safeguard against inducing or prolonging hypoglycemia with clinical use. These actions lead to robust dose-dependent reductions in postprandial glucose when bolus subcutaneous injections of exenatide were administered prior to a meal47 and to reductions in fasting glucose when administered during an extended fast.47 When exenatide was subcutaneously infused for 24 hours in patients with type 2 diabetes, dose-dependent reductions in both fasting and postprandial glucose were observed.47 In phase 2 dose-ranging studies, gastrointestinal adverse events (nausea and diarrhea) appear to be the dose-limiting side effects.
Native exenatide is now commercialized as a twice-daily injection administered at breakfast and dinner under the trade name Byetta®. This twice-daily formulation (exenatide BID) was shown to reduce A1C by approximately 1% in long-term controlled trials with robust postprandial glucose lowering, modest improvements in fasting glucose and reductions in bodyweight.48-50 Mild to moderate nausea was the most common side effect (40-50% incidence in patients that titrate to the 10 µg high dose), typically occurring early in treatment and dissipating with time.48 Approximately 10% of subjects had recurring nausea through 30 weeks of therapy and discontinuation due to nausea was <5%.48-50 Consistent with the glucose-dependent effects of exenatide, hypoglycemia is rare with long-term treatment, although the incidence increases when combined with an agent that induces hypoglycemia, such as a sulfonylurea.48, 50

In order to improve patient convenience and to enhance the effects of exenatide on fasting glucose, an extended-release formulation was developed that could provide continuous exenatide exposure with once-weekly administration. The formulation utilizes biodegradable polymeric microspheres, composed of exenatide in a poly lactide-co-glycolide (PLG) polymeric matrix. PLG is a common biodegradable medical polymer used in absorbable sutures and sustained-release pharmaceuticals.51 This extended-release formulation is injected subcutaneously and slowly releases native exenatide into the subcutaneous space through a complex process of polymer hydration and degradation. Thus, this absorption rate-limited formulation allows for continuous systemic exposure to exenatide without modification of the native peptide. Once absorbed, the general pharmacokinetics properties of exenatide are unchanged.52-54

The extended-release formulation of exenatide (exenatide once weekly or EQW) was approved in Europe in June 2011 and is currently under review at the US Food & Drug Administration (FDA). In 26 and 30 week controlled trials, EQW resulted in A1C reductions from baseline ranging from 1.5% to 1.9% with reductions in bodyweight similar to those observed with exenatide BID (2-4 kg).52-55 The improved reductions in A1C relative to exenatide BID are due to more pronounced reductions in fasting plasma glucose. The greater effect of EQW on fasting glycemia is likely due to the altered pharmacokinetic profile as plasma concentrations of exenatide are present in the fasting state with EQW, but not with exenatide BID. Reductions in the postprandial glucose increment above pre-prandial values are less with EQW than with exenatide BID, due, at least in part, to a reduced effect on gastric emptying.52 Notably, less gastrointestinal side effects are observed with EQW treatment compared to exenatide BID treatment.52-53

Liraglutide
Liraglutide is a DPP-4 resistant GLP-1 analog that achieves slowed absorption and increased half-life through the substitution of arginine for lysine at position 34 and the addition of a C16 fatty acid change at position 26 resulting in reversible binding to albumin.56-59 The half-life of liraglutide is 11-15 hours allowing for once-daily administration.56 Although the potency of liraglutide is reduced 100-fold relative to GLP-1 (albumin binding is 98-99%), it retains the basic actions of GLP-1.15 Long-term treatment with liraglutide 1.8 mg results in reductions in A1C of 1.0% to 1.5% and robust reductions in fasting plasma glucose (FPG).60-64
Liraglutide treatment is also associated with weight loss at a magnitude similar to that observed with exenatide. Like EQW, liraglutide’s continuous exposure results in robust reductions in fasting plasma glucose with a less robust effect to suppress the postprandial glucose increment compared to exenatide BID. Liraglutide has been available since 2010 in both the US and Europe, under the trade name Victoza.

**Comparison of GLP-1 Based Therapies**

Although the GLP-1 mediated therapies have several similarities, there are differences in their pharmacology brought about, at least in part, by differences in pharmacokinetics and in differences in the concentration of GLP-1 or GLP-1 agonist available to receptors. As depicted in Figure 1, lower concentrations of “GLP-1 equivalents” are required to induce effects on insulin and glucagon secretion, acting directly on β-cells and α-cells and/or through afferent nervous system pathways. Thus, all GLP-1 based therapies induce these effects. Reductions in gastric emptying, appetite, and food intake (with resulting weight loss) require higher concentrations that are not achieved with DPP-4 inhibition (plasma concentrations of GLP-1 increase 2-fold with chronic treatment) but are achieved with all three GLP-1R agonists. Finally, gastrointestinal side effects are dose-dependent and thus, therapies that achieve high concentrations of “GLP-1 equivalents” have a higher risk of nausea or vomiting. This summary is, however, over-simplified as differences in portal concentration, off target effects, and tachyphylaxis of effect all contribute to the clinical differences observed with these molecules. In addition, the individual GLP-1R agonists have differences in their plasma free concentration due to differences in immunogenicity and/or protein binding. Liraglutide is 98%-99% bound to albumin which reduces its potency by approximately 100-fold. Both therapies induce antibodies that may bind drug and reduce the available concentration of the agonist, but the incidence of antibodies is higher in exenatide-treated subjects than in liraglutide-treated subjects.

![Figure 1. Dose-response of GLP-1 Mechanisms of Action and the Corresponding Concentrations of GLP-1 or GLP-1R Agonist Available in Plasma](image-url)
Outline of the Thesis

While native exenatide has a high potential as a therapeutic based on its pharmacokinetic and pharmacodynamic properties, the clinical development plan needed to determine 1) the dose(s) that provided an optimal balance between tolerability and effect size and 2) the optimal dose timing and dose frequency that provided a balance between patient convenience and overall effect. Additionally, as there is a strong dose-dependence to nausea and vomiting, strategies to reduce gastrointestinal adverse effects needed to be considered. Chapter 2 describes the first 28-day clinical study of this class of type 2 diabetes therapies (GLP-1 receptor antagonists) and was designed to study the effects of twice-daily (BID) injections of exenatide at breakfast and dinner or breakfast and bedtime and three times a day (TID) injections at breakfast, dinner, and bedtime. Lunch injections were not studied as compliance for mid-day administration is typically poor. The bedtime injection was studied to determine if night-time exenatide exposure could improve the next day’s fasting glucose level despite exenatide’s relatively short (6-7 hour) pharmacokinetic profile. Chapter 3 describes a clinical study designed to determine if dose titration can be used to induce tolerance to the gastrointestinal side effects observed with high doses of exenatide. A second statistically focused manuscript was also published that evaluated the pros and cons of the various study designs we considered to address this question. Results from the study described in Chapter 3 were used to design the drug initiation strategy for phase 3 studies and ultimately for commercial use.

Chapter 4 describes the single dose and multiple dose clinical studies of the exenatide extended-release formulation. These studies were designed to determine the optimal dose regimen that would provide continuous exenatide exposure in the therapeutic range as defined in the exenatide BID development program. The chapter also includes an assessment of the plasma concentrations of exenatide necessary to achieve a postprandial glucose lowering effect compared to a fasting glucose lowering effect.

Chapter 5 provides a review of the differential effects of GLP-1 mediated therapies (DPP-4s, short-acting GLP-1R agonists and GLP-1R agonists that provide continuous exposure) on fasting and postprandial glucose. The chapter also provides support for the hypothesis that continuous GLP-1 receptor activation results in tachyphylaxis of the gastric emptying effect, thus reducing the effect on postprandial glucose. The clinical implications of this hypothesis are also discussed.

Lastly, peptide and protein therapeutics have the potential to induce an immune response even when the drug is identical to the endogenous human peptide, as reported for exogenously administered human insulins. The consequences of such a response vary widely and include possible effects on efficacy, safety, and on endogenous systems if the antibody cross-reacts with an endogenous peptide. Thus, it is crucial to characterize the immune response to any peptide or protein therapeutic. Chapter 6 provides an extensive characterization of the incidence, time course, and clinical consequence of anti-exenatide antibodies resulting from chronic administration of exenatide BID and exenatide once weekly.

Throughout this manuscript, the terms exenatide, exendin-4, and AC2993 (the chemical compound number for exenatide) are used interchangeably.
References

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6. IDF. The Economic Impacts of Diabetes, 2011.


60. Marre M, Shaw J, Brandle M, Bebakar WM, Kamraduddin NA, Strand J, Zdravkovic M, Le Thi TD, Colaguiri S. Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). Diabetic Medicine. 2009;26:268-78.


Chapter 2

Effect On Glycemic Control of Synthetic Exendin-4 (AC2993) Additive to Existing Metformin and/or Sulfonylurea Treatment in Patients with Type 2 Diabetes

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ABSTRACT

OBJECTIVE - AC2993 (synthetic exendin-4; exenatide) is a peptide which enhances glucose-dependent insulin secretion, suppresses inappropriately elevated glucagon secretion, and slows gastric emptying. AC2993 also promotes β-cell proliferation and neogenesis in vitro and in animal models. This study examines the activity and safety of subcutaneously injected AC2993 in patients with type 2 diabetes currently treated with diet and/or oral antidiabetic agents (OAAs).

RESEARCH DESIGN AND METHODS – A total of 109 patients treated with diet and a sulfonylurea and/or metformin were enrolled in a blinded study. Patients were randomly assigned to one of three subcutaneously (SC) injected regimens of AC2993 (0.08 µg/kg) or placebo for 28 days.

RESULTS – All three AC2993 regimens led to significant reductions in serum fructosamine relative to placebo (p ≤ 0.004). Mean reductions ranged from 39 to 46 µmol/l. All AC2993 groups had reductions in HbA1c ranging from 0.7 to 1.1% (p ≤ 0.006). An end-of-study HbA1c <7% was achieved by 15% of AC2993 patients, versus 4% of placebo patients, confirming AC2993 effects on fasting and postprandial glycemia. On Days 14 and 28, the β-cell index (homeostasis model assessment) for patients treated with AC2993 was 50%-100% higher than baseline, contrasting with unchanged levels for placebo. The most common adverse event was transient mild-to-moderate nausea.

CONCLUSIONS - AC2993 is a promising therapeutic for patients with type 2 diabetes. In this study, it had significant activity on HbA1c levels in patients not currently achieving optimal glucose control with diet and/or OAAs.

Key Words: exendin-4, AC2993, diabetes, glycosylated hemoglobin, plasma glucose, HOMA
INTRODUCTION

The natural history of type 2 diabetes is characterized by the emergence of postprandial and subsequently, fasting hyperglycemia (1). In most individuals, hyperglycemia results from a failure of β-cell insulin secretory capacity to adequately compensate for insulin resistance in peripheral tissues (2). Results from the U. K. Prospective Diabetes Study (UKPDS) indicate that β-cell failure is progressive, despite therapy with diet, insulin, sulfonylurea, or metformin (3, 4). In addition, a reduction in glycosylated hemoglobin A₁c (HbA₁c) lowered the risk of vascular complications, suggesting that any reduction in HbA₁c may be beneficial to subjects with type 2 diabetes. As β-cell dysfunction is progressive, many type 2 patients eventually require insulin as primary therapy to achieve glycemic goals (5, 6). Glucose control in this population remains inadequate, as average HbA₁c values reported in most epidemiologic studies are well above 8% as compared to normal levels <6%, despite the availability of a number of therapeutic agents (7, 8).

AC2993 (synthetic exendin-4; exenatide), a 39-amino acid peptide, exhibits many desirable features of a novel antidiabetic therapy, including glucose-dependent enhancement of insulin secretion (9-11), suppression of inappropriately high glucagon secretion (12), and slowing of gastric-emptying rate (13), which may be paradoxically accelerated in people with diabetes (14). At least some of these antidiabetic actions (e.g., enhancement of insulin secretion) may be mediated by binding of AC2993 to the pancreatic glucagon-like peptide-1 (GLP-1) receptor (15). Also, in animal and in vitro models, AC2993 and GLP-1 have been shown to promote β-cell proliferation and neogenesis from precursor cells (16-18). Data obtained in animal models also indicate that AC2993 reduces food intake, causes weight loss, and has an insulin-sensitizing effect (9, 19, 20). The present study was undertaken to evaluate the effectiveness of AC2993 in improving glycemic control among patients with type 2 diabetes who are treated with sulfonylureas or metformin, alone or in combination, over a 28-day period.

METHODS

Study Subjects
A total of 116 patients with type 2 diabetes were recruited from 24 sites throughout the U.S. Males, or females who were surgically sterile or postmenopausal, aged 18-65 years were eligible for enrollment. Patients were to be treated with diet and a regimen of sulfonylurea or metformin (alone or in combination) that was stable for at least the prior 6 months. The entry oral antidiabetic agent regimen was continued throughout the study. Planned entry HbA₁c was between 8.0 and 11.0%. Actual enrolled HbA₁c ranges are given in Table 1. The target BMI at enrollment was 27-40 kg/m², inclusive. The following Institutional Review Boards approved this study: The Western IRB, Olympia, Washington; University of North Carolina, Chapel Hill, North Carolina; Henry Ford Hospital, Detroit, Michigan; Washington Hospital, Freemont, California; Medstar Research Institute, Washington, D.C.; and Scripps Clinic, San Diego, California. All patients provided written informed consent.
Table 1. Demographic and Baseline Characteristics of Patients Treated With AC2993 or Placebo*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AC2993 BID (bd) (N = 26)</th>
<th>AC2993 BID (bs) (N = 27)</th>
<th>AC2993 TID (bds) (N = 28)</th>
<th>Placebo (N = 28)</th>
</tr>
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<tbody>
<tr>
<td>Sex - % (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>62 (16)</td>
<td>63 (17)</td>
<td>57 (16)</td>
<td>75 (21)</td>
</tr>
<tr>
<td>Women</td>
<td>38 (10)</td>
<td>37 (10)</td>
<td>43 (12)</td>
<td>25 (7)</td>
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<tr>
<td>Race - % (N)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>54 (14)</td>
<td>44 (12)</td>
<td>54 (15)</td>
<td>68 (19)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>27 (7)</td>
<td>30 (8)</td>
<td>21 (6)</td>
<td>25 (7)</td>
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<tr>
<td>Black</td>
<td>13 (3)</td>
<td>22 (6)</td>
<td>18 (5)</td>
<td>7 (2)</td>
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<td>Other</td>
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<td>4 (1)</td>
<td>7 (2)</td>
<td>0 (0)</td>
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<tr>
<td>Age (years)</td>
<td>54 ± 8</td>
<td>51 ± 9</td>
<td>50 ± 9</td>
<td>51 ± 9</td>
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<td>Weight (kg)</td>
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<td>98.2 ± 16.0</td>
<td>96.8 ± 17.5</td>
<td>97.6 ± 16.7</td>
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<td>Body-mass index (kg/m²)</td>
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<td>Fasting plasma glucose (mmol/l)</td>
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<td></td>
<td></td>
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<tr>
<td>Day –1</td>
<td>11.2 ± 2.8</td>
<td>11.6 ± 3.3</td>
<td>10.9 ± 2.4</td>
<td>12.3 ± 3.6</td>
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<tr>
<td>Day 1</td>
<td>11.1 ± 3.4</td>
<td>11.7 ± 3.6</td>
<td>10.7 ± 2.5</td>
<td>12.0 ± 3.4</td>
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<td>Day 28</td>
<td>9.4 ± 2.5</td>
<td>10.2 ± 3.5</td>
<td>9.3 ± 3.2</td>
<td>10.9 ± 3.5</td>
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<tr>
<td>Serum fructosamine (µmol/l) †</td>
<td>344 ± 74</td>
<td>340 ± 71</td>
<td>331 ± 59</td>
<td>346 ± 56</td>
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<tr>
<td>HbA₁c - % † (Range)</td>
<td>9.1 ± 1.2 (7.7 - 12.0)</td>
<td>9.3 ± 1.0 (7.6 - 12.6)</td>
<td>9.2 ± 1.1 (7.7 - 11.7)</td>
<td>9.4 ± 1.3 (7.7 - 11.6)</td>
</tr>
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<td>Oral agent use - % (N)</td>
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<td></td>
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<tr>
<td>Sulfonylurea alone</td>
<td>12 (3)</td>
<td>15 (4)</td>
<td>36 (10)</td>
<td>18 (5)</td>
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<tr>
<td>Metformin alone</td>
<td>23 (6)</td>
<td>22 (6)</td>
<td>21 (6)</td>
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<td>Sulfonylurea + metformin</td>
<td>65 (17)</td>
<td>63 (17)</td>
<td>43 (12)</td>
<td>61 (17)</td>
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<tr>
<td>Anti-hypertensive agent - % (N)</td>
<td>42 (11)</td>
<td>63 (17)</td>
<td>54 (15)</td>
<td>46 (13)</td>
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</table>

* Plus-minus values are means ±SD.
† The upper limit of the normal range for serum fructosamine is 285 µmol/l. The upper limit of the normal range for glycosylated hemoglobin is 6.0%.
b = breakfast, d = dinner, s = bedtime

Study Design

This was a randomized, triple-blind (with respect to subject, investigator, and sponsor), parallel-group, placebo-controlled study designed to assess glucose control and evaluate safety in patients receiving subcutaneously (SC) injected AC2993 (0.08 µg • kg⁻¹ • injection⁻¹) or placebo for 28 days. After a 2-week, single-blind, placebo lead-in, patients were randomly assigned to one of three AC2993 treatment groups: twice daily (BID) [breakfast (b) and dinner (d)]; BID [b and bedtime (s)]; thrice daily (TID) [bds]; or placebo TID [bds]. To maintain the study blind, patients treated BID received a third injection of placebo. Appropriate volumes of study medication [0.1 mg/ml AC2993 (Star Biochemicals, now Mallinckrodt, Inc., Torrance, CA)] or placebo, both manufactured according to current good manufacturing practices, were administered using an insulin syringe.

Activity assessments included changes from baseline to day 28 in serum fructosamine and postprandial plasma glucose, HbA₁c, fasting plasma glucose, body weight, and fasting and postprandial lipids. Baseline was defined as Day –1 for glucose and lipid measures and...
as Day 1 for all other measures. Subjects were required to fast each day after midnight. After at least 14 days of single-blind placebo lead-in, subjects returned for a standardized meal tolerance test (Day -1). The test was performed in the morning after the all-night fast, 15 min after injection of placebo. Blood samples were collected at 15 and 5 min before the injection, and at 0.5, 1, 1.5, 2, 3, 4 and 6 h after the injection. The 6-h sample was only assayed for AC2993. The following morning (day 1), after another all-night fast, subjects were randomized to placebo or one of three AC2993 treatments. Subjects also underwent identical standardized meal tolerance tests with timed blood sampling on Days 1, 14, and 28. The standardized meal had a composition of 55% carbohydrate, 15% protein, and 30% fat. Patients consumed one bagel, one cheese slice, orange juice, soft corn margarine, and 2% milk. The quantity of liquids and margarine were adjusted based on body weight to maintain equal caloric intake per kilogram and activity level across the patient cohort.

Quantitation of plasma glucose, fructosamine, insulin, HbA1c, lipids, and cortisol were performed by Esoterix Laboratory (Calabasas Hills, CA) according to well-established methods. Quantitation of AC2993 and anti-AC2993 antibodies was done by Amylin Pharmaceuticals, Inc. (San Diego, CA). Glucose was measured using the Roche Glucose/HK Reagent Set (Roche Diagnostics, Indianapolis, IN) for glucose analysis. Fructosamine was measured using a colorimetric assay (21). Insulin was measured using a two-site immunoenzymometric assay. Total hemoglobin and HbA1c were measured using high-performance liquid chromatography (HPLC), with a normal reference range of 4.2-5.9%. The assay provides values comparable to those obtained in the Diabetes Control and Complications Trial (22). Cortisol was measured by radioimmunoassay. Plasma AC2993 concentrations were measured using an immunoenzymometric assay. Briefly, microtiter plates were coated with an anti-AC2993 monoclonal antibody (Amylin Pharmaceuticals) at 4°C for 1-3 days. Nonspecific binding sites were blocked using 1% nonfat dried milk diluted in 0.05 M carbonate buffer (pH 9.5). Samples were added to wells and incubated for 1-2 h at room temperature. A biotinylated secondary monoclonal antibody (Amylin Pharmaceuticals) preincubated with streptavidin-alkaline phosphatase was added to each well and incubated at room temperature for 1-2 h. Wells were washed with Tris-buffered saline/Tween (0.05 mol/l Tris, 0.15 mol/l sodium chloride, 0.02% sodium azide, 0.1% Tween-20) between incubation steps. The assay was developed using 50 μl of 0.1 mg/ml 4-methylumbelliferyl phosphate solution. The reaction was stopped using 0.1 mol/l sodium phosphate/1.5 mol/l sodium chloride.

Measurement of anti-AC2993 antibodies was performed using a solid-phase enzyme-linked immunosorbent assay. Briefly, AC2993 (5 μg/ml in 0.05 mol/l carbonate buffer, pH 9.5) was added to wells of microtiter plates, except for control wells coated with anti-human Ig (10 μg/ml in the same buffer). The plates were incubated 1-3 days at 4°C, then incubated with MegaBloc3 (1:500; Cell Associates, The Sea Ranch, CA) for 1 h at room temperature. Samples were added and incubated for 1 h. Between incubation steps, wells were washed with PBS/0.1% Tween-20. Anti-human Ig/horseradish peroxidase was added and the mixture incubated for 1 h. The assay was then developed using 0-phenylenediamine (Sigma, St. Louis, MO) in citrate buffer (0.055 mol/l phosphate/0.024 mol/l citrate, pH 5.0). The reaction was stopped by 4N sulfuric acid. Absorbance was read at 490 nm.
Homeostasis model assessment (HOMA) (23) was conducted to assess β-cell function at baseline and at Days 14 and 28. The HOMA scores (24) were calculated as follows:

\[
\text{HOMA β-cell index} = \frac{20 \times \text{fasting insulin (µU/ml)}}{\text{fasting glucose (mmol/l)} - 3.5}
\]

Safety was evaluated using spontaneous adverse event reporting and monitoring of clinical laboratory measures and vital signs. Nausea was assessed as part of the routine adverse event gathering process.

**Statistical Analysis**

All summaries and analyses were based on the intent-to-treat population. A total of 109 patients were randomized and received at least one dose of study medication. Twelve subjects withdrew from the study [3 in placebo, 4 in AC2993 BID (bd), 2 in AC2993 BID (bs), and 3 in AC2993 TID], but were included in the statistical analyses up until the time of their withdrawal. Efficacy endpoints were analyzed using a one-way ANOVA to test the null hypothesis of no difference among treatment groups versus the alternative hypothesis of a difference among treatment groups. No data imputation was employed. Pairwise comparisons were also conducted between the AC2993 arms and placebo. P values ≤0.05 were considered significant. Specific P values are given in the text.

The study was powered to detect a statistically significant difference (\(\alpha = 0.05\)) of 30 µmol/l in the change in fructosamine from baseline between one of the AC2993 treatment groups and placebo. The sample size also provided ~80% power to detect a statistically significant difference (\(\alpha = 0.05\)) of 1.7 mmol/l change in average postprandial plasma glucose from baseline between one of the AC2993 groups and placebo.

Time-weighted averages over all post-meal time points were computed for Days -1, 1, and 28 postprandial glucose concentration values. The time-weighted average was computed as the area under the concentration-time curve (AUC) value obtained using the trapezoidal rule divided by the sampling duration. Time-weighted averages were also calculated for postprandial lipids.

**RESULTS**

**Study Subjects**

A total of 116 patients with type 2 diabetes were recruited from 24 sites throughout the U.S., resulting in 109 subjects randomized to the intent-to-treat population. Of the seven excluded subjects, four did not meet the inclusion/exclusion criteria after screening, two withdrew consent, and one had an adverse event prior to randomization (motor vehicle accident). Baseline and demographic characteristics were well balanced across the four treatment groups (Table 1).
Serum Fructosamine and HbA1c
AC2993 treatment led to statistically significant (P ≤ 0.004) reductions in serum fructosamine at Day 28 [45, 39, and 46 µmol/l for BID (bd), BID (bs), and TID (bds), respectively] relative to placebo (5 µmol/l; Fig. 1A). Similarly, statistically significant (P ≤ 0.006) reductions in HbA1c were observed after all AC2993 regimens [1.1%, 0.7%, and 1.0% for BID (bd), BID (bs), and TID, respectively] compared with placebo (0.3%) during the same treatment period (Fig. 1B). The overall reduction for the three AC2993 groups combined was ~0.9%. End of study HbA1c of <7% was achieved by 15% of all AC2993 patients compared with 4% of placebo. The proportion of patients with entry HbA1c ≥8% achieving A1C<8% was 43% and 5% for AC2993 and placebo patients, respectively.

**Figure 1.** (A) Mean (±SE) change in fructosamine from day 1 to day 28 of treatment with 0.08 µg/kg AC2993 or placebo, summarized for each treatment group separately (intent-to-treat population). (B) Mean (±SE) change in HbA1c from baseline (day –1) to day 28 of treatment with 0.08 µg/kg AC2993 or Placebo, summarized for each treatment group separately (intent-to-treat population). (C) Mean (±SE) β cell index calculated by HOMA at baseline (day –1), day 1, day 14 and day 28 of treatment with 0.08 µg/kg AC2993 or Placebo, summarized for each treatment group separately (intent-to-treat population). b, breakfast; d, dinner; s, bedtime.

**β-cell Function**
AC2993 treatment appeared to enhance β-cell function, as assessed by HOMA analysis (Fig. 1C). The β-cell index for all regimens ranged from 50-100% greater at Days 14 and 28 of AC2993 treatment compared to baseline (day –1) and day 1. The β-cell index for placebo-treated patients remained unchanged.
Plasma Glucose
Statistically significant reductions (P ≤ 0.004) from baseline to day 28 in mean postprandial plasma glucose concentration were observed with AC2993 treatment [4.4, 3.2, and 3.4 mmol/l for BID (bd), BID (bs), and TID, respectively] compared with placebo (0.6 mmol/l). These results were similar to the reductions seen on the first day of treatment (Fig. 2). Mean reductions in fasting plasma glucose after AC2993 treatment at day 28 (range of means: 1.2-1.7 mmol/l) were not significantly different from placebo (1.1 mmol/l), although there was a trend toward reduced fasting glucose throughout the study. Compared with day 1, the AC2993 plasma glucose profile on day 28 showed reduced fasting glucose concentrations. On day 28, there was a modest rise in postprandial glycemia after AC2993 treatment; however this change was markedly lower than in the placebo treatment arm on day 28.

Body Weight
There was no significant effect of AC2993 treatment on change in body weight from day 1 to day 28 (range of means −0.8 to +0.1 kg) compared with placebo treatment arm (+0.9 kg). There was no detectable influence of baseline HbA1c, baseline body weight, or concomitant antidiabetic therapy on weight loss; however the overall size of the cohort and the duration of the trial were insufficient to definitively state that these were not covariates.

Lipids
There were no notable differences among the treatment groups in fasting concentrations of triglycerides, HDL cholesterol, LDL cholesterol, or apolipoprotein B at day 28. While not statistically significant, incremental (baseline-adjusted) time-weighted average postprandial triglyceride concentrations tended to be decreased at day 28 compared to day −1 in the AC2993...
treatment groups (change from day –1 to day 28, range -0.25 to –0.35 mmol/l) compared with placebo (change from day –1 to day 28, 0.14 mmol/l).

**AC2993 Pharmacokinetics**

Plasma AC2993 for all patients increased steadily until reaching peak concentrations 2-3 h after administration of 0.08 μg/kg SC and were still detectable 6 h post-dose. For subjects with undetectable anti-AC2993 antibodies, $t_{\text{max}}$ was 202 ± 182 min. on day 1 and 226 ± 170 min on day 28 with corresponding $C_{\text{max}}$ values of 163 ± 86 and 159 ± 81 pg/ml, respectively ($N = 63$; mean ± SD). For subjects with anti-AC2993 antibodies at any time during the study, $t_{\text{max}}$ was 125 ± 42 min on day 1 and 373 ± 250 min on day 28 with corresponding $C_{\text{max}}$ values of 172 ± 57 and 357 ± 215 pg/ml, respectively ($n = 18$ for all AC2993 arms combined).

**Vital Signs and Clinical Laboratory Assessments**

No notable differences were observed for the change in vital signs (blood pressure and heart rate) from day –1 to day 28 among any treatment groups, which included a substantial proportion of patients (~50%) receiving concomitant medications for preexisting hypertension. No patient withdrew prematurely from the study due to a vital sign abnormality.

There were no clinically relevant changes in hematology, clinical chemistry, or urinalysis analyte values from day –1 to day 28 in any of the treatment groups.

There were no statistically significant effects of AC2993 on postprandial plasma cortisol on days 1 and 28 (data not shown). The range of means was 246-480 nmol/l. There was a small, acute, transient increase in serum cortisol concentrations after dosing with AC2993 on day 1, compared with placebo. There was no rise in post-dose mean cortisol values in any of the treatment groups at day 28.

Fifteen (19%) of the 81 AC2993-treated patients developed low-titer anti-AC2993 antibodies during the study. There was no evidence that patients with an antibody response had a diminished glycemic response.

**Adverse Events**

The most common treatment-emergent adverse events reported were nausea (31% overall incidence) and hypoglycemia (15% overall incidence). The normal range for plasma glucose is 3.9-5.8 mmol/l. Values <3.3 mmol/l were considered hypoglycemic. The majority (91%) of the reported nausea and all hypoglycemia were mild or moderate in intensity, with no reports of hypoglycemia requiring the assistance of another individual. Hypoglycemia only occurred in patients who were taking a sulfonylurea.

Nausea was only observed in the AC2993 arms and was most pronounced during the initial days of treatment. Thereafter, the incidence of nausea declined to ~13% by the end of the 28-day treatment period. Four (3.7%) of the 109 patients withdrew due to nausea, all within the first 12 days of the study.

There was no evidence of adverse events that could be associated with an allergic reaction to study medication for patients with anti-AC2993 antibodies.
DISCUSSION

Exendin-4 was originally isolated from the salivary secretions of the lizard Heloderma suspectum (Gila monster), in which it circulates after ingestion of a meal (25), and may have endocrine functions related to metabolic control. Exendin-4 has a 53% amino acid sequence overlap with mammalian GLP-1. However, exendin-4 is the product of a separate gene distinct from the proglucagon gene from which GLP-1 is expressed (26). Unlike GLP-1, which is degraded within 1-2 minutes by dipeptidyl peptidase-IV (DPP-IV) when administered SC, exendin-4 is resistant to DPP-IV degradation and, thus, is more readily available for exerting beneficial metabolic effects similar to those of GLP-1 (27). These properties confer upon AC2993 (synthetic exendin-4, exenatide) a very high (>1000-fold) in vivo potency relative to GLP-1 (10, 28). However, not all actions of exendin-4 are predictable based on the known pharmacology of GLP-1. For example, GLP-1, but not exendin-4, suppresses gastric acid secretion (29). Moreover, while intraportal GLP-1 infusion triggers the hepatic vagal afferents, exendin-4 does not (30).

The current study indicates that 28 days of AC2993 treatment reduces HbA1c by ~0.9% compared to baseline in patients with type 2 diabetes not attaining HbA1c goals with oral agent therapy or diet. In addition, the proportion of patients achieving the American Diabetes Association target HbA1c <7% (31) was fourfold greater in the AC2993 arms. Given that HbA1c only fully reflects a change in glycemia 3 months after a sustained change has occurred, this reduction in HbA1c and enhanced ability to achieve clinically relevant HbA1c target values over 1 month are highly clinically significant.

Glucose profiles during ingestion of a mixed meal demonstrated a marked acute effect of AC2993 to reduce postprandial glycemia that was sustained over the 28-day observation period. This postprandial effect is likely mediated via three key actions of AC2993 that have been observed in animal models of diabetes: 1) increased release of insulin and amylin from the β-cell (10); 2) suppression of the paradoxically high glucagon secretion observed in animals with diabetes (12); and 3) slowing of the rate of gastric emptying (13). While no statistically demonstrable effect on fasting plasma glucose was observed at day 28, day 14 fasting plasma glucose in the AC2993 group was significantly reduced compared to placebo (data not shown). The ability of AC2993 to reduce fasting plasma glucose has previously been reported to be secondary to enhanced insulin secretion (10) and suppression of inappropriately elevated glucagon secretion (12). In patients with type 2 diabetes, GLP-1 has also been shown in a 6-week study to lower both fasting and postprandial glucose (32). However, in contrast to AC2993, GLP-1 needed to be continuously infused (32).

The effects of AC2993 on postprandial, and to a lesser extent, fasting glycemia were the key factors leading to the changes in fructosamine and HbA1c (indicators of average glycemic control over the prior 2 weeks and 3 months, respectively). It is important to note that the study was not designed to assess differences among the various treatment groups. Across the different regimens, 28 days of AC2993 resulted in fructosamine concentrations approaching the upper limit of normal (285 μmol/l) and HbA1c reductions of ~0.9%. This clinically significant improvement in glycemic indexes over 28 days in patients not previously achieving glycemic control with metformin and/or sulfonylurea treatment is remarkable, as the magnitude of improvement is difficult to achieve with the simple addition of a second or third oral agent (33). Moreover, while insulin therapy can be used to achieve this outcome,
A vast literature documents that this approach is generally associated with significant weight gain (4), increased hypoglycemia (4), and attendant morbidities (34). Interestingly, AC2993 treatment was associated with no weight gain in the face of improved glycemic control. Consistent with this observation, exendin-4 has been reported to acutely reduce food intake in healthy human subjects (35) and cause weight loss in animal models of obesity (9).

HOMA analysis revealed improved β-cell secretory function following AC2993 therapy. It is noteworthy that fasting values of plasma glucose and insulin, which were used to calculate HOMA, were obtained before the morning dose of AC2993 when plasma concentrations of AC2993 were negligible, suggesting a fundamental alteration in β-cell function following AC2993 exposure. These data are consistent with the extensive literature documenting enhanced β-cell function after treatment with exendin-4 in animal models of diabetes (16-18).

AC2993 treatment was also associated with a strong trend towards reduced postprandial serum triglyceride concentrations, as was also seen in previous, short-term clinical studies (36). While fasting lipid parameters tended to improve, there were no differences compared with placebo.

Safety was evaluated using spontaneous adverse event reporting and monitoring of clinical laboratory measures and vital signs. The most common adverse events encountered were mild to moderate nausea and hypoglycemia. Nausea tended to occur mainly upon initiation of therapy and subsided over the first week. Hypoglycemia was mostly undocumented, mild to moderate, and did not require assistance from another person. Importantly, there were no reports of hypoglycemia in AC2993-treated patients receiving metformin alone—consistent with the notion that sulfonylureas are inherently hypoglycemic independent of the prevailing glucose concentration. Although one mechanism of AC2993 action is to slow gastric emptying to better match the rate of nutrient inflow with the rate of glucose disposal, there was no clinical evidence from this study that AC2993 treatment resulted in the induction or exacerbation of conditions such as gastroparesis.

While a small, acute, and transient rise in serum cortisol was observed on the first day of AC2993 treatment, similar to that seen with GLP-1 (37), the assessment on day 28 revealed no such rise in any study patient. There were no clinically relevant effects of AC2993 on other clinical laboratory analytes, blood pressure, or heart rate.

In conclusion, these data demonstrate for the first time in a randomized, triple-blinded trial that AC2993 administered BID or TID for 28 days in patients with type 2 diabetes failing oral agent therapy causes a marked reduction in HbA1c.

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REFERENCES

Chapter 3

Effectiveness of Progressive Dose-escalation of Exenatide (Exendin-4) in Reducing Dose-limiting Side Effects in Subjects with Type 2 Diabetes

Mark S. Fineman, Larry Z. Shen, Kristin Taylor, Dennis D. Kim and Alain D. Baron

ABSTRACT

BACKGROUND: Exenatide (exendin-4) exhibits dose-dependent glucoregulatory activity, but causes dose-limiting nausea and vomiting. This study was designed to formally assess the possibility of inducing tolerance to the side effects of nausea and vomiting at therapeutic doses of exenatide, using a dose-escalation methodology.

METHODS: In this two-arm, triple-blind, multicenter study, 123 subjects with type 2 diabetes were enrolled and randomized; 99 (80.5%) of them completed the study. Subjects in the exenatide-primed arm received subcutaneous exenatide, starting at 0.02 µg/kg three times a day (TID) and increasing in 0.02 µg/kg per dose increments every 3 days for 35 days. Subjects in the exenatide-naive arm received placebo TID for 35 days. At the end of this 35-day regimen, subjects in both arms received the same highest dose of exenatide (0.24 µg/kg TID) for 3 days. Thus, the exenatide-naive arm received exenatide for the first time on Day 35.

RESULTS: The exenatide-primed arm had a lower proportion of subjects experiencing nausea and vomiting in response to exposure to the highest dose of exenatide (27% vs 56% in the exenatide-naive arm; p = 0.0018). Kaplan-Meier estimates of cumulative incidence were 0.28 in the exenatide-primed arm, compared with 0.68 in the exenatide-naive arm (p≤0.001). As predicted by the study design, fewer subjects in the exenatide-primed arm reported severe nausea (29%) and vomiting (10%) than in the exenatide-naive arm (48% and 31%, respectively). In the exenatide-primed arm, fasting serum glucose progressively declined over the first 35 days of dosing, but was unchanged in the exenatide-naive arm (placebo phase) during the same interval.

CONCLUSION: Gradual dose-escalation of exenatide successfully reduced the proportion of subjects experiencing dose-limiting nausea and vomiting, with no loss of glucoregulatory activity, thus demonstrating the value of gradual dose-escalation in mitigating the gastrointestinal side effects of exenatide.

Keywords: exenatide; exendin-4; type 2 diabetes; incretin mimetic; dose-escalation; mitigation of side effects; reduction of adverse events
INTRODUCTION

Exenatide (exendin-4) is an incretin mimetic having glucoregulatory activities similar to those of native glucagon-like peptide-1 (GLP-1). These actions include glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, and slowing of gastric emptying [1-7]. At least some of these antidiabetic actions (e.g., enhancement of insulin secretion) may be mediated by binding of exenatide to the GLP-1 receptor [8]. While GLP-1 exhibits a half-life of less than 1 min, exendin-4 has a half-life of approximately 20 min when given intravenously, thus making it an attractive potential drug candidate. In animal models of diabetes and in insulin-secretory cell lines, exenatide and GLP-1 reportedly improve β-cell function by increasing the expression of key genes involved in insulin secretion, by increasing insulin biosynthesis, and by augmenting β-cell mass through multiple mechanisms [4]. Data obtained in animal models also indicate that exenatide and GLP-1 reduce food intake, cause weight loss, and have an insulin-sensitizing effect [4].

Exenatide is a 39-amino acid synthetic peptide currently undergoing clinical evaluation. It has demonstrated potentially beneficial antidiabetic actions at doses of 0.05 μg/kg to 0.4 μg/kg [1-4]. However, exenatide also causes dose-limiting nausea and vomiting. Data from previous short-term controlled studies suggested that the incidence of nausea and vomiting caused by exenatide decreased in parallel with increasing duration of treatment. It is not uncommon to observe drug-induced side effects that subside with increasing duration of drug exposure, a phenomenon known as tolerance [9]. While this phenomenon is relatively well established for small molecule therapeutics, it is less well established for peptide hormones. Moreover, tolerance induction has largely been established by clinical practice rather than by formal assessment.

A critical step in drug development is establishing the correct dose(s) of a therapeutic agent. Dose-limiting side effects can potentially restrict the use of optimally effective doses of therapeutic agents. Thus, during drug development, and early approach to establish the potential for inducing tolerance to dose-limiting side effects of a therapeutic agent could be useful. This study was designed to formally assess the potential induction of tolerance to the nausea and vomiting associated with exenatide administration. The methodology utilized herein may have broad applicability in managing dose-limiting side effects during Phase 2 clinical trials of other drugs to facilitate establishment of optimal dosing regimens.

MATERIALS AND METHODS

Study Subjects
A total of 123 patients with type 2 diabetes mellitus were recruited at 31 sites in the United States. Subjects were males, or females who were surgically sterile or postmenopausal, aged 18 to 65 years, with the following characteristics: (1) fasting blood glucose (FPG) ≤240 mg/dL; (2) diabetes, treated with a regimen of diet and/or metformin and/or thiazolidinediones (TZDs), which was stable for at least 3 months prior to screening; (3) HbA1c between 6.5% and 11.0%, inclusive; (4) body mass index (BMI) between 27 kg/m² and 40 kg/m², inclusive;
(5) no history of diabetic ketoacidosis or blood C-peptide ≤1 ng/mL; (6) no clinically significant abnormal laboratory test values except for those consistent with type 2 diabetes; and (7) no untreated/unstable cardiovascular disease. Subjects were excluded (1) if they had clinically significant comorbid conditions; (2) if they were treated with insulin or glyburide/metformin within 3 months of screening; (3) if they were treated with miglitol, acarbose, repaglinide, or nateglinide within 4 weeks of screening; or (4) if they had chronic use of systemic corticosteroids.

**Study Design**

This was a two-arm, randomized, placebo-controlled, triple-blind (subjects, investigators, and sponsor), multicenter study. The study was designed to compare the proportion of subjects experiencing nausea and vomiting in subjects after receiving a target dose of exenatide that was known to cause nausea and vomiting (0.24 μg/kg), delivered either in a dose-escalation regimen (exenatide-primed arm) or as a first-time exposure (exenatide-naive arm; Table 1). In the exenatide-primed arm, subjects received progressively increasing doses of exenatide 3 times a day (TID), starting from 0.02 μg/kg TID and increasing by 0.02 μg/kg increments per dose every 3 days until reaching a maximum dose of 0.24 μg/kg TID on approximately Day 35±1 day (11 total dose levels). The final dose of 0.24 μg/kg TID was continued for 3 days. The exenatide-naive arm received equivalent escalating volumes of placebo TID for approximately 35 days (±1 day for each visit window). At the end of the placebo phase, the exenatide-naive arm received a first-time dose of 0.24 μg/kg exenatide TID for 3 days. Each dose of the study medication was injected subcutaneously in the abdominal region 15 min before breakfast and dinner, and at bedtime. This design led to comparable blinded exposure to the study procedures between the two arms, and therefore allowed unbiased comparison of the proportion of subjects experiencing dose-limiting side effects between a dose-escalation procedure and acute introduction of a high dose of exenatide. The primary outcome measurement of the study was the proportion of subjects experiencing severe nausea, nausea leading to withdrawal, or vomiting, occurring by the last dosing date. Secondary outcome measurements included the proportion of subjects with all degrees of nausea occurring by the last dosing date, the safety and tolerability of the study medication, and other physical indicators of study medication activity. The intensity of an adverse event was classified as mild (usually transient, requiring no special treatment, and not interfering with the subject’s daily activities), moderate (caused a low level of inconvenience or concern, may interfere with daily activities, but is usually ameliorated by simple therapeutic measures) or severe (interrupted the subject’s usual daily activities and generally required systemic drug therapy or other treatment).

The conduct of this study was approved by an Institutional Review Board for each study site. All patients provided written informed consent. This study was conducted in accordance with the recommendations provided in the Declaration of Helsinki (1964), including all amendments up to and including the South Africa revision (1996).
### Table 1. Dosing schedule

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</table>

*Clinic Visits 3 to 6 were to occur on the day specified, ± 1 day. The termination visit was to occur 4 to 8 days following discontinuation of study medication.

### Statistical Analysis

Statistical analyses were performed on the intent-to-treat population, consisting of all subjects who were randomized and received at least one dose of randomized treatment. Subjects in the intent-to-treat population who withdrew before the last dose because of reasons other than nausea and vomiting were included in the analysis, but were considered as not having completed the study. Fisher’s Exact Test was used to compare the exenatide-primed arm and the exenatide-naive arm with respect to the overall proportion of subjects experiencing severe nausea, nausea leading to withdrawal, and vomiting. The study was designed to provide approximately 80% power to detect a statistically significant reduction in the above incidence (α=0.05) by Day 37±1. It was assumed the proportion of subjects experiencing dose-limiting side effects would be at least 60% in the exenatide-naive arm and no more than 30% in the exenatide-primed arm. To account for early dropout, the Kaplan-Meier method was used to estimate the overall incidence of the side effects over the course of the entire study. The standard errors (SEM) of the Kaplan-Meier estimates were calculated using Greenwood’s formula [10]. Owing to variability associated with the ±1 day window around study visits, analyses were performed on study data out to Day 43. Change in body weight and serum glucose concentrations were evaluated using ANOVA.

### Assays

Serum glucose concentrations were quantitated by Esoterix Center for Clinical Trials (Calabasas Hills, CA) using the Roche Glucose/HK Reagent Set (Roche Diagnostics, Indianapolis, IN).
RESULTS

Study Subjects
A total of 123 subjects were enrolled and randomized, of which 99 (80.5%) completed all dosing days and 24 (19.5%) withdrew prior to receiving the last scheduled dose. Baseline demographics were comparable across treatment arms (Table 2). In the exenatide-naive arm, 77.0% of the subjects completed the study compared with 83.9% in the exenatide-primed arm. Of the subjects who withdrew from the study, 8 in the exenatide-naive arm and 3 in the exenatide-primed arm did so because of a protocol violation, withdrawal of consent, or an administrative problem. The remaining withdrawals, 6 in the exenatide-primed arm and 7 in the exenatide-naive arm, were due to an adverse event.

The oral antidiabetic agents taken concomitantly were metformin [38 subjects (62.3%) in the exenatide-naive arm and 43 subjects (69.4%) in the exenatide-primed arm] and TZDs [9 subjects (14.8%) in the exenatide-naive arm and 10 subjects (16.1%) in the exenatide-primed arm]. Other medications commonly used during the study were ACE-inhibitors (16 in each arm), analgesics (14 in the exenatide-naive arm and 19 in the exenatide-primed arm), antithrombotic agents (16 in the exenatide-naive arm and 13 in the exenatide-primed arm), and serum lipid reducing agents (18 in the exenatide-naive arm and 20 in the exenatide-primed arm).

Table 2. Baseline demographics and study disposition for intent-to-treat subjects.

<table>
<thead>
<tr>
<th></th>
<th>Exenatide-naive (N = 61)</th>
<th>Exenatide-primed (N = 62)</th>
<th>All Subjects (N = 123)</th>
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<tbody>
<tr>
<td>Gender:</td>
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<td></td>
</tr>
<tr>
<td>Female</td>
<td>32 (52.5%)</td>
<td>38 (61.3%)</td>
<td>70 (56.9%)</td>
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<tr>
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<td>29 (47.5%)</td>
<td>24 (38.7%)</td>
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<tr>
<td>Race:</td>
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<td>44 (72.1%)</td>
<td>43 (69.4%)</td>
<td>87 (70.7%)</td>
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<td>8 (12.9%)</td>
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</tr>
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<td>7 (5.7%)</td>
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<td>1 (0.8%)</td>
</tr>
<tr>
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<td>12 (9.8%)</td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>HbA₁c (%)</td>
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<td>7.8±1.5</td>
<td>7.7±1.4</td>
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<td>47 (77.0%)</td>
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<td>Withdrew</td>
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<td>10 (16.1%)</td>
<td>24 (19.5%)</td>
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<td>Reason for Withdrawal</td>
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<td></td>
<td></td>
</tr>
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<td>Withdrawal of Consent</td>
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<td>2 (1.6%)</td>
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<tr>
<td>Adverse Event</td>
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<td>7 (11.3%)</td>
<td>13 (10.6%)</td>
</tr>
<tr>
<td>Investigator Decision</td>
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<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Protocol Violation</td>
<td>2 (3.3%)</td>
<td>1 (1.6%)</td>
<td>3 (2.4%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
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<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>Administrative¹</td>
<td>4 (6.6%)</td>
<td>2 (3.2%)</td>
<td>6 (4.9%)</td>
</tr>
</tbody>
</table>

¹Included discontinuation of study site participation in the study.

Mean±SD.
Clinical Outcomes

There was a statistically significant difference between treatment arms in the proportion of subjects experiencing severe nausea, nausea leading to withdrawal, or vomiting by the date of the last dose (p=0.0018). As shown in figure 1A, 34 subjects (55.7%) in the exenatide-naive arm and 17 subjects (27.4%) in the exenatide-primed arm experienced events in this category. Kaplan-Meier estimates on Day 43 (the day after the last dose day for the last subject) were 0.68 for the exenatide-naive arm and 0.28 for the exenatide-primed arm (p≤0.001). Because the study design allowed a ±1 day window for each visit, and because patients often did not adhere to the protocol’s visit structure, there was some variation in the actual day the subjects in the exenatide-naive arm received the initial 0.24 μg/kg exenatide dose, as well as some variation in the exenatide-primed arm (dosage factor). This variation resulted in a pronounced ‘stepping’ pattern in the proportion of subjects experiencing nausea and vomiting between Days 31 and 36. Figure 1B shows the Kaplan-Meier plot for the proportion of subjects experiencing nausea and vomiting, corrected for this dosage factor. The stepping pattern was reduced (prior to receiving exenatide), however a small but discernable stepping pattern remained after correction for the dosage factor. The remaining stepping pattern was likely attributable to preconditioning during the informed consent process where it was explained that subjects could expect to experience nausea and vomiting during the study.

Subject dropout rates due to nausea or vomiting were estimated on the basis of the raw proportion of subjects who withdrew owing to nausea and/or vomiting by the date of the last dose. Prior to receiving the 0.24 μg/kg dose, 3 subjects (4.9%) in the exenatide-naive arm and 7 subjects (11.3%) in the exenatide-primed arm withdrew because of nausea and/or vomiting. Within the exenatide-primed arm, subjects withdrew at the following doses: 0.02 μg/kg (1), 0.10 μg/kg (1), 0.12 μg/kg (2), 0.16 μg/kg (1), 0.2 μg/kg (1), 0.22 μg/kg (1). As predicted, more subjects in the exenatide-naive arm reported severe nausea (47.5%) and severe vomiting (31.1%) than subjects in the exenatide-primed arm (29.0% and 9.7%, respectively).

The proportion of subjects experiencing all nausea is shown in Figure 2. At Day 43, the proportion of subjects experiencing nausea was higher in the exenatide-naive arm (62.3%) than in the exenatide-primed arm (46.8%). Kaplan-Meier analysis out to Day 43 yielded significantly different values of 0.73 for the exenatide-naive arm and 0.48 for the exenatide-primed arm (p<0.0047).
Figure 1. Kaplan-Meier plots for the proportion of subjects experiencing severe nausea, nausea leading to withdrawal, or vomiting by the date of the last dose. (A) Event incidence was significantly lower in the exenatide-primed arm (p=0.0018). Analysis up to Day 43 (the day after the last dose day for the last subject) yielded values of 0.68 for the exenatide-naive arm and 0.28 for the exenatide-primed arm (p=0.0001). Because the study design allowed a ±1-day window for each visit, there was some variation in the actual day the subjects in the exenatide-naive arm received the initial 0.24 μg/kg exenatide dose (dosage factor). (B) Graph for proportion of subjects experiencing nausea and vomiting, corrected for dosage factor.

Figure 2. Kaplan-Meier plots for the proportion of subjects experiencing all nausea. Nausea incidence at Day 43 was lower in the exenatide-primed arm. Analysis up to Day 43 (the day after the last dose day for the last subject) yielded values of 0.73 for the exenatide-naive arm and 0.48 for the exenatide-primed arm (p=0.0047). Because the study design allowed a ±1-day window for each visit, there was some variation in the actual day the subjects in both arms received the initial 0.24 μg/kg exenatide dose.

Summary of Adverse Events
No serious adverse events were observed. A total of 108 subjects experienced 508 treatment-emergent adverse events distributed similarly across treatment arms. Fifty-seven subjects (93.4%) in the exenatide-naive arm experienced 231 adverse events and 51 subjects (82.3%) in the exenatide-primed arm experienced 277 adverse events. The most common treatment-emergent adverse events were gastrointestinal disorders, with 88 subjects (71.5%) experiencing 318 events, primarily nausea or vomiting. The next most frequent gastrointestinal adverse event was diarrhea, reported in 6 subjects (9.8%) in the exenatide-naive arm and 10 subjects (16.1%) in the exenatide-primed arm. Of the subjects reporting diarrhea, 2 subjects in the exenatide-primed arm reported 3 severe events. All other diarrhea events were rated mild or moderate.
Treatment-emergent adverse events in other system/organ classes that occurred in more than 5% of subjects were headache in 14 subjects (11.4%; 7 subjects each arm), decreased appetite in 10 subjects (8.1%; 5 subjects each arm), dizziness (excluding vertigo) in 9 subjects (7.3%; 4 in exenatide-naive arm), and mild-to-moderate injection-site bruising in 9 subjects (7.3%; 6 in exenatide-naive arm). Only one incident of headache was rated severe (exenatide-naive arm); the remainder were rated mild or moderate in intensity.

**Fasting Serum Glucose Profile**

Fasting serum glucose concentrations progressively decreased in the exenatide-primed arm during the first 35-day dosing phase in parallel with exenatide dose increments of 0.02 μg/kg TID every 3 days (Figure 3). All fasting serum glucose concentrations after the initiation of dosing were significantly lower than at baseline (p≤0.001). By the end of the 35-day dosing phase, fasting serum glucose in the exenatide-primed arm was 34% lower than that at baseline (115 mg/dL on day 35 versus 174 mg/dL on Day 1; p≤0.001). In contrast, no change in fasting serum glucose concentrations was observed in the exenatide-naive arm during the 35-day dosing phase when this arm received placebo.

**Body Weight**

There was a significantly greater reduction in body weight in the exenatide-primed arm than in the exenatide-naive arm (p≤0.001). The change in weight was -1.54±0.25 kg in the exenatide-primed arm and -0.38±0.20 kg in the exenatide-naive arm.

**DISCUSSION**

The reduction in number of drug-induced side effects with increasing duration of drug exposure, a phenomenon known as tolerance, is well documented in medical literature [9]. In addition, the induction of drug tolerance does not appear to be limited to any particular chemical class of therapeutic compounds or disease targets [9]. The α-glucosidase inhibitor, acarbose, and the biguanide metformin are two examples of antidiabetic agents with a recommended schedule of stepwise dose-escalation at the initiation of treatment for the
purpose of building tolerance to dose-limiting gastrointestinal side effects [11-14]. In both cases, the recommendation to slowly escalate the doses came from post hoc analyses of clinical trial data or from clinical experience. For example, in a retrospective analysis of 677 elderly subjects (>65 years old) with diabetes, subjects who initiated acarbose treatment at a higher daily dose (>100 mg) were 1.4-times more likely to discontinue treatment than were those who started at a lower dose [15]. On the basis of similar experiences, an acarbose study demonstrated that the proportion of subjects experiencing gastrointestinal distress decreased from 41% at Week 4 to 25% at Week 24 on a dosing regimen of 50 mg TID for the first 4 weeks, which was increased to a maintenance dose of 100 mg TID thereafter [16]. Dose-escalation is also commonly used for other therapeutic classes such as antihypertensives (alpha blockers) and psychotropics (selective serotonin reuptake inhibitors). In addition, anecdotal evidence in the medical literature supports the use of gradual dose-escalation to mitigate side effects. For example, Ohira et al. [17] reported a patient with dose-limiting reactions to the protein erythropoietin who responded to dose-escalation with complete amelioration of adverse side effects. While clinical practice experience is important in suggesting how best to dose a given drug safely, it is also important to better understand the therapeutic feasibility and magnitude of effect of dose-escalation. Clearly, establishing that dose-escalation reduces dose-limiting adverse events can serve to design better treatment algorithms that minimize adverse events and maximize therapeutic dose.

The proportion of subjects experiencing dose-limiting nausea and vomiting caused by acute administration of exenatide doses greater than 0.2 μg/kg approached 70% in early single dose-rising trials. The purpose of the current study was to use a rigorous study design from both clinical and statistical perspectives to evaluate whether a dose-escalation regimen could reduce the primary dose-limiting adverse events of exenatide. We used the current design because it offered subjects in the two treatment arms comparable exposure to study procedures and captured the placebo effect in the exenatide-naive arm. Most importantly, this study design showed that after approximately 40 days of cumulative exposure to exenatide, the cumulative incidence of nausea and vomiting remained lower than that observed in the exenatide-naive arm with only 3 days of exposure to exenatide. As predicted, more subjects reported severe nausea and vomiting when exposed to a first-time dose of 0.24 μg/kg exenatide (exenatide-naive arm) than did subjects who were ultimately receiving the same dose following dose-escalation, thus confirming the utility of the dose-escalation strategy to ameliorate side effects to this peptide. Therefore, this dose-escalation approach was effective.

Fasting serum glucose concentrations progressively decreased in the exenatide-primed arm during the first 35-day dosing phase, in parallel with incremental increases in exenatide dose. By the end of the 35-day dosing phase, mean fasting serum glucose in the exenatide-primed arm was 34% lower than at baseline. In contrast, no change in fasting glucose concentrations were observed in placebo-treated subjects during the 35-day dosing phase. These results further support the glucoregulatory effects of exenatide reported in humans [1,2]. Most importantly, the progressive reduction in fasting serum glucose observed in the exenatide-primed arm underscores the important observation that the dose-escalation did not appear to induce tolerance or tachyphylaxis to the potent glucoregulatory activities of exenatide. These results demonstrate the value and feasibility of this study design in assessing the possibility of tolerance induction to adverse events, with no apparent loss of therapeutic activity, before initiating pivotal Phase 3 clinical trials.
It is important to keep in mind that the design of this study does not represent a recommendation of the treatment protocol for initiation of exenatide treatment, but simply a proof-of-principle that dose-escalation has the desired effect to reduce the proportion of subjects experiencing nausea. It would be unrealistic to expect patients to adopt such a multi-stepped dose-escalation scheme. Nevertheless, these data were valuable in designing a dosing scheme for Phase 3 clinical trials for exenatide. Indeed, if one assumes that induction of tolerance is related to total drug exposure, one can derive a less complex dose-escalation regimen.

In summary, a dose-escalation regimen to a target dose of exenatide reduced the proportion of subjects experiencing dose-limiting nausea and vomiting compared to acute exposure to the same target dose. In addition, exenatide treatment in the exenatide-primed arm was associated with reduced fasting plasma glucose concentrations, with no apparent loss of the glucoregulatory activity of the drug. This study design could potentially be generally applicable to other drugs with dose-limiting side effects.

ACKNOWLEDGEMENTS

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REFERENCES


Chapter 4

Pharmacokinetics and Pharmacodynamics of Exenatide Extended-release After Single- and Multiple-dosing

Mark Fineman, Shawn Flanagan, Kristin Taylor, Maria Aisporna, Larry Z. Shen, Kenneth F. Mace, Brandon Walsh, Michael Diamant, Brenda Cirincione, Prajakti Kothare, Wen I Li, Leigh MacConell

ABSTRACT

BACKGROUND AND OBJECTIVES: Exenatide is a glucagon-like peptide-1 receptor agonist, available in an immediate-release (IR), twice-daily formulation, which improves glycemic control through the enhancement of glucose-dependent insulin secretion, suppression of inappropriately elevated postprandial glucagon secretion, slowing of gastric emptying and reduction of food intake. The objectives of these studies were to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of an extended-release (ER) exenatide formulation in patients with type 2 diabetes mellitus.

PATIENTS AND METHODS: Patients with type 2 diabetes participated in either a single-dose trial (n=62) or a repeated-administration trial (n=45). The pharmacokinetic and safety effects of single-dose subcutaneous administration of exenatide ER (2.5 mg, 5 mg, 7 mg, or 10 mg) versus placebo were studied over a period of 12 weeks in patients with type 2 diabetes. These results were used to predict the dose regimen of exenatide ER required to achieve steady-state therapeutic plasma exenatide concentrations. A second clinical study investigated the pharmacokinetics, pharmacodynamics and safety of weekly exenatide ER subcutaneous injections (0.8 mg or 2 mg) versus placebo in patients with type 2 diabetes over a period of 15 weeks. Furthermore, population-based analyses of these studies were performed to further define the exposure-response relationships associated with exenatide ER.

RESULTS: Exenatide exposure increased with dose (2.5 mg, 5 mg, 7 mg, or 10 mg) and exhibited a multiple-peak profile over approximately 10 weeks. Multiple-dose pharmacokinetics were predicted from superpositioning of single-dose data; weekly administration of exenatide ER 0.8 mg and 2 mg for 15 weeks confirmed predictions. Weekly dosing resulted in steady-state plasma exenatide concentrations after 6-7 weeks. Fasting plasma glucose was reduced similarly with both doses after 15 weeks (-42.7±15.7 mg/dL with the 0.8 mg dose and -39.0±9.3 mg/dL with the 2 mg dose; both p<0.001 vs. placebo), and the integrated exposure-response analysis demonstrated that the drug concentration producing 50% of the maximum effect (EC₅₀) on fasting plasma glucose was 56.8 pg/mL (a concentration achieved with both the 0.8 mg and 2 mg doses of exenatide ER). The 2 mg dose reduced bodyweight (-3.8±1.4 kg; p<0.05 versus placebo) and postprandial glucose excursions. Glycosylated haemoglobin (HbA₁c) was reduced with the 0.8 mg dose (-1.4±0.3%; baseline 8.6%) and with the 2 mg dose (-1.7±0.3%; baseline 8.3%) [both p<0.001 vs placebo]. Adverse events were generally transient and mild to moderate in intensity.

CONCLUSION: These studies demonstrated that (i) a single dose of exenatide ER resulted in dose-related increases in plasma exenatide concentrations; (ii) single-dose exposure successfully predicted the weekly-dosing exposure, with 0.8 mg and 2 mg weekly subcutaneous doses of exenatide ER eliciting therapeutic concentrations of exenatide; and (iii) weekly dosing with either 0.8 and 2 mg exenatide ER improved fasting plasma glucose control, whereas only the 2 mg dose was associated with improved postprandial control and weight loss.
INTRODUCTION

In preclinical and clinical studies, the glucagon-like peptide (GLP)-1 receptor agonist exenatide has been demonstrated to improve glycaemic control through the enhancement of glucose-dependent insulin secretion, suppression of inappropriately elevated postprandial glucagon secretion, slowing of gastric emptying, and reduction of food intake\(^1\)-\(^4\). A subcutaneous, twice-daily, immediate-release (IR) formulation of exenatide (elimination half-life \(t_{1/2}\) 2.4 hours, time to reach the maximum plasma concentration \(T_{\text{max}}\) 2.1 hours\(^5\)) which is approved for the treatment of type 2 diabetes mellitus, is administered within 60 minutes of the morning and evening meals. Thus, the glucose-lowering actions of this formulation are most evident during postprandial periods. In placebo-controlled trials, twice-daily administration of exenatide IR resulted in mean reductions of approximately 1% in glycosylated haemoglobin (HbA\(_{1c}\)) levels, with bodyweight reductions ranging from -1.5 to -2.8 kg\(^6\)-\(^9\).

An alternative, long-acting formulation of exenatide (exenatide extended-release [ER]; also referred to as exenatide QW) has recently been developed that utilizes biodegradable polymeric microspheres, composed of exenatide in a poly lactide-co-glycolide (PLG) polymeric matrix. PLG is a common biodegradable medical polymer with an extensive history of human use in absorbable sutures and extended-release pharmaceuticals\(^10\). This sustained release technology allows for continuous systemic exposure to exenatide without modification of the native peptide. Following subcutaneous injection, exenatide is slowly released from the microspheres through diffusion and erosion. Given the ER profile of this formulation of exenatide, a weekly regimen would be expected to result in sustained steady-state plasma concentrations that provide 24-hour glucose control (i.e., improved fasting plasma glucose [FPG] and postprandial glucose [PPG] levels).

The present studies were designed to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneous administration of exenatide ER in patients with type 2 diabetes. First, the pharmacokinetic and safety effects of single dose administration of exenatide ER were studied over a period of 12 weeks in patients with type 2 diabetes. These results were used to predict the dose regimen of exenatide ER required to achieve steady-state therapeutic plasma exenatide concentrations. A second clinical study\(^11\) investigated the pharmacokinetics, pharmacodynamics, and safety of weekly exenatide ER subcutaneous injections in patients with type 2 diabetes over a period of 15 weeks. Furthermore, population-based analyses of these studies were performed to further define the exposure-response relationships associated with exenatide ER.
METHODS

Study Patients
All patients were diagnosed with type 2 diabetes and had been treated for at least 3 months (prior to screening) with diet and exercise, and/or metformin. Patients in both trials had an HbA1c level ranging from 7.0% to 11.0%, were between 18 and 75 years of age, and had a body mass index between 25 and 45 kg/m². Patients who had previously received exenatide treatment were excluded. All patients provided written informed consent prior to participation, and the study was conducted in accordance with the principles described in the Declaration of Helsinki, including all amendments through the 1996 South Africa revision12. The Institutional Review Boards for the single-dose study were Quorum Review Inc. (Seattle, WA, USA), Independent Investigational Review Board, Inc. (Plantation, FL, USA), Southern Institutional Review Board (Miami, FL, USA), and Crescent City Institutional Review Board (New Orleans, LA, USA). Chesapeake Research Review, Inc (Columbia, MD, USA) and Oregon Health and Science University (Portland, OR, USA) were the institutional review boards responsible for the multiple-dosing study [Clinicaltrials.gov Identifier: NCT00103935].

Study Design
Single-dose administration of exenatide extended-release (ER)
The single-dose exenatide ER trial was divided into a 14-day, open-label, lead-in period and a 12-week, double-blind (patient and investigator) assessment period, with no changes in pre-existing antidiabetic regimens (diet and exercise, and/or metformin). As dose titration has been shown to improve gastrointestinal tolerability13, patients (n=68) self-administered a low dose (5 ug) of exenatide IR three times daily for 14 days during the lead-in period prior to exenatide ER administration. Lead-in medication was administered prior to morning and evening meals, and the third daily injection was administered at bedtime, at least 4 hours after the previous injection. After the lead-in period, patients (n = 62) were randomized, via an interactive voice response system, to receive placebo (n = 15; microspheres with 0.5% ammonium sulfate instead of exenatide) or a single subcutaneous injection of exenatide ER (2.5 mg [n = 14], 5 mg [n = 10], 7 mg [n = 12], or 10 mg [n = 11]). The study medication was administered into the anterior abdominal wall, after an overnight fast (8 hours), immediately prior to a standardized liquid meal of Ensure Plus (7 mL/kg, 0.0015 kcal/mL). Blood samples for pharmacokinetic evaluation were obtained at -0.25, 0.25, 0.5, 1, 2, 4, 8, 12, 16 24, 30, 36, and 48 hours relative to injection, and weekly thereafter until study termination. Of the 62 patients, 45 (73%) received metformin (500-2,550 mg daily) during the study.

Weekly-dosing administration of exenatide ER
The weekly-dose exenatide ER trial11, conducted in a separate cohort of patients from the single-dose trial, was divided into a 3-day, double-blind (patient and investigator) lead-in period; a 15-week, double-blind, treatment period; and a 12-week follow-up period. The patients were randomized to a treatment group via contact with an interactive voice response system, and the study medication was administered at the study sites by study personnel. During the 3-day lead-in period, patients (n=45) self-administered 5 µg of exenatide IR or placebo via subcutaneous injection twice daily. On day 1 and weekly thereafter, patients...
received subcutaneous injections of placebo (n = 14; at volumes corresponding to exenatide doses) or exenatide ER (0.8 mg [n = 16] or 2 mg [n = 15]) for 15 weeks with no changes in pre-existing antidiabetic regimens (diet and exercise and / or metformin). Pharmacokinetic, pharmacodynamic, and safety measures were collected for 27 weeks (a 15-week treatment period and a 12-week follow-up period). Patients were given glucose meters and instructed to perform measurements by fingerstick at the fingertip for self-monitored blood glucose (SMBG) profiles. SMBG profiles were recorded on three separate days at both baseline and at week 15; preprandial glucose was measured 15 minutes before each meal, and PPG levels 1.5-2 hours after each meal. Of the 45 patients, 27 (60%) patients received metformin (500-2,550 mg per day) prior to the lead-in period and continued to receive metformin during the treatment and follow-up periods.

**Laboratory Values**

Plasma exenatide concentrations were determined using a validated two-site sandwich ELISA that only detects intact peptide. The assay employs a capture antibody (EXE4:2-8.4) that recognizes the c-terminal end of exenatide and a detection antibody (GLP1:3-3.1) that recognizes the n-terminal region. The assay does not cross-react with GLP-1 and has a lower limit of quantitation (LLQ) of 10 pg/ml. The assay was performed at Amylin Pharmaceuticals, Inc. (San Diego, CA, USA) for the single dose study and Millipore, Inc. (St. Charles, MO, USA) for the multi-dosing study. HbA\textsubscript{1c} levels were quantitated by Quintiles Laboratories Ltd (Smyrna, GA, USA) using a high-performance liquid chromatography method certified at level 1 by the National Glycohemoglobin Standardization Program\textsuperscript{14, 15}.

**Pharmacokinetic/Pharmacodynamic Assessments**

Pharmacokinetic endpoints were calculated using standard noncompartamental methods. Nonparametric superpositioning techniques were performed using the single-dose administration data to predict plasma exenatide concentrations with weekly dosing. Preliminary exposure-response analyses were conducted in NONMEM Version VI of software to evaluate the relationship between exenatide concentration and either FPG levels, PPG levels, or change in bodyweight. Due to the short duration of the trial and the small number of patients, covariate analyses were not performed. Data was pooled from both trials to evaluate the relationship between FPG levels and exenatide concentrations. Because of study design differences, only the multiple-dosing study was utilized for the evaluation of exenatide exposure on PPG excursions and bodyweight responses. PPG excursion exposure-response modelling utilized the breakfast meal data. Initial model development for all three endpoints utilized an inhibitory maximum effect (E\textsubscript{max}) model based on previous population exposure-glycaemic response modeling efforts and visual displays of the current data. The mean daily PPG level was calculated from postprandial SMBG measurements obtained on three separate days at baseline and at week 15 in evaluable patients. PPG excursions were calculated by subtracting the preprandial value from the postprandial value for breakfast, lunch, and dinner, and then averaged over 3 separate days at baseline and at week 15.
Statistical Analysis

In the single-dose exenatide ER trial, safety endpoints were reported using the intent-to-treat (ITT) population (n = 62; all patients who were assigned to a treatment group and received the single injection of study medication), and pharmacokinetic endpoints were summarized for the evaluable population (n = 58; all ITT patients who completed the study procedures in compliance with the protocol and had adequate data for reliable evaluation of pharmacokinetic parameters). An additional four patients were excluded from the pharmacokinetic analysis because they had high exenatide concentrations (>100 pg/mL; 10x the LLQ) at baseline (prior to administration of study medication). Descriptive statistics for plasma exenatide concentrations were presented by treatment and time point. Pharmacokinetic parameters were derived using the noncompartmental approach. The area under the plasma concentration-time curve (AUC) values were calculated using the linear trapezoidal method over the sampling period, with a minimum of three values required. The time-weighted mean concentration over the sampling period (C_{ave}) was calculated as AUC/sampling period. The C_{max} was defined as the maximum plasma concentration observed during the sampling period. All pharmacokinetic parameter calculations required a minimum of three non-missing values. Statistical evaluations to assess dose proportionality were conducted.

In the multiple-dosing exenatide ER trial, safety measurements, HbA_1c levels, FPG levels and bodyweight were summarized for the ITT population (n = 45; all randomized patients who received at least one injection of randomized lead-in medication [placebo or exenatide IR 5 µg]), and pharmacokinetic endpoints were summarized for the evaluable population (n = 43; all ITT patients who completed the study procedures through 15 weeks in compliance with the protocol and had adequate data for reliable evaluation of pharmacokinetic parameters). Pharmacokinetic parameters were calculated using standard noncompartmental methods. Post hoc analyses were performed to compare the 0.8 mg and 2.0 mg exenatide ER groups with the placebo group with respect to the change from baseline in HbA_1c levels, FPG levels and bodyweight. Statistical testing was done at α=0.05.

RESULTS

Patient Demographics and Disposition

Patient demographics in both the single-dose and multiple-dosing studies are presented in table I. Baseline characteristics were well balanced across treatment arms within each trial. In both studies, the majority of patients randomized in the ITT population completed the study (94% completed the single-dose study, 96% completed the multiple-dosing study). Withdrawal rates were generally comparable across treatment groups. In the single-dose trial, four ITT patients withdrew from the trial. One patient in the placebo group withdrew due to an adverse event (coronary artery disease) and three patients were lost to follow-up (one in each of the placebo, 2.5 mg, and 10 mg groups). Two patients withdrew from the multiple-dosing trial; both were randomized to the placebo group (one experienced an adverse event [dizziness] in the lead-in period, and the other experienced loss of glucose control during the treatment period).
Table I. Patient demographic and other baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Single-Dose Exenatide ER Trial</th>
<th>Weekly-Dosing Exenatide ER Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ITT N=62 Evaluable N=58</td>
<td>ITT N=45 Evaluable N=43</td>
</tr>
<tr>
<td>Sex, Female / Male (%)</td>
<td>52/48</td>
<td>40/60</td>
</tr>
<tr>
<td>Age (y)</td>
<td>53 ± 8</td>
<td>53 ± 11</td>
</tr>
<tr>
<td>Race, Caucasian / Black / Hispanic / Other (%)</td>
<td>26/11/44/19</td>
<td>60/16/20/4</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>90 ± 19</td>
<td>106 ± 20</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>32.3 ± 4.6</td>
<td>35.7 ± 5.7</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>8.1 ± 0.9</td>
<td>8.5 ± 1.2</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>172 ± 42</td>
<td>179 ± 41</td>
</tr>
<tr>
<td>Duration of diabetes (y)</td>
<td>5.7 ± 4.5</td>
<td>4.5 ± 4.0</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

Exenatide ER Pharmacokinetics and Pharmacodynamics

Single-Dose Exenatide ER Pharmacokinetics

The concentration-time profile following administration of the single dose of exenatide ER was biphasic, reflecting the mechanism of drug release in this formulation (figure 1). The initial phase of drug release was observed within the first few hours (figure 1 inset; \( T_{\text{max},0-48\,h} \) range: 2.1-5.1 hours). Two additional phases of release were observed with peak exenatide concentrations at approximately 2 and 7 weeks after dose administration. The initial release from this formulation was well controlled. The AUC\(_{0-48\,h}\) (geometric mean ± standard error [SE]) values were 460 ±57, 996 ±125, 1498 ±425, and 1745 ±247 pg • h/mL for exenatide ER 2.5 mg, 5 mg, 7 mg, and 10 mg, respectively, representing approximately 1-2% of the total AUC of the profile (figure 1 inset).

Plasma exenatide concentrations increased with increasing dose (AUC\(_{\text{Day1- study end}}\) [geometric mean±SE] 40378±4903, 89907±10416, 95336±17176, and 110263±24769 pg•h/mL for exenatide ER 2.5 mg, 5 mg, 7 mg, and 10 mg, respectively), and were not found to be dose proportional (\( p<0.001 \)). The mean \( T_{\text{max},(\text{Day1- study end})} \) occurred 6-7 weeks post-injection (mean: 39-48 days; figure 1). The \( C_{\text{max}}/C_{\text{av}} \) ratio for all exenatide ER treatment groups was approximately 3 (3.36 for 2.5 mg, 3.00 for 5 mg, 3.08 for 7 mg, and 2.93 for 10 mg), indicating a similar release profile over a 4-fold dose range. Exenatide plasma concentrations were sustained for longer than 60 days post-injection in all doses, and single doses of 5 mg, 7 mg, or 10 mg resulted in plasma concentrations above the known minimally effective concentration (50pg/mL) to lower FPG [16].

The \( C_{\text{max}} \) achieved with a 10 µg subcutaneous dose of the twice-daily exenatide IR formulation in clinical studies (mean [10\text{th}, 90\text{th} percentile] 211 [100, 385] pg/mL) was considered a desirable therapeutic concentration target for steady-state exenatide ER concentrations. Nonparametric superpositioning techniques were performed using the single-dose administration data to predict plasma exenatide concentrations with weekly dosing (figure 2; shaded area). These results suggested that a 2 mg dose administered at a weekly dosing
interval would sustain average steady-state exposure within the $C_{\text{max}}$ 10th to 90th percentile range observed with the twice-daily exenatide IR formulation. This 2 mg dose was therefore selected for the weekly-dosing study as a maximally efficacious dose, considering both efficacy and side effects. A dose of 0.8 mg weekly was expected to provide concentrations in the lower end of the target therapeutic range and was selected as a minimally efficacious dose that would help characterize the lower portion of the exposure-response curve and further inform model-based dose selection.

Figure 1. Mean + standard deviation (SD) plasma exenatide concentrations after a single injection of exenatide extended-release (ER) [evaluable population: n=54]. The inset graph shows plasma exenatide concentrations from 0 to 48 h after a single exenatide ER injection.

Figure 2. Mean + standard deviation (SD) plasma exenatide concentrations following a single dose of exenatide immediate-release (IR) and multiple doses of exenatide extended-release (ER) [intent-to-treat population for exenatide ER: n=31; exenatide IR data: n=39]. The grey shaded area represents 2 mg weekly-dosing predictions based on single-dose data.
Multiple-Dosing Pharmacokinetics and Pharmacodynamics of Exenatide ER

Weekly injections of exenatide ER 0.8 mg and 2 mg resulted in dose-related increases in plasma exenatide concentrations that approached steady state after week 6-7 (figure 2). By week 2, plasma exenatide concentrations with the 2 mg dose exceeded the minimally effective concentration (~50 pg/mL) shown to reduce FPG levels\[16\]. Exenatide concentrations were within the target therapeutic range throughout the majority of the treatment period, as was predicted from superpositioning of single-dose pharmacokinetic data (figure 2; shaded area). The decline in exenatide plasma concentrations during the follow-up period was consistent with single-dose pharmacokinetics, with mean plasma exenatide concentrations approaching the minimally effective level (~50 pg/mL) approximately 6 weeks after discontinuation of therapy and approaching the LLQ (10 pg/mL) approximately 10 weeks after discontinuation of therapy. Weekly administration of exenatide ER 0.8 mg and 2 mg for 15 weeks elicited positive effects on FPG levels (table II and figure 3a). Patients who received either 0.8 mg or 2 mg exenatide ER experienced gradual reductions in FPG levels that reached a nadir at approximately 4 weeks. At the end of the 15-week treatment period, both active groups demonstrated a FPG reduction of approximately 40 mg/dL (figure 3a) that was temporally aligned with exenatide exposure (figure 2). In contrast, placebo-treated patients experienced a slight mean increase in FPG levels.

Patients who received weekly doses of exenatide ER experienced a reduction in mean PPG levels (-36.4±20.1 mg/dL and -56.8±13.3 mg/dL with exenatide ER 0.8 mg [n=15] and 2 mg [n=15], respectively) compared to an increase in PPG levels with placebo (21.4±14.4 mg/dL; n=12). However, the 2 mg dose of exenatide ER was the only dose associated with an attenuated rise in PPG levels from the preprandial values (mean PPG excursions; table II). Both doses of exenatide ER resulted in improvements in HbA1c levels after 15 weeks of treatment (table II and figure 3b).

Weekly injections of exenatide ER 2 mg were also associated with progressive bodyweight loss (figure 3c). After 15 weeks of treatment with exenatide ER 2 mg, but not with exenatide ER 0.8 mg, bodyweight was significantly reduced compared with placebo (table II; figure 3). Weight loss achieved with exenatide ER 2 mg did not appear to plateau during the treatment period.

Exposure-Response Modeling

A total of 1514 time-matched pairs of FPG levels and plasma exenatide concentrations from both studies (105 total patients) were pooled for exposure-response analysis. An inhibitory $E_{\text{max}}$ model best described the relationship between FPG levels and exenatide concentrations with a population mean estimated exenatide concentration that produced 50% of the $E_{\text{max}}$ ($EC_{50}$) of 56.8 pg/mL and a maximum glucose response ($E_{\text{max}}$) of 57.0 mg/dL (figure 4). The exposure-response analyses evaluating bodyweight change and PPG excursions from the multiple-dosing study resulted in poor precision, probably because of the small number of samples and short duration of the study. However, these preliminary modeling efforts did suggest that higher concentrations of plasma exenatide are required to observe effects on bodyweight (Δ body weight $EC_{50}$: 184 pg/mL [coefficient of variation 12.7%]). Parameter estimates could not be attained with any degree of precision for PPG excursions, probably because the $E_{\text{max}}$ was not achieved, which also implies that greater exenatide exposure may be required for a sustained postprandial effect.
Figure 3. Mean ± standard error (SE) change in (a) fasting plasma glucose (FPG) levels; (b) glycosylated haemoglobin (HbA\textsubscript{1c}) levels; and (c) bodyweight with weekly administration of exenatide extended-release (ER) [intent-to-treat population: n=45].
Table II. Pharmacodynamics of weekly administration of exenatide extended-release (ER)

Values are expressed as mean ± SE ITT population, except for PPG excursions (evaluable population: n=12. N=16 for 0.8 mg and n=15 for 2 mg).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Exenatide ER 0.8 mg</th>
<th>Exenatide ER 2.0 mg</th>
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<tbody>
<tr>
<td></td>
<td>BL</td>
<td>Wk 15</td>
<td>change</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>183.7±11.1</td>
<td>197.5±15.8</td>
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<td>PPG excursions (mg/dL)</td>
<td></td>
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<td></td>
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<tr>
<td>breakfast</td>
<td>40.4±12.7</td>
<td>48.8±13.1</td>
<td>13.8±17.3</td>
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<tr>
<td>lunch</td>
<td>31.4±13.7</td>
<td>45.1±16.1</td>
<td>13.8±18.9</td>
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<tr>
<td>dinner</td>
<td>34.2±11.9</td>
<td>43.6±11.4</td>
<td>9.3±9.1</td>
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<tr>
<td>HbA₁c (%)</td>
<td>8.6±0.4</td>
<td>9.0±0.4</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>101.2±5.4</td>
<td>101.7±5.7</td>
<td>0±0.7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE

ITT population, except for PPG excursions (evaluable population: n=12. N=16 for 0.8 mg and n=15 for 2 mg).

BL=baseline; FPG=fasting plasma glucose; HbA₁c=glycosylated haemoglobin; ITT=intent-to-treat; PPG=postprandial plasma glucose; SE=standard error.
Safety and Tolerability of Exenatide ER

In the single-dose exenatide ER trial, frequent (>10% incidence) treatment-emergent adverse events in patients who received placebo microspheres were injection-site reactions (injection-site erythema [n=7; 47%]; injection-site pain [n=1; 7%], injection-site swelling [n=1; 7%]), nausea (n=3; 20%), abdominal pain (n=2; 13%), and diarrhoea (n=2; 13%). Eight patients (17%) receiving exenatide ER experienced one or more injection site reactions (injection-site erythema [n=2; 4%]; injection-site bruising [n=3; 6%]; injection site pain [n=2; 4%], and injection-site pruritus [n=3; 6%]). The incidence of injection-site reactions was similar among exenatide ER treatment groups. The only other frequent treatment-emergent adverse event in the population of patients who received exenatide ER was upper respiratory tract infection (n=8 [17%]). Other treatment-emergent adverse events observed in patients receiving exenatide ER included mild or moderate hypoglycaemia in two patients (4%; n=1 with the 7 mg dose, n=1 with the 10 mg dose) and mild or moderate nausea in two patients (4%; n=1 with the 7 mg dose, n=1 with the 10 mg dose).

Nausea, the most frequently reported adverse event during weekly administration of exenatide ER, was reported in 4 patients (25%) at the 0.8 mg dose and 4 patients (27%) at the 2 mg dose, compared with 2 patients (14%) receiving placebo. Other frequent treatment-emergent adverse events (>10% incidence) for each treatment group were injection site pruritus (n=2; 14%), bronchitis (n=2; 14%) and rash (n=2; 14%) with placebo; gastroenteritis (n=3; 19%), hypoglycaemia (n=4; 25%), injection site bruising (n=2; 13%), diarrhoea (n=2; 13%), hypertension (n=2; 13%) and tooth abscess (n=2; 13%) with exenatide ER 0.8 mg; and gastroenteritis (n=2; 13%), sinusitis (n=2; 13%) and arthralgia (n=2; 13%) with exenatide ER 2 mg. There were no events of severe hypoglycaemia. No adverse events led to the withdrawal of patients from weekly-dosing exenatide ER treatment arms.
DISCUSSION

Single dose administration of exenatide ER 2.5 mg, 5 mg, 7 mg, and 10 mg resulted in dose-related increases in plasma exenatide concentrations, with sustained exenatide exposure lasting for approximately 9-11 weeks. As demonstrated in figure 1, exenatide ER exhibits a biphasic release pattern, as is observed with similar drug-delivery systems\(^ {17}\), with an initial release observed on the day of dosing, followed by a more sustained release phase. This initial release of exenatide constitutes a small percentage of overall exposure, with \(\text{AUC}_{0-48\text{ h}}\) accounting for approximately 1-2% of overall \(\text{AUC}_{\text{Day 1-study end}}\). Of note, the \(\text{AUC}\) of exenatide ER did not exhibit linear dose dependency, as demonstrated by the \(\text{AUC}\) ratios of the 5 mg, 7 mg, and 10 mg doses compared with the 2.5 mg dose (2.2, 2.4, and 2.7, respectively). The small initial release of this formulation allows for the use of larger weekly doses of exenatide without inducing poor acute tolerability. Superpositioning of single-dose exposure was used to predict the dosing regimen necessary to achieve steady-state plasma concentrations of exenatide within the range that is known to be efficacious with the IR formulation. Weekly dosing with exenatide ER 0.8 mg and 2 mg achieved therapeutic plasma exenatide concentrations (>50 pg/mL) with optimal results observed with the 2 mg dose. Therapeutic exposure was achieved after 2 weeks of treatment with exenatide ER 2 mg (figure 2), and steady-state plasma concentrations of exenatide were achieved after approximately 6-7 weeks.

Consistent with the expected effects of continuous GLP-1 receptor activation, weekly exenatide ER dosing resulted in an improvement in FPG levels (as previously reported\(^ {11}\)), and these improvements were temporally related to exenatide exposure with both the 0.8 mg and 2 mg weekly doses of exenatide ER (figures 2 and 3). Exposure-response analysis demonstrated that the \(\text{EC}_{50}\) for FPG was 56.8 pg/mL (figure 4), a concentration achieved with both the 0.8 mg and 2 mg doses of exenatide ER. Indeed, weekly administration of the 0.8 mg and 2 mg doses resulted in similar FPG reductions of approximately 40 mg/dL after 15 weeks of treatment, suggesting that the maximally effective concentrations were achieved for FPG reduction.

Fifteen weeks of treatment with either dose of exenatide ER also resulted in reduced mean PPG levels. However, it should be noted that the improvement in PPG levels with the 0.8 mg dose was primarily due to reduced FPG and preprandial glucose levels, and PPG excursions were relatively unaltered with placebo and 0.8 mg exenatide ER (table II). In contrast, exenatide ER 2 mg reduced the magnitude of PPG excursions in a manner similar to that observed following exenatide IR administration, and probably contributed to the larger mean reduction in HbA1c levels observed with the 2 mg dose (-0.3%; p-value not significant vs. exenatide ER 0.8 mg). The exposure-response modelling of PPG excursions was of limited precision but did suggest that the \(\text{EC}_{50}\) for PPG effects was higher than that calculated for FPG (56.8 pg/mL).

The improvements in glucose control achieved with exenatide IR are often accompanied by a reduction in bodyweight. In the present study, weekly administration of exenatide ER 2 mg was associated with a bodyweight reduction of 3.8 kg after 15 weeks of treatment. Weight loss appeared to be progressive throughout the 15-week treatment period (figure 3c). In a 30-week clinical trial of exenatide ER, similar reductions in bodyweight were observed during the first 14 weeks of treatment, after which time, weight loss continued at a slower rate for the
remainder of the 30-week treatment period [18]. No weight loss was observed in those patients receiving the lower dose of exenatide ER, consistent with the calculated EC$_{50}$ of 179 pg/ml. Overall, these analysis suggest that higher circulating concentrations are required to elicit the mechanisms of action that affect PPG levels and bodyweight (slowing of gastric emptying and reduction in food intake, respectively), which are thought to be predominantly mediated through a central effect [19-22]. In contrast, much lower plasma concentrations of exenatide are required to elicit the predominantly peripheral effects of exenatide that result in reductions in FPG (enhancement of insulin secretion and suppression of glucagon secretion).

It is important to note that the available data from these studies, while sufficient to characterize the dose-response of FPG levels, PPG levels and bodyweight, have limited utility for fully characterizing exposure-response. This was most evident when modelling PPG levels and weight loss, as both models demonstrated poor precision. This is likely due to the small sample size available for describing the upper end of the exposure-response relationship. Despite this limitation, these preliminary models were able to demonstrate that the exposure-response relationship of FPG levels is different from that of PPG levels and weight loss. Additional modelling from larger studies will be necessary to fully characterize the extent of these differences.

These studies demonstrated that a single dose of exenatide ER resulted in dose-related increases in plasma exenatide concentrations. Single-dose exposure successfully predicted weekly-dosing exposure, with exenatide ER 0.8 mg and 2 mg weekly eliciting therapeutic concentrations of exenatide that improved 24-hour glucose control. Furthermore, weekly treatment with exenatide ER 2 mg in patients with type 2 diabetes was associated with the improved PPG control and weight loss observed with the IR formulation of exenatide administered twice daily.
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