ABSTRACT

Greater understanding of incretin biology has led to the development of new approaches to the treatment of type 2 diabetes and new avenues to enhancing incretin action. Although the direct effects of glucagon-like peptide-1 (GLP-1) are mediated by GLP-1 receptors on the β cell, indirect effects are partially mediated through activation of complex ascending pathways that engage centers in the central nervous system with the pancreas, liver, and peripheral tissues. Clinical studies have demonstrated that the beneficial effects of continuously administered exogenous GLP-1 do not significantly diminish over 3 months of treatment. Studies have also shown that in addition to stimulating insulin secretion from β cells, GLP-1 reprograms defective β cells to become more sensitive to glucose. Enhancement of incretin action is the foundation for myriad new options in the treatment of type 2 diabetes. Although several incretin-based approaches are currently under study, 1 incretin-enhancing agent has already received US Food and Drug Administration approval for the treatment of type 2 diabetes. Incretin biology and associated therapeutic strategies are discussed in detail in this article. (Adv Stud Med. 2006;6(7A):S586-S594)

A clearer understanding of incretin biology has led to the identification of new therapeutic targets for the treatment of type 2 diabetes. The predominant incretin hormone, glucagon-like peptide-1 (GLP-1), binds to GLP-1 receptors on the surface of β cells. These receptors are coupled with a signal transduction pathway that, when activated, results in increased insulin biosynthesis and secretion, thereby enhancing the function of the islet. Activation of the GLP-1 receptor also results in the expression of specific gene and protein products that increase β-cell proliferation and reduce β-cell apoptosis. Classic therapeutic agents for type 2 diabetes do not activate these pathways.

In addition to enhancing insulin secretion, GLP-1 has acute effects on glucagon secretion and gastric emptying, which can be observed within minutes of administration of a GLP-1 receptor agonist. As a result of these activities, blood glucose levels are reduced and metabolic milieu is improved. These actions indirectly reduce glucotoxicities and lipotoxicities, thereby enhancing β-cell health. Thus, the antihyperglycemic actions of GLP-1 include enhancing glucose-dependent insulin secretion, inhibiting postprandial glucagon secretion, slowing gastric emptying, and enhancing satiety. Two observations have supported this avenue of therapy: subjects with type 2 diabetes have reduced GLP-1 levels, and exogenously administered GLP-1 improves glycemia.

The long-term goal of managing diabetes is to prevent acute and chronic complications of diabetes, including microvascular and macrovascular complications. Clinical studies have shown that a 1% reduction in glycated hemoglobin A1c used as a measure of blood glucose control over time reduced microvascular complications by 30% to 35%. However, studies consistently reveal that the vast majority of patients with type 2 diabetes fail to achieve acceptable target goals...
for A1c. In 2000, data from the National Health and Nutrition Examination Survey showed that only 37% of participants had reached an A1c level of less than 7%. The following year, the American College of Endocrinologists revisited recommendations for glycemic control and lowered the target threshold for A1c levels to 6.5%.

In 2005, a US study of 157,000 subjects with type 2 diabetes reported that 66% of subjects were above this 6.5% threshold, again demonstrating a significant failure of glycemic control despite advances in understanding and managing diabetes. Suboptimal control with traditional therapies may partly be attributed to difficulties in attenuating the postprandial rise in glycemia, a progressive decline in β-cell function, and the association of many antihyperglycemic treatments with weight gain. The goal of incretin-based research is to engage the incretin pathway in the β cell to produce beneficial metabolic effects and improved A1c levels with well-tolerated, long-term therapies that greatly improve upon the current state of diabetes care.

INCRETIN BIOLOGY

Although GLP-1 effects on the β cell are directly mediated through interaction with GLP-1 receptors, many other incretin effects are mediated through indirect pathways. For example, activation of ascending vagal pathways engages centers in the central nervous system, which ultimately results in communication with the pancreas, liver, and peripheral tissue, such as muscle and fat (Figure 1). Although this complex physiological pathway is difficult to study in vitro, studies in animals and humans have furthered our understanding of incretin action.

The blood glucose-lowering actions of GLP-1 are associated with the normal control mechanisms used by β cells and α cells (responsible for glucagon secretion) to regulate their secretion. GLP-1 infusion leads to a progressive lowering of blood glucose levels to normal, with an associated increase in insulin secretion and suppression of glucagon secretion. However, as blood glucose levels return to normal, insulin secretion is terminated and glucagon suppression is alleviated—mechanisms that are thought to reduce the chance of developing hypoglycemia. Thus, the response to GLP-1 is distinct from that of current secretagogues of the sulfonylurea or glitinide class, which continue to stimulate insulin secretion even as glucose returns to normal levels.

In 1997, a study by Rachman et al demonstrated the effectiveness of continuous intravenous administration of GLP-1 for the treatment of type 2 diabetes. GLP-1 significantly reduced diurnal glucose excursions in the fasting and postprandial states, returning diurnal glucose concentrations virtually to that of control subjects. The pivotal study underscoring the utility of a GLP-1 approach was conducted in patients with type 2 diabetes who were administered GLP-1 continuously for 6 weeks. Enrolled patients had been diabetic for a mean of 4.6 years, with a mean A1c level of 9.1% and a mean body mass index of 33.5 kg/m². Patients received a continuous (24 hours a day, 7 days a week) subcutaneous infusion of GLP-1 or saline control via a portable insulin pump for 6 weeks. Patients receiving GLP-1 showed a significant (P < .001) and sustained improvement in blood glucose in the fasting and postprandial states compared with patients receiving saline control (Figure 2). Additionally, GLP-1 treatment was associated with significantly reduced total body weight, A1c levels, improved insulin sensitivity, and better β-cell function.

**Figure 1. How the GLP-1 Receptor Signaling Pathway Protects the Vulnerable β Cell in Type 2 Diabetes**

The effect on body weight resulted from increased satiety and was not attributed to potential side effects, such as nausea or vomiting. GLP-1 was well tolerated, with reports of nausea and vomiting similar between GLP-1 and saline-treated groups. The study also reported findings supporting increased sensitivity of $\alpha$ cells and $\beta$ cells. Therefore, GLP-1 not only stimulated insulin secretion from the $\beta$ cell, but also reprogrammed defective $\beta$ cells to become more sensitive to glucose. This effect on improving $\beta$-cell function and sensitivity is a property of the incretin mimetics, exenatide and liraglutide. The Zander study also demonstrated the feasibility of chronic GLP-1 administration, which has since been extended for up to 3 months without significant reduction in the clinical response. To date, no clinical tachyphylaxis or receptor desensitization has been observed with chronic GLP-1 administration. These studies and others provided critical support for GLP-1 approaches for the treatment of type 2 diabetes.

**NEW THERAPEUTIC APPROACHES**

The demonstrated safety and efficacy of chronic GLP-1 administration in pilot studies of patients with type 2 diabetes have led to several areas of new research targeting incretin pathways. The ubiquitous protease, dipeptidyl peptidase-IV (DPP-IV), cleaves the N-terminus of GLP-1 and gastric inhibitory polypeptide (GIP) within minutes of their secretion, producing inactive peptides. Therefore, new therapeutic strategies for incretin biology in the treatment of type 2 diabetes have focused on overcoming the short half-life of incretins. This involves the use of DPP-IV inhibitors and GLP-1 analogs, which are resistant to DPP-IV inactivation through fusion of natural GLP-1 with albumin or use of nonhydrolyzable structural analogs of GLP-1, the incretin mimetics.

**INHIBITION OF DPP-IV**

In approaching treatment by inhibiting DPP-IV, investigators have questioned whether the protease activity is physiologically essential for the control of blood glucose. DPP-IV activity resides on the extracellular domain of a larger glycoprotein, CD26. Using CD26 knockout mice, Marguet et al reported normal fasting blood glucose levels but reduced glycemic excursion after a glucose challenge. Researchers also noted increased levels of glucose-stimulated insulin secretion and GLP-1 in these mice after a glucose challenge. This study demonstrated that CD26 plays a fundamental role in the control of blood glucose and underscores its utility as a pharmacologic target in the treatment of type 2 diabetes.

**MECHANISM OF ACTION OF DPP-IV INHIBITORS**

The mechanism by which DPP-IV inhibitors work is unclear, but an approach to understanding how they operate has been examined in a study comparing the activity of different DPP-IV inhibitors (Val-Pyr, LAF-237, SYR106124, and TP8211) in normal mice versus mice lacking GIP receptors, GLP-1 receptors, or both.
Investigators reported that the glucose-lowering actions of the DPP-IV inhibitors remained intact in mice lacking the GLP-1 or GIP receptor alone, but was absent in mice missing both receptor types. Similar data have not yet been reported in humans, but studies of the role of GLP-1 and GIP as determinants of DPP-IV inhibitor action are ongoing.

**DPP-IV INHIBITORS**

**Vildagliptin**

Vildagliptin (NVP-LAF237) is a long-acting DPP-IV inhibitor that prolongs the activity of endogenous GLP-1. A phase II study of vildagliptin in subjects with type 2 diabetes reported improvements in glucose excursion and enhanced meal-stimulated GLP-1 levels. At the end of this 4-week study, insulin levels were similar between vildagliptin-treated and placebo-treated subjects, but vildagliptin-treated subjects had significantly reduced glucose and glucagon excursions. These results demonstrated a significantly improved insulin-to-glucose ratio with DPP-IV inhibitor treatment. A 12-week, double-blind, placebo-controlled study of vildagliptin in metformin-treated patients with type 2 diabetes with an extension to 52 weeks reported additional benefits of DPP-IV inhibition. Investigators reported a decrease in A1c levels (Δ -0.6 ± 0.1%) from baseline in the vildagliptin-treated group, but no change in the placebo group. At 52 weeks of treatment, the vildagliptin group maintained the glycemic benefit, whereas the placebo group incurred further loss of glycemic control. In addition, vildagliptin was associated with a low incidence of hypoglycemia, tolerability comparable to that of placebo, and weight neutrality. During the first 12 weeks, body weight decreased a mean of 0.4 ± 0.2 kg in the vildagliptin group compared with 0.5 ± 0.2 kg in the placebo group. At the end of 52 weeks, the weight change was -0.2 kg in both treatment groups.

**Sitagliptin**

Sitagliptin (MK-0431) is another DPP-IV inhibitor entering late-stage clinical development. In two 12-week, double-blind, placebo-controlled, dose-ranging studies of patients with type 2 diabetes, sitagliptin treatment reduced placebo-subtracted A1c levels by 0.4% to 0.8% with no change in body weight. **GLP-1 ANALOGS**

**CJC-1131**

Another approach to lengthening the half-life of GLP-1 is through analogs that are not readily hydrolyzed by DPP-IV. CJC-1131 is an analog consisting of GLP-1 covalently bound to albumin, which has a circulating half-life of 11 to 14 days. As an albumin conjugate, CJC-1131 has a half-life of 10 days in humans. Although it binds and activates the GLP-1 receptor, it is resistant to cleavage by DPP-IV. CJC-1131 has been shown to significantly reduce glycaemia and body weight in patients with type 2 diabetes. A 12-week, double-blind, placebo-controlled study was conducted in patients with type 2 diabetes who were inadequately controlled with metformin alone or in combination with sulfonylurea. In this study, patients were removed from sulfonylurea but remained on metformin. Once-daily CJC-1131 treatment produced a greater mean decrease in A1c levels from baseline than placebo treatment (Δ -1.1%; P <.0001). Combined CJC-1131 and metformin treatment was associated with nausea (overall incidence: 25%) and weight loss (mean: 2.5 kg vs 1.6 kg for placebo; P <.05).

**Albugon**

Created by the fusion of human albumin and GLP-1 genes, Albugon (GlaxoSmithKline, Triangle Park, NC) is a human recombinant albumin GLP-1 hybrid protein that exhibits an extended half-life similar to that of albumin while also activating GLP-1 receptors. In vitro studies have demonstrated the efficacy of Albugon to stimulate GLP-1 receptors with somewhat less potency than that of the incretin mimetic exendin-4 (Figure 3).

**Incretin Mimetics**

**Liraglutide**

Liraglutide is a long-acting, acylated GLP-1 analog currently in phase III clinical trials. Structural modifications from GLP-1 render it resistant to DPP-IV and include a fatty acid moiety that allows noncovalent binding to albumin. This latter property extends the circulating half-life and pharmacokinetic profile of liraglutide, thus allowing once-daily administration. In a study of subjects with type 2 diabetes, Chang et al reported that a single dose of liraglutide resulted in restoration of β-cell sensitivity to glucose. In addi-
tion, two 12-week, double-blind studies have demonstrated the efficacy and safety of liraglutide on enhancing insulin sensitivity and controlling blood glucose in patients with type 2 diabetes.\textsuperscript{21,22} In the study of 193 patients with type 2 diabetes by Madsbad et al, all but the lowest dose of liraglutide examined substantially decreased $A_1C$ levels (Figure 4).\textsuperscript{22,23} The 0.75-mg dose of liraglutide decreased $A_1C$ by 0.75 percentage points compared with placebo ($P < .0001$). No consistent effect of liraglutide on weight was noted, but liraglutide was associated with a low incidence of hypoglycemia, a dose-dependent incidence of nausea, and other gastrointestinal events (eg, diarrhea, vomiting, and constipation), which were generally mild and transient. In the Feinglos et al study, 210 patients with type 2 diabetes previously treated with an oral antidiabetic drug were randomized to receive metformin with either placebo or 1 of 5 doses of liraglutide.\textsuperscript{21} At once-daily doses of 0.45 mg to 0.75 mg, liraglutide was shown to improve glycemic control and weight similar to metformin. Both drugs were similarly tolerated with no major hypoglycemic events.

**EXENATIDE**

Exenatide is a 39-amino acid peptide that is structurally identical to native exendin-4, a peptide isolated from the salivary secretions of the gila monster that shares many properties of GLP-1.\textsuperscript{24} Exenatide is rapidly absorbed after subcutaneous injection and binds the GLP-1 receptor with similar affinity as GLP-1. However, it is resistant to inactivation by DPP-IV. Exenatide is a twice-daily injection that was approved by the US Food and Drug Administration in 2005 for the treatment of patients with type 2 diabetes who do not achieve adequate glycemic control with metformin and/or a sulfonylurea.

Exenatide has been shown to improve $\beta$-cell function and glucose control in multiple studies. In a study of 12 fasting patients with type 2 diabetes who were previously treated with diet or oral agents, exenatide dose-dependently increased insulin secretion with a resultant fall in glucose concentrations.\textsuperscript{25} Insulin secretion was attenuated as the glucose levels normalized, a
characteristic of GLP-1 analogs that is thought to confer a reduced risk for hypoglycemia. Furthermore, in a 28-day study of 109 patients with type 2 diabetes who failed to achieve optimal glycemic control with diet and/or an oral antidiabetic drug, subcutaneously injected exenatide significantly reduced A1c ($P \leq 0.006$) and serum fructosamine ($P \leq 0.006$), and attenuated the postprandial rise in plasma glucose ($P \leq 0.004$) compared with placebo. Exenatide had no effect on body weight.

Fehse et al examined the effect of exenatide on the first- and second-phase insulin response to intravenous glucose in 13 patients with type 2 diabetes and 12 patients with normal glucose tolerance. Exenatide increased insulin ($P < 0.005$) and C-peptide area under the curves ($P < 0.005$) during the first (0–10 minute) and second (10–120 minute) phases after glucose challenge by 2- to 3-fold. Exenatide-treated patients with type 2 diabetes had a similar secretory pattern and higher insulin secretion rates than normal glucose tolerance subjects, whereas placebo-treated patients with type 2 diabetes had a blunted first-phase insulin response compared with normal glucose tolerance subjects (Figure 5). This is a critical finding given that the diminished first-phase secretory response is the initial metabolic failure in type 2 diabetes. Thus, exenatide acutely improved β-cell function and restored the first- and second-phase insulin response in patients with type 2 diabetes.

In a series of 3 pivotal phase III studies (the AMIGO trials), exenatide was shown to improve glycemic control in patients with type 2 diabetes who had failed to achieve glycemic control with metformin monotherapy, sulfonylurea monotherapy, or combination therapy with these agents. These studies were 30-week, triple-blind, placebo-controlled trials randomizing 733, 336, and 377 patients in a 2-to-1 fashion in favor of exenatide. Postprandial glucose levels were improved in the exenatide-treated groups compared with placebo-treated groups (Figure 6). Across these trials,
baseline A1c ranged from 8.2% to 8.7% and decreased approximately 1% with exenatide compared with placebo. A mean weight loss of approximately 2 kg was observed in all 3 studies, with the greatest loss occurring in patients receiving metformin plus exenatide. An A1c goal of less than 7% was reached by 46%, 41%, and 35% of patients receiving 10 µg of exenatide twice daily in the metformin monotherapy study, the sulfonylurea monotherapy study, and the combination metformin and sulfonyl-urea study (representing a more advanced type 2 diabetes population), respectively.

In 52-week, open-label extensions of the AMIGO trials, all patients were treated with exenatide 5 µg twice daily for the first 4 weeks of the open-label extension before titrating to 10 µg twice daily thereafter. Patients who had received placebo during the double-blind studies showed a rapid decrease of A1c upon initiation of exenatide treatment to levels similar to the active treatment arms of the double-blind studies. At 82 weeks, mean changes from baseline in A1c were similar across groups (at least -1.1%), and an A1c goal of 7% or less was achieved by 51% of patients receiving exenatide 10 µg twice daily.

Patients who had received placebo during the double-blind studies also achieved significant weight loss with exenatide treatment during the open-label study, achieving similar weight loss as the active treatment groups during the first 30 weeks. Mean reductions in weight from baseline were 4.5 kg with 82 weeks of exenatide treatment and 3.3 kg with 30 weeks of exenatide treatment (Figure 7). The benefits of exenatide on glycemic control and body weight were durable over the 82 weeks of study.

In the 30-week exenatide trial of metformin-treated patients with type 2 diabetes, similar rates of hypoglycemia were reported between treatment groups (5.3%, 4.5%, and 5.3% for 10 micrograms exenatide, 5 µg exenatide, and placebo, respectively). This result would be expected for an agent producing glucose-dependent insulin secretion. However, in the studies with patients who were treated with sulfonylurea alone or in combination with metformin, exenatide was associated with an increase in hypoglycemia compared with placebo. There were no serious episodes—but there was 1 severe episode—of hypoglycemia in these studies.

Study investigators hypothesized that the hypoglycemia resulted from the exenatide-induced improvement in glycemia coupled with the nonglucose-dependent effects of sulfonylurea, suggesting a need for sulfonylurea dose management when using exenatide.

As shown in Figure 8, the most common adverse events in the 30-week trials were generally gastrointestinal in nature. Nausea was mostly mild to moderate in intensity and occurred with a higher incidence during the first 8 weeks of therapy, with declining incidence thereafter. Nausea was cited as the cause for study discontinuation in 3% of exenatide- and 1% of placebo-treated patients. Exenatide-associated weight loss progressed over time and was observed with patients who did not report nausea, indicating that weight loss was not primarily due to nausea.

CONCLUSIONS

Research into incretin action in addition to the knowledge that GLP-1 is deficient in type 2 diabetes and that GLP-1 receptor activation improves glycemic control and body weight were durable over the 82 weeks of study. The benefits of exenatide on glycemic control and body weight were durable over the 82 weeks of study.

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After week 30, both placebo and exenatide groups received open-label exenatide (5 µg twice daily for 4 weeks, 10 µg twice daily thereafter) until week 82. Reprinted with permission from Blonde et al. Exenatide (exendin-4) reduced A1c and weight over 82 weeks in overweight patients with type 2 diabetes. Paper presented at: the 65th Scientific Sessions of the American Diabetes Association; June 10-14, 2005; San Diego, Calif. Abstract 477-P.
control have together led to new therapeutic approaches. DPP-IV inhibitors and GLP-1 analogs, including the synthetic exendin-4 (exenatide), have been in development, with exenatide receiving US Food and Drug Administration approval in 2005. To date, clinical trial results have demonstrated the efficacy of these agents to improve glycemia with mild-to-moderate side effects. Importantly, these agents have not been associated with the characteristic weight gain of most antihyperglycemic therapies. In fact, significant weight loss has been reported with exenatide treatment. Studies have also suggested the ability of these agents to preserve or enhance β-cell function and/or mass. Ongoing research continues to develop these and similar compounds to enhance incretin action, with great promise for new options in the treatment of type 2 diabetes.

REFERENCES