Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes

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Incretin mimetics are a new class of pharmacological agents with multiple antihyperglycemic actions that mimic the actions of incretin hormones such as glucagon-like peptide (GLP)-1. Dipeptidyl peptidase (DPP-IV) inhibitors suppress the degradation of many peptides, including GLP-1, thereby extending their bioactivity. Several incretin mimetics and DPP-IV inhibitors are undergoing late-stage clinical trials for the treatment of type 2 diabetes. These agents appear to have multiple mechanisms of action, including some or all of the following: enhancement of glucose-dependent insulin secretion; suppression of inappropriately elevated glucagon secretion; slowing of gastric emptying; and decreased food intake (i.e. appetite suppression). Based on preliminary clinical data, incretin mimetics and DPP-IV inhibitors show potential for treating type 2 diabetes.

Type 2 diabetes is characterized by the emergence of postprandial (post-meal) and, subsequently, fasting hyperglycemia (fasting plasma glucose >125 mg/dl) [1,2]. Hyperglycemia results from pancreatic β-cells secreting inadequate insulin to compensate for insulin-resistance in peripheral tissues [3,4]. The increasing worldwide prevalence of type 2 diabetes has major implications for healthcare systems and affected individuals, particularly because of the vascular complications associated with this disease.

The aim of pharmacological therapy is to control hyperglycemia and, ultimately, to avert the devastating complications associated with sustained tissue exposure to excessively high glucose concentrations. However, because of the complex nature of the disease and the progressive deterioration in pancreatic β-cell function, glycemic control in type 2 diabetes patients remains difficult. As a result, long-term therapy with currently available agents (Table 1) is often associated with an inability to maintain adequate glycemic control [2]. Here, two new classes of potential antidiabetic agents that are undergoing human clinical testing peptidase (DPP)-IV inhibitors. These agents elicit glucoregulatory actions similar to those of the incretin hormone glucagon-like peptide (GLP)-1 but through different mechanisms.

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Incretins

The observation that insulin secretion from pancreatic β-cells was more robust after an oral glucose bolus than after an equivalent intravenous glucose bolus led to the elucidation of the role of intestinal peptides in the regulation of postprandial insulin secretion [5]. This 'incretin effect' was attributed to the insulinotropic action of gut hormones, specifically glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. Patients with type 2 diabetes generally lack the glucose-lowering response to GIP. By contrast, the insulinotropic response to GLP-1 is typically intact in this patient population, but circulating levels of postprandial GLP-1 are deficient. Therefore, therapeutic interventions have focused on exerting a pharmacological GLP-1 effect.

In mammals, GLP-1 is derived from the proglucagon

TABLE 1

Overview of type 2 diabetes therapy

Pharmacologic treatment classes	Mechanism of action	Beneficial effects and characteristics	Adverse events
Available therapeutic agents			
Biguanides	Decrease hepatic glucose production Decrease intestinal glucose absorption Increase glucose uptake by skeletal muscle and fat	Reduce blood glucose concentration Increase sensitivity to insulin Reduce blood lipid levels Oral administration	Diarrhea Nausea Vomiting Can cause lactic acidosis
SFUs	Stimulate pancreatic β-cell insulin secretion	Reduce blood glucose concentration Lower risk of hypoglycemia than insulin Oral administration	Hypoglycemia Weight gain Nausea Vomiting
TZDs	Improve target cell response to insulin Decrease hepatic glucose output Increase insulin-dependent glucose uptake in skeletal muscle and fat	Reduce blood glucose concentration Beneficial alteration of blood lipid levels Possible beneficial effects on pancreas Oral administration	Alteration in liver function indicators Anemia Detrimental cardiac effects Edema Weight gain
Meglitinides and D-phenylalanine derivatives	Stimulate glucose-mediated insulin secretion	Reduce blood glucose concentration Reduce postprandial glucose excursions Stimulates insulin secretion Oral administration	Hypoglycemia Diarrhea Weight gain
α-Glucosidase inhibitors	Inhibit pancreatic α-amylase and membrane-bound α-glucosidase enzymes Delay glucose absorption by inhibiting intestinal disaccharide metabolism	Reduce blood glucose concentration Reduce postprandial glucose excursions Stabilize daytime glucose concentrations Disperse calories over time Weight neutral Oral administration	Abdominal pain Diarrhea Flatulence Possible elevated liver enzymes
Insulin and analogues	Replace or supplement endogenous insulin hormone to correct deficiency	Reduce blood glucose concentration Subcutaneous injection	Hypoglycemia Weight gain
Potential therapeutics			
Incretin mimetics (clinical testing)	Replace or supplement endogenous incretin hormone(s) glucoregulatory activity	Reduce blood glucose concentration Reduce postprandial glucose excursions Enhance glucose-dependent insulin secretion Suppress inappropriately elevated glucagon secretion Reduce food intake Slow gastric emptying Weight reduction or weight neutral Possible beneficial effects on pancreas and insulin sensitivity Subcutaneous injection	Nausea Vomiting
DPP-IV inhibitors (clinical testing)	Inhibit peptide hormone metabolism by DPP-IV enzyme, thus increasing blood concentrations of endogenous bioactive forms of GLP-1 and other peptides	Reduce blood glucose concentration Weight neutral Possible beneficial effects on insulin sensitivity Oral administration	Not known: awaiting Phase III clinical data

and the insulinotropic activity of GLP-1 is mediated through GLP-1 receptors on pancreatic β-cells. The release of GLP-1 in response to a meal occurs rapidly (within 10 min) in healthy individuals and is highly correlated with insulin secretion into the circulatory system [5]. In individuals with type 2 diabetes or impaired glucose tolerance circulating concentrations of postprandial GLP-1 and a blunted insulin secretory response to food intake [5,7].

The biological activities of GLP-1 [5,7,8] include: (i) mediating glucose-dependent insulin secretion to aid plasma glucose uptake by tissue; (ii) suppression of postprandial glucagon secretion to reduce hepatic glucose release; (iii)

circulation with glucose as food is absorbed from the gut; and (iv) suppression of food intake (i.e. appetite). In addition, animal data suggest that regulation of maintenance of pancreatic β-cell mass is a normal physiological function of GLP-1.

The activity of GLP-1 in controlling glucose excursions in preclinical diabetes models led to a series of GLP-1 clinical trials in humans [8]. In patients with type 2 diabetes, continuous infusion of exogenous GLP-1 via either the intravenous or the subcutaneous route was reported to nearly normalize glycemia by enhancing glucose-mediated insulin secretion, suppressing glucagon secretion and slowing gastric emptying. The insulinotropic and glucagonostatic actions of GLP-1 were shown to be glucose-dependent (i.e. GLP-1 action abated as euglycemia was restored) [8]. Therefore, long-term exogenous GLP-1 therapy would not be expected to induce severe hypoglycemia when administered alone. Unfortunately, the pharmacokinetic properties of GLP-1 limit the feasibility of this approach, because GLP-1 is rapidly degraded by DPP-IV [half-life ($t_{1/2}$) of <2 min] [9,10]. Zander *et al.* [11] reported that although subcutaneous infusion of GLP-1 lowered plasma glucose, continuous administration was required.

Incretin mimetics

Incretin mimetics are a new class of pharmacological agents with multiple antihyperglycemic actions that mimic some effects of endogenous incretin hormones, including the glucose-dependent enhancement of insulin secretion. Although these agents can exhibit glucoregulatory effects similar to those of GLP-1, their actions might not be mediated solely through the pancreatic GLP-1 receptor. Therefore, the class name 'incretin mimetic' is intended to emphasize the glucoregulatory and metabolic effects of these agents, rather than their specific mechanisms of action.

Several GLP-1 analogues with resistance to degradation by DPP-IV are currently undergoing human clinical trials. Liraglutide (Novo Nordisk) and CJC1131 (ConjuChem) have undergone the most extensive testing to date: liraglutide is expected to start Phase III clinical trials in 2005 or 2006 (www.novonordisk.com/investors/rd_pipeline/rd_pipeline.asp?showid=4). Exenatide (Amylin Pharmaceuticals and Eli Lilly and Company), a synthetic version of the naturally occurring hormone exendin-4 from the Gila Monster lizard, is at a more advanced stage of development, recently completing three pivotal Phase III clinical trials that formed the basis of a new drug application (NDA) submitted in June 2004 to the FDA (http://investors.amylin.com/phoenix.zhtml?c=101911&p=irol-newsArticle&ID=608621&highlight=). Another incretin mimetic, ZP10 (Zealand Pharma and Aventis), is currently in early-stage clinical trials (www.zp.dk/news/ZP10%20Licensing%20deal_UK%20version%20(Final).pdf). In preclinical investigations, ZP10 suppressed glucose excursions and enhanced insulin secretion

Liraglutide

Liraglutide is a GLP-1 derivative synthesized by the covalent coupling of a GLP-1 analogue with a fatty acid [13]. Liraglutide has been reported to have multiple glucoregulatory actions, including reduction of hyperglycemia through stimulation of insulin secretion, suppression of inappropriate glucagon secretion, slowing of gastric emptying and enhancement of β-cell function and mass [14–23]; to date, the effect of liraglutide on β-cell function and mass has only been studied in animal models. Liraglutide also reduced food intake and body weight in obese or prediabetic rats [15,16,22], but had no durable effects in obese or diabetic mice [14]. No long-term data have been reported on the possible effects of liraglutide on food intake and body weight in humans, and short-term trials have yielded mixed results [20,21].

Several Phase I and II studies have reported on liraglutide administration to humans [17–21,24–26]. In a study with 11 subjects with type 2 diabetes, a single liraglutide injection (10 μg/kg) resulted in the reduction of fasting plasma glucose over 12 h, and subsequently suppression of glucose excursions and slowing of gastric emptying after a meal [19]; beneficial effects on insulin secretion and glucagon suppression postmeal were also reported. Nausea was the most common treatment-related adverse event noted. In a study with ten patients with type 2 diabetes, Chang *et al.* [18] demonstrated that dosing with 7.5 μg/kg liraglutide restores the glucose-dependent insulin response to glucose infusion to a level similar to that recorded in ten healthy volunteers subjected to the same experimental procedure without liraglutide. In another study, 13 subjects with type 2 diabetes were dosed with 6 μg/kg liraglutide or placebo for 7 days [19], followed by physiological assessments using a glucose 'clamp' procedure [27]. This study reported that liraglutide suppressed postprandial glucose excursions, reduced fasting plasma glucose concentrations, enhanced first-phase insulin response after meals and suppressed postprandial plasma glucagon concentrations, with no effect on the rate of gastric emptying. HOMA-B analysis [28], a measure of β-cell function, suggested a beneficial effect on β-cell function during fasting. Liraglutide had a clearance $t_{1/2}$ of ~18 h [17]. The three subjects with the highest serum levels of liraglutide experienced nausea and abdominal pain that dissipated over the course of the treatment period.

A 12-week study in 190 patients with type 2 diabetes compared liraglutide doses ranging from 0.045 to 0.750 mg/day concomitantly administered with the antidiabetic sulfonylurea (SFU) glimepiride or placebo [21]. Generally, dose-dependent reductions in A1C, which is the percentage of glycosylated hemoglobin in circulating red blood cells', were reported in all but the lowest liraglutide dosing group, with a maximum change of −0.75% compared with placebo, which was comparable to the results observed in subjects receiving glimepiride treatment. HOMA-B analysis [28]

function) suggested significant improvement in the 0.75 mg/day liraglutide group compared with placebo, and HOMA-B improvement was similar to that observed with glimepiride. Mild-to-moderate nausea and headache were the most common adverse events related to liraglutide exposure in this study. Other gastrointestinal events (e.g. diarrhea, vomiting and constipation) also occurred, but at a lower incidence. One subject treated with liraglutide experienced mild hypoglycemia compared with four subjects in the glimepiride group experiencing the same adverse event.

In another Phase II study, 33 patients with type 2 diabetes were dosed with liraglutide (0.6 mg/day) or placebo for 8 weeks [20]. Liraglutide treatment was associated with a decrease in A1C (–0.8%; $p = 0.028$) and fasting serum glucose (–2.2 mmol/l; $p = 0.002$) compared with placebo. Furthermore, insulin sensitivity, as measured by HOMA-S, increased ($p = 0.015$) and gastric emptying appeared to be unaffected. Transient episodes of nausea and diarrhea were the most frequent adverse events associated with liraglutide exposure; hypoglycemia was not observed.

In human subjects with type 2 diabetes, liraglutide significantly reduced body weight after 12 weeks of treatment at a dose of 0.45 mg/day, but not at lower (0.045 and 0.225 mg/day) or higher (0.60 and 0.75 mg/day) doses [21]. No members of the liraglutide group gained weight, which is in contrast to the SFU control group. In a 33 patient study, 8 weeks at a dose of 0.6 mg/day, liraglutide had no apparent effect on body weight or food intake [20].

CJC1131

CJC1131 is a GLP-1 analogue containing a reactive linker that facilitates covalent (irreversible) binding to serum albumin [29]. The resultant GLP-1–albumin complex retains the activities of GLP-1, together with increased resistance to DPP-IV-mediated inactivation and prolonged duration of action *in vivo* [30]. A study showed that the CJC1131–albumin complex bound to Chinese hamster ovary cells transfected with human recombinant pancreatic GLP-1 receptor with a K_i of 12 nM (compared with a K_i of 5.2 nM for GLP-1) [29]. In the same assay, the CJC1131–albumin complex activated cyclic AMP with an EC_{50} of 11–13 nM, which is similar to GLP-1 [29]. Published literature indicates that the conjugate molecule can reduce basal and postprandial plasma glucose concentrations in normoglycemic and hyperglycemic (diabetic) mice. Postglucose challenge plasma glucose excursions were not suppressed in GLP-1 receptor knockout mice, which is not the case in wild-type mice, suggesting the dependence of this activity of CJC1131 on a functional GLP-1 receptor. In the same series of experiments, CJC1131 slowed gastric emptying and suppressed food intake in rats, a trait shared with native GLP-1 [31].

In a clinical trial using healthy human volunteers, single subcutaneous injections of CJC1131 (1.5–20.5 μg/kg) determined that the $t_{1/2}$ of this compound is 9–15 days, with dose-proportional area under curve [$AUC_{(0-inf)}$] and T_{max} ranging from 23 to 76 h [32]. In a recently reported Phase II clinical trial with 206 subjects, with type 2 diabetes, each subject was injected with a once daily subcutaneous dose of CJC1131 that was escalated periodically during a 28-day titration (www.goodmedia.com/equicom/conjuchem/web/presspop.cfm?newsID=3022). Subsequently, subjects were randomized to administration of CJC1131 once daily over 56 days at a rate of one, two or three times a week, and doses were adjusted individually to maintain an undisclosed concentration of compound in the bloodstream. Mild-to-moderate transient nausea and vomiting were the most frequent side effects reported in CJC1131-treated subjects and were dose-limiting. Several measures of glycemia (A1C and postprandial plasma glucose concentrations) were reduced (as was body weight) to varying degrees by the different CJC1131 treatment regimens. In a separate Phase II trial, 22 obese patients with type 2 diabetes were treated with CJC1131 for 14 days at a dose of 2, 4 or 8 μg/(kg day) or for 20 days with 12 μg/(kg day), with dose-escalation in some groups to enable tolerance-induction to the higher doses [33]. Mild-to-moderate transient nausea and vomiting were again the most frequent side effects reported. Overall glycemia was reduced by variable amounts, as was body weight, depending on dose and treatment duration. Rechallenge with two subcutaneous injections of CJC1131 (6 weeks apart) in healthy human volunteers exposed to the GLP-1 conjugate 6–12 months earlier was not associated with adverse immune responses, including no evidence of neutralizing antibodies. The absence of neutralizing antibodies after exposure to CJC1131 has also been described in patients with type 2 diabetes [34]. ConjuChem is currently exploring the activity of CJC1131 in combination therapy with metformin, thiazolidinediones (TZDs) or SFUs (www.goodmedia.com/equicom/conjuchem/web/presspop.cfm?newsID=2887).

Exenatide

Exenatide and GLP-1 share particular glucoregulatory activities, including glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying and reduction of food intake [11,35–43]. In addition, exenatide has been shown to restore first-phase insulin secretion [44,45] and to promote β-cell proliferation and islet neogenesis from precursor cells in *in vitro* and *in vivo* models of diabetes [46].

Exploratory dose-ranging Phase II clinical trials with

*Normal healthy human A1C values range from 5% to 6% and provide an accurate measure of average blood glucose concentrations over the preceding 2–3 months. Higher than normal A1C values indicate prolonged periods of ambient hyperglycemia and A1C values in individuals with poorly controlled diabetes (i.e. those with fluctuating blood glucose levels

dose range of 0.05–0.20 μg/kg administered subcutaneously, with transient nausea and vomiting as the reported dose-limiting adverse events [37,38]. Pharmacokinetic profiles suggested minimal dependence on body weight, supporting Phase III development of fixed dosage regimens [37,38]. In addition, early clinical experience suggested a Phase III study design strategy for mitigating the transient nausea and vomiting side effects by including a dose initiation period of 1 month at 5 μg twice daily, followed by a maintenance dose of 10 μg twice daily [47].

Three dosing regimens of exenatide were compared to placebo in a 28-day, Phase II study enrolling 109 patients with type 2 diabetes that were treated with metformin and/or a SFU [48]. Patients received 0.08 μg/kg exenatide before breakfast and evening meal, before breakfast and bedtime or before all 3 events. All exenatide groups had significant reductions in A1C compared with placebo ($p < 0.006$), ranging from −0.7 to −1.1%. An end-of-study A1C of <7% was achieved by 15% of exenatide-treated subjects, compared with 4% of placebo-treated subjects. In addition, HOMA-B analysis [28] revealed increased β-cell function at days 14 and 28 compared with baseline (day −1) and day 1. However, β-cell function was unchanged in the placebo group. The most common adverse events reported were mild-to-moderate nausea and hypoglycemia.

Another Phase II study assessed whether glucose-dependent insulin secretion and the normal physiological response to hypoglycemia were preserved during continuous intravenous infusion of exenatide or placebo [43]. Eleven healthy volunteers were evaluated using a clamp procedure [27] to modulate plasma glucose concentrations. During euglycemia, although insulin secretory rates were significantly higher (~3.5-fold) in subjects treated with exenatide compared with those receiving placebo, they rapidly decreased as plasma glucose concentrations were lowered. Exenatide suppressed inappropriate glucagon secretion during euglycemia, but did not block the counterregulatory rise in glucagon, cortisol, epinephrine, norepinephrine and growth hormone during hypoglycemia. In addition, recovery rates after the release of the hypoglycemic clamp were equivalent in exenatide- and placebo-treated subjects.

Patients with type 2 diabetes usually have a dramatic reduction in first-phase (i.e. early phase) insulin secretion – the insulin normally secreted by pancreatic β-cells within 10 min after a sudden rise in plasma glucose concentrations [49]. This defect is important because the first-phase of insulin secretion is postulated to have the greatest impact on postprandial plasma glucose excursions [49]. In subjects with type 2 diabetes, treatment with exenatide increased plasma insulin ($p < 0.005$) during the first (0–10 min) and second (10–120 min) phases of glucose-stimulated insulin secretion by a range of 180 to 310% and also increased insulin secretion rates, relative to placebo [45]. Exenatide-treated patients with type 2 diabetes had a in plasma insulin concentrations was accompanied by a significant increase in the glucose disappearance constant ($p = 0.0031$), supporting the metabolic activity of the insulin released in this typical biphasic pattern. Thus, exenatide stimulated both first- and second-phase insulin secretion after glucose challenge in patients with type 2 diabetes and the most common adverse events were two cases of moderate nausea and one case of mild hypoglycemia.

More recently, the results of three exenatide Phase III clinical trials in subjects with type 2 diabetes treated with metformin and/or an antidiabetic SFU were presented [39,50–55]. Thirty weeks of exenatide dosing (5 or 10 μg twice daily) significantly reduced A1C, fasting plasma glucose levels and postprandial glucose excursions. Mean A1C reductions from baseline in the 10 μg exenatide groups ranged from −0.9 to −0.8% compared with a mean range of +0.1 to +0.2% in the placebo groups. HOMA-B and proinsulin:insulin ratio indicated improvement in the exenatide-treated patients. In addition, progressive reductions in body weight were observed, with means ranging from −2.8 to −1.6 kg in the 10 μg dosing groups after 30 weeks of treatment, compared with means ranging from −0.9 to −0.3 kg in the placebo group ($p < 0.05$). In an interim assessment of ongoing, open-label extensions of these trials, patients with 52 weeks of 10 μg exenatide exposure had A1C reductions of $-1.1 \pm 0.1\%$ ($n = 162$) from baseline, suggesting durability of glycemic control in this group [56]. This same patient cohort had weight reductions of -3.6 ± 0.5 kg ($n = 162$) from baseline, suggesting that weight loss in this group was progressive. Exenatide was generally well-tolerated by patients cotreated with metformin and/or an antidiabetic SFU. Mild-to-moderate nausea was the most common adverse event related to exenatide exposure and the incidence of nausea decreased with continued treatment. Mild hypoglycemia was most commonly observed in patients also treated with a SFU.

DPP-IV inhibitors

The enzyme DPP-IV, also known as CD26, is a transmembrane and circulating serine protease responsible for cleaving polypeptides containing proline or alanine residues in the penultimate N-terminal position, thus releasing dipeptides from the parent molecules and thereby altering their biological function [57]. As well as being found in blood plasma, DPP-IV is expressed constitutively on epithelial cells of the kidney, intestine, liver (bile duct) and pancreas, on endothelial cells in the vasculature, on fibroblasts in skin, synovia, and mammary gland, on cells contacting the cerebrospinal fluid and on subsets of immune cell leukocytes (e.g. T cells, B cells, natural killer cells and macrophages).

DPP-IV knockout mice are fertile and generally healthy, with normal fasting plasma glucose levels [58]. In the initial report on this mouse strain, plasma concentrations of

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