Perspectives in Diabetes

Therapeutic Strategies Based on Glucagon-Like Peptide 1

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Glucagon-like peptide (GLP)-1 is an incretin hormone with potent glucose-dependent insulinotropic and glucagonostatic actions, trophic effects on the pancreatic β-cells, and inhibitory effects on gastrointestinal secretion and motility, which combine to lower plasma glucose and reduce glycemic excursions. Furthermore, via its ability to enhance satiety, GLP-1 reduces food intake, thereby limiting weight gain, and may even cause weight loss. Taken together, these actions give GLP-1 a unique profile, considered highly desirable for an antidiabetic agent, particularly since the glucose dependency of its antihyperglycemic effects should minimize any risk of severe hypoglycemia. However, its pharmacokinetic/pharmacodynamic profile is such that native GLP-1 is not therapeutically useful. Thus, while GLP-1 is most effective when administered continuously, single subcutaneous injections have short-lasting effects. GLP-1 is highly susceptible to enzymatic degradation in vivo, and cleavage by dipeptidyl peptidase IV (DPP-IV) is probably the most relevant, since this occurs rapidly and generates a noninsulinotropic metabolite. Strategies for harnessing GLP-1's therapeutic potential, based on an understanding of factors influencing its metabolic stability and pharmacokinetic/pharmacodynamic profile, have therefore been the focus of intense research in both academia and the pharmaceutical industry. Such strategies include DPP-IV–resistant GLP-1 analogs and selective enzyme inhibitors to prevent in vivo degradation of the peptide. *Diabetes* 53:2181–2189, 2004

When the gene encoding glucagon, the mammalian pancreatic hormone, was cloned, the structure of its precursor, proglucagon, was deduced and shown to contain the sequences of two additional peptides, named glucagon-like peptide (GLP)-1 and -2 because of their considerable sequence homology to glucagon (1). It was a further several years before two endogenous peptides, GLP-1 (7-36)amide (2) and GLP-1(7-37) (3) were identified. When these peptides were demonstrated to be highly potent insulinotropic agents (2–4), interest in GLP-1 research grew significantly.

GLP-1 possesses a number of properties that make it a potentially ideal antidiabetic agent. It is released from the intestinal L-cell in response to orally ingested nutrients and has effects on the endocrine pancreas, on the gastrointestinal tract, and in the brain (rev. in 5). Thus, in the pancreas, GLP-1 acts as an incretin hormone, stimulating meal-induced insulin secretion. This effect is glucose dependent, meaning that any risk of hypoglycemia during exogenous peptide administration is practically eliminated. GLP-1 not only stimulates insulin exocytosis, but it also promotes all steps in insulin biosynthesis (6). More recently, direct effects on β-cell growth and survival have been identified, with GLP-1–stimulated proliferation (7,8) and differentiation of new β-cells (9,10) leading to increased β-cell mass. There is also increasing evidence that GLP-1 receptor signaling results in a reduction of β-cell apoptosis (11–14), which will further contribute to increased β-cell mass. Moreover, GLP-1 inhibits glucagon secretion, which, notably, is also glucose dependent (15), meaning that GLP-1 administration is unlikely to impair the glucagon counterregulatory response to hypoglycemia. In the gastrointestinal tract, GLP-1 inhibits motility and secretion (16), thereby contributing to reduce the glucose excursion by delaying the passage of nutrients to the small intestine. Indeed, under physiological circumstances in healthy subjects, this effect appears to outweigh its insulinotropic effect (16). In humans, peripherally administered GLP-1 has a satiating effect (see 17), and when given over a prolonged period (6 weeks) by continuous subcutaneous infusion, patients with type 2 diabetes reported a reduction in appetite, which led to significant reductions in body weight (Fig. 1) by the end of the study (18). A decreased gastric emptying rate seems to be involved (17), but a reduced sensation of appetite during GLP-1 in the fasting state, before meal ingestion (19), suggests other mechanisms may also contribute. Central administration of GLP-1 inhibits food intake in rodents (20), raising the possibility that peripherally released GLP-1 may have direct effects on the brain, because circulating GLP-1 can access GLP-1 receptors in brain areas (subfornical organ, area postrema) that participate in the regulation of appetite and energy homeostasis (21). However, it is also relevant that gastric distension activates GLP-1–containing neurons in the caudal nucleus of the solitary tract, suggesting a role for centrally expressed GLP-1 as an

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Received for publication 1 April 2004 and accepted in revised form 27 May 2004.

C.F.D. is a paid consultant for Novo Nordisk.

DPP-IV, dipeptidyl peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; NEP, neutral endopeptidase; OAA, oral antidiabetic agent.

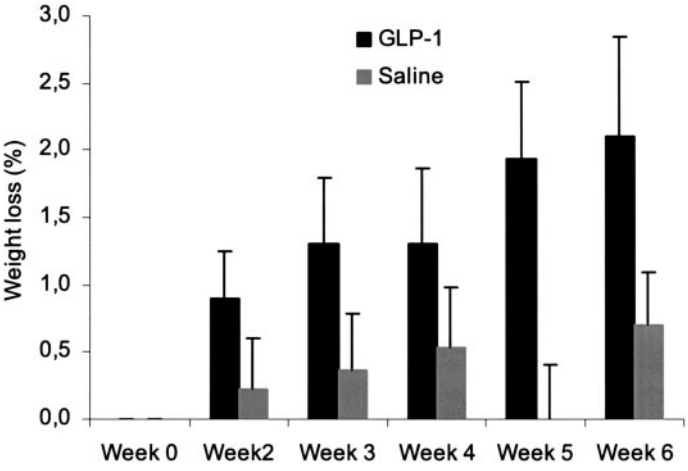

FIG. 1. Changes in body weight (expressed as a percentage of the pretreatment [week 0] weight) in type 2 diabetic patients assigned to continuous subcutaneous infusion of placebo (saline) or GLP-1 (4.8 pmol · kg^{-1} · min^{-1}) for 6 weeks. Treatment with GLP-1 for 6 weeks resulted in a significant (P = 0.02) reduction in body weight relative to pretreatment weight, whereas body weight after placebo treatment was not changed (P = 0.4). Reprinted with permission from Elsevier (Zander M, et al., *Lancet* 359:824–830, 2002).

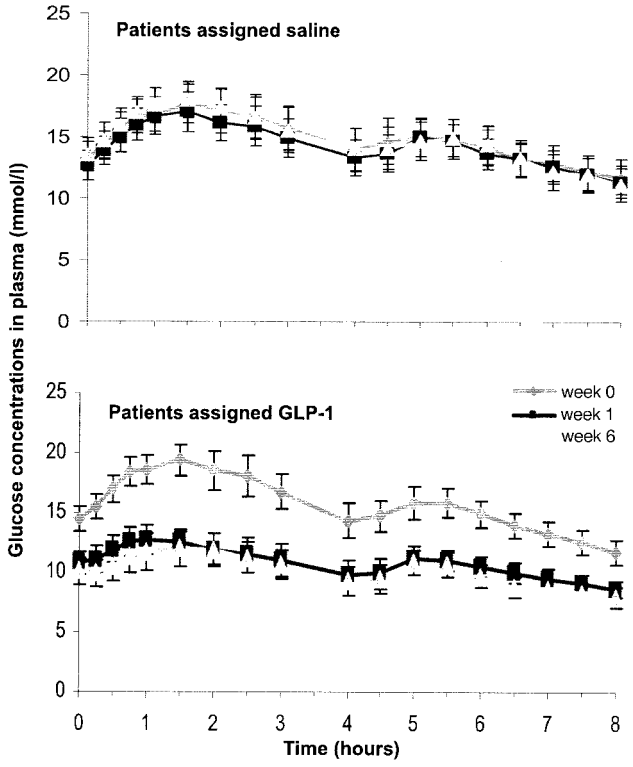

FIG. 2. Eight-hour plasma glucose profiles in type 2 diabetic patients assigned to continuous subcutaneous infusion of placebo (saline) or GLP-1 (4.8 pmol · kg^{-1} · min^{-1}) for 6 weeks, measured before treatment (week 0), and after 1 and 6 weeks of treatment. Treatment with GLP-1 led to significant reductions in fasting plasma glucose (P < 0.0001) and 8-h mean glucose concentrations (P < 0.001), but these parameters did not change significantly on placebo treatment (P = 0.13 and 0.95, respectively). Reprinted with permission from Elsevier (Zander M, et al., *Lancet* 359:824–830, 2002).

inhibitor of food intake (22). Interestingly, central administration of the GLP-1 receptor antagonist, exendin (9-39), increases food intake (20), suggesting that GLP-1 produced locally within the brain may exert a tonic satiating effect.

GLP-1 and diabetes. The incretin effect is known to be reduced in patients with type 2 diabetes, resulting in inappropriately low insulin secretion following oral ingestion of nutrients (23). More recent studies have indicated that GLP-1 secretion is also impaired in these subjects, suggesting that a reduced meal-related GLP-1 response may contribute to the decreased incretin effect (24). GLP-1 is effective in patients with type 2 diabetes, increasing insulin secretion and normalizing both fasting and postprandial blood glucose when given as a continuous intravenous infusion (25), even in subjects with advanced type 2 diabetes long after sulfonylurea secondary failure (26). Unexpectedly, the effects of a single subcutaneous injection of GLP-1 were disappointing. Although high plasma levels of immunoreactive GLP-1 were achieved, insulin secretion rapidly returned to pretreatment values and blood glucose concentrations were not normalized (27). Nevertheless, the effect of repeated subcutaneous administration on fasting blood glucose is as good as that of intravenous administration (27), while continuous subcutaneous administration for 6 weeks reduces fasting and postprandial glucose concentrations (Fig. 2) and lowers HbA_{1c} concentrations (18).

Incretin hormone metabolism. A possible explanation for the short-lived effectiveness of single subcutaneous injections of GLP-1 was indicated when it was shown that GLP-1 (and the other incretin, glucose-dependent insulinotropic polypeptide [GIP]) was metabolized by plasma in vitro and that the enzyme dipeptidyl peptidase-IV (DPP-IV) was capable of mediating this degradation (28). DPP-IV is a membrane-bound ectoenzyme, found in numerous sites, including the kidney, intestine, and capillary endothelium. It cleaves an NH_2-terminal dipeptide from peptides where the penultimate amino acid residue is proline or alanine (Fig. 3), and since these residues in GLP-1 (and GIP) are important for receptor activation, it was suggested that DPP-IV may be involved in regulating their biological activity (28). Indeed, pharmacological studies suggested that the GLP-1 metabolite [GLP-1 (9-36)amide] can behave as an antagonist at the pancreatic GLP-1 receptor (29), although subsequent in vivo studies demonstrated that it does not antagonize the insulinotropic effects of GLP-1 (30,31). Given the interest in developing an antidiabetic therapy based on GLP-1, these in vitro studies (28) spurred research into incretin hormone metabolism. Thus, Deacon et al. (32) reported that DPP-IV inhibition prevented GLP-1 degradation by human plasma in vitro and identified the truncated metabolite as an endogenous circulating peptide in humans, whereas Kieffer et al. (33) showed in vivo degradation of exogenous GLP-1 (and GIP) in normal rats, but not in a mutant strain lacking DPP-IV. Subsequently, exogenous GLP-1, particularly after subcutaneous injection, was demonstrated to be NH_2-terminally degraded in healthy and diabetic subjects (34). Taken together with the knowledge of DPP-IV's widespread distribution and the suggestion that the truncated metabolites may be unable to activate the respective incretin receptors (28), an important role for DPP-IV in the physiological regulation of GLP-1 and GIP activity was suggested (28,33,34). The finding that the enzyme is localized in the endothelium of capillaries actually adjacent to GLP-1–containing L-cells (35) and the demonstration that over half of newly synthe-

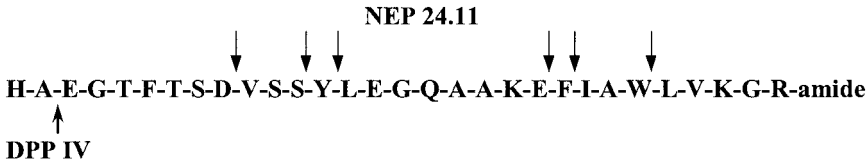

FIG. 3. The amino acid sequence of GLP-1 (7-36)amide, showing the cleavage sites of DPP IV and NEP 24.11 (from 18 and 23).

sized (intact) GLP-1 is NH_2-terminally degraded even before it leaves the local capillary bed (35) further underscore the relevance of DPP-IV in incretin biology.

The involvement of a second enzyme in incretin hormone metabolism was suggested when GLP-1 was demonstrated to be a substrate for neutral endopeptidase (NEP) 24.11 in vitro (36,37). NEP 24.11 is a membrane-bound zinc metallopeptidase that cleaves peptides at the NH_2-terminal side of aromatic or hydrophobic amino acids, and six potential cleavage sites in GLP-1 were identified (Fig. 3) (36). GIP was also degraded by NEP 24.11, albeit more slowly, and it was suggested that its larger size (42 vs. 30 amino acids for GLP-1) may be one factor determining its suitability as a substrate, since the enzyme has a preference for smaller peptides (36), but the physiological significance was not examined in vivo. Since NEP 24.11 has a widespread tissue distribution and is found in particularly high concentration in the kidneys, it could be speculated to be involved in the renal clearance of peptide hormones.

Therapeutic strategies based on GLP-1. In our study showing that exogenous GLP-1 was rapidly NH_2-terminally degraded in both healthy and type 2 diabetic subjects, we discussed the potential physiological role of DPP-IV in incretin hormone metabolism and suggested that "inhibition of dipeptidyl peptidase IV may prove a useful adjunct in the management of type 2 diabetes" because "inhibition of GLP-1 (7-36)amide would . . . increase the availability of the biologically active peptide," and it was additionally suggested that DPP-IV–resistant GLP-1 analogs may also have therapeutic potential (34,38). Subsequently, with the confirmation of the pivotal role of DPP-IV and the postulated role of NEP 24.11 in incretin hormone metabolism, coupled with ongoing studies into the possibility of using GLP-1 therapeutically, many studies have addressed the possibility of manipulating the in vivo survival of GLP-1 as a novel approach to the treatment of diabetes. In this context, two separate approaches can be envisaged: *1*) the development of analogs of GLP-1 that are not susceptible to enzymatic degradation and *2*) the use of selective enzyme inhibitors to prevent in vivo degradation and enhance levels of the intact, biologically active peptides.

Enzyme-resistant GLP-1 analogs. This approach has been investigated experimentally, and promising compounds are now in the final stages of clinical development. Initial studies examined the effect of simply substituting the penultimate alanine in GLP-1 to render it more DPP-IV resistant. These analogs maintain their affinity for the GLP-1 receptor and are more stable in vivo (39,40), resulting in greater potency than native GLP-1 (40), but—although they are not degraded by DPP-IV—they are still cleared relatively quickly from the plasma by other mechanisms, meaning their usefulness in the clinical setting is likely to be limited.

Exendin-4 is a GLP-1 receptor agonist, originally isolated from the venom of the Gila monster, which shares 53% sequence homology with native GLP-1. It is resistant to DPP-IV (because of the penultimate NH_2-terminal glycine instead of alanine as in GLP-1) and survives longer in the circulation (plasma half-life of 26 min in humans [41] compared with 1–2 min for intact biologically active GLP-1 [42]). This may partly be due to exendin-4 being a poor substrate for NEP 24.11, because although the NH_2-terminal regions of both peptides show high sequence homology, several potential NEP 24.11 cleavage sites present in GLP-1 are absent in exendin-4 (36). In addition, by virtue of its COOH-terminal extension, exendin-4 is larger (39 amino acids) than GLP-1, which may contribute to it being a poorer substrate, because, as mentioned above, NEP 24.11 has a preference for smaller substrates. In contrast to GLP-1, which is cleared more rapidly, the metabolic clearance of exendin-4 in humans is similar to the glomerular filtration rate (41), suggesting that the kidneys are important in clearing exendin-4. In insulin-resistant diabetic mice, repeated administration of exendin-4 for 13 weeks increased plasma insulin and reduced blood glucose and HbA_{1c} concentrations (43). In Zucker rats, 8 weeks of exendin-4 treatment was associated with both reduced glycemia and insulin levels, suggesting improved glucose tolerance (44), and in addition, body weight gain was reduced. More recently, the effects of exendin-4 were examined in Goto-Kakizaki (GK) rats. In these animals, a genetic neonatal β-cell mass deficit is considered to be the primary defect leading to basal hyperglycemia and subsequent development of diabetes, but exendin-4 treatment during the first postnatal week (the pre-diabetic period) increases the β-cell mass, with subsequent improvements in glycemic control at adult age (45). In *db/db* mice, exendin-4, given in the pre-diabetic period, expands the functional β-cell mass via effects on both proliferation and apoptosis, delaying the development of diabetes (46), while neonatal GLP-1 or exendin-4 treatment stimulates β-cell neogenesis in newborn streptozotocin-injected rats (a model of β-cell regeneration), leading to both short- and long-term effects on β-cell mass recovery and glucose homeostasis (47). When given neonatally, exendin-4 prevents the subsequent development of diabetes in the intrauterine growth retarded rat by normalizing PDX (a pancreatic growth factor) levels and β-cell proliferation rates and preventing the progressive reduction in β-cell mass that usually occurs in this model (48). In healthy humans, acute intravenous infusions of exendin-4 are insulinotropic and reduce both fasting and postprandial glucose concentrations (41). Exenatide (AC2993, synthetic exendin-4) has now reached phase 3 of clinical development. In a placebo-controlled study in type 2 diabetic patients, exenatide reduces fasting glucose when given acutely, and postprandial glucose when given twice daily over 5 days before breakfast and dinner (49). However, in the 5-day study, there was no significant effect on pre-breakfast fasting glucose levels, suggesting that the duration of action of the previous evening's dose was

insufficient to maintain an antiglycemic effect overnight. This was confirmed in a 1-month study, where once-daily injections did not maintain satisfactory glucose control, but twice-daily treatment significantly improved HbA_{1c}, relative to pretreatment levels, even though full 24-h blood glucose control was still not achieved (50). When given in combination with ongoing oral antidiabetic agents (OAAs) (metformin and/or a sulfonylurea) two or three times daily, exenatide leads to further reductions in serum fructosamine and HbA_{1c} compared with OAAs alone (51). Preliminary findings from an ongoing clinical trial, in which twice-daily exenatide injections in addition to existing OAAs was compared to the baseline period with OAAs alone, indicate significant improvements in fasting plasma glucose and HbA_{1c} by 4 weeks that were maintained up to 20 weeks (52). Weight changes were not noted in the shorter-duration studies (50,51), but by the 20th week reductions in body weight were seen (52). There were some cases of hypoglycemia (15% overall), but notably only in patients also taking sulfonylureas, and none were reported as being severe (52). Some patients (19%) developed anti-exenatide antibodies, but these appeared not to influence glycemic control, and apart from mild/moderate nausea, no serious side effects were reported (51,52).

LY307161-SR is a sustained release formulation of a DPP-IV–resistant GLP-1 analog. Single daily injections of this compound for 12 weeks significantly improves both fasting and postprandial glucose concentrations in type 2 diabetic patients (53). However, many patients experienced adverse injection site reactions, leading to reduced compound exposure (53), and development has now been put on hold.

Another analog, liraglutide (NN2211; 97% homologous to native GLP-1), which is in late phase 2 of clinical development, has been designed to overcome both the effects of DPP-IV degradation and the short plasma survival time (54). Acylation with a fatty acid chain in liraglutide promotes binding to albumin, thereby reducing access to the NH_2-terminal by DPP-IV and allowing the molecule to escape renal filtration. Combined with delayed absorption from the injection site, this results in a stable analog with a plasma elimination half-life of around 12 h in humans, giving a pharmacodynamic profile suitable for once-daily dosing (55). Liraglutide reduces glycemia in insulin-resistant murine models of diabetes and is associated with increased β-cell mass and proliferation after 2 weeks of treatment (56). Similar findings were obtained in diabetic rats, in which β-cell mass changes correlated positively with the degree of hyperglycemia so that where normoglycemia was attained, the hyperglycemia-induced increase in β-cell mass was prevented (57). In acute (single-dose) placebo-controlled crossover clinical studies, liraglutide reduces fasting and postprandial glucose concentrations in type 2 diabetic patients (55) and is associated with restoration of β-cell responsiveness to physiological hyperglycemia (58). Results from longer-duration studies have only recently been reported. Thus, studies in type 2 diabetic patients indicated that 1-week treatment with once-daily liraglutide significantly reduces 24-h glucose concentrations and improves β-cell function compared with placebo (59), and the beneficial effects appear to be maintained, with patients showing significant improvements in glycemic control and a trend toward weight reduction after 12 weeks, as compared with sulfonyurea treatment (60). Mild initial and transient nausea/vomiting was reported, but otherwise no serious adverse side effects were noted.

Other approaches involving covalent binding to albumin (e.g., CJC-1131), resulting in a plasma elimination half-life of around 2 weeks in humans (corresponding to the circulating half-life of albumin itself), have also recently been reported (61). CJC-1131 lowers blood glucose in diabetic mice, and the effect persists up to 1 week following discontinuation of treatment (62).

From the available data, protease-resistant GLP-1 analogs appear to be associated with remarkably few undesirable side effects. Nausea and vomiting seem to be the most commonly reported adverse reaction, as might be expected from compounds based on a naturally occurring peptide with known gastrointestinal effects. However, it is noteworthy that even this symptom is generally reported as being transient, occurring primarily during the first week and then disappearing, suggesting that tachyphylaxis to the gastrointestinal effects may occur. Importantly, no severe hypoglycemic events have been reported. In the 5-day study with exenatide as monotherapy, no hypoglycemic events were reported (49), while after 1 month, only 9 (of >2,000) measurements revealed blood glucose levels of 3.6 mmol/l or less (50). There were no cases of severe hypoglycemia during 12 weeks of liraglutide monotherapy, and only 1 patient (of 135) reported minor hypoglycemia (60). In a study specifically designed to address this question, liraglutide was demonstrated not to impair glucagon-mediated hypoglycemia counterregulation (63). Even when exenatide was combined with patients' existing OAAs, those taking exenatide and metformin reported no hypoglycemic events (blood glucose <3.3 mmol/l), and although some (~19%) receiving exenatide and a sulfonylurea (with or without metformin) experienced mild-to-moderate hypoglycemia, none had severe hypoglyemia (51).

Enzyme inhibitors. The alternative approach, inhibiting degradation of endogenous GLP-1, has also been the focus of much interest. In particular, with the finding that GLP-1 is uniquely sensitive to DPP-IV cleavage in vivo, development of selective compounds to inhibit DPP-IV activity (thereby enhancing biologically active incretin concentrations) has been undertaken by a number of pharmaceutical companies, and several potent orally active DPP-IV inhibitors have been described. The use of such compounds has allowed substantiation of our initial hypothesis that DPP-IV inhibition may influence GLP-1 metabolism in vivo and lead to improvements in glucose tolerance (34). Thus, we demonstrated that the prototypal DPP-IV inhibitor, valine-pyrrolidide, eliminated NH_2-terminal degradation of GLP-1 in vivo, improving the metabolic stability of the intact biologically active peptide and potentiating its insulinotropic and antihyperglycemic effects in anesthetized pigs (64), whereas Pederson et al. (65) reported that another inhibitor, isoleucine-thiazolidide, improved glucose tolerance in rats. Subsequently, these results were corroborated in acute studies demonstrating that DPP-IV inhibition is effective in animal models of impaired glucose

tolerance (66,67). The mechanism of action appears to involve enhancement of endogenous, intact, biologically active GLP-1, because these levels increase following DPP-IV inhibition (66,67). However, valine-pyrrolidide also improves glucose tolerance in mice lacking the GLP-1 receptor (68), suggesting that DPP-IV inhibition may affect other substrates involved in glucose homeostasis. GIP is also a DPP-IV substrate (28,33), and DPP-IV inhibition reduces degradation of exogenous GIP, enhancing its insulinotropic and antihyperglycemic effects in anesthetized pigs (69), and increases intact endogenous GIP concentrations in conscious dogs (70), suggesting that preservation of intact GIP is likely to contribute to the improved glucose tolerance seen after DPP-IV inhibition. Indeed, after acute DPP-IV inhibition, it appears that all the beneficial effects on glucose tolerance are mediated via GLP-1 and GIP receptor signaling, since the glucose-lowering actions of DPP-IV inhibitors were eliminated in the double incretin receptor knockout (DIRKO) mouse (71), although it remains unknown whether other substrates may contribute after longer-term DPP-IV inhibition.

Data describing effects of long-term DPP-IV inhibition are now also available. In a 12-week study in Vancouver Zucker diabetic fatty (ZDF) rats, chronic DPP-IV inhibition with isoleucine-thiazolidide was associated with sustained improvements in glucose tolerance and β-cell responsiveness, which appeared to improve with time, and interestingly, by the end of the study, inhibitor-treated animals had lower body weights (72). Moreover, the same authors also demonstrated that chronic DPP-IV inhibition improves not only β-cell function, but also both hepatic and peripheral insulin sensitivity (73). The longer-acting inhibitor, FE 999-011, given twice daily, continuously inhibits plasma DPP-IV activity and was found to normalize the glucose excursion after oral glucose administration in Zucker obese rats (74). In ZDF rats, this compound actually delayed the onset of hyperglycemia and restored food and water intake to pre-diabetic levels. Active GLP-1 and pancreatic GLP-1 receptor mRNA levels were increased, suggesting the possibility that the inhibitor led to a GLP-1–mediated improvement in β-cell function (74). Together with other studies demonstrating that DPP-IV inhibition preserves islet function in diabetic mice (75) and improves β-cell survival and islet cell neogenesis in streptozotocin-induced diabetic rats (76), these results support the suggestion that DPP-IV inhibition may be able to prevent the transition from impaired glucose tolerance to overt type 2 diabetes (38).

In human studies, single doses of a DPP-IV inhibitor reduce the glucose excursion in healthy and diabetic subjects (77,78). The first chronic study, with two or three times daily administration of the short-acting inhibitor, NVP DPP728, to patients with mild type 2 diabetes gave clinical proof of the concept that DPP-IV inhibition is a viable approach to treating diabetes. Fasting and postprandial glucose concentrations were significantly reduced, and HbA_{1c} levels were lowered compared with placebo, even after only 4 weeks of treatment (79). NVP DPP728 was well tolerated, with only minor adverse events, including pruritis and nasopharyngitis, being reported; these adverse effects were described as being short lived and transient and did not lead to treatment being discontinued. Moreover, they appear to be drug specific and unrelated to DPP-IV inhibition per se, since similar symptoms were not reported for another inhibitor, LAF237, which has now reached phase 3 clinical development (80). LAF237 is longer acting than NVP DPP728, and once-daily treatment for 4 weeks significantly improves metabolic control. Fasting and postprandial glucose concentrations and HbA_{1c} levels were significantly reduced compared with placebo, insulin secretion was sustained, and postprandial levels of active GLP-1 were increased. Moreover, glucagon concentrations were significantly reduced by LAF237 (80), suggesting that GLP-1–mediated inhibition of glucagon secretion, in addition to its insulinotropic effects, contributes to mediating the effects of DPP-IV inhibition. To date, there are no reports of changes in body weight in humans after DPP-IV inhibitor treatment.

There has been some debate over whether DPP-IV inhibitor monotherapy will be as effective as GLP-1 receptor agonist therapy and indeed whether it will be effective enough to be clinically useful at all. This was largely based on the assumption that the mechanism of action of DPP-IV inhibitors was predominately reliant on preventing degradation of endogenous GLP-1, raising the question of whether this would be sufficient to have a significant effect in type 2 diabetes. However, it is now clear that in addition to GLP-1, intact (active) endogenous GIP levels are also enhanced (70) and glucagon levels are lowered (80), although whether this is secondary to increased GLP-1 is unclear. It therefore seems likely that DPP-IV inhibitors exert their beneficial effects on glucose tolerance via effects on several different endogenous substrates. Preclinical studies indicate that DPP-IV inhibitors do have positive effects on glucose tolerance in animal models of diabetes, and the only chronic studies in humans reported so far have also yielded promising results, suggesting that DPP-IV inhibitor monotherapy is a feasible treatment option. How DPP-IV inhibitors will compare with GLP-1 receptor agonists in terms of efficacy is, as yet, unknown, and direct comparison in matched patient groups will be required before this question can be answered.

The limited human data suggest that DPP-IV inhibitors are well tolerated, and DPP-IV inhibition does not seem to be associated with hypoglycemic events. Four (of 61) patients treated with NVP DPP728 for 28 days reported symptoms suggestive of hypoglycemia, but only 1 had a blood glucose level of <3.3 mmol/l (79), whereas no hypoglycemic incidences were reported for LAF237 (80). Preliminary studies with LAF237 indicate that it does not significantly increase the risk for hypoglycemia when given together with the sulfonylurea, glibenclamide (81).

The possibility of other side effects unrelated to incretin hormone metabolism has also been the subject of some concern. The incretin hormones are not the only substrates for DPP-IV, raising the possibility that inhibition of the cleavage of other endogenous DPP-IV substrates may give rise to undesirable side effects. Among the additional substrates identified in kinetic studies are a number of neuropeptides, including pituitary adenylylate cyclase–activating polpeptide (PACAP), vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), neuropeptide Y (NPY), and growth hormone–releasing hormone (GHRH), other regulatory peptides (such as GLP-2

and peptide YY [PYY]), as well as a number of chemokines and cytokines (rev. in 82). However, it should be noted that it is unknown how many of the potential substrates identified in kinetic studies are actually endogenous substrates in vivo or moreover whether DPP-IV is the major mediator of their elimination or whether they are metabolized by other enzymes. DPP-IV is also found as a membrane-associated molecule on the surface of T-cells (where it is known as CD26). Here, it contributes to T-cell activation and proliferation via its interaction with other membrane-expressed antigens such as CD45 (83), raising the possibility that DPP-IV inhibition may compromise immune function, although it is unclear whether the catalytic activity per se is required for CD26's immune role or even whether its role is essential. In these contexts, it is relevant that both Fischer rats with mutations in the catalytic site and mice with a targeted deletion of the gene encoding CD26 are completely viable and seem to suffer no ill effects because of the lack of DPP-IV (33,68). Furthermore, no adverse side effects were reported during chronic DPP-IV inhibition in rodents (72–76), while early indications from the 4-week clinical trials also show good tolerability with few adverse events (79,80), suggesting that DPP-IV inhibition may be a safe and effective treatment, although longer-term studies are needed to confirm this.

As discussed above, other enzymes may additionally be involved in determining the metabolic stability of GLP-1; Hupe-Sodmann et al. (36,37) demonstrated that GLP-1 is a substrate for NEP 24.11 in vitro. Studies from the author's laboratory have indicated that NEP 24.11 may indeed have a physiological role in GLP-1 metabolism, because the selective NEP 24.11 inhibitor, candoxatril, increases the plasma half-life of GLP-1. By itself, this has only a modest effect in potentiating the antihyperglycemic effect of exogenous GLP-1, resulting in a small reduction in the glucose excursion following intravenous glucose in anesthetized pigs, presumably because GLP-1 is still susceptible to NH_2-terminal truncation by DPP-IV (A. Plamboeck and C.F.D., unpublished observations). However, when DPP-IV and NEP 24.11 inhibitors are administered concomitantly, the combined effect is greater than the effect of either inhibitor alone, resulting in significant improvements in the antihyperglycemic and insulinotropic effects of exogenous GLP-1 (84). Results of studies demonstrating effects on endogenous GLP-1 concentrations together with potential effects on glucose tolerance are awaited. Of interest, the first preliminary report of a compound possessing potent dual DPP-IV and NEP 24.11 inhibitory activity has recently been presented (85).

CONCLUSIONS

The studies discussed above support the idea that a GLP-1–based therapy will be a safe and effective treatment for type 2 diabetes. The clinical studies reported so far indicate that this approach, whether achieved by DPP-IV inhibition or by GLP-1 receptor agonists, has the potential to reduce and maybe even normalize both fasting and postprandial glucose concentrations, without having an adverse effect on weight gain. Moreover, the preclinical studies raise the hope that such a therapy may be able to delay or even halt the progression of the disease, or possibly even prevent its development, by providing a means of safely treating subjects with impaired glucose tolerance. Finally, but by no means least, this approach may turn out to be inherently safer than existing insulin secretagogues, because of its glucose dependency. Thus, GLP-1 receptor agonists and DPP-IV inhibitors have not been associated with any incidences of severe hypoglycemia, even when given in combination with existing OAAs, while when given as monotherapy, virtually no hypoglycemic events have been reported.

Although the two approaches (GLP-1 receptor agonists and DPP-IV inhibitors) can be described as being "GLP-1 based," there are clear differences between them, the most obvious of which is their route of administration. The GLP-1 receptor agonists described so far are all based on the native peptide, meaning that they are not orally available, whereas DPP-IV inhibitors are low–molecular weight compounds suitable for oral administration. However, future developments may provide alternative means of administration of GLP-1 analogs, in analogy with the possibility of intrapulmonary administration or buccal and skin uptake of insulin (rev. in 86). In this context, buccal absorption of native GLP-1, resulting in blood glucose reductions in diabetic patients, has been described (87). However, other differences mean that DPP-IV inhibitors cannot be regarded as being an "oral GLP-1." GLP-1 analogs, by virtue of their enhanced plasma survival time, have kinetic profiles that elevate plasma levels into the therapeutic range for prolonged periods, giving 24-h antihyperglycemic coverage, while DPP-IV inhibitors are likely to potentiate the natural diurnal rhythms of their substrates (e.g., enhancing meal-stimulated intact incretin levels). Secondly, the dose of the GLP-1 analogs can be titrated according to the patient's need, whereas DPP-IV inhibition only preserves the endogenously secreted peptide from degradation, meaning there is a limit to how far plasma levels of the active peptide can increase, although it might be possible to combine a DPP-IV inhibitor with a GLP-1 secretagogue in order to raise GLP-1 levels further. Thirdly, all of the effects of the analogs are mediated via the GLP-1 receptor, while emerging data suggest that DPP-IV inhibitors are likely to be multifactorial in their mechanism of action. Finally, and in contrast to OAAs like sulfonylureas, chronic treatment of type 2 diabetic patients with native GLP-1 (18) and the GLP-1 analogs (52,60) seems to be associated with beneficial body weight reductions, whereas until longer-term clinical studies are reported, it is unknown how DPP-IV inhibitors will fare in this respect. Only direct comparison in the clinical setting will reveal how (or whether) these differences between the two approaches will *1*) affect their ability to treat the symptoms of the diabetic phenotype effectively and safely and *2*) show which patient groups are most likely to benefit. In the meantime, both GLP-1 receptor agonists and DPP-IV inhibitors represent promising new approaches to therapy of type 2 diabetes.

NOTE ADDED IN PROOF

At the recent 64th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 4–8 June 2004, encouraging preliminary clinical results with LAF237 as 12 weeks' monotherapy and for 1 year in combination with metformin were presented [Pratley R, Galbreath E:

Twelve-week monotherapy with the DPP-4 inhibitor, LAF237 improves glycemic control in patients with type 2 diabetes (T2DM) (Abstract). *Diabetes* 53 (Suppl. 2):A83, 2004; Ahrén B, Gomis R, Standl E, Mills D, Schweizer A: Prolonged efficacy of LAF237 in patients with type 2 diabetes (T2DM) inadequately controlled with metformin (Abstract). Late-breaking abstract 7-LB]. At the same meeting, preclinical data suggested that inhibition of DPP-8 and/or DPP-9 gave rise to some toxicological signs and immunological effects [Leiting B, Nichols E, Biftu T, Edmonsen S, Ok H, Weber AE, Zaller D, Thornberry NA: Inhibition of dipeptidyl peptidase IV does not attenuate T cell activation in vitro (Abstract). *Diabetes* 53 (Suppl. 2):A2, 2004; Lankas G, Leiting B, Roy RS, Eierman G, Biftu T, Kim D, Ok H, Weber AE, Thornberry NA: Inhibition of DPP8/9 results in toxicity in preclinical species: potential importance of selective dipeptidyl peptidase IV inhibition for the treatment of type 2 DM (Abstract). *Diabetes* 53 (Suppl. 2):A2, 2004], indicating that selectivity for DPP-IV versus other related enzymes may be of considerable relevance with respect to their therapeutic application.

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