Acute and Chronic Effects of the Incretin Enhancer Vildagliptin in Insulin Resistant Rats

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Running title: DPP-4 inhibition in Zucker rats

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Nonstandard Abbreviations:
dipeptidyl peptidase IV DPP-4

glucagonlike peptide-1 GLP-1

minimum effective dose MED

oral glucose tolerance test OGTT

carboxymethylcellulose CMC

area under the curve AUC

immunoreactive insulin IRI

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ABSTRACT

The enzyme dipeptidyl peptidase-IV (DPP-4) inactivates the incretin hormone GLP-1. Because GLP-1 has therapeutic effects in patients with type 2 diabetes but its potential is limited by a short half-life, DPP-4 inhibition is a promising approach to diabetes treatment. This study examined acute (single dose) and chronic (once-a-day dosing for 21 days) effects of the DPP-4 inhibitor, vildagliptin (0.03–10 mg/kg), on plasma DPP-4 activity, intact GLP-1, glucose and insulin after an oral glucose load in insulin-resistant Zucker fatty rats, and acute effects in mildly insulin-resistant, high fat fed normal rats. A single oral dose of vildagliptin in Zucker rats produced a rapid and dose-related inhibition of DPP-4: the minimum effective dose (MED) was 0.3 mg/kg. Glucose-induced increases of intact GLP-1 were greatly but similarly enhanced by vildagliptin at doses ≥0.3 mg/kg. Post-load glucose excursions decreased and the insulinogenic index (Δinsulin/Δglucose at 10 minutes) increased, with an MED of 0.3 mg/kg and a maximally effective dose of 3 mg/kg. The effects of vildagliptin after chronic treatment were nearly identical to those of acute administration, and vildagliptin had no effect on body weight. In fat-fed normal rats, vildagliptin (3 mg/kg) also decreased post-load glucose excursions and increased the insulinogenic index, but these effects were smaller than those in Zucker rats. Conclusion: vildagliptin is an orally-effective incretin enhancer with antihyperglycemic activity in insulin-resistant rats and exhibits no tachyphylaxis. GLP-1-mediated augmentation of glucose-induced insulin release appears to make the major contribution to the antidiabetic properties of vildagliptin.
INTRODUCTION

The incretin hormone, glucagonlike peptide-1 (GLP-1), has multiple metabolic effects that would be desirable attributes of an oral antidiabetic agent (Holst, 2002). These include glucose-dependent stimulation of insulin and suppression of glucagon release (Ahren et al., 1997), slowing of gastric emptying (Willms et al., 1996), stimulation of non–insulin-mediated glucose uptake (D’Alessio et al., 1994), suppression of endogenous glucose production independent of pancreatic hormones (Prigeon et al., 2003) and possibly appetite suppression (Naslund et al., 1999). However, due to its peptidic nature and short plasma half-life (~2 minutes), GLP-1 must be administered parenterally and continuously to exert its therapeutic actions (Zander et al., 2002). Recognition that the enzyme dipeptidyl peptidase-IV (DPP-4) is responsible for the degradation and inactivation of GLP-1 (Mentlein et al., 1993) raised the possibility of developing small molecule inhibitors of this enzyme to leverage the antidiabetic effects of endogenous GLP-1 while avoiding the need for parenteral administration.

Several orally available, specific inhibitors of DPP-4 have been described and have been reported to improve glucose metabolism in various animal models of type 2 diabetes (Balkan et al., 1999; Pederson et al., 1998; Pospisilik et al., 2002a; Reimer et al., 2002; Tourrel et al., 2002; Sudre et al., 2002; Pospisilik et al., 2003) and recently, in diabetic patients (Ahren et al., 2002). Vildagliptin (formerly known as LAF237) is a new stable, selective, and orally effective inhibitor of DPP-4 (Villhauer et al., 2003). In preliminary studies, a single oral dose of vildagliptin was found to augment insulin release and to reduce glucose excursions during an oral glucose tolerance test (OGTT) in Zucker fatty (fa/fa) rats.

The purpose of the present study was to explore a full dose-response of vildagliptin in Zucker fatty rats, to determine if the primary or secondary pharmacodynamics of the compound
exhibit tachyphylaxis with 3-week daily administration, to assess potential effects on body weight gain, and to compare effects of the compound in the severely insulin resistant and glucose intolerant fa/fa rat to those in normal rats rendered mildly insulin resistant by high-fat feeding.

METHODS

Animals and surgical procedures. Three studies were performed with adult male rats weighing 355 to 572 g, approximately 3 months of age. They were individually housed in plastic flat-bottom shoebox cages with wood chip bedding at 20 ± 2º C with a reverse light cycle (lights off 8:00 am to 8:00 pm). Zucker fatty rats (fa/fa) and their lean (FA/?) littermates (Charles River Laboratories, Cambridge, MA) were given ad libitum access to standard rodent chow and water, and the normal Sprague Dawley rats (Charles River Laboratories, Cambridge, MA) were maintained on a high-fat diet (57% of calories from saturated fat) (Purina Laboratories, Richmond, IN) for 3 weeks before experimentation.

Prior to study, silastic catheters were implanted in a jugular vein using aseptic technique and Ketamine:Xylazine:Acepromazine anesthesia (30:6:1, 0.5–0.7 ml/kg, i.m.). The tip of the cannula was placed in the right atrium, fixed with a suture and externalized at the nape of the neck. Cannulae were filled with a 55% polyvinylpyrrolidone solution (in 500 IU/ml heparin). Body weights were monitored and studies were performed only after the rats regained their pre-surgery body weights (generally 5 days).

Dosing and oral glucose tolerance tests. Study 1 examined the acute effects of vildagliptin (0.03 to 10 mg/kg in vehicle, 1 ml/kg) or vehicle (0.5% carboxymethylcellulose [CMC] in 0.2% Tween 80, 1 ml/kg) in 46 Zucker fatty (fa/fa) rats (n = 6 to 10 rats/group); 6 lean littermates (FA/?) received vehicle. In Study 2, these rats continued once-daily treatment for another 20 days. Study 3 examined the effects of a single oral dose of vildagliptin (3 mg/kg, n =
8) or vehicle (1 ml/kg, n = 6) in high fat fed normal Sprague Dawley rats. In all studies, vildagliptin or vehicle was administered by oral gavage at between 8:00 and 9:00 am. For the chronic study (Study 2), rats received vehicle or vildagliptin in vehicle at the specified doses in their home cages on Days 2 through 20.

In all studies, oral glucose tolerance tests (OGTTs) were performed following an overnight fast and samples were obtained for measurement of plasma DPP-4 activity, glucose, insulin and intact (N-terminally detected) GLP-1. For the OGTTs, rats were moved to the experiment room at 8:00 am. The atrial catheters were then connected to sampling tubing and filled with saline. After 30 to 40 minutes of acclimation, a 0.5 ml baseline blood sample was taken (T = –15 minutes for studies in Zucker rats and T = –30 minutes for studies in Sprague Dawley rats) and rats immediately received vehicle or vildagliptin by gavage. A post-dose, pre-glucose sample was obtained at 0 minutes, then an oral glucose load was administered (10% glucose, 10 ml/kg). Post-load blood samples were obtained at 1, 3, 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes. The volume of blood removed was replaced at each sampling time with heparinized donor blood, which was obtained via cardiac puncture.

Sample handling and analysis. Samples (0.5 ml) for analysis of plasma glucose, insulin and DPP-4 activity were obtained at each time-point and placed in chilled Eppendorf tubes containing 50 μL EDTA:Trasylol (25 mg/ml of 10,000 KIU/ml Trasylol, FBA Pharmaceuticals, Westhaven, CT). Larger blood samples (0.75 ml) were collected at –15, 0, 5, 10, 15, and 30 minutes for measurement of intact GLP-1 [7-36 amide]. These samples were placed in tubes containing the DPP-4 inhibitor, valine pyrrolidide at a final concentration of 1 μM.

Plasma glucose was measured with a glucose oxidase method (Sigma Diagnostics, St. Louis, MO). Insulin was measured with a double antibody RIA and intact GLP-1 [7-36 amide]
was measured with an ELISA (Linco Research, St. Charles, MO). This assay employs an N-terminally directed antibody and does not cross-react with the degradation product, GLP-1 [9-36 amide]. Plasma DPP-4 activity was measured as described previously (Villhauer et al., 2003).

All data were expressed as the mean ± SEM for each group of rats. Areas under the curve were calculated with a trapezoidal method. Comparisons between two groups were made by unpaired T-test. Comparisons among 3 or more groups were made with a one-way ANOVA with Dunnett’s post-ANOVA test to compare each treatment group with the vehicle-treated control group or Tukey’s post-ANOVA test to make multiple pairwise comparisons among all treatment groups.

RESULTS

Acute dose response in fa/fa rats – Study 1. Figure 1 depicts the primary pharmacology (plasma DPP-4 activity, Panel A) and plasma levels of intact GLP-1 (Panel B) in Zucker fatty rats following oral administration of increasing doses of vildagliptin (0.03 to 10 mg/kg) or vehicle, together with data from vehicle-treated lean (FA/?) Zucker rats during OGTTs (1 g/kg glucose administered at Time 0). Baseline levels of plasma DPP-4 activity did not differ among groups (mean at Time –15 = 7.8 ± 0.3 mU/ml, n = 44) and was expressed as the percentage of each animal’s baseline level. As shown in Figure 1A, plasma DPP-4 activity in vehicle-treated fa/fa and FA/? rats decreased modestly with time, to 76%–79% of baseline at 90 minutes post-dose, and the lowest dose of vildagliptin (0.03 mg/kg) had no significant effect. At higher doses, vildagliptin produced a rapid and dose-related inhibition of plasma DPP-4 activity, with both 3 and 10 mg/kg producing nearly complete (>90%) inhibition that was maintained throughout the sampling period. As shown in Figure 1B, there was no significant difference among groups in
the pre-dose (T = –15 min) or pre-glucose (T = 0 min) levels of intact GLP-1. Following the oral glucose load, intact GLP-1 increased very modestly, by 1 or 2 pM in vehicle-treated fa/fa and FA/? rats, and in the fa/fa rats receiving 0.03 mg/kg vildagliptin. At doses of 0.3 to 10 mg/kg, vildagliptin greatly and similarly augmented plasma levels of intact GLP-1 during OGTT. Peak plasma levels of intact GLP-1 occurred at 5 minutes post-glucose, with values ranging from 23 ± 3 pM (3 mg/kg) to 33 ± 9 pM (1 mg/kg) and there were no statistically significant differences among groups treated with effective doses of vildagliptin.

Figure 2 depicts the glucose (Panel A) and insulin (Panel B) profiles during OGTTs in these Zucker rats following acute administration of vehicle or vildagliptin. As shown in Figure 2A, vehicle-treated fa/fa rats had modestly elevated glucose levels in the fasted state and had markedly impaired glucose tolerance relative to the lean FA/? rats. Vildagliptin produced a dose-related improvement of glucose tolerance, and the 3 mg/kg dose appeared to elicit the maximum effect. As illustrated in Figure 2B, the obese fa/fa rats were hyperinsulinemic relative to the lean FA/? rats, reflecting their insulin resistant state, and at doses of 0.3 mg/kg and above, vildagliptin augmented the insulin response to oral glucose. The peak plasma insulin level occurred at 10 minutes post-glucose and the 3 mg/kg dose appeared to elicit the maximum effect.

To allow a better appreciation of the various effects of different doses of vildagliptin, several efficacy parameters were calculated and are summarized in Table 1. These include 1) plasma DPP-4 activity at Time 0, 2) plasma levels of intact GLP-1 at 5 minutes post-glucose, 3) the 90-minute incremental area under the curve for glucose, 4) the 45-minute incremental area under the curve for immunoreactive insulin (IRI) and 5) the insulinogenic index (Δ IRI/Δ glucose) at 10 minutes post-glucose. The time-points chosen for each parameter were selected to maximally differentiate between the doses. The minimum effective dose for the insulin area.
under the curve was 1 mg/kg and for all other parameters, the MED was 0.3 mg/kg. A monotonic dose-response for DPP-4 inhibition was apparent, but augmentation of active GLP-1 appeared to be “all or nothing”. The maximally effective dose for the glucose area under the curve and the insulinogenic index, a measure of β-cell function, was 3 mg/kg, and at the highest dose tested (10 mg/kg), the effects of vildagliptin on the insulin area under the curve and the insulinogenic index failed to achieve statistical significance.

**Chronic effects in fa/fa rats – Study 2.** To determine if vildagliptin exhibited tachyphylaxis, the animals used in Study 1 continued daily treatment for an additional 20 days. Figure 3 depicts plasma DPP-4 activity (panel A) and plasma levels of intact GLP-1 (panel B) in fa/fa rats during OGTTs performed on Day 1 and Day 21 of dosing with vildagliptin (3 mg/kg) or vehicle. The profiles of plasma DPP-4 activity on Day 1 and Day 21 were virtually superimposable in vildagliptin-treated rats, and glucose-stimulated plasma levels of intact GLP-1 were somewhat higher on Day 21 than on Day 1. Figure 4 illustrates the glucose (Panel A) and insulin profiles (Panel B) during OGTTs performed on Day 1 and Day 21 of dosing with vildagliptin (3 mg/kg) or vehicle. As shown in Figure 4, the glucose and insulin profiles in vildagliptin-treated rats were essentially indistinguishable on Day 1 and Day 21 of dosing. Thus, there was no evidence of tachyphylaxis for the effects of vildagliptin on any of the parameters measured. Body weight gain in the fa/fa rats treated for 21 days with vildagliptin (3 mg/kg) averaged 96 ± 9 g (4.5 ± 0.4 g/day). This was not significantly different from weight gain in the vehicle-treated fa/fa rats, which averaged 104 ± 7 g (5.0 ± 0.3 g/day, data not shown).

The full dose-response was examined in Study 2 after 21-day treatment with vildagliptin and the OGTT-derived parameters are reported in Table 2, together with fasting plasma glucose (FPG) levels (ie, prior to receiving the Day 21 dose). Vildagliptin had no significant effect on
FPG after 3-week daily dosing, although there was a slight trend toward a reduction at the higher doses. The dose-response characteristics of the OGTT-derived parameters were very similar with acute and chronic treatment. Although the suppression of DPP-4 activity with the 0.3 mg/kg dose at Time 0 was not statistically significant, a substantial and significant inhibition was observed at 20, 45, and 90 minutes post-glucose in rats receiving 0.3 mg/kg vildagliptin for 21 days (data not shown). Thus, the MED for effects of vildagliptin on all parameters was 0.3 mg/kg and the maximum effect on glucose tolerance was seen with a dose of 3 mg/kg. At the highest dose tested (10 mg/kg) the effects of vildagliptin on glucose area under the curve, IRI area under the curve, and the insulinogenic index on Day 21 of treatment failed to achieve statistical significance. There was no significant difference between the acute and chronic effects of any dose of vildagliptin when assessed by ANOVA followed by post-hoc pair-wise comparisons.

To examine the likelihood of a causal relationship between inhibition of DPP-4, augmentation of intact GLP-1, increased β-cell function and reduction of glucose excursions, correlation analyses were performed using individual data obtained on Day 1 and Day 21 of treatment. As reported in Table 3, with both acute and chronic administration of vildagliptin, there was a significant inverse relationship between plasma DPP-4 activity and intact GLP-1 best described by similar negative exponential functions on Day 1 and Day 21 (r = 0.699, P<0.001 on both days). There were also significant, positive correlations between plasma levels of intact GLP-1 and the incremental insulin area under the curve and the 10-minute insulinogenic index, best fit by logarithmic functions (correlation coefficients ranging from 0.360 to 0.517, P-values ranging from 0.02 to 0.001). Further, there was a strong negative relationship between plasma levels of active GLP-1 and the incremental glucose area under the curve best described by very
similar logarithmic functions (r = 0.551 on Day 1, r = 0.614 on Day 21, \( P<0.001 \) for both days). There was also a strong inverse relationship between the insulinogenic index and the incremental glucose area under the curve best described by similar negative exponential functions on Day 1 (r = 0.495, \( P<0.001 \)) and Day 21 (r = 0.674, \( P<0.001 \)).

*Acute effects in fat-fed normal rats – Study 3.* The acute effects of a single oral dose of vildagliptin (3 mg/kg, po) were then examined in normal Sprague Dawley rats that had been maintained on a high-fat diet for the previous 3 weeks. Figure 5 depicts plasma levels of DPP-4 activity (panel A), glucose (panel B), and insulin (panel C) during an OGTT in these fat-fed rats. As shown in Figure 5A, in rats given vildagliptin, DPP-4 activity decreased rapidly and markedly and remained below 10% of baseline throughout the 90-minute sampling period (\( P \leq 0.001 \) vs vehicle at all time-points). In vehicle-treated rats, plasma DPP-4 activity was somewhat more variable and tended to increase with time. As shown in Figure 5B, baseline plasma glucose levels were nearly identical in rats assigned vildagliptin (92 ± 3 mg/dl) and vehicle (93 ± 1 mg/dl) and did not change appreciably until the exogenous glucose load was administered. In rats receiving vildagliptin, the glucose excursion was reduced relative to that in vehicle-treated rats. Thus, both mean peak glucose (173 ± 5 mg/dl) and the incremental area under the curve (AUC\(_{0-90\text{min}}\)) [1.6 ± 0.2 mg/dl•min] were significantly lower in rats receiving vildagliptin than in vehicle-treated rats (190 ± 5 mg/dl, \( P=0.032 \) and 2.4 ± 0.1 mg/dl•min, \( P=0.001 \), respectively). Fasting insulin levels in these fat-fed rats (~45 µU/ml) were substantially less than those seen in the fa/fa rats, illustrating their more modest degree of insulin resistance relative to the Zucker fatty rats. As depicted in Figure 5C, baseline and post-drug levels of insulin were similar in vehicle and vildagliptin-treated fat-fed rats. Although the insulin response to glucose tended to be greater in rats receiving vildagliptin than in vehicle-treated rats, neither
the mean peak insulin level (481 ± 72 μU/ml) nor the 45 minute incremental area under the curve (6.0 ± 0.5 mU/ml•min) was significantly greater in rats receiving vildagliptin than in vehicle-treated rats (296 ± 42 μU/ml and 4.5 ± 0.6 mU/ml•min, respectively) using a 2-tailed test. However, the 10-minute insulinogenic index was significantly higher in rats receiving vildagliptin (5.3 ± 0.8) than in those receiving vehicle (2.5 ± 0.3, P< 0.05), suggesting an improvement in β-cell function. Samples for measurement of GLP-1 were not obtained in this study.
DISCUSSION

The incretin hormone GLP-1 is released in response to nutrient ingestion and serves a physiologic role in the maintenance of normal glucose homeostasis, due in part to its action to augment glucose-stimulated insulin release (Kreymann et al., 1987). Exogenously administered GLP-1 exerts powerful antidiabetic effects (Zander et al., 2002) and indeed, several analogs of GLP-1 are being developed for the treatment of type 2 diabetes (Fineman et al., 2003). (Juhl et al., 2002) However, due to their peptidic nature, GLP-1 analogs must be administered parenterally. Moreover, native GLP-1 has a very short plasma half-life (~2 minutes) because the enzyme, DPP-4 cleaves the N-terminal dipeptide from intact GLP-1 [7-36 amide], yielding GLP-1 [9-36 amide], which has none of the beneficial effects of the intact peptide, and may act as an antagonist (Thorens et al., 1993).

Several orally effective inhibitors of DPP-4 have been described and have been reported to have antidiabetic actions in animals (Balkan et al., 1999; Sudre et al., 2002; Deacon et al., 2001; Deacon et al., 2002; Pospisilik et al., 2002b) and in humans (Ahren et al., 2002). The focus of the current study, vildagliptin, is a recently discovered, potent, and specific inhibitor of DPP-4 that has a longer duration of action than its predecessor, NVP-DPP728 (1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(S)-pyrrolidine), but only one single-dose acute study has confirmed its antidiabetic properties in glucose-intolerant rats (Villhauer et al., 2003). The present work describes both the acute and chronic (3-week) effects of a broad dose-range of vildagliptin in Zucker fatty rats and compares the acute effects to those in the more mildly insulin-resistant model produced by high-fat feeding in normal rats.

From the acute study it was clear that vildagliptin rapidly inhibited plasma DPP-4 activity in Zucker fatty rats in a dose-related manner. This action of vildagliptin was accompanied by 1)
a marked increase in the glucose-stimulated levels of intact GLP-1, 2) enhanced glucose-stimulated insulin levels, and 3) a marked decrease in glucose excursions following an oral glucose challenge. The minimum effective dose of vildagliptin to inhibit DPP-4, to augment intact GLP-1, to improve β-cell function and to reduce glucose excursions was 0.3 mg/kg, and a dose of 3 mg/kg exerted maximal effects on all parameters. These findings are consistent with those of several earlier studies using other DPP-4 inhibitors in glucose intolerant rodents including Zucker fatty rats (Balkan et al., 1999; Pederson et al., 1998), high-fat–fed rats (Mitani et al., 2002a) and mice (Ahren et al., 2000), and aged rats (Mitani et al., 2002b), although no previous studies reported on the dose-response characteristics of DPP-4 inhibitors.

Based on the known physiology of GLP-1, it is likely that blockade of DPP-4 caused the increase of intact GLP-1, that the elevated GLP-1 enhanced insulin secretion and that the elevated insulin levels contributed to the improvement of glucose tolerance. This interpretation is supported by the present findings of a strong negative correlation between DPP-4 activity and plasma levels of intact GLP-1, positive correlations between intact (active) GLP-1 and the incremental insulin area under the curve or the insulinogenic index, and a strong negative relationship between intact GLP-1 or the insulinogenic index and post-load glucose excursions.

In earlier studies with a structurally related DPP-4 inhibitor, it was shown that the compound-mediated improvement of glucose tolerance and augmentation of the insulin response to oral glucose in rats fed a high fat diet were not observed in a DPP-4 deficient strain of rats fed a high fat diet (Mitani et al., 2002a). (Mitani et al., 2002b) Such findings strongly support the conclusion that inhibition of DPP-4 underlies the metabolic effects of vildagliptin seen in the present study. However, metabolically important peptides other than GLP-1 (such as GIP) are
also degraded by DPP-4 (Mentlein et al., 1993, Mentlein et al., 1999), hence substrates other than GLP-1 may contribute to the metabolic effects of DPP-4 inhibitors.

Indeed, alternative substrates of DPP-4, together with possible differences in tissue penetration, and thus sites of action of vildagliptin, may provide some explanation for the apparently reduced effectiveness of the highest dose of vildagliptin. For example, since DPP-4 is ubiquitous enzyme with a widespread tissue distribution, and its expression varies greatly between tissues (Lambeir, et al. 2003), although plasma DPP-4 activity was fully and equally inhibited (in both acute and chronic studies) in rats receiving either the 3 or the 10 mg/kg dose, it is possible that only the 10 mg/kg dose achieved levels sufficient to penetrate a compartment or tissue where another DPP-4 substrate, with effects opposing those of GLP-1 is produced. Clearly a different experimental approach will be necessary to resolve the possible mechanisms and mediators of the apparently reduced efficacy of high doses of vildagliptin.

It was recently reported that the insulinotropic and antihyperglycemic actions of a DPP-4 inhibitor were absent in double incretin receptor knockout (DIRKO) mice, again supporting a critical role for the incretin peptides to mediate the effects of DPP-4 inhibitors (Hansotia et al., 2004). However, in single incretin receptor knockout mice (lacking either GIP or GLP-1), a DPP-4 inhibitor was at least partially effective, suggesting that both incretin peptides contribute to improvements of insulin secretion and glucose tolerance elicited by DPP-4 inhibition, at least in rodents.

Given the current findings, that vildagliptin was highly effective to inhibit DPP-4, increase active GLP-1, improve β-cell function and reduce post-load glucose levels it appears to be unnecessary to invoke additional mechanisms to explain the glucose-lowering effects of vildagliptin. Nonetheless, it should be acknowledged that GLP-1 is known to have several
actions that can lead to an overall glucose lowering effect, including suppression of glucagon secretion (Ahrén et al., 1997), slowing of gastric emptying (Willms et al., 1996), enhanced insulin-independent glucose disposal (D’Alessio et al., 1994) and pancreas-independent reduction of endogenous glucose production (Prigeon et al., 2003). Therefore, further mechanistic studies would be of considerable interest and may be important for understanding the therapeutic effects of DPP-4 inhibitors in patients with type 2 diabetes.

The second experiment described here (ie, the 21-day study) aimed to determine if the effects of vildagliptin exhibited tachyphylaxis. Again, a relatively straightforward interpretation of those findings seems warranted. Since the DPP-4, glucose, and insulin profiles in rats given 3 mg/kg vildagliptin were nearly identical on Day 1 and Day 21 of dosing, and plasma levels of intact GLP-1 were if anything higher on Day 21 than on Day 1, it may be concluded that vildagliptin does not exhibit tachyphylaxis when given orally once daily for 3 weeks. The minimum effective dose on Day 21 for all parameters was again 0.3 mg/kg and maximum effects on glucose tolerance were seen with a dose of 3 mg/kg.

Several long-term studies with other DPP-4 inhibitors have examined their effects not only on glucose tolerance (Pospisilik et al., 2002a;Reimer et al., 2002) but on insulin sensitivity (Pospisilik et al., 2002a;Pospisilik et al., 2002b), β-cell mass (Tourrel et al., 2002;Pospisilik et al., 2003) and even on the progression from impaired glucose tolerance to overt diabetes in prediabetic rodents (Sudre et al., 2002). The absence of tachyphylaxis, as directly demonstrated by the present study, is consistent with (indeed, a prerequisite for) the long-term effectiveness of DPP-4 inhibitors and fulfillment of their therapeutic promise for the treatment of type 2 diabetes. It will be of great interest to determine if vildagliptin can also exert a disease-modifying, “pancreas-sparing” effect in diabetic, or even pre-diabetic patients, as predicted by preclinical
studies with other DPP-4 inhibitors, and most recently, by findings that vildagliptin stimulates β-cell replication, inhibits apoptosis and increases β-cell mass when given for 5 days to neonatal rats (Duttaroy et al., 2005).

The last small study described here assessed the effectiveness of vildagliptin in normal rats rendered modestly insulin resistant by a high-fat diet. Although the compound was fully effective to inhibit plasma DPP-4, there was only a small improvement in glucose tolerance, no significant effect on plasma insulin levels and a modest improvement of β-cell function as reflected by an increase in the insulinogenic index. These findings are reminiscent of an earlier demonstration that another DPP-4 inhibitor had very minor effects in lean Zucker rats (Balkan et al., 1999) and suggest that the magnitude of effect is proportional to the degree of metabolic derangement. In other words, DPP-4 inhibition has anti-hyperglycemic, not hypoglycemic, actions.

In summary, vildagliptin is an orally effective inhibitor of DPP-4 that greatly augments glucose-stimulated circulating levels of intact, biologically active GLP-1. It exerts dose-related effects on DPP-4, glucose tolerance and on β-cell function as reflected by the insulinogenic index. None of the effects of vildagliptin exhibit tachyphylaxis, suggesting its efficacy will be maintained during long-term treatment. Finally, the effects of vildagliptin are anti-hyperglycemic, not hypoglycemic, predicting a better safety profile than those of direct insulin secretagogues such as the sulfonylureas. We conclude that the incretin enhancer vildagliptin holds promise for the long-term treatment of type 2 diabetes, and the many potential mechanisms underlying its antidiabetic actions merit further exploration.
REFERENCES


FOOTNOTES

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FIGURE LEGENDS

**Figure 1:** Plasma DPP-4 activity (Panel A) and plasma levels of intact (N-terminally-detected) GLP-1 (Panel B) during oral glucose tolerance tests (1 g/kg glucose given at Time 0) performed in conscious male Zucker fatty rats (fa/fa) or in vehicle-treated lean littermates (FA/?) after acute oral administration of vehicle or increasing doses of vildagliptin. Doses of vildagliptin in mg/kg are used as labels and numbers of rats per treatment group are shown in parentheses. Mean ± SEM.

**Figure 2:** Plasma levels of glucose (Panel A) and immunoreactive insulin (IRI, Panel B) during oral glucose tolerance tests (1 g/kg glucose given at Time 0) performed in conscious male Zucker fatty rats (fa/fa) or vehicle-treated lean littermates (FA/?) after acute oral administration of vehicle or increasing doses of vildagliptin. Doses of vildagliptin in mg/kg are used as labels and numbers of rats per treatment group are shown in parentheses. Mean ± SEM.

**Figure 3:** Plasma DPP-4 activity (Panel A) and plasma levels of intact (N-terminally-detected) GLP-1 (Panel B) during oral glucose tolerance tests (1 g/kg glucose given at Time 0) performed in conscious male Zucker fatty rats (fa/fa) on Day 1 and Day 21 of daily oral administration of vildagliptin (VILD, 3 mg/kg, n = 8) or vehicle (n = 10). Mean ± SEM.

**Figure 4:** Plasma levels of glucose (Panel A) and immunoreactive insulin (IRI, Panel B) during oral glucose tolerance tests (1 g/kg glucose given at Time 0) performed in conscious male Zucker fatty rats (fa/fa) on Day 1 and Day 21 of daily oral administration of vildagliptin (3 mg/kg, n = 8) or vehicle (n = 10). Mean ± SEM.
Figure 5: Plasma levels of DPP-4 activity (Panel A), glucose (Panel B) and immunoreactive insulin (IRI, Panel C) during oral glucose tolerance tests (1 g/kg glucose given at Time 0) performed in conscious, high-fat fed normal male rats after acute oral administration of vildagliptin (3 mg/kg) or vehicle. Numbers of rats per treatment group are shown in parentheses, mean ± SEM.
Table 1: Plasma DPP-4 activity, plasma levels of intact GLP-1[7-36 amide] and OGTT-derived parameters in Zucker fatty rats (fa/fa) or lean littermates (FA/?) given vehicle or vildagliptin (VILD) fifteen minutes prior to an oral glucose load (1 g/kg) Mean ± SEM. *p<0.05, **p<0.01 vs vehicle.

<table>
<thead>
<tr>
<th>Treatment (dose in mg/kg)</th>
<th>DPP-4 activity (% baseline at T = 0)</th>
<th>GLP-1 [7-36 amide] at T = 5 min (pM)</th>
<th>Incremental Glucose area under the curve (g/dl•min)</th>
<th>Incremental IRI AUC(0-45min) (mU/ml•min)</th>
<th>Insulinogenic Index at T = 10 min</th>
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<td>Vehicle</td>
<td>83 ± 13</td>
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<td>VILD (0.03)</td>
<td>81 ± 14</td>
<td>7 ± 2</td>
<td>7.8 ± 0.5</td>
<td>5.3 ± 2.9</td>
<td>4.6 ± 1.7</td>
</tr>
<tr>
<td>VILD (0.3)</td>
<td>42 ± 8*</td>
<td>23 ±5**</td>
<td>4.5 ± 0.5**</td>
<td>23.6 ± 6.1</td>
<td>11.5 ± 1.5**</td>
</tr>
<tr>
<td>VILD (1.0)</td>
<td>29 ± 8**</td>
<td>33 ± 9**</td>
<td>3.5 ± 0.7**</td>
<td>23.0 ± 5.1**</td>
<td>12.1 ± 2.0**</td>
</tr>
<tr>
<td>VILD (3.0)</td>
<td>6 ± 1**</td>
<td>23 ± 3*</td>
<td>2.9 ± 0.4**</td>
<td>21.0 ± 3.9*</td>
<td>14.2 ± 3.2**</td>
</tr>
<tr>
<td>VILD (10.0)</td>
<td>4 ± 1**</td>
<td>28 ± 4**</td>
<td>3.4 ± 0.7**</td>
<td>14.7 ± 6.8</td>
<td>7.9 ± 1.8</td>
</tr>
<tr>
<td>FA/? Vehicle</td>
<td>89 ± 4</td>
<td>6 ± 2</td>
<td>3.5 ± 0.5**</td>
<td>3.6 ± 1.0</td>
<td>2.7 ± 0.9</td>
</tr>
</tbody>
</table>
Table 2: DPP-4 activity, plasma levels of intact GLP-1[7-36 amide] fasting plasma glucose (FPG) and OGTT-derived parameters in Zucker fatty rats (fa/fa) given vehicle or vildagliptin (VILD) once daily for 21 days. Mean ± SEM. *p<0.05, **p<0.01 vs vehicle. NA = data not available.

<table>
<thead>
<tr>
<th>Treatment (dose in mg/kg)</th>
<th>DPP-4 activity (% baseline at T = 0)</th>
<th>GLP-1 [7-36 amide] at T = 5 min (pM)</th>
<th>FPG (mg/dl)</th>
<th>Incremental Glucose AUC(0-90min) (g/dl•min)</th>
<th>Incremental IRI AUC(0-45min) (mU/ml•min)</th>
<th>Insulinogenic Index at T = 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>73 ± 10</td>
<td>11 ± 2</td>
<td>106 ± 3</td>
<td>7.0 ± 0.4</td>
<td>7.7 ± 1.4</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>VILD (0.03)</td>
<td>82 ± 13</td>
<td>15 ± 6</td>
<td>111 ± 5</td>
<td>7.0 ± 0.6</td>
<td>11.5 ± 2.2</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>VILD (0.3)</td>
<td>51 ± 11</td>
<td>28 ± 5**</td>
<td>109 ± 3</td>
<td>4.1 ± 0.4**</td>
<td>28.8 ± 10.0**</td>
<td>14.8 ± 3.9**</td>
</tr>
<tr>
<td>VILD (1)</td>
<td>33 ± 8**</td>
<td>36 ± 4**</td>
<td>101 ± 6</td>
<td>3.4 ± 0.5**</td>
<td>20.2 ± 2.7</td>
<td>11.3 ± 1.8</td>
</tr>
<tr>
<td>VILD (3)</td>
<td>9 ± 2**</td>
<td>41 ± 6**</td>
<td>101 ± 3</td>
<td>3.3 ± 0.5**</td>
<td>24.6 ± 5.5**</td>
<td>13.8 ± 1.9**</td>
</tr>
<tr>
<td>VILD (10)</td>
<td>13 ± 4**</td>
<td>31 ± 3**</td>
<td>98 ± 3</td>
<td>5.5 ± 0.2</td>
<td>16.9 ± 6.4</td>
<td>7.1 ± 1.5</td>
</tr>
<tr>
<td>FA/?</td>
<td>NA</td>
<td>6 ± 2</td>
<td>76 ± 1**</td>
<td>3.5 ± 0.5</td>
<td>5.0 ± 1.3</td>
<td>2.7 ± 0.9</td>
</tr>
</tbody>
</table>
Table 3: Correlations between calculated parameters.

<table>
<thead>
<tr>
<th>y: GLP-1</th>
<th>x: DPP-4</th>
<th>Day 1</th>
<th></th>
<th></th>
<th>Day 21</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td>r</td>
<td>p</td>
<td>Function</td>
<td>r</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>y = 28.4e^{-0.016(x)}</td>
<td>0.699</td>
<td>&lt;0.001</td>
<td>y = 37.2e^{-0.015(x)}</td>
<td>0.699</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>y: IRI AUC</td>
<td>x: GLP-1</td>
<td>Function</td>
<td>r</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y = 7658•Ln(x) - 5096</td>
<td>0.456</td>
<td>&lt;0.002</td>
<td>y = 9874•Ln(x) - 11221</td>
<td>0.517</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>y: I/G</td>
<td>x: GLP-1</td>
<td>Function</td>
<td>r</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y = 3.6•Ln(x) - 0.56</td>
<td>0.466</td>
<td>&lt; 0.02</td>
<td>y = 2.3•Ln(x) + 2.26</td>
<td>0.360</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>y: Gluc AUC</td>
<td>x: GLP-1</td>
<td>Function</td>
<td>r</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y = -1689•Ln(x) + 9562</td>
<td>0.551</td>
<td>&lt;0.001</td>
<td>y = -1542•Ln(x) + 9622</td>
<td>0.614</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>y: Gluc AUC</td>
<td>x: I/G</td>
<td>Function</td>
<td>r</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y = 6803e^{0.051(x)}</td>
<td>0.495</td>
<td>&lt;0.001</td>
<td>y = 7431e^{0.048(x)}</td>
<td>0.674</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

A) DPP-4 Activity (% Baseline) vs. Time (min)

B) GLP-1[7-36 amide] (pM) vs. Time (min)

- Vehicle (10)
- 0.03 mg/kg (6)
- 0.3 mg/kg (7)
- 1 mg/kg (8)
- 3 mg/kg (8)
- 10 mg/kg (7)
- FA/ (6)
Figure 3

A

DPP-4 Activity (% Baseline)

Time (min)

B

GLP-1 [7-36amide] (pM)

Time (min)
Figure 4

**A**

Glucose (mg/dl)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vehicle Day 1</th>
<th>Vehicle Day 21</th>
<th>VILD Day 1</th>
<th>VILD Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B**

IRI (µU/ml)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vehicle Day 1</th>
<th>Vehicle Day 21</th>
<th>VILD Day 1</th>
<th>VILD Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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</tr>
<tr>
<td>30</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>60</td>
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</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5

A. DPP-4 Activity (% baseline)

- Vehicle (6)
- VILD (8)

B. Glucose (mg/dl)

- Vehicle (6)
- VILD (8)

C. Glucose (µU/ml)

- Vehicle (6)
- VILD (8)