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Vildagliptin Reduces Glucagon during Hyperglycemia and Sustains Glucagon Counterregulation during Hypoglycemia in Type 1 Diabetes

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Context: The dipeptidyl peptidase-4 inhibitor, vildagliptin, inhibits glucagon secretion at hyperglycemia but appears to enhance glucagon counterregulation during hypoglycemia in type 2 diabetes.

Objective: The objective of the investigation was to study whether vildagliptin also improves β-cell function in type 1 diabetes (T1D).

Patients and Methods: The study was a single-center, double-blind, randomized, placebo-controlled crossover study involving 28 patients with C-peptide negative and antibody positive T1D [21 males, seven females, glycosylated hemoglobin 57.9 mmol/mol (7.5%)]. Patients received vildagliptin (50 mg twice a day) or placebo as an add-on to their insulin therapy for 4 wk each. On d 28 of the respective treatment period, patients were served a standard meal (500 kcal) to raise the circulating incretin hormone levels followed by a hyperinsulinemic hypoglycemic clamp at 2.5 mmol/liter.

Main Outcome Measure: The increase in plasma glucagon levels during the 30-min hypoglycemic clamp (min 165–195 of the test) was measured.

Results: During the meal, glucagon levels were lower with vildagliptin than with placebo (120 min area under the curve glucagon 2.4 ± 0.2 vs. 2.6 ± 0.2 nmol/liter × minutes, P = 0.022 for between group difference). In contrast, during hypoglycemia, the glucagon counterregulation was not reduced by vildagliptin (increase in glucagon 1.5 ± 1.0 pmol/liter with vildagliptin vs. 1.7 ± 0.8 pmol/liter with placebo, P = NS). In addition, the counterregulatory responses in epinephrine, norepinephrine, cortisol, and pancreatic polypeptide were not different between the treatments. During the 4-wk treatment period, vildagliptin reduced the mean glycosylated hemoglobin, whereas there was no change with placebo [between group difference was −3.4 ± 1.0 mmol/mol (−0.32 ± 0.09%; P = 0.002)] from baseline of 57.9 mmol/mol (7.5%).

Conclusions: Vildagliptin, although inhibiting glucagon secretion during hyperglycemia, does not compromise the glucagon counterregulatory response during hypoglycemia in T1D. (J Clin Endocrinol Metab 97: 0000–0000, 2012)
ciated with improved glycemia in patients with type 2 diabetes (T2D), both when vildagliptin is given in monotherapy (8, 9) and when given in combination with metformin (10, 11), thiazolidinediones (12, 13), sulfonylureas (14), or insulin (15). The mechanism of the reduced glycemia is exerted mainly by increasing insulin secretion and reducing glucagon secretion in hyperglycemia (7, 16). The glucose dependency of the effects to reduce glycemia is one mechanism whereby vildagliptin is associated with a low risk of hypoglycemia (17).

Another important mechanism behind the low risk of hypoglycemia is a sustained counterregulation to hypoglycemia (18). This was demonstrated in an earlier report showing that when given as monotherapy in drug-naïve patients with T2D, vildagliptin, although inhibiting the glucagon response to meal ingestion, the glucagon response during a hypoglycemic clamp at 2.5 mmol/liter appeared to be at least normal, if not slightly improved (18). Because glucagon counterregulation is an important mechanism for the defense against hypoglycemia (19, 20), this mechanism would prevent from hypoglycemia. It is possible that the enhanced counterregulation through the stimulation of glucagon secretion by GIP contributes to this reduced risk for hypoglycemia (5).

In type 1 diabetes (T1D), relative hyperglucagonemia contributes, in association with insulin deficiency, to the hyperglycemia (21). This would suggest that incretin-based therapy could be added to ongoing insulin therapy also in T1D, provided that it reduces glucagon levels also in this disease. The latter was suggested in an earlier report showing that vildagliptin reduces glucagon levels after a meal ingestion in subjects with T1D (22). However, if the combination of vildagliptin and insulin will be a potential therapy in T1D, as in T2D, does not compromise the glucagon counterregulation to hypoglycemia. To study this, we examined the effects of vildagliptin on glucagon levels during a test with the initial meal ingestion (to raise incretin hormone levels) followed by hypoglycemia (during a hypoglycemic clamp at 2.5 mmol/liter) after 4 wk of treatment in patients with T1D in a placebo-controlled crossover study.

Materials and Methods

Study design

The study was a single-center, double-blind, randomized, placebo-controlled crossover study involving 28 patients with T1D. Patients received vildagliptin (50 mg twice a day) or placebo as add-on to insulin for 4 wk in random order with a 4-wk washout in between. On d 28 of the respective treatment, patients were served a standard meal (500 kcal) followed by a hyperinsulinemic hypoglycemic clamp at 2.5 mmol/liter. Each patient attended one screening visit (wk − 4), during which inclusion/exclusion criteria were assessed. Eligible patients were randomized at visit 2 (d 1) and were expected to complete two treatment periods, receiving a different blinded study medication during each period (vildagliptin 50 mg twice a day or placebo, in random order). At the baseline (d 1) visit, the study medication was dispensed for 4 wk for outpatient treatment. The test procedure (see Study assessments) was performed after an overnight fast on d 28 of the first treatment period. Study medication was then discontinued, and a 4-wk washout period occurred before the alternative 4-wk treatment period. The test procedure was repeated after an overnight fast on d 28 of the second treatment period.

Study population

The study enrolled male and female patients (females of child-bearing potential were required to use a medically approved birth control method) aged 18 yr old or older, with a diagnosed C-peptide-negative and antibody-positive T1D of 2- to 20-yr duration and a glycosylated hemoglobin (HbA1c) of 48–68 mmol/mol (6.5–8.5%). Patients were excluded if they were pregnant or lactating, had T2D, had acute infection during the 4 wk preceding the study, had severe hypoglycemia within 2 wk before the study, had severe liver disease, were a blood donor, or were treated with GH or an oral steroid during the 2 months preceding the study.

Study assessments

The test procedure (performed after an overnight fast, after the placement of a cannula in an antecubital vein) began with a baseline blood sample being taken (at time −20 min), and then study medication was given 15 min before the standard breakfast meal was provided (at time 0 min). A premeal blood sample was obtained (at time −5 min), and the meal was consumed within 10 min. The standard breakfast meal comprised 180 ml orange juice, two slices (60 g) bread, 30 g jam, 15 g butter or margarine, 120 ml whole milk (or equivalent amount of cheese plus 120 ml water), and decaffeinated coffee or tea, supplying 500 kcal (60% carbohydrate, 30% fat, 10% protein). This was followed by a hyperinsulinemic hypoglycemic clamp (glucose 2.5 mmol/liter). During the meal test, samples for the determination of glucose, glucagon, and intact GLP-1 were obtained at times 0, 5, 15, 30, 45, 60, 90, and 120 min (with time 120 min serving as baseline for the hypoglycemic clamp). For the hypoglycemic clamp, patients received a primed, continuous infusion of insulin [Actrapid (Novo Nordisk A/S, Bagsvaerd, Denmark) 600 pmol/m²·min, 0–4 min; 500 pmol/m²·min, 4–7 min; 400 pmol/m²·min, 7–10 min; and 300 pmol/m²·min thereafter], with glucose infused (20%) at a variable rate to achieve 2.5 mmol/liter glucose. Frequent samples for real-time glucose measurements were taken to adjust the glucose infusion rate [performed by the glucose dehydrogenase technique with a HemoCue device (HemoCueAB, Angelholm, Sweden)] during the clamp. At 75 min after the start of insulin infusion for the hypoglycemic clamp, the insulin infusion was discontinued; glucose was infused if necessary and plasma glucose levels were allowed to recover. During the hypoglycemic clamp period (120–195 min), samples were taken at times 135, 150, 165, 180, and 195
min for the measurement of glucose, glucagon, pancreatic polypeptide (PP), epinephrine, norepinephrine, and cortisol.

Assays
Cortisol, epinephrine, norepinephrine, HbA1c, fasting plasma glucose (FPG), and safety laboratory assessments were made by the Department of Clinical Chemistry (Skåne University Hospital, Malmö, Sweden), and glucagon, GLP-1, and PP were measured at the Department of Clinical Sciences Lund (Lund University). Glucagon concentrations were analyzed with a double-antibody RIA using guinea pig antihuman glucagon antibodies specific for pancreatic glucagon (Millipore, Billerica, MA). PP was determined with sandwich immunoassay technique (ELISA) using rabbit antihuman PP antibodies (Millipore). Norepinephrine and epinephrine concentrations were determined by HPLC. Cortisol was determined with the BeckmanCoulter Access immunoassay system (Fullerton, CA). Intact GLP-1 was determined by sandwich immunoassay technique (ELISA) using monoclonal antibodies specific for the intact form of GLP-1 (Millipore). HbA1c, FPG, and safety laboratory assessments were performed according to standardized and validated procedures and good laboratory practice.

Data analysis
From samples obtained during the standard meal test, the 120-min areas under the curve (AUC) were calculated by the trapezoidal method for glucose, glucagon, and GLP-1. During the hypoglycemic clamp step, the change from time 165 to time 195 min (when glucose was clamped at 2.5 mmol/liter) was calculated for all analytes. Changes in HbA1c were assessed during the two treatment periods. Between-treatment differences in each of the aforementioned variables and for measures at individual time points were estimated with paired t tests in the completers population. The completers population was defined as all randomized patients who received at least one dose of study drug and had a valid assessment of the primary variable at the end of each treatment period (n = 28).

Ethics and good clinical practice
The protocol was approved by the Ethics Committee of Lund University and the Swedish Medical Product Agency, and all subjects gave written informed consent before entering the study. The study was conducted using good clinical practice and in accordance with the Declaration of Helsinki.

Results
Patients studied
Thirty-six patients were screened and 29 patients were randomized (14 to treatment sequence A and 15 to treatment sequence B). Twenty-eight patients completed the study (14 in each treatment sequence) and comprised the completers population defined earlier. Table 1 summarizes the demographic and baseline characteristics of the completers population. There were no differences between the subjects in the two treatment sequences in any of the variables. Patients were all Caucasian and predominantly male, with a mean age, body mass index, disease duration, baseline HbA1c, and baseline FPG of approximately 30 yr, 24.8 kg/m², 11 yr, 57.9 mmol/mol (7.5%), and 10.5 mmol/liter, respectively. Twenty-six patients were treated with daily basal-bolus injections; their mean insulin dose was 30 U/d (long acting insulin; 0.37 ± 0.07 U/kg) and 31 U/d (short acting insulin; 0.37 ± 0.09 U/kg). Two patients were treated with a continuous sc insulin infusion [daily dose 60 and 36 U (0.67 and 0.52 U/kg), respectively].

Meal test
Figure 1 shows the blood glucose levels during the tests in which a standardized meal was served followed by the hypoglycemic clamp. Glucose levels were lower during the meal test after treatment with vildagliptin. The 120-min AUCglucose was 1.21 ± 0.09 mmol/liter × minutes with vildagliptin vs. 1.39 ± 0.08 mmol/liter × minutes with placebo; however, the between-treatment difference in

| TABLE 1. Demographic and baseline characteristics of the completers population |
|---------------------------------|-----------------|-----------------|--------------|
|                                 | Sequence A (vildagliptin/placebo) | Sequence B (placebo/vildagliptin) | All          |
| Age (yr)                        | 29.8 ± 4.1      | 30.2 ± 5.1      | 30.0 ± 4.6   |
| Males                           | 14 (78.6%)      | 10 (71.4%)      | 21 (75.0%)   |
| Caucasians                      | 14 (100%)       | 14 (100%)       | 28 (100%)    |
| BMI (kg/m²)                     | 25.4 ± 1.9      | 24.0 ± 4.2      | 24.8 ± 3.3   |
| HbA1c (mmol/mol)                | 58.3 ± 6.1      | 57.6 ± 4.8      | 57.9 ± 5.5   |
| HbA1c (%)                       | 7.53 ± 0.60     | 7.46 ± 0.48     | 7.49 ± 0.55  |
| FPG (mmol/liter)                | 10.7 ± 4.0      | 10.4 ± 4.5      | 10.5 ± 4.3   |
| Disease duration (yr)           | 11.6 ± 4.1      | 10.4 ± 4.6      | 11.0 ± 4.3   |
| Daily long-acting insulin (U)²  | 28.6 ± 8.7      | 30.9 ± 8.5      | 30.1 ± 8.5   |
| Daily long-acting insulin (U/kg)| 0.35 ± 0.07     | 0.38 ± 0.07     | 0.37 ± 0.07  |
| Daily short-acting insulin (U)² | 31.4 ± 9.8      | 29.8 ± 13.8     | 30.6 ± 11.60 |
| Daily short-acting insulin (U/kg)| 0.37 ± 0.08   | 0.36 ± 0.10     | 0.37 ± 0.09  |

a Two patients on continuous sc insulin infusion were not included here (their daily dosage of rapid acting insulin was 60 and 36 U, respectively, 0.67 and 0.52 U/kg). Data are expressed as mean ± SD or number (percent).
AUC_{glucose} (-0.18 ± 0.13 mmol/liter × minutes) did not reach statistical significance (P = 0.175).

Figure 2 shows the glucagon and GLP-1 responses to meal ingestion after treatment with vildagliptin or placebo. The premeal levels of glucagon were 19.6 ± 1.5 pmol/liter with vildagliptin and 29.4 ± 1.4 pmol/liter with placebo (P = 0.557 for between treatment difference). Although there was an increase in glucagon levels in both groups after the meal ingestion, the glucagon levels after meal ingestion were lower after treatment with vildagliptin than after placebo. The 120-min AUC_{glucagon} was 2.4 ± 0.2 nmol/liter/minutes with vildagliptin vs. 2.6 ± 0.2 nmol/liter/minutes with placebo, which corresponds to a reduction by vildagliptin of −9.1 ± 3.5% (P = 0.022). The fasting GLP-1 levels were higher after vildagliptin treatment (5.9 ± 0.8 pmol/liter) than after placebo treatment (4.0 ± 0.5 pmol/liter; P = 0.032 for the between treatment difference). The GLP-1 response to the meal was larger and was maintained for a longer period after vildagliptin than placebo treatment, resulting in a markedly elevated 120 min AUC_{GLP-1} (291 ± 98 pmol/liter/minutes with vildagliptin vs. 64 ± 27 pmol/liter/minutes with placebo, P = 0.034 for the between treatment difference).

**Hypoglycemic clamp**

Glucose levels were clamped at 2.5 mmol/liter with no difference in glucose levels between the two tests (Fig. 1). There was no significant difference in glucose infused to maintain the hypoglycemic glucose level during min 120–195 (92 ± 8 μg/kg after vildagliptin vs. 110 ± 10 μg/kg after placebo; P = 0.15 for between treatment difference). During hypoglycemia, levels of glucagon, cortisol, norepinephrine, epinephrine, and PP increased, with no difference between the two treatments (Fig. 3 and Table 2). The GLP-1 levels were slightly higher during the hypoglycemic clamp with vildagliptin than with placebo. During the recovery phase during the hour after the hypoglycemia, from min 195 to min 255, glucose was higher after placebo treatment than after vildagliptin, the final glucose level at 255 min being 6.4 ± 0.3 mmol/liter after vildagliptin treatment and 7.6 ± 0.6 mmol/liter after placebo treatment (P = 0.047 for between treatment difference).

**Glycemic control**

During the 4-wk treatment with vildagliptin, the mean HbA1c was significantly reduced from 58.1 ± 1.1 mmol/mol (7.51 ± 0.11%) to 54.7 ± 1.0 mmol/mol (7.17 ± 0.10%), i.e. by −3.6 ± 0.7 mmol/mol (−0.34 ± 0.10%; P < 0.001), whereas there was no significant
change in HbA1c during the 4-wk treatment with placebo [change from 57.6 ± 0.9 mmol/mol (7.46 ± 0.09%) to 57.4 ± 0.9 mmol/mol (7.44 ± 0.09%), i.e. by −0.2 ± 0.7 mmol/mol (−0.02 ± 0.07%)], corresponding to a between-group difference in change of HbA1c of −3.4 ± 1.0 mmol/mol (−0.32 ± 0.09%, P = 0.002). The dosage of insulin was not significantly altered during the study periods.

Safety and tolerability
The overall adverse event (AE) profile during treatment with vildagliptin was similar to that during placebo administration. In total, 46 AE were reported during the study, of which 34 occurred during treatment with study medication [18 with vildagliptin (in 16 patients), 16 with placebo (in 16 patients)] and 12 during the washout period between the treatments (eight in the vildagliptin/placebo treatment sequence and four in the placebo/vildagliptin treatment sequence). The only AE that was reported by more than three patients was the common cold, which was reported in 11 patients during treatment with study medication (four with vildagliptin, seven with placebo) and in five patients during the washout period (three in the vildagliptin/placebo treatment sequence and two in the placebo/vildagliptin treatment sequence). Four patients reported mild hypoglycemia (two with vildagliptin, one with placebo and one in the washout period) with no serious hypoglycemia. One patient receiving placebo discontinued therapy after having abdominal pain at the fifth day after start of study medication; there were no discontinuations on vildagliptin treatment or in the washout period. No serious AEs were reported in the study.

Discussion
The main finding of this study is that the DPP-4 inhibitor, vildagliptin, reduced glucagon levels during meals and at the same time preserved glucagon counterregulation during hypoglycemia after 4 wk of therapy in T1D.

It has previously been discussed that incretin-based therapy would be suited for add-on to insulin therapy in T1D (23), and, for example, the GLP-1 receptor agonist, liraglutide, has been shown to improve glycemia in T1D.

| TABLE 2. Glucagon, GLP-1, cortisol, norepinephrine, epinephrine, and PP responses to hypoglycemia |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------------------------|-----------------|
| Mean ± SD                                      | Mean ± SD                                      | Difference Mean (95% CI)                         | P value                                      |
| Glucagon (pmol/liter)                          | 1.5 ± 1.0                                     | 1.7 ± 0.8                                      | −0.2 (−0.9 1.3)                              | 0.895           |
| Cortisol (nmol/liter)                          | 192 ± 160                                     | 148 ± 183                                      | 51 (−2.84)                                   | 0.311           |
| Norepinephrine (nmol/liter)                    | 0.46 ± 0.53                                   | 0.50 ± 1.0                                     | −0.04 (−0.16 0.08)                           | 0.356           |
| Epinephrine (nmol/liter)                       | 1.10 ± 1.21                                   | 1.07 ± 1.0                                     | 0.03 (−1.19 1.25)                            | 0.148           |
| PP (pmol/liter)                                | 99 ± 24                                       | 73 ± 25                                        | 25 (−4.29)                                   | 0.394           |
| GLP-1 (pmol/liter)                             | 3.3 ± 3.8                                     | 1.4 ± 2.6                                      | 1.9 (1.0 2.8)                                | 0.033           |

Data show the change in levels from min 165 to min 195 [means ± SD or mean (95% confidence interval)]. CI, Confidence interval.
One rationale for such therapy would be reduction of the elevated glucagon levels in the disease, as previously demonstrated for vildagliptin (22). In the present study, we reproduced that glucagon levels after meal ingestion is lower after vildagliptin treatment than after placebo treatment. The main focus of this study was, however, to examine the counterregulatory responses to hypoglycemia. The counterregulatory response to hypoglycemia is of importance because poor counterregulation would increase the likelihood for development of hypoglycemic events, which would limit the usefulness of incretin based therapy in T1D. Thus, it has been repeatedly demonstrated that hypoglycemia is the limiting factor achieving good glycemic control in T1D (25). We found that during a hypoglycemic clamp when glucose levels were lowered to 2.5 mmol/liter during 30 min, glucagon levels were increased along with increases in cortisol, norepinephrine, epinephrine, and PP. Vildagliptin did not compromise these responses because they were not different after vildagliptin and placebo treatment. Hence, also in T1D, as has been previously demonstrated in drug-naïve patients with T2D (12), vildagliptin inhibits glucagon secretion when glucose levels are elevated and does not inhibit any counterregulatory glucagon response to hypoglycemia.

The sustainment of glucagon secretion during hypoglycemia despite the clear suppression of glucagon levels during hyperglycemia suggests that vildagliptin has different actions on α-cell secretion depending on the glucose levels. At hyperglycemia, the inhibition of glucagon is important for initiating improved glucose control. This inhibition may result from a direct action of GLP-1 on glucagon secretion (which, however, has been questioned because there is a very low GLP-1 receptor expression on α-cells; for review see Ref. 26), from enhanced glucose sensitivity in the α-cells, thereby augmenting the inhibitory action of glucose on glucagon secretion, through an indirect effect by GLP-1 to stimulate somatostatin secretion, which would inhibit glucagon secretion (27), or through stimulation of β-cell secretion, which would inhibit glucagon secretion through insulin or other products from the β-cells (28). The opposing effect of vildagliptin to sustain, and not inhibit, the glucagon response to hypoglycemia might be explained by enhanced glucose sensitivity in α-cells, thereby promoting and not inhibiting the direct effect of low glucose to stimulate glucagon secretion (7). It might, however, also be explained by increased autonomic activation of glucagon secretion, which is an important mechanism during hypoglycemia (29, 30), and also through glucose-dependent insulinotropic polypeptide (GIP), the concentration of which also is increased by vildagliptin (6), and which stimulates glucagon secretion during hypoglycemia (5). We also determined the circulating PP during the hypoglycemic clamp. PP levels are considered an index of parasympathetic autonomic nervous system activity (31), which is involved in the glucagon counterregulation to hypoglycemia (29). We found that PP levels increased during the hypoglycemic phase and that this increase was sustained after vildagliptin treatment, suggesting that autonomic nerve activation is not compromised by the DPP-4 inhibitor. More studies are now required to further analyze the mechanism of the glucose-dependent influence of vildagliptin on glucagon secretion in T1D. In our previous study in T2D, vildagliptin was found to increase the glucagon counterregulatory response to hypoglycemia (7). In that study, the glucagon response to hypoglycemia was higher than in the present study in T1D, which is a well-known difference between the two forms of diabetes (32, 33) and which might explain why vildagliptin seemed to have a more pronounced effect in T2D vs. in T1D.

In the present study, fasting and prandial GLP-1 levels were shown to be elevated by vildagliptin, which confirms effects of vildagliptin in T2D (6, 34). We also found that during the entire hypoglycemic phase during the clamp, GLP-1 levels were higher after vildagliptin treatment than after placebo treatment. A further, interesting observation was that during the end of the hypoglycemic clamp period, there was a slight, but significant, increase in GLP-1 levels and this increase was augmented by vildagliptin. This finding might suggest that hypoglycemia lasting for more than 15–20 min increases not only glucagon secretion but also GLP-1 secretion, which is a finding requiring further studies. Finally, an important finding in this study was also that during the recovery period after the end of the hypoglycemic clamp, glucose levels increased less after vildagliptin treatment than after placebo treatment. This reinforces the finding that vildagliptin reduces the glycaemia in T1D because the difference in glucose levels after the recovery phase was similar to the difference in fasting glucose after vildagliptin vs. placebo.

Although this study was not designed to assess the clinical utility of adding vildagliptin to insulin therapy in T1D, it is worth noting that after only 4 wk of vildagliptin treatment, HbA1c levels were reduced without increasing hypoglycemia, which is similar to what has been demonstrated with vildagliptin plus insulin treatment in T2D (15, 35). In summary, the present study demonstrates that in patients with T1D, the DPP-4 inhibitor vildagliptin improves glycaemia and inhibits glucagon levels during meal ingestion but sustains glucagon counterregulation during hypoglycemia. This would suggest that vildagliptin might be examined in further, more long-term clinical studies as
a potential therapy of T1D as an add-on to insulin therapy without increasing the risk for hypoglycemia.

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