Dipeptidyl Peptidase 4 Inhibitor Sitagliptin Maintains β-Cell Function in Patients With Recent-Onset Latent Autoimmune Diabetes in Adults: One Year Prospective Study

Yunjuan Zhao,* Lin Yang,* Yufei Xiang, Lingjiao Liu, Gan Huang, Zhaofeng Long, Xia Li, R. David Leslie, Xiangbing Wang, and Zhiguang Zhou

Diabetes Center (Y.Z., L.Y., Y.X., L.L., G.H., Z.L., X.L., Z.Z.), Institute of Metabolism and Endocrinology, The Second Xiangya Hospital and Key Laboratory of Diabetes Immunology, Ministry of Education, Central South University, Changsha, Hunan 410011, People's Republic of China; Department of Diabetes and Metabolic Medicine (R.D.L.), Blizard Institute, London E1 2AT, United Kingdom; and Division of Endocrinology, Metabolism, and Nutrition (X.W.), Rutgers University-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903

Context: Dipeptidyl peptidase 4 (DPP-4) inhibitors have been widely used in type 2 diabetes. An important unanswered question concerns the effect of DPP-4 inhibition on β-cell function in patients with autoimmune diabetes.

Objective: The objective of the study was to investigate the effects of the DPP-4 inhibitor on β-cell function in patients with recent-onset latent autoimmune diabetes in adults (LADA).

Design and Setting: This study was an open-label, randomized-controlled study conducted in the Department of Endocrinology at the Second Xiangya Hospital.

Patients and Intervention: Thirty recently diagnosed LADA patients were randomized 1:1 to receive insulin therapy with 100 mg/d sitagliptin (group A, n = 15) or without sitagliptin (group B, n = 15) for 12 months.

Main Outcome Measures: Fasting and 2-hour postprandial blood samples were obtained at baseline and after 3, 6, 9, and 12 months of treatment to determine blood glucose, glycosylated hemoglobin, and C-peptide levels.

Results: There were no differences in the clinical baseline data between the two groups. During the 12 months of follow-up, there were no significant differences in glucose and glycosylated hemoglobin levels between the two groups. At 12 months, fasting C-peptide (FCP), 2-hour postprandial C-peptide (CP), and ΔCP (ΔCP = 2 h CP−FCP) levels were not different in group A ($P > .05$) compared with baseline, whereas in group B the levels of FCP, 2-hour CP and ΔCP were significantly decreased compared with baseline ($P < .05$). Levels of 2-hour CP were higher in group A than group B at 12 months ($P < .05$).

Conclusions: LADA patients treated with sitagliptin and insulin maintained β-cell function by comparison with insulin alone. **(*J Clin Endocrinol Metab* 99: E876–E880, 2014)**

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* Y.Z. and L.Y. contributed equally to this work.

Abbreviations: Ab, antibody; BMI, body mass index; CP, postprandial C-peptide; DPP-4, dipeptidyl peptidase-4; FCP, fasting C-peptide; GAD, glutamic acid decarboxylase; GADA, GAD antibody; HbA_{1C}, glycosylated hemoglobin; LADA, latent autoimmune diabetes in adults.

Dipeptidyl peptidase 4 (DPP-4) inhibitors are a new class of oral antidiabetic agent that has been shown to preserve β-cell function in mouse models of type 2 diabetes (1, 2), patients with type 2 diabetes (3, 4), and even in patients with impaired fasting glucose (5). Linagliptin, a DPP-4 inhibitor, has a direct protective effect on β-cell function under diabetogenic conditions in vitro (6). Furthermore, DPP-4 inhibition reduces insulitis and stimulates β-cell function in a nonobese diabetic mouse model of autoimmune diabetes, a classic model of type 1 diabetes (7, 8). There was increased serum DPP-4 activity (9) and increased DPP-4 expression on terminally differentiated $CD4^+$ T-cells (10) in patients with type 1 diabetes. These observations provided a rationale to test the effects of DPP-4 inhibition in type 1 diabetes. A recent trial has shown that sitagliptin significantly improves glycemic control in adult patients with insulin-dependent type 1 diabetes (11). An important unanswered question concerns the effect of DPP-4 inhibition on β-cell function in patients with less severe autoimmune diabetes.

Latent autoimmune diabetes in adults (LADA) is a form of type 1 diabetes that comprises about 6%–10% of patients initially diagnosed with type 2 diabetes (12). LADA is a good model for investigation of the effects of DPP-4 inhibition on β-cell function in autoimmune diabetes. In the present study, we investigated the effects of a widely used DPP-4 inhibitor, sitagliptin, on the preservation of β-cell function in patients with LADA.

Materials and Methods

Patient selection

The trial was designed as a single-center, randomized-controlled study conducted in LADA patients in the Department of Endocrinology at the Second Xiangya Hospital. Stratified randomization was performed by professional statisticians without involvement of the study investigators. Patients with recent-onset LADA were selected according to the following criteria: 1) diabetes diagnosed according to the report of the World Health Organization in 1999, 2) age of 25–70 years, 3) glutamic acid decarboxylase (GAD) antibody (GADA) positive, 4) insulin independent within the first 6 months after the diagnosis of diabetes, 5) fasting C-peptide (FCP) level of 200 pmol/L or greater or a 2-hour postprandial C-peptide (CP) ≥400 pmol/L or greater, and 6) duration of diabetes of 3 years or less. The exclusion criteria were as follows: 1) evidence of any other autoimmune diseases; 2) insulin requirement of more than 0.8 U/kg·d; 3) evidence of chronic or acute infection; 4) a history of any malignancy, congestive heart failure, or secondary diabetes; 5) renal disease or renal dysfunction with serum creatinine of 1.5 mg/dL or greater for males and 1.4 mg/dL or greater for females; 6) women who were pregnant, had frequent abortion, or were breastfeeding; and 7) patients unable to follow the treatment protocol.

Of 72 LADA patients screened from June 2009 to June 2011, 30 LADA patients met eligibility criteria and were recruited. All patients were given oral and written information, and informed content was obtained in accordance with the Declaration of Helsinki. The research protocols were approved by the Ethics Committee in the Second Xiangya Hospital, Central South University. This study has been registered online [www.clinicaltrials.gov/ (identifier NCT01159847)].

Treatment protocol

After a 3-month washing-in period, patients (n = 40) with a glycosylated hemoglobin (HbA_{1C}) of 7.5%–11% (58–97 mmol/mol) entered a 1-month single insulin therapy run-in period. Thirty patients with adequate compliance during the run-in period had baseline assessments and were randomized in a 1:1 ratio to insulin with sitagliptin 100 mg daily (group A, n = 15) or without sitagliptin (group B, n = 15). The intervention lasted for 12 months, and the follow-up visits took place at baseline and after 3, 6, 9, and 12 months of treatment. All 30 participants finished the treatment protocol and attended the visits at all five time points.

GAD-antibody (Ab) assay

GAD-Ab was tested by radioligand assay. Having GADA positivity was defined as 18 U/mL or greater (World Health Organization units) (12). The assay has been confirmed by Diabetes Antibody Standardization Program (2012) and is sponsored by the Immunology of Diabetes Society (IDS). The interassay and intraassay variation coefficients were 7.1%–10.8% and 4.9%–8.3%, respectively. The sensitivity and specificity of the GAD-Ab assay were 82% and 98%, respectively.

Assessment of β-cell function and HbA_{1C}

A standard 543.6-kcal, mixed-meal tolerance test (44.4% of calories as carbohydrate, 47.7% as fat, and 7.9% as protein) was administered at baseline and at 3, 6, 9, and 12 months of treatment. In every visit time, sitagliptin dose was held (washed out) for 24 hours before the standard-meal test, and insulin injection was withheld on the day of a standard-meal test. Plasma glucose and C-peptide levels were measured at times 0 and 120 minutes after the administration of the standard meal. C-peptide was detected by a chemiluminescence method (Adiva Centaur System). The inter- and intraassay variation coefficients were 3.7–4.1% and 1.0–3.3%, respectively. The HbA_{1C} levels were measured by automated liquid chromatography (HLC-723G8; Tosoh). The inter- and intraassay variation coefficients were less than 3% and less than 1%, respectively.

Safety assessments

Safety and tolerability were assessed from adverse experiences, physical examinations, vital signs, and clinical laboratory tests throughout the study. Adverse experiences were evaluated by investigators for the frequency, intensity, and relationship to the study drug. Hypoglycemia is defined as a blood glucose level of less than 3.9 mmol/L (13).

Statistical analysis

Data are presented as the mean ± SEM. A one-way ANOVA was used to examine the baseline data and the changes in fasting, 2-hour postprandial glucose, HbA_{1C}, and C-peptide between groups. A paired *t* test was used for a comparison of the C-peptide changes from the baseline within the same group. Categor-

Table 1. Baseline Demographic and Clinical Characteristics (Mean ± SEM)

	Group A (n = 15)	Group B (n = 15)	*P* Value
Age, y	48.0 ± 2.8	46.9 ± 3.7	.81
Gender (female/male)	5/10	7/8	.71
Duration, y	1.4 ± 0.2	1.4 ± 0.2	.96
BMI, kg/m^2	23.2 ± 0.9	23.5 ± 0.8	.78
Systolic blood pressure, mm Hg	119.3 ± 3.6	120.0 ± 3.5	.89
Diastolic blood pressure, mm Hg	69.8 ± 1.9	73.0 ± 2.7	.33
Total insulin daily dose, U	14.9 ± 1.5	17.9 ± 1.9	.22
Insulin dose, units/kg · d	0.2 ± 0.1	0.3 ± 0.1	.23
GADA titer, U/mL	330.5 (72.0–732.6)	174.0 (61.4–929.3)	.76
HbA_{1C}, %	6.4 ± 0.2	6.5 ± 0.2	.81
HbA_{1C}, mmol/mol	46.7 ± 2.3	47.5 ± 2.3	.81
Fasting blood glucose, mmol/L	6.5 ± 0.3	6.4 ± 0.2	.75
Two-hour postprandial glucose, mmol/L	12.8 ± 1.1	13.1 ± 1.0	.81
Fasting C-peptide, pmol/L	414.2 ± 50.7	389.3 ± 52.5	.73
Two-hour postprandial C-peptide, pmol/L	1553.9 ± 183.3	1530.0 ± 205.4	.93
ΔC-peptide, pmol/L	1139.7 ± 152.1	1140.7 ± 167.0	.99
Total cholesterol, mmol/L	4.6 ± 0.1	4.7 ± 0.2	.95
Triglycerides, mmol/L	1.3 ± 0.1	1.1 ± 0.1	.28
HDL-cholesterol, mmol/L	1.3 ± 0.1	1.4 ± 0.1	.13
LDL-cholesterol, mmol/L	2.9 ± 0.1	2.7 ± 0.1	.32

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein. The differences were detected by one-way ANOVA except that gender difference was detected by χ^2 test.

ical variables were compared by χ^2 test. A value of $P < .05$ was considered to be significant.

Results

Baseline characteristics

There were no significant differences between the two groups in age, gender, duration of diabetes, body mass index (BMI), GADA titers, glucose levels, HbA_{1C}, FCP, the 2-hour CP levels, blood pressure, and lipid profiles at baseline. Baseline demographic and clinical characteristics are shown in Table 1.

Effect of DPP-4 inhibition on GADA titers, glycemic control, and insulin dosages

There were no significant differences between groups A and B in BMI, body weight, lipid profiles (Supplemental Data, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org), insulin dosage, fasting glucose, 2-hour postprandial glucose, and HbA_1c levels at baseline and after 3, 6, 9, and 12 months of treatment ($P > .05$). At 12 months, the fasting glucose levels were 6.2 ± 0.3 mmol/L in group A and 6.2 ± 0.7 mmol/L in group B ($P = .99$) and 2-hour postprandial glucose levels (10.8 ± 1.2 vs 13.0 ± 1.2 mmol/L, $P = .21$), HbA_{1C} levels (6.2% ± 0.2% vs 6.2% ± 0.1%, $P = 1.00$) and insulin dose (16.7 ± 1.8 vs 22.0 ± 2.8 U, $P = .11$). There was no significant difference between group A and group B in the GADA titers after 12 months of treatment ($P = .45$).

Effects of DPP-4 inhibition on islet β-cell function

There were no significant differences in the levels of FCP, 2-hour CP and ΔCP (ΔCP = 2 h CP−FCP) in group A at 12 months compared with baseline ($P = .28$-$.88$). In group B, the level of FCP continually decreased from a baseline of 389.3 ± 52.5 pmol/L to 321.0 ± 56.1 pmol/L at 3 months ($P < .01$); 300.4 ± 35.2 pmol/L at 6 months ($P = .06$); 287.1 ± 30.9 pmol/L at 9 months ($P < .05$); and 279.7 ± 46.5 pmol/L at 12 months ($P < .05$). The 2-hour CP levels in group B continually decreased from 1530.0 ± 205.4 pmol/L at baseline to 1124.6 ± 176.2 pmol/L at 3 months ($P < .01$); 1101.0 ± 121.7 pmol/L at 6 months ($P < .05$); 962.6 ± 108.0 pmol/L at 9 months ($P < .05$); and 843.0 ± 120.5 pmol/L at 12 months ($P < .01$). Similarly, the mean ΔCP levels in group B gradually decreased from the baseline of 1140.7 ± 167.0 pmol/ L to 803.6 ± 138.0 pmol/L at 3 months ($P < .01$); 800.6 ± 93.4 pmol/L at 6 months ($P < .05$); 675.5 ± 95.5 pmol/L at 9 months ($P < .05$); and 563.3 ± 91.9 pmol/L at 12 months ($P < .01$). The levels of 2-hour CP and ΔCP in group A were significantly higher than those in group B at 12 months ($P < .05$; one way ANOVA). The trends of fasting, 2-hour CP, and ΔCP of patients in group A and group B are shown in Figure 1.

Safety and tolerability

Sitagliptin and insulin were generally well tolerated and the incidence of drug-related adverse experiences including the incidence of hypoglycemia was low and similar between the two groups. There were no severe hypoglycemia events requiring intervention and no other severe side effects.

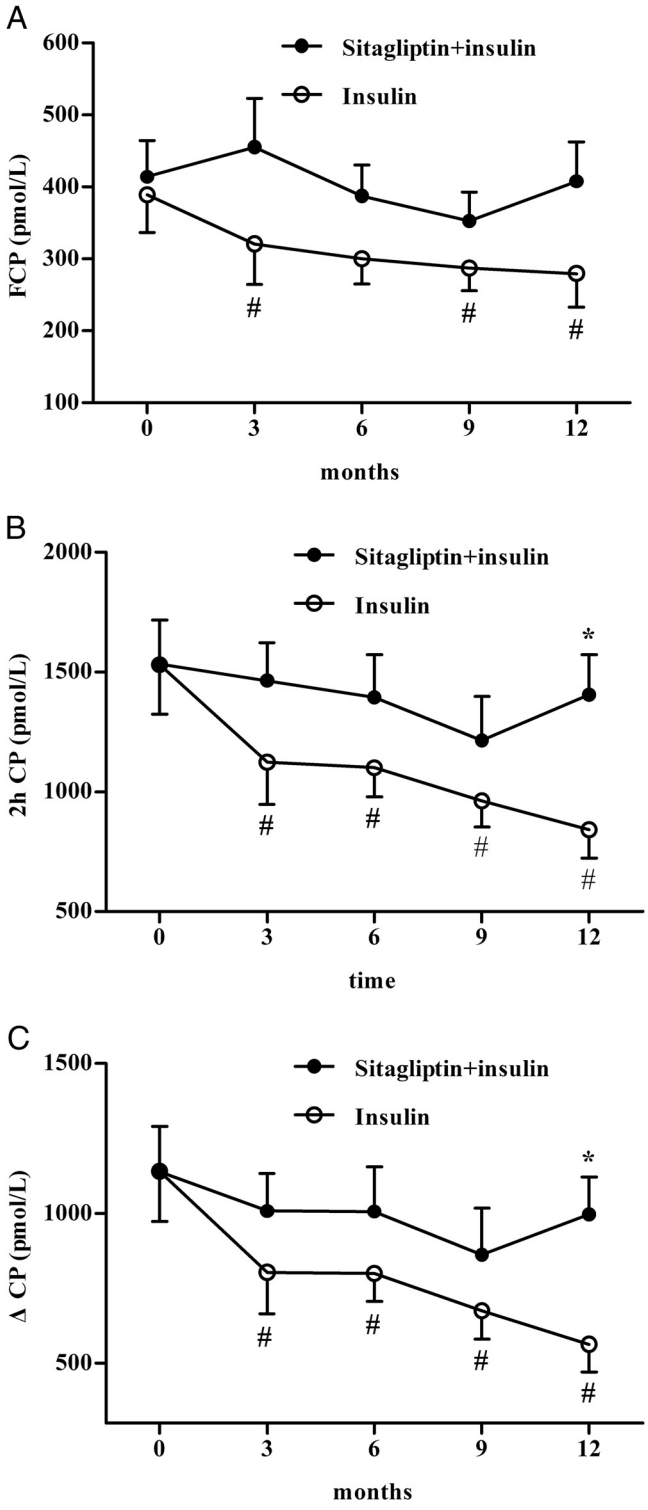

Figure 1. Effect of sitagliptin on β-cell function. Black circles, Sitagliptin + insulin group; white circles, insulin group. FCP (A), 2-hour CP (B), and ΔCP (C) were measured at the indicated month during the treatment period. Data are presented as means ± SEM. *, $P < .05$ vs the control group (one way ANOVA); #, $P < .05$ vs the baseline (pair *t* test).

Discussion

This study demonstrates that the addition of sitagliptin to the treatment of LADA patients receiving insulin was well tolerated and provided improvements in the parameters of islet function. Existing studies indicate that DPP-4 inhibitors have the potential to protect β-cell function (3, 4) in patients with type 2 diabetes as well as in a mouse model of autoimmune diabetes (7). Our present results are consistent with that hypothesis.

High-titer GADA [defined as ≥180 U/mL (12)] or insulin therapy could influence β-cell loss in patients with LADA (14), but in our study, there was no difference in GADA titers or insulin dosage between the two groups. Therefore, the maintained β-cell function in group A is likely due to the addition of sitagliptin.

The Diabetes Control and Complications Trial study indicated that changes of glucose levels may affect β-cell function (15). In our study, we called every patient weekly and adjusted insulin dosage if their fasting blood glucose was 6.1 mmol/L or greater and/or their 2-hour postprandial blood glucose was 7.8 mmol/L or greater. After 12 months of treatment, most of the patients kept the HbA_{1C} target of 7% or less (53 mmol/mol). The addition of sitagliptin to patients in group A did not increase the risk of hypoglycemia. Thus, in our study of LADA patients, sitagliptin treatment delayed the loss of β-cell secretion despite unaltered glucose levels. Because most subjects in our study had recent-onset diabetes with less than 3 years of disease, we conclude that sitagliptin treatment maintains the β-cell function in recent-onset LADA patients.

A recent report by Johasen et al (16) found that another DPP-4 inhibitor, linagliptin, preserved β-cell function in patients with LADA during a 2-year study. The β-cell function of those subjects in group B decreased significantly despite insulin treatment, most likely due to autoimmune β-cell destruction. However, the lack of decline of β-cell function in group A treated with insulin plus sitagliptin could be metabolic or immune mediated. Our preliminary data suggest sitagliptin has some effects on T-cell modulation (our unpublished data). DPP-4 is the lymphocyte cell surface protein CD26, critical in T-cell biology as a marker of T-cell activation (17). DPP-4 inhibition increased regulatory T-cells and reversed recent-onset diabetes in a nonobese mouse model of autoimmune diabetes mice (7). DPP-4 was considered to link metabolic disturbance with immunological dysregulation in patients with chronic hepatitis C virus infection (18). However, the beneficial metabolic effect of DPP-4 inhibitors, additive to insulin therapy, could occur independently of immune effects secondary to reduced β-cell stress, reduced glucagon, or β-cell regeneration (19). It is evident that insulin alone is inadequate in the treatment of LADA and our present results highlight that inadequacy (20).

In conclusion, our study presents evidence that LADA patients treated with the DPP-4 inhibitor sitagliptin had

maintained β-cell function, in addition to any benefits from insulin alone. This observation has important therapeutic implications for the use of DPP-4 inhibitors in autoimmune diabetes. Further investigation in a larger cohort is warranted to assess clinical outcomes as well as to more thoroughly explore the mechanism of β-cell preservation in these patients.

Acknowledgments

Y.Z. collected the data, researched and analyzed the data, and wrote the manuscript. L.Y. designed the project and monitored the trial. Y.X. researched the data and contributed to the discussion. L.L. and Z.L. contributed to the data collection. G.H. and X.L. contributed to the discussion. R.D.L. reviewed and edited the manuscript. Z.Z. and X.W. designed the project and wrote, revised, and edited the manuscript.

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Address all correspondence and requests for reprints to: Zhiguang Zhou, MD, PhD, 139 Renmin Zhong Road, Changsha, Hunan 410011, People's Republic of China. E-mail: zhouzg@hotmail.com; or Xiangbing Wang, MD, PhD, MEB 384B, 1 RWJ Place, PO Box 19, New Brunswick, NJ 08093. E-mail: xw030912@aol.com.

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