Complementary glucagonostatic and insulinotropic effects of DPP-4 inhibitors in the glucose-lowering action in Japanese patients with type 2 diabetes

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Abstract The dipeptidyl peptidase-4 (DPP-4) inhibitors have a low risk of causing hypoglycemia as monotherapy. However, insulin administration is frequently required, particularly in patients with type 2 diabetes and with reduced insulin secretory capacity. The effects of adding DPP-4 inhibitors were evaluated using continuous glucose monitoring (CGM) in Japanese patients with type 2 diabetes who were insufficiently controlled by basal insulin with biguanide. The effects of adding DPP-4 inhibitors on blood glucose and plasma insulin and glucagon levels were evaluated. Δ glucagon showed a significant association with post-prandial glucose increase in the group with diminished insulin secretory capacity, C-peptide index (CPI) <0.8 ($p = 0.016$), while Δ C-peptide reached significant association in the group with relatively intact insulin secretory capacity, CPI ≥ 0.8 ($p = 0.017$). The mean plasma glucose levels and M values were similarly improved in patients treated with the three DPP-4 inhibitors. Hypoglycemia did not occur in any of the DPP-4 inhibitor groups. In conclusion, complementary glucagonostatic and insulinotropic effects of adding DPP-4 inhibitors are involved in the glucose-lowering action of Japanese patients with type 2 diabetes according to their insulin secretory capacity. Such combination therapy may well be a superior therapeutic option for the treatment of diabetes in Japanese patients who often exhibit reduced insulin secretory capacity.

Keywords Type 2 diabetes mellitus · DPP-4 inhibitors · Hypoglycemia · Glucagon · Insulin

Abbreviations

DPP-4	Dipeptidyl peptidase-4
CGM	Continuous glucose monitoring
BOT	Basal supported oral therapy
SUIT	Secretory units of islets in transplantation
CPI	C-peptide index
GLP-1	Glucagon like peptide-1
GIP	Glucose-dependent insulinotropic polypeptide
FPG	Fasting plasma glucose
PPG	Postprandial plasma glucose
CPR	C-peptide immunoreactivity
IRG	Immunoreactive glucagon
ANOVA	Analysis of variance
NPH	Neutral protamine hagedorn

Introduction

In elderly Japanese type 2 diabetes patients with insufficient glycemic control, it is critical to protect the pancreatic β -cells, which have intrinsically lower capacity of insulin secretion in Japanese than in Caucasian [1], by basal-supported oral therapy (BOT), which combines an oral hypoglycemic drug and a small amount of insulin therapy from an early stage. However, insulin therapy is not provided at the appropriate time for many patients due to concern for hypoglycemia, which results in depleted insulin secretion in these patients. Since insulin secretion becomes especially diminished in elderly patients with type 2 diabetes who have had diabetes for a long time, many patients

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require concomitant insulin therapy to control their blood glucose levels.

BOT is a convenient insulin induction therapy by which once daily basal insulin is applied as a combination therapy in type 2 diabetes patients who show insufficient therapeutic effects with the sulfonylurea drugs, which aim mainly to suppress the fasting blood glucose levels. Although the fast-acting insulin and the mixed insulin preparation induction therapies were more effective in reducing blood glucose levels than the intermediate-acting insulin and the long-acting insulin induction therapies, higher frequencies of hypoglycemia and body weight gain were reported in the patients treated with the fast-acting insulin and the mixed insulin preparation induction therapies [2]. Because of the aging population with diabetes, in recent years, safer treatments having a lower risk of hypoglycemia are desired. It also has been reported that hypoglycemia can lead to dementia in elderly people [3]. In practice, basal insulin using long-acting insulin is used more often because of the lower risk of hypoglycemia. However, the sulfonylurea drugs when concurrently administered with the conventional BOT increase the risk of hypoglycemia, especially during fasting, because they promote insulin secretion irrespective of food intake [4]. The action of dipeptidyl peptidase-4 (DPP-4), an enzyme that degrades GLP-1 and GIP induced by food intake, is inhibited by DPP-4 inhibitors. Accordingly, the effects of the DPP-4 inhibitors as insulin secretagogues and their glucagon-antisecretory action are blood glucose-dependent [5]. A recent clinical study has shown that hypoglycemia and postprandial blood glucose increase are associated with cardiovascular events [6]. Hypoglycemia is often caused by sulfonylurea drugs, but DPP-4 inhibitors effectively suppress the postprandial increase in blood glucose and, with monotherapy, less frequently cause hypoglycemia. It is considered that by using DPP-4 inhibitors as the concomitant drugs administered with BOT instead, it should be possible to manage the blood glucose levels with less fluctuation and a smaller risk of fasting hypoglycemia, since they stimulate insulin secretion only as the blood glucose rises.

To date, there has been no detailed report on the effects of concurrent administration of basal insulin and DPP-4 inhibitors on improvement of blood glucose or insulin and glucagon secretion in Japanese patients with type 2 diabetes. In this study, the effects of an additional dose of DPP-4 inhibitor on improving blood glucose levels were evaluated using continuous glucose monitoring (CGM) in Japanese patients with type 2 diabetes, who were 60 years of age or older and half of whom had reduced insulin secretory capacity, insufficiently controlled by BOT involving biguanides. The effects of adding the DPP-4

inhibitors to BOT on plasma glucagon levels and insulin levels were evaluated.

Materials and methods

We obtained a unique number of the institutional review board in Gifu University and also obtained the written, informed consent form from all participants. The study protocol was approved by the institutional review board of Gifu University (No. 25-124).

In this study, dietary and insulin therapies were administered to 21 Japanese patients 60 years of age or older with type 2 diabetes and who were hospitalized to control their glucose levels. Long-acting insulin analogues or intermediate-acting insulin analogues were administered to the patients as the basal insulin therapy, and only biguanides were concomitantly administered as the oral hypoglycemic agents. We used long-acting insulin as basal insulin for patients with reduced insulin secretion capacity, while we used NPH intermediate insulin, especially to lower the fasting blood glucose, for patients with relatively intact insulin secretion capacity. The DPP-4 inhibitors were then administered to patients who had insufficient blood glucose management. The concurrently administered DPP-4 inhibitors were vildagliptin in 12 patients, sitagliptin in six patients, and teneligliptin in three patients. Day 1 was defined as the day when the plasma glucose level in the patient was stabilized by the basal insulin therapy. The plasma glucose level, serum C-peptide immunoreactivity (CPR), and immunoreactive glucagon (IRG) levels (SRL, Tokyo, Japan) were measured in the blood samples obtained before and at 2 h after breakfast (Energy 2.2 ± 0.1 kJ, Carbohydrate 59.0 ± 2.0 %E; Protein 14.9 ± 1.6 %E; Fat 24.3 ± 2.1 %E). We used Glucagon RIA [SML] kit (Euro-DiagnosticaAB, Sceti Medical Labo, Tokyo, Japan) in this study. On day 2 and day 3, the DPP-4 inhibitor (vildagliptin $50 \text{ mg} \times 2$, sitagliptin $50 \text{ mg} \times 1$ or teneligliptin $20 \text{ mg} \times 1$) was administered to the patients. On day 3, plasma glucose, CPR, and glucagon levels were measured in the blood samples obtained during fasting and at 2 h after the breakfast. The SUI (secretory units of islets in transplantation) index, which is correlated with β -cell mass and acute insulin response to glucagon, was calculated by the formula: $250 \times \text{fasting CPR (nM)} / (\text{FPG (mM)} - 3.43)$ [7]. The CPI (C-peptide index) was calculated by the formula: $\text{fasting CPR (nM)} / \text{FPG (mM)} \times 16.7$ [8, 9]. The plasma glucose level was evaluated using the CGM (iPro[®]2, Medtronic MiniMed, Inc.) on day 1, day 2, and day 3 (Fig. 1). The change in the glucose level was estimated by mean glucose over 24 h, M value index ($110 \times \log_{10} \text{PG} / 1001^3$), standard deviation (SD), area under the curve (AUC $\geq 10 \text{ mM day}$, and AUC $< 3.3 \text{ mM day}$ [10, 11]. The basal

insulin doses were not changed in any of the patients during the study.

Statistical analyses

Wilcoxon signed-rank test was used for comparison between before and after administration of DPP-4 inhibitors. Mann–Whitney *U* test was performed for comparison between the once and twice daily treatment groups, and ANOVA on ranks (Kruskal–Wallis test) was performed for multiple comparisons among the three groups of each of the DPP-4 inhibitors. Multiple regression analysis was performed by stepwise method to investigate correlation between post prandial glucose increase and age, sex, BMI, Δ glucagon, and Δ C-peptide and also performed in two groups divided based on the insulin secretory capacity, C-peptide index (CPI <0.8 and CPI $\geq$ 0.8). For the best model, the smallest RMSE (root mean squared error) was selected. Statistical analysis was performed with JMP 11.0 software (SAS Institute, Inc., Cary, NC, USA).

Fig. 1 Study protocol of the present study Δ : Blood examination was performed before and at 2 h after breakfast on day 1 and day 3. *DPP4i* Dipeptidyl peptidase-4 inhibitor. *CGM* Continuous Glucose Monitoring

Results

The clinical characteristics of the study patients are shown in Table 1. Twelve men and nine women were included in the study. The mean [data are presented as the mean ($\pm$ SD)] age was 73.0 (6.6) years old. The mean hemoglobin A1c (HbA1c) level at admission was 9.2 (1.6) % [NGSP] or 78 (18) mmol/mol [IFCC]. In this study, NGSP values were used for HbA1c throughout. The mean basal insulin dose was 8.5 U/day: insulin glargine in six patients, insulin detemir in four patients, and neutral protamine hagedorn (NPH) in 11 patients. The mean ($\pm$ SD) dose of the concomitantly administered biguanide (metformin) was 523.8 (335.4) mg/day.

The mean plasma glucose levels over the 24 h before administration of the DPP-4 inhibitors was 9.5 (1.6) mM, the SD value was 2.6 (1.0) mM, and the *M* value was 21.3 (13.7). The AUC $\geq$ 10 was 1.1 (0.9) mM day and AUC <3.3 was 0 mM day. The mean plasma glucose level over the 24 h at 2 days after the DPP-4 inhibitor therapy was 8.5 (1.4) mM, SD value was 2.0 (0.8) mM, and the *M* value was 12.3 (9.3) (in all cases $p < 0.01$). The AUC $\geq$ 10 mM was 0.5 (0.5) mM day ($p < 0.01$) and AUC <3.3 mM was 0 mM day. The nocturnal *M* value (SD) before administration of the DPP-4 inhibitors was 6.7 (9.6), not significantly different from that after administration of the DPP-4 inhibitors 4.3 (5.0), ($p > 0.05$) (Table 2). We divided subjects into two groups based on the insulin secretory capacity, C-peptide index ($n = 11$ for CPI <0.8 and $n = 10$ for CPI $\geq$ 0.8) because CPI value of 0.8 was reported to be a critical level for insulin dependence in Japanese patients [8, 9]. These data are similar in the CPI <0.8 and CPI $\geq$ 0.8 groups.

CPR and glucagon data before and after DPP-4 inhibitors therapy are shown in Table 3. Although the fasting CPR levels were elevated significantly ($p = 0.0037$), the fasting glucose level did not show a significant decrease

Table 1 Clinical characteristics of the patients in each DPP-4 inhibitor group

	Vildagliptin	Sitagliptin	Teneligliptin
Age (years)	70.5 $\pm$ 5.5	75.3 $\pm$ 4.2	78.6 $\pm$ 9.1
Gender: male/female	8/4	4/2	0/3
BMI (kg/m ²)	23.1 $\pm$ 4.6	22.9 $\pm$ 2.9	22.5 $\pm$ 3.1
HbA1c (%) [NGSP]	9.0 $\pm$ 1.8	9.3 $\pm$ 0.9	10.0 $\pm$ 1.5
(mmol/mol) [IFCC]	75 $\pm$ 20	78 $\pm$ 10	86 $\pm$ 17
Duration of diabetes (years)	13.5 $\pm$ 9.0	12.4 $\pm$ 14.6	18.0 $\pm$ 9.8
Basal insulin (U)	7.9 $\pm$ 2.8	9.8 $\pm$ 3.1	8.6 $\pm$ 3.3
Metformin (mg)	500.0 $\pm$ 381.8	625.0 $\pm$ 190.9	416.6 $\pm$ 311.8
u-CPR (μ g/day)	110.1 $\pm$ 86.4	60.5 $\pm$ 39.5	63.3 $\pm$ 44.2

Data are presented as mean $\pm$ SD

Not significant among the three groups by Kruskal–Wallis test

HbA1c glycated hemoglobin, u-CPR urinary C-peptide immunoreactivity

Table 2 The CGM data before and after DPP-4 inhibitors therapy

	Day 1	Day 3	<i>p</i> value
Mean glucose (mM)	9.5 ± 1.6	8.5 ± 1.4	0.00020
SD (mM)	2.6 ± 1.0	2.0 ± 0.8	0.0015
AUC ≥10 (mM day)	1.1 ± 0.9	0.5 ± 0.5	0.00030
AUC <3.3 (mM day)	0	0	
<i>M</i> value	21.3 ± 13.7	12.3 ± 9.3	0.00010
<i>M</i> value (10PM–8AM)	6.7 ± 9.6	4.3 ± 5.0	0.32

Data are presented as mean ± SD

CGM Continuous glucose monitoring, DPP-4 Dipeptidyl peptidase-4
p value by Wilcoxon signed-rank test

Table 3 C-peptide and glucagon data before and after DPP-4 inhibitors therapy

	Day 1	Day 3	<i>p</i> value
FPG (mM)	6.57 ± 1.08	6.26 ± 1.16	0.099
PPG (mM)	12.68 ± 3.12	10.58 ± 2.97	0.00040
F-CPR (nM)	0.39 ± 0.27	0.47 ± 0.34	0.0037
PP-CPR (nM)	1.57 ± 0.84	1.69 ± 0.96	0.16
Δ CPR (nM)	1.18 ± 0.60	1.22 ± 0.67	0.53
SUIT index	31.3 ± 19.6	41.4 ± 25.1	<0.0001
CPI	0.96 ± 0.58	1.21 ± 0.80	<0.0001
F-IRG (ng/l)	158.8 ± 29.6	161.9 ± 33.5	0.069
PP-IRG (ng/l)	175.9 ± 39.8	164.8 ± 35.5	0.0023
Δ IRG (ng/l)	19.1 ± 21.1	2.86 ± 16.3	0.00090

Data are presented as mean ± SD

FPG fasting plasma glucose, PPG postprandial plasma glucose, F-CPR fasting C-peptide immunoreactivity, PP-CPR postprandial C-peptide immunoreactivity, ΔCPR (PP-CPR)–(F-CPR), Δ IRG: (PP-IRG)–(F-IRG)

p value by Wilcoxon signed-rank test

(*p* = 0.099). The postprandial glucose levels showed a significant decrease (*p* = 0.0004). The SUIT (secretory units of islets in transplantation) index, which is correlated with β-cell mass and the acute insulin response to glucagon, showed a significant increase and CPI showed significant increase before and after administration of the DPP-4 inhibitors (*p* < 0.0001 and *p* < 0.0001). These differences were significant even in the group with reduced insulin secretory capacity of CPI < 0.8 (*p* = 0.0020 and *p* = 0.019) (Table 4).

The fasting glucagon levels before administration of the DPP-4 inhibitors were not significantly different from those after administration of the DPP-4 inhibitors (*p* = 0.069). The postprandial glucagon levels before the administration of the DPP-4 inhibitors, however, differed significantly from those after administration of the DPP-4 inhibitors (*p* = 0.0023). The mean change in the glucagon levels from before to after breakfast before

Table 4 C-peptide and glucagon data before and after DPP-4 inhibitors therapy (CPI < 0.8) (*n* = 11)

	Day 1	Day 3	<i>P</i> value
FPG (mM)	6.32 ± 0.84	5.93 ± 1.17	0.10
PPG (mM)	12.86 ± 3.51	10.73 ± 3.33	0.0059
F-CPR (nM)	0.20 ± 0.09	0.25 ± 0.09	0.17
PP-CPR (nM)	1.11 ± 0.44	1.18 ± 0.48	0.37
Δ CPR (nM)	0.91 ± 0.39	0.93 ± 0.44	0.76
SUIT index	17.4 ± 5.8	26.4 ± 10.6	0.0020
CPI	0.53 ± 0.18	0.68 ± 0.22	0.019
F-IRG (ng/l)	159.9 ± 29.6	165.7 ± 30.9	0.14
PP-IRG (ng/l)	184.4 ± 30.0	171.4 ± 30.1	0.041
Δ IRG (ng/l)	24.6 ± 19.0	5.7 ± 20.1	0.020

Data are presented as mean ± SD

FPG fasting plasma glucose, PPG postprandial plasma glucose, F-CPR fasting C-peptide immunoreactivity, PP-CPR postprandial C-peptide immunoreactivity, Δ CPR (PP-CPR)–(F-CPR), Δ IRG: (PP-IRG)–(F-IRG)

p value by Wilcoxon signed-rank test

Table 5 C-peptide and glucagon data before and after DPP-4 inhibitors therapy (CPI ≥ 0.8) (*n* = 10)

	Day 1	Day 3	<i>p</i> value
FPG (mM)	6.87 ± 1.30	6.63 ± 1.10	0.82
PPG (mM)	12.50 ± 2.81	10.42 ± 2.71	0.054
F-CPR (nM)	0.60 ± 0.26	0.72 ± 0.36	0.014
PP-CPR (nM)	2.08 ± 0.90	2.27 ± 1.06	0.32
Δ CPR (nM)	1.48 ± 0.67	1.54 ± 0.77	0.59
SUIT index	46.7 ± 17.9	58.1 ± 26.5	0.049
CPI	1.44 ± 0.48	1.80 ± 0.81	0.0059
F-IRG (ng/l)	153.6 ± 30.8	157.9 ± 37.5	0.39
PP-IRG (ng/l)	166.7 ± 48.5	157.7 ± 41.2	0.12
Δ IRG (ng/l)	13.1 ± 22.8	−0.28 ± 11.0	0.055

Data are presented as mean ± SD

FPG fasting plasma glucose, PPG postprandial plasma glucose, F-CPR fasting C-peptide immunoreactivity, PP-CPR Postprandial C-peptide immunoreactivity, Δ CPR (PP-CPR)–(F-CPR), Δ IRG: (PP-IRG)–(F-IRG)

p value by Wilcoxon signed-rank test

administration of the DPP-4 inhibitors was 19.1 (21.1) ng/L and that after administration of the DPP-4 inhibitors was 2.86 (16.3) ng/L, which was significantly different (*p* = 0.0009). This difference was significant only in the group with reduced insulin secretory capacity of CPI < 0.8 (*p* = 0.020) (Table 4 and 5). We then investigated the correlation between the indices of glycemic control and insulin and glucagon secretion. Multiple regression analysis was performed by stepwise method to investigate the correlation between postprandial glucose increase and BMI, sex, age, Δ glucagon, and Δ CPR. Δ

Table 6 Multiple regression analysis of post prandial glucose increase (PPPG-FPG)_{Day3} – (PPPG-FPG)_{Day1} with clinical characteristics

R^2	Effect (mean)	p value
0.59		
Age	–3.50	0.017
Sex	–9.75	0.21
BMI	3.18	0.12
Δ IRG (Day3–Day1)	–1.22	0.0021
Δ CPR (Day3–Day1)	14.5	0.064

Δ IRG (Day3–Day1): Δ IRG_{Day3} – Δ IRG_{Day1} = {(PP-IRG)–(F-IRG)}_{Day3} – {(PP-IRG)–(F-IRG)}_{Day1}

Δ CPR (Day3–Day1): Δ CPR_{Day3} – Δ CPR_{Day1} = {(PP-CPR)–(F-CPR)}_{Day3} – {(PP-CPR)–(F-CPR)}_{Day1}

BMI body mass index

Table 7 Multiple regression analysis of post prandial glucose increase (PPPG-FPG)_{Day3} – (PPPG-FPG)_{Day1} with clinical characteristics (CPI <0.8) ($n = 11$)

R^2	Effect (mean)	p value
0.79		
Age	–7.83	0.010
Sex	–27.7	0.041
BMI	3.69	0.22
Δ IRG (Day3–Day1)	–1.80	0.016

Δ IRG (Day3–Day1): Δ IRG_{Day3} – Δ IRG_{Day1} = {(PP-IRG)–(F-IRG)}_{Day3} – {(PP-IRG)–(F-IRG)}_{Day1}

For the best model, only the smallest RMSE (root mean squared error) was selected

BMI body mass index

glucagon showed significant association with the postprandial plasma glucose (PPG) at p value of 0.0021, while Δ CPR did not reach significant association at p value of 0.064 (Table 6). When divided into the two groups based on the insulin secretory capacity, C-peptide index (CPI <0.8 and CPI $\geq$ 0.8), Δ glucagon showed a significant association at p value of 0.016 in the CPI <0.8 group; Δ CPR reached significant association at p value of 0.017 in the CPI $\geq$ 0.8 group (Table 7 and 8).

The improvements in the mean plasma glucose levels, SD values, AUC $\geq$ 10 mM, and M values were found to be similar among the three groups of patients treated with each DPP-4 inhibitor and also between the once and twice daily treatment groups (data not shown). No patients with hypoglycemia AUC <3.3 mM were found in any DPP-4 inhibitor group. We defined the patients with improvement in mean blood glucose levels, SD values, or M values by less than 10 % as non-responders. Three patients were non-responders in this study. Their clinical features are shown in Table 9.

Table 8 Multiple regression analysis of post prandial glucose increase (PPPG-FPG)_{Day3} – (PPPG-FPG)_{Day1} with clinical characteristics (CPI $\geq$ 0.8) ($n = 10$)

R^2	Effect (mean)	p value
0.86		
Age	–0.699	0.35
BMI	4.92	0.013
Δ CPR (Day3–Day1)	12.0	0.017

Δ CPR (Day3–Day1): Δ CPR_{Day3} – Δ CPR_{Day1} = {(PP-CPR)–(F-CPR)}_{Day3} – {(PP-CPR)–(F-CPR)}_{Day1}

For the best model, only the smallest RMSE (root mean squared error) was selected

BMI body mass index

Table 9 Clinical features of the non-responders to the DPP-4 inhibitors

	Case 1	Case 2	Case 3
Age (years)	65	73	83
Sex	M	F	M
BMI (kg/m ²)	26	20	20.6
HbA1c (%) [NGSP]	8.7	8.5	9.9
(mmol/mol) [IFCC]	72	69	85
Duration of diabetes (years)	20	6	40
u-CPR (μ g/day)	78	43	100
Retinopathy	(–)	(–)	(–)
Neuropathy	(+)	(+)	(+)
Nephropathy	(–)	(–)	(–)
Drug	Vildagliptin	Vildagliptin	Sitagliptin

BMI body mass index, *u-CPR* urinary C-peptide immunoreactivity

Discussion

In the previous study, the effects of concurrent administration of sitagliptin with basal insulin in Japanese patients were evaluated using a 7-point monitor of glucose per day and showed improved mean glucose level, M value, and coefficient of variation 2 months after the beginning of sitagliptin treatment [12]. Although it has been shown that combination therapy with basal insulin and sulfonylurea drugs is effective in improving fasting blood glucose levels, there is insufficient efficacy in the management of postprandial glucose levels in patients with type 2 diabetes [13]. DPP-4 inhibitors have insulin secretory and glucagon-antiseecretory actions dependent on the glucose level, which are thought to have a sufficient effect together on improving the postprandial blood glucose level when combined with basal insulin therapy. However, there are no reports evaluating the effects of DPP-4 inhibitors on improvement of glucose levels and hypoglycemia prevention in detail using CGM with special reference to both insulin and glucagon levels in Japanese.

In this study, with reference to the postprandial plasma glucose (PPG) increase, significant difference of increase in Δ CPR was found between before and after administration of the DPP-4 inhibitors only in patients with a relatively intact insulin secretory capacity of $\text{CPI} \geq 0.8$, while significant difference of decrease in the Δ glucagon levels was found only in patients with reduced insulin secretory capacity of $\text{CPI} < 0.8$. Indeed, antidiabetic actions of endogenous and exogenous GLP-1 in type 1 diabetic patients has been reported [14]. When we compared the decremental postprandial glucose level between two groups of $\text{CPI} < 0.8$ and $\text{CPI} \geq 0.8$, no significant difference was found. This suggested that the glucagon anti-secretory effect on postprandial glucose level is independent of the insulin secretory effect. Thus, the beneficial effects of BOT with DPP-4 inhibitors in improving blood glucose levels result from complementary glucagonostatic and insulinotropic effects.

It has been reported that the inhibitory mechanism of glucagon secretion is impaired in the postprandial state in patients with type 2 diabetes [15]. Postprandial glucagon levels in elderly patients are relatively higher than those in younger patients [16]. Furthermore, the increase in postprandial glucose levels in elderly patients is more prominent than that in younger patients [17]. It is therefore thought that suppression of postprandial glucagon secretion is essential to sufficiently improve the glucose levels, although measurement of glucagon cannot be entirely accurate due to lack of specific antibody and there is no precise account of daily profiling of glucagon. The major treatment strategies and explanations of the pathogenesis of diabetes have been primarily focused on the regulation of the insulin levels. However, a recent study in glucagon receptor knockout mice has shown that diabetes-associated hyperglycemia, the depletion of glycogen, an increase in gluconeogenesis, and an increase in ketone bodies all are found only in the presence of the actions of glucagon [18]. In patients with type 2 diabetes, postprandial glucagon secretion is increased in addition to the fasting glucagon secretion [15]. Release of glucose from the liver by glycogenolysis is, therefore, thought to be an important therapeutic target for the management of postprandial glucose levels.

Metformin, concurrently used in this study, increases the concentration of activated GLP-1 after a meal [19]. A recent study has shown that metformin inhibits glucose synthesis in liver by decreasing intracellular cAMP levels, which attenuates glucagon actions [20]. Suppression of glucagon actions is a promising therapeutic target for treatment of diabetes in the future. While the glucagonostatic and insulinotropic effects of GLP-1 have been reported to contribute equally to its glucose-lowering

action in middle-aged obese Europeans with type 2 diabetes [21], we show in this study that the complementary glucagonostatic and insulinotropic effects of the DPP-4 inhibitors in reducing post prandial glucose levels likely depend on residual insulin secretory capacity in non-obese Japanese patients with type 2 diabetes.

In addition to their action as insulin secretagogues and their glucagon-antiseecretory action, the DPP-4 inhibitors have additional effects that include the suppression of peristalsis, suppression of appetite, and protection of the pancreatic β -cells [22]; however, it is reported that there is a subpopulation of patients who are refractory to treatment with DPP-4 inhibitors [23, 24]. This study using CGM found that three of 21 patients (14 %) had no improvement in blood glucose excursions at least during the early treatment phase. We compared responders with non-responders with reference to Δ C-peptide and Δ glucagon; however, no significant difference was observed (p value: 0.37 and 0.27). As the three non-responders belonged to the $\text{CPI} < 0.8$ group, patients with relatively intact insulin secretory capacity might be more amenable to DPP-4 inhibitors treatment via mechanisms other than insulinotropic and/or glucagonostatic action. There were no consistent tendencies in the clinical characteristics of the three patients. A differential response of GLP-1 due to a variation of the GLP-1 receptor has been reported in non-responders to the DPP-4 inhibitors in Europe and the United States [25]. However, the effects of the DPP-4 inhibitors are also affected by lifestyle, such as dietary patterns [26], and the actual frequency of non-responders remains to be investigated.

It has been reported that hypoglycemia is associated with cardiovascular events [6, 27] and dementia [3, 28]. As the frequency of hypoglycemia unawareness is high in elderly people [29], CGM is especially useful for detecting hypoglycemia in such patients, especially at nocturnal times [30]. In the present study, the DPP-4 inhibitors were administered in addition to the basal insulin, and the data showed that there were no patients with hypoglycemia.

In conclusion, the present study reveals that complementary glucagonostatic and insulinotropic effects of DPP-4 inhibitors contribute according to the endogenous insulin secretory capacity, at least in the acute effect, to the improvement of the glucose levels in combination with basal insulin therapy in Japanese patients. Such combination therapy also improved the management of the glucose levels, including a lower risk of hypoglycemia and a smaller fluctuation in glucose levels. Such combination therapy may be a superior therapeutic option in the treatment of diabetes in elderly Japanese patients who generally have poor insulin secretory capacity.

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Compliance with ethical standards

Conflict of interest YH received honoraria for lectures from Astellas Pharma Inc. and scholarship grants from MSD. JT received honoraria for lectures from Astellas Pharma Inc., Sanofi K.K., Ono Pharmaceutical Co. Ltd., Novo Nordisk Pharma Ltd., Eli Lilly Japan K.K., Daiichi Sankyo Co., Ltd., Takeda Pharmaceutical Co., Ltd., MSD., Daiippon Sumitomo Pharma Co.,Ltd., Mitsubishi Tanabe Pharma Corporation, Boehringer Ingelheim, Taisho Toyama Pharmaceutical Co.Ltd. and Kowa Company, Ltd., and scholarship grants from Boehringer Ingelheim, Sanofi K.K., Ono Pharmaceutical Co. Ltd., Novo Nordisk Pharma Ltd., Novartis Pharma K.K., Sanwa Kagaku Kenkyusho Co., Ltd., Astellas Pharma Inc., Takeda Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Eli Lilly Japan K.K. Taisho Toyama Pharmaceutical Co.Ltd., MSD., Kowa Company, Ltd. and Kyowa Hakko Kirin Co.Ltd. K.H declares that he has no conflict of interest.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revision. Informed consent or substitute for it was obtained from all patients for being included in the study.

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