Efficacy and safety of repaglinide added to sitagliptin in Japanese patients with type 2 diabetes: A randomized 24-week open-label clinical trial

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Abstract. Although sitagliptin and repaglinide monotherapies improve postprandial hyperglycemia, the long-term effects and safety of their combination has not been examined. In this randomized 24-week trial of Japanese patients with poor control (HbA1c 7.0-8.5%) by sitagliptin, we divided 40 patients randomly into two equal groups of the repaglinide add-on to sitagliptin (ADD-ON, n=20), or sitagliptin switched to repaglinide (SWITCH, n=20). The meal tolerance test was carried out at weeks 0 and 24. The primary outcomes were changes in HbA1c and area under the curves (AUC) of glucose from the baseline to week 24. The mean change in HbA1c from baseline to week 24 was larger in the ADD-ON (-0.87±0.63%, mean±SD), compared with the SWITCH (0.03±0.65%, p=0.000). Significant improvements were noted in the mean changes in fasting glucose and AUCs of glucose in the ADD-ON *vs.* SWITCH (p=0.007 and p=0.000). Insulin secretion relative to glucose elevation (ISG; defined as AUC insulin/AUC glucose) increased significantly in the ADD-ON, although the mean change in fasting insulin level was significantly decreased in the ADD-ON (p=0.015 and p=0.026). The AUC of glucagon was significantly lower at 24-week relative to baseline in the ADD-ON, but was not significant in the two groups (p=0.047 and p=0.056, respectively). The combination therapy produced significant reductions in HbA1c, AUC of glucose and fasting glucose compared with switching to repaglinide without weight gain or severe hypoglycemia. The improved glycemic control with this combination therapy may be at least in part due to augmentation of repaglinide-induced insulin secretion by sitagliptin.

Key words: Dipeptidyl peptidase-IV inhibitors, Glinides, Meal tolerance test, Insulin secretion, Glucagon-like peptide-1 (GLP-1)

IN PATIENTS with type 2 diabetes mellitus (T2DM), deterioration of glucose homeostasis progresses from postprandial to fasting hyperglycemia [1] and postprandial hyperglycemic spikes play a major role in glycemic control especially in patients with HbA1c <8.5% [2]. Hence, improvement of postprandial hyperglycemia is necessary to attain appropriate glycemic goal (*i.e.*, HbA1c <7%) [3]. Furthermore, postprandial hyperglycemia is an independent risk factor for cardio-

vascular disease; large cohort studies have indicated that not fasting plasma glucose but 2-hour plasma glucose after a 75-g oral glucose tolerance test is strongly associated with all-cause and cardiovascular mortality [4-6]. At this stage, however, no prospective interventional trial has provided evidence to support the hypothesis that lowering postprandial glucose leads to lower risk of cardiovascular disease.

Dipeptidyl peptidase-4 (DPP-4) inhibitors improve both postprandial and fasting hyperglycemia through various mechanisms. Recent studies showed that DPP-4 inhibitors improve glycemic control not by increasing postprandial insulin secretion but by lowering the appearance of oral glucose, postprandial endogenous glucose release and glucagon response, and by improving insulin sensitivity and beta-cell glucose

sensing [7-10]. This group of drugs also has other beneficial effects including improvement of endothelial function, reduction of inflammatory markers, minimization of oxidative stress ischemia/reperfusion injury and atherogenesis in experimental models, though the effect on cardiovascular outcome remains uncertain in clinical settings [11]. In addition, DPP-4 inhibitors are more effective in Asian patients compared with Caucasian patients [12] and improve not only glycemic control but also blood pressure, lipid profiles, and quality of life [13], with a resultant increase in the prescription of these drugs in Japan [14]. Because T2DM is a progressive disease with continuous deterioration of β-cell function, many patients fail to maintain appropriate glycemic control, including postprandial glucose level and HbA1c. These patients need drug therapy intensification using another type of hypoglycemic agents.

Another treatment option to improve postprandial hyperglycemia includes the use of glinides, alpha-glucosidase inhibitors, GLP-1 receptor agonists, and rapid-acting insulin preparations. However, alpha-glucosidase inhibitors are weaker than the others with regard to their hypoglycemic effect, and GLP-1 receptor agonists cannot be used as add-on therapy to DPP-4 inhibitors, while rapid-acting insulin preparations carry the risks of severe hypoglycemia and weight gain. Moreover, both GLP-1 receptor agonists and rapid-acting insulin preparations cannot be taken orally.

Glinide drugs rapidly increase postprandial insulin secretion shortly after a meal and correct postprandial hyperglycemia without weight gain or severe hypoglycemia. Among the glinides, repaglinide provides greater and significant glycemic improvement than nateglinide when used alone [15, 16] as well as in combination with metformin [17]. Relative to sulfonylurea, repaglinide is as effective as glibenclamide or glimepiride and more effective than glipizide [18]. Furthermore, mortality and cardiovascular risks in repaglinide treatment are not statistically different from metformin treatment whereas almost all sulfonylureas, with the exception of gliclazide, are associated with increased mortality and cardiovascular risk [19]. Based on the above background, we hypothesized that the combination of DPP-4 inhibitors and repaglinide is a potentially promising option for treatment of T2DM patients with inadequate glycemic control using DPP-4 inhibitors alone. To our knowledge,

such hypothesis has not been tested before and there are no long-term clinical trials using DPP-4 inhibitors and repaglinide. To test our hypothesis, we compared in this prospective clinical trial the long term efficacy and safety of the combination of repaglinide and sitagliptin to that of repaglinide monotherapy in Japanese T2DM patients who had been poorly controlled with sitagliptin.

Materials and Methods

Patients and study design

The study was a multicenter, prospective, randomized 24 week open-label trial (University Hospital Medical Information Network Clinical Trials Registry System (UMIN-CTR) ID UMIN000011420). The period of this study was between August 8, 2013 and June 30, 2015. The study subjects were 40 patients with T2DM who were followed up at the Diabetes Outpatient Clinic of our hospital who fulfilled the following criteria: *1)* age more than 20 years, *2)* HbA1c at study entry between 7.0 and 8.5% with <0.5% fluctuation over a period of more than 3 months, *3)* previous treatment with diet, exercise, and 50 mg of sitagliptin over the past 3 months. Patients treated with metformin or pioglitazone were also included, as long as the doses of these drugs were not changed during the study period. None of the study patients was being treated with sulfonylurea, glinides, alpha-glucosidase inhibitors, glucagon like protein-1 (GLP-1) receptor agonists or sodium glucose transporter 2 (SGLT2) inhibitors. *4)* free of concomitant chronic diseases, such as liver disease (aspartate aminotransferase ≥100 IU/L or alanine aminotransferase >100 IU/L), kidney disease (serum creatinine >2.0 mg/dL), heart disease (apparent heart failure or myocardial infarction within 3 months), pancreatic disease, any active malignancy, anemia (hemoglobin <11.0 g/dL), thrombocytopenia (platelet count <10,000 /mm^3), infectious disease (including hepatitis B or C virus), or gastrointestinal disease, *5)* free of serious diabetic complications at study entry, such as progressive neuropathy or proliferative retinopathy, and *6)* self-reported estimated alcohol consumption of less than 40 g per day. The study excluded pregnant and lactating women and those judged by the attending physicians to be unfit or not suitable for participation in a clinical trial.

Fig. 1 provides an outline of the study protocol. Clinical follow-up at the outpatients clinic confirmed
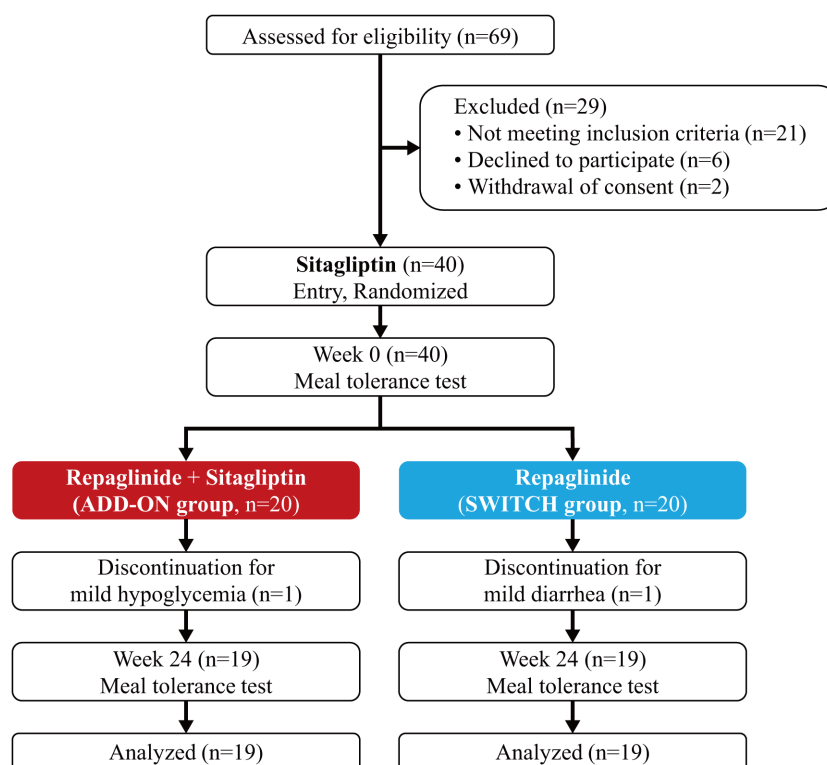
Fig. 1 Study protocol

The meal tolerance test was carried out at weeks 0 and 24.

that all 40 participating patients were inadequately controlled with sitagliptin 50 mg once daily (HbA1c 7.0-8.5%). At study entry, they were randomized (at 1:1 ratio) by a computer-generated assignment into the repaglinide as an add-on therapy to sitagliptin (ADD-ON group: 0.5 mg of repaglinide 3 times/day + 50 mg of sitagliptin once daily, n=20), or were switched from sitagliptin to repaglinide (SWITCH group: 0.5 mg of repaglinide 3 times/day and no sitagliptin, n=20) between August 2013 and December 2014. All patients underwent a meal tolerance test at weeks 0 and 24 and were clinically followed-up at regular intervals at the Diabetes Outpatients Clinic at Toho University School of Medicine (Omori) (Tokyo, Japan) or Toranomon Hospital (Tokyo, Japan).

The study protocol was reviewed and approved by the ethics committee of Toho University School of Medicine with the identifier 24-258 and by the ethics committee of Toranomon Hospital with the identifier shouningai-38. Written informed consent was obtained from each participant before study entry. The study was performed in accordance with the Declaration of Helsinki.

Study outcomes

The primary outcomes were change in HbA1c and area under the curve (AUC) of glucose from the baseline to week 24. The secondary outcomes were changes in AUCs of insulin and glucagon from baseline to week 24, and safety and tolerability of repaglinide in combination with sitagliptin. Safety endpoints included adverse events, which included subjective symptoms and objective findings, laboratory tests, physical findings and vital signs. All instances of hypoglycemia were reported as adverse events.

Meal tolerance test

A standard meal tolerance test was conducted at baseline and 24 weeks. The participant attended the Diabetes Clinic at 0900 h after a 12-h fast and was provided with 50 mg of sitagliptin at baseline test for both groups, and 0.5 mg of repaglinide with or without 50 mg of sitagliptin at week 24 test, just before the standard oral meal load test. A standardized test meal, which was formulated in coordination with the Japan Diabetes Society [20], was provided for breakfast, irrespective of differences in physique. The test

meal provided 460 kcal, and comprised 56.5 g carbohydrates, 18.0 g fat and 18.0 g protein [with a total of 51.4 energy% (E%) from carbohydrate, 33.3 E% from fat and 15.3 E% from protein]. All subjects consumed the meal within 15 min after receiving the above glucose-lowering agents. Blood samples were obtained during the test for determination of plasma glucose, insulin, glucagon, triglyceride, LDL-cholesterol and HDL-cholesterol at 0, 15, 30, 60, 120 and 180 minutes and active GLP-1 at 0 and 30 minutes.

Laboratory tests

HbA1c was measured using standard high-performance liquid chromatography. The HbA1c value was expressed as a National Glycohemoglobin Standardization Program equivalent value calculated by the following equation: HbA1c (%) = 1.02 × HbA1c (Japan Diabetes Society) (%) + 0.25 (%) [21]. Plasma glucose concentration was determined by the hexokinase method. Determination of serum insulin level was outsourced to Special Reference Laboratories (SRL) Inc. (Tachikawa, Japan) and determined by the chemiluminescent enzyme immunoassay (CLEIA) system. Serum glucagon was measured with a radioimmunoassay (RIA) kit (Euro-Diagnostica Inc., Malmö, Sweden). This kit was reported to be the best-performing assay for glucagon among several commercially available assay kits at the time when the study began (August 2013) [22]. Plasma active GLP-1 (GLP-1 (7-36) amide) was measured by enzyme-linked immunoassay kit (EGLP-35K, Millipore, Billerica, MA) using blood samples collected in tubes containing dipeptidyl peptidase IV inhibitor (BD P700, Becton Dickinson) to prevent proteolytic cleavage. The estimated glomerular filtration rate (eGFR) was determined by the following equation: eGFR (mL/min/1.73m^2) = 194 × [serum creatinine]$^{-1.094}$ × Age$^{-0.287}$ (× 0.739 for women) [23].

Statistical analysis

All data were expressed as mean±standard deviation (SD) or median (interquartile range). All variables were tested for distribution pattern using the Shapiro-Wilks normality test. Differences between the ADD-ON and SWITCH groups were determined by the chi-square test, two sample *t*-test (for normally distributed variables) and Mann-Whitney U test (for variables with skewed distribution). Differences between baseline and week 24 were tested by chi-square test,

paired *t*-test (for normally distributed variables) and Mann-Whitney U test (for variables with skewed distribution). A *p* value less than 0.05 was considered statistically significant.

The AUC and incremental (baseline subtracted) AUC (iAUC) were calculated by the trapezoidal method. To adjust for hyperglycemia-induced insulin secretion, insulin secretion relative to glucose elevation (ISG) was used. ISG was defined as AUC insulin / AUC glucose. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) statistical program (version 22, SPSS Japan Inc., Tokyo).

Because this study is a pilot study, the sample size was determined based on our previous trial [24] which reported statistically significant HbA1c changes between the combination therapy of vildagliptin (DPP-4 inhibitor) and nateglinide (glinides) and the vildagliptin monotherapy in a sample of 20 participants per group. As this study compares the combination therapy of sitagliptin (DPP-4 inhibitor) and repaglinide (glinides) compared with the repaglinide-monotherapy, the final sample size was determined as 40 participants.

Results

Patient characteristics

Table 1 summarizes the clinical characteristics of patients of the two treatment groups baseline. The two groups were comparable with regard to sex, body mass index (BMI), duration of diabetes, glycemic control, eGFR and medications, including oral glucose-lowering agents. However, patients of the ADD-ON group were significantly younger than the SWITCH group (*p*=0.021). Only one patient in each group failed to complete 24 weeks of therapy due to adverse events (Fig. 1).

Effects of treatments on HbA1c, body weight and other parameters

Fig. 2 illustrates changes in indexes of glycemic control. The mean change in HbA1c from the baseline to week 24 was -0.87% in the ADD-ON group, which was significantly better than the change in the SWITCH group (0.03%, *p*=0.000) (Fig. 2a, 2b). At the end of the study, HbA1c was <7.0% in 58% of the patients of the ADD-ON group, compared with only 26% of the SWITCH group (*p*=0.049, Fig. 2c).

Table 1 Clinical characteristics of the enrolled patients at baseline

	SWITCH group	ADD-ON group	*p* value
Age, years	65.2 ± 6.4	59.4 ± 8.5	0.021
Male sex, n (%)	15 (75)	14 (70)	0.723
Body mass index, kg/m^2	26.6 ± 3.9	25.2 ± 3.3	0.226
Abdominal circumference, cm	88.0 (85.5-98.0)	88.5 (85.0-94.0)	0.728
Systolic blood pressure, mmHg	125.5 ± 12.6	123.0 ± 11.5	0.507
Diastolic blood pressure, mmHg	73.8 ± 6.6	73.4 ± 10.8	0.889
Heart rate, beats/min	72.1 ± 6.6	74.3 ± 11.6	0.485
Hypertension, n (%)	14 (70)	10 (50)	0.197
Dyslipidemia, n (%)	14 (70)	17 (85)	0.451
Cardiovascular disease, n (%)	4 (20)	2 (10)	0.661
Diabetes duration, years	8.5 (6-13)	6.8 (4.75-11)	0.201
Glycated hemoglobin (HbA1c),%	7.6 ± 0.4	7.6 ± 0.4	0.940
Fasting plasma glucose, mmol/L	8.5 ± 1.7	8.6 ± 1.5	0.841
Fasting plasma insulin, μIU/L	7.3 ± 5.8	9.5 ± 7.7	0.309
eGFR, mL/min/1.73 m^2	70.9 ± 14.6	72.4 ± 10.2	0.699
Uric acid, mg/dL	5.5 ± 1.2	5.3 ± 0.9	0.571
Medications			
Metformin, n (%)	14 (70)	12 (60)	0.507
Pioglitazone, n (%)	3 (15)	2 (10.5)	1.000
Statins, n (%)	7 (35)	13 (65)	0.058
Fibrates, n (%)	2 (10)	0 (0)	0.487
Ezetimibe, n (%)	1 (5)	0 (0)	1.000
Eicosapentaenoic acid, n (%)	1 (5)	1 (5)	1.000
ACEI/ARB, n (%)	8 (40)	5 (25)	0.311
Calcium channel blocker, n (%)	7 (35)	5 (25)	0.490
Diuretics, n (%)	2 (10)	0 (0)	0.487
Anti-platelet agents, n (%)	3 (15)	3 (15)	1.000

Data are mean±standard deviation, median (interquartile range) or n (%). eGFR, estimated glomerular filtration rate; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockers.
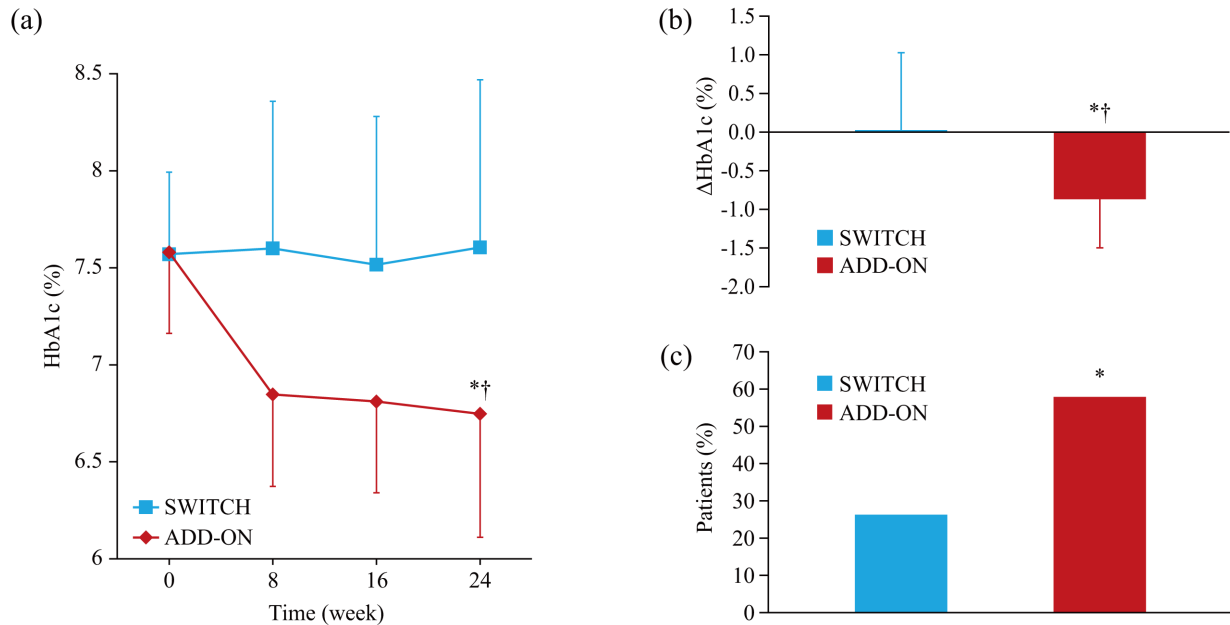
Fig. 2 Changes in HbA1c

(**a**) Serial changes in glycated hemoglobin (HbA1c) during the 24 weeks in the ADD-ON group (repaglinide + sitagliptin) and SWITCH group (repaglinide). (**b**) Mean changes in HbA1c at week 24 in the two groups. (**c**) Percentage of patients who achieved HbA1c <7%. Data are mean±SD. * *p*<0.05 *vs*. SWITCH group, by the two sample *t*-test or chi-square test; † *p*<0.05 *vs*. baseline, by the paired *t*-test. HbA1c, glycated hemoglobin.

There were no significant changes in body weight, BMI, blood pressure and abdominal circumference at 24 weeks (relative to the baseline) in both groups (Table 2).

Meal tolerance test

Fig. 3a shows the serial changes in mean plasma glucose concentrations after the standard meal load. Significant improvements were noted in mean changes in fasting glucose (-1.30 *vs.* 0.16 mmol/L, *p*=0.007) and AUCs of glucose (-329.4 *vs.* 26.4 mmol-min/L, *p*=0.000) in the ADD-ON group relative to the SWITCH group (Fig. 3b, 3c).

Fig. 4a shows the serial changes in mean serum insulin concentrations after the standard meal load. Significant difference were noted between the ADD-ON and SWITCH groups with regard to the mean change in fasting insulin level (-2.00±4.18 *vs.* 1.19±4.30 μIU/L, *p*=0.026, Fig. 4b). On the other hand, the mean change in ISG was significantly higher in the ADD-ON group than the SWITCH group (1.5±1.3 *vs.* 0.5±1.1 μIU /mmol, respectively, *p*=0.015, Fig. 4c).

The AUC of glucagon was significantly lower at the end of treatment compared with the baseline in the ADD-ON group, but the difference between the ADD-ON and SWITCH groups was not significant (-4,075±8,348 *vs.* 2,001±10,528 pg-min/mL, *p*=0.056) (Fig. 5a, 5b).

Fig. 6 shows the mean increments in plasma active GLP-1 concentrations after the meal load (0 to 30 minutes) from the baseline to week 24. The increment in plasma active GLP-1 concentration was significantly lower at 24 weeks compared with the baseline in the SWITCH group and there was a significant difference between the ADD-ON and SWITCH groups (1.92±5.46 *vs.* -3.94±5.46 pmol/L, *p*=0.002).

The Supplementary Table 1 provides more details on the results of the meal tolerance test. Significant improvements were noted in mean changes in AUCs of glucose (0 to 30 minutes) (-33.3 *vs.* 2.7 mmol-min/L,

p=0.018) and AUC of glucose (0 to 15 minutes) (-17.5 *vs.* 1.9 mmol-min/L, *p*=0.011) in the ADD-ON group relative to the SWITCH group. The mean change in ISG (0 to 30 minutes) and ISG (0 to 15 minutes) were significantly higher at the end of treatment compared with the baseline in the ADD-ON group, but the differences between the ADD-ON and SWITCH groups were not significant (0.7±0.9 *vs.* 0.2±0.7 μIU/ mmol, *p*=0.052 and 0.3±0.6 *vs.* 0.07±0.7 μIU/mmol, *p*=0.260). There were no significant changes in fasting triglyceride, LDL-cholesterol, HDL-cholesterol, AUC of triglyceride, AUC of LDL-cholesterol and AUC of HDL-cholesterol, respectively.

Adverse events

None of the patients developed severe hypoglycemia requiring the assistance of another person and none had nocturnal hypoglycemia. Mild postprandial hypoglycemic episodes were observed in 4 patients of the ADD-ON group and 2 patients of the SWITCH group. All the 6 patients noted hypoglycemia only after eating fewer meals with regular oral glucose-lowering agents. One patient of the ADD-ON group with mild hypoglycemia withdrew from the study. One patient of the SWITCH group did not complete the study because of mild diarrhea. No physical, hematological, biochemical or urinary abnormalities were detected throughout the study. The mean weight changes and other physical findings were similar in both groups as described above.

Discussion

In the present study, the combination therapy of sitagliptin and repaglinide significantly improved HbA1c, fasting plasma glucose and AUC of glucose, compared with switching from sitagliptin to repaglinide.

The mean improvement in HbA1c was -0.87% in the ADD-ON group and 57% of patients of the ADD-ON group achieved HbA1c <7.0%. The mean change in

Table 2 Changes in physical parameters

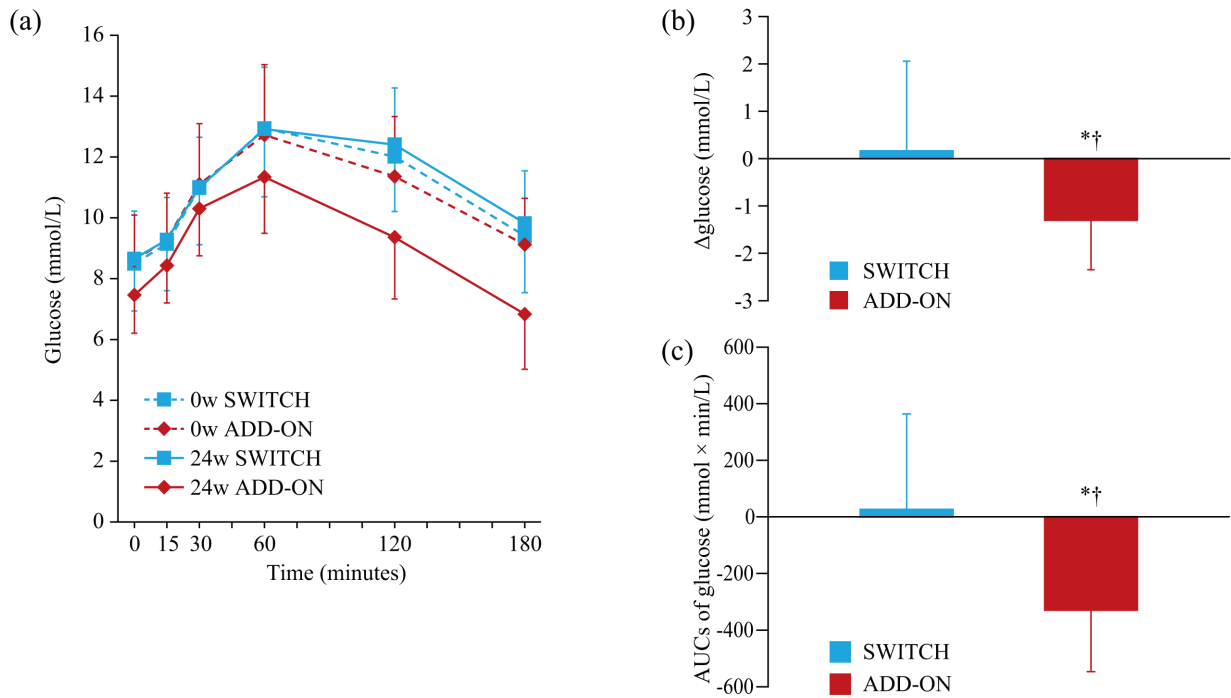|	SWITCH group	ADD-ON group	*p* value
Δbody weight, kg	0.45 ± 1.30	0.57 ± 1.47	0.781
Δbody mass index, kg/m^2	0.15 ± 0.49	0.21 ± 0.56	0.763
Δabdominal circumference, cm	0.91 ± 3.55	-0.42 ± 2.47	0.198
Δsystolic blood pressure, mmHg	5.78 ± 18.74	-2.26 ± 6.39	0.099
Δdiastolic blood pressure, mmHg	4.00 ± 7.68	-0.32 ± 6.66	0.076

Data are mean ± standard deviation.

Fig. 3 Changes in plasma glucose concentrations after standard meal load

(**a**) Serial changes in plasma glucose during the meal tolerance test. (**b**) Mean changes in fasting plasma glucose from the baseline to week 24. (**c**) Mean changes in AUCs of plasma glucose from the baseline to week 24. Data are mean±SD. * $p<0.05$ *vs*. SWITCH group, by the two sample *t*-test or chi-square test; † $p<0.05$ *vs*. baseline, by the paired *t*-test. AUC, area under the curve.
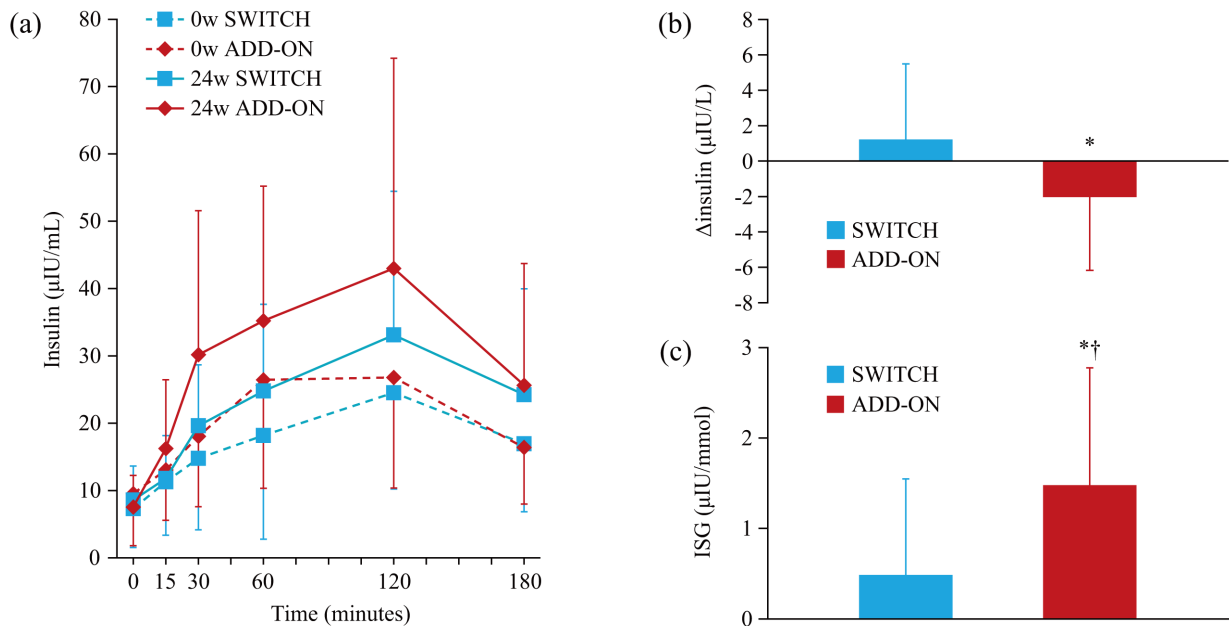

Fig. 4 Changes in serum insulin concentrations after standard meal load

(**a**) Serial changes in serum insulin during the meal tolerance test. (**b**) Mean changes in fasting serum insulin from the baseline to week 24. (**c**) Mean changes in ISG from the baseline to week 24. Data are mean±SD. * $p<0.05$ *vs*. SWITCH group, by the two sample *t*-test or chi-square test; † $p<0.05$ *vs*. baseline, by the paired *t*-test. ISG, insulin secretion relative to glucose elevation (AUC insulin/AUC glucose); AUC, area under the curve.
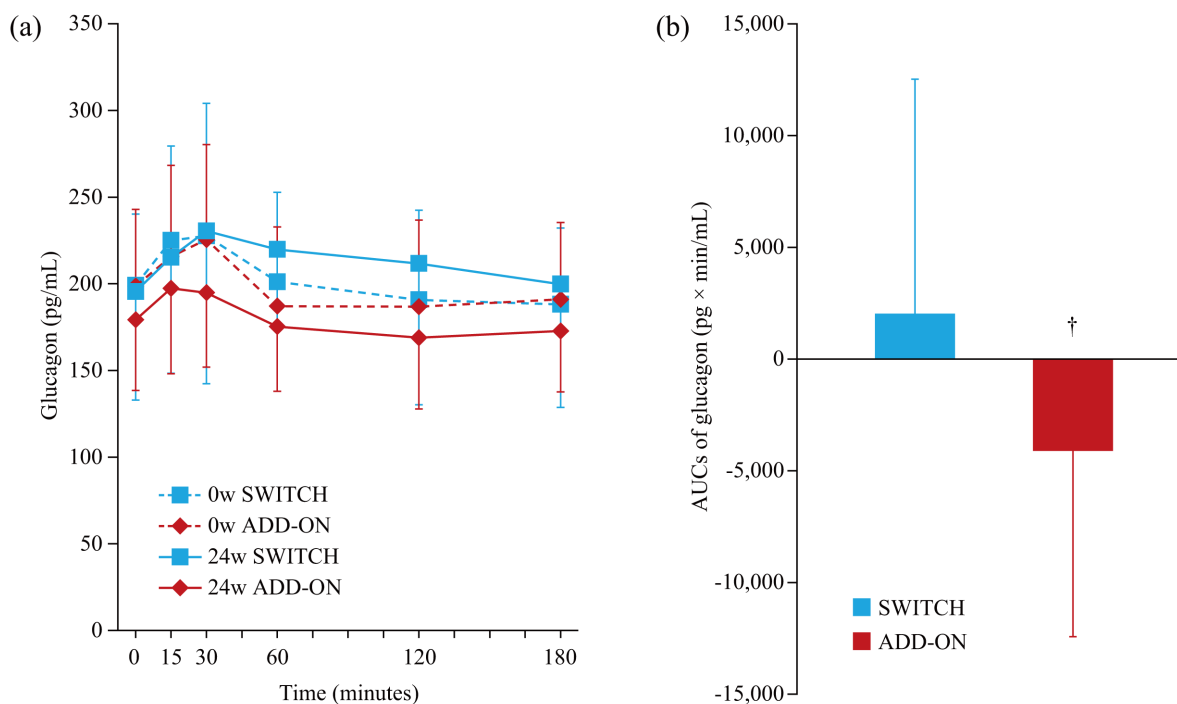
(a)

(b)

Fig. 5 Changes in serum glucagon concentrations after standard meal load

(**a**) Serial changes in serum glucagon during the meal tolerance test. (**b**) Mean changes in AUCs of serum glucagon from the baseline to week 24. Data are mean±SD. * $p<0.05$ *vs*. SWITCH group, by the two sample *t*-test or chi-square test; † $p<0.05$ *vs*. baseline, by the paired *t*-test. AUC, area under the curve.

Fig. 6 Changes in AUCs of plasma aGLP-1 from the baseline to week 24

Data are mean±SD. * $p<0.05$ *vs*. SWITCH group, by the two sample *t*-test or chi-square test; † $p<0.05$ *vs*. baseline, by the paired *t*-test. AUC, area under the curve; aGLP-1, active glucagon-like peptide-1.
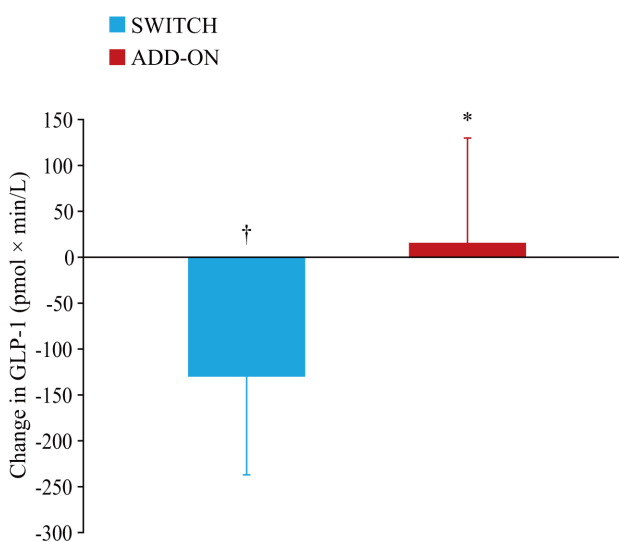
fasting plasma glucose improved significantly in the ADD-ON group compared with the baseline and with the SWITCH group. These results were similar to the previous uncontrolled trial which added repaglinide to the treatment of Japanese patients with T2DM who were poorly controlled with sitagliptin [25]. In this regard, previous trials that examined the efficacy of repaglinide monotherapy [15] or the combination of repaglinide and metformin [17] showed greater HbA1c reduction rates (-1.57% and -1.28%, respectively). The different reduction rates in HbA1c may be explained by the higher baseline HbA1c and the maximal dose of repaglinide used in the previous studies.

Our results showed a significant improvement in AUC of glucose in the ADD-ON group relative to the baseline and the SWITCH group. Although both repaglinide and sitagliptin improve postprandial glucose excess when used alone or in combination with metformin [15, 17, 26, 27], only a few clinical studies examined the postprandial effects of long-term combination therapy of DPP-4 inhibitor and glinides [24, 25]. We reported previously a randomized controlled trial

in which 24-week vildagliptin added to nateglinide in Japanese T2DM patients resulted in significant improvement in the AUC of glucose [24]. The present study showed similar improvement in postprandial glucose elevation, suggesting that the combination of DPP-4 inhibitor and glinides is a potentially effective treatment option for T2DM.

Our results also showed that the 24-week ADD-ON therapy significantly increased ISG compared to the baseline and the SWITCH group. Since ISG represents insulin secretion adjusted for hyperglycemia, this finding suggests that the combination therapy improved beta-cell function. Since sitagliptin itself neither increases AUC of insulin [7, 8] nor ISG [9, 10] but improves insulin sensitivity and beta-cell glucose sensing [7], it may be reasonable that sitagliptin augments repaglinide-induced insulin secretion, based on the increase in ISG in the ADD-ON group. That such augmentation is possible is supported by the finding of significant difference in the active GLP-1 level between the two groups, as shown in Fig. 6. However, since previous studies reported that DPP-4 inhibitors augmented insulin secretory responses both after oral glucose and during isoglycemic intravenous glucose infusions [7, 28], another mechanism including removal of glucose toxicity might explain the increased ISG in the ADD-ON group.

Only a few studies assessed the effect of the combination of incretin-based therapy and glinides on postprandial insulin and incretin secretion [24, 29, 30]. Two studies assessed the postprandial insulin and incretin secretion following a single administration of incretin-based therapy and glinides [29, 30]. In the first study, mitiglinide and sitagliptin significantly increased the insulinogenic index and the AUC of plasma intact GLP-1, compared with mitiglinide monotherapy [29]. In the second study, AUCs of serum insulin and plasma active GLP-1 were significantly higher in the nateglinide and exogenous GLP-1 combination compared with nateglinide alone [30]. Although these studies examined the effects of a single administration, their results are similar to the results of the present 24-week study. Thus, our study suggests at least medium term effect for the combination therapy on insulin secretion.

On the other hand, the fasting insulin level was significantly decreased in the ADD-ON group compared with the SWITCH group. The significant decrease in fasting plasma glucose level in the ADD-ON group

might result in decreased glucose-dependent fasting insulin secretion.

The mean change in AUC of glucagon decreased significantly in the ADD-ON group compared with the baseline, but the difference between the ADD-ON and the SWITCH groups was not significant (p=0.047 and p=0.056, respectively). Previous studies reported that sitagliptin decreased AUC of glucagon after a single administration [31] and after 12-week treatment [32] through an increase in active GLP-1 [33]. Similarly, repaglinide monotherapy decreased AUC of glucagon although the actual mechanism remains unknown [15]. Since both repaglinide and sitagliptin decrease AUC of glucagon, the mean AUC of glucagon decreased in the ADD-ON group and did not increase in the SWITCH group. This result suggests that the combination therapy may be effective in suppressing inappropriate postprandial glucagon secretion in T2DM patients. In contrast, nateglinide is reported to enhance glucagon secretion both *in vitro* [34] and after a single dose [35]. These studies indicate that the glucagon-suppressive effect of repaglinide might not be due to increased insulin secretion but rather to the difference in the molecular binding site between these insulin secretagogues. The actual mechanism of the glucagon suppressive effect in the combination therapy remains to be established.

In the present clinical trial, none of the patients developed severe hypoglycemia or nocturnal hypoglycemia although 4 patients of the ADD-ON group and 2 patients of the SWITCH group noted mild postprandial hypoglycemia. This result suggests that repaglinide can be added to sitagliptin to achieve better glycemic control without increased risk of severe hypoglycemia. A previous Japanese report suggested that the combination therapy of sulfonylurea and DPP-4 inhibitor carries the risk of severe hypoglycemia by activating Epac2 to Rap1 signaling [36]. However, because repaglinide does not activate this signaling [37] and because the fasting insulin level was significantly decreased rather than increased in the ADD-ON group, adding repaglinide to sitagliptin should not increase the risk of severe hypoglycemia and may be safer than the addition of sulfonylurea.

This study has several limitations. First, the relatively small sample size could affect the results especially those of the AUCs of glucagon, which tended to be different though not significant. A much larger sample study will be needed in future studies. Second, the dose of repaglinide was not titrated. According to

the approved dose in Japan, the starting dose of repaglinide is 0.25 mg thrice a day, followed by step-up increases to a maxim dose of 1.0 mg thrice a day in the absence of any safety problems. This might underestimate the effect of repaglinide on glycemic parameters and the incidence of hypoglycemic events after titration in both groups. On the other hand, the fixed starting repaglinide dose of 0.5 mg thrice a day in both groups might increase the incidence of hypoglycemia because all hypoglycemic events were observed within 1 to 2 months after starting repaglinide. Careful titration of repaglinide starting with 0.25 mg thrice a day might reduce the risk of hypoglycemia.

In conclusion, the combination therapy of repaglinide and sitagliptin produced significant reductions in HbA1c, AUC of glucose and fasting glucose, compared with switching to repaglinide, without any weight gain and no incidence of severe hypoglycemia. The improved glycemic control is probably the result of augmentation of repaglinide-induced insulin secretion by sitagliptin.

Supplementary Table 1 Results of the meal tolerance test

	SWITCH group				ADD-ON group				*p* value [*]
	Baseline	24 weeks	Δchange	*p* value [†]	Baseline	24 weeks	Δchange	*p* value [†]	
FPG, mmol/L	8.5 ± 1.7	8.7 ± 1.7	0.2 ± 1.9	0.721	8.6 ± 1.5	7.5 ± 1.3	-1.3 ± 1.0	0.000 [†]	0.007 [*]
Fasting IRI, μIU/mL	7.3 ± 5.8	8.6 ± 5.1	1.2 ± 4.3	0.244	9.5 ± 7.7	7.6 ± 4.7	-2.0 ± 4.2	0.052	0.026 [*]
Glucose AUC (0-180), mmol × min/L	2,045.5 ± 337.7	2,072.0 ± 322.2	26.4 ± 337.5	0.737	2,020.7 ± 252.2	1,691.3 ± 274.2	-329.4 ± 217.7	0.000 [†]	0.000 [*]
IRI AUC (0-180), μIU × min/mL	3,373.7 ± 2,088.0	4,484.1 ± 2,289.8	1,110.4 ± 1,726.0	0.012 [†]	4,031.2 ± 1,809.3	5,900.9 ± 3,205.6	1,869.7 ± 2,245.9	0.002 [†]	0.25
IRI iAUC (0-180), μIU × min/mL	2,041.6 ± 1,306.4	2,938.3 ± 1,925.5	896.7 ± 1,453.2	0.015 [†]	2,312.9 ± 1,757.7	4,541.9 ± 2,781.8	2,229.0 ± 2,548.9	0.001 [†]	0.055
ISG (0-180), μIU /mmol	1.8 ± 1.3	2.2 ± 1.2	0.5 ± 1.1	0.068	2.0 ± 0.9	3.5 ± 1.8	1.5 ± 1.3	0.000 [†]	0.015 [*]
Glucose AUC (0-30), mmol × min/L	283.9 ± 48.1	286.6 ± 50.8	2.7 ± 53.9	0.828	293.0 ± 40.1	259.7 ± 37.7	-33.3 ± 32.5	0.000 [†]	0.018 [*]
IRI AUC (0-30), μIU × min/mL	328.1 ± 234.6	388.4 ± 186.9	60.3 ± 124.7	0.049 [†]	397.7 ± 212.7	526.4 ± 298.5	128.7 ± 213.3	0.017 [†]	0.237
IRI iAUC (0-30), μIU × min/mL	119.6 ± 99.1	126.2 ± 108.6	6.7 ± 126.1	0.835	104.5 ± 220.4	299.9 ± 227.7	145.5 ± 198.7	0.009 [†]	0.023 [*]
ISG (0-30), μIU /mmol	1.2 ± 1.0	1.4 ± 0.7	0.2 ± 0.7	0.200	1.4 ± 0.7	2.1 ± 1.3	0.7 ± 0.9	0.002 [†]	0.052
Glucose AUC (0-15)	132.5 ± 24.7	134.5 ± 25.1	1.9 ± 27.2	0.761	136.7 ± 19.7	119.2 ± 18.1	-17.5 ± 15.2	0.000 [†]	0.011 [*]
IRI AUC (0-15), μIU × min/mL	143.9 ± 103.1	156.3 ± 80.9	12.4 ± 61.5	0.402	171.0 ± 97.8	180.0 ± 106.0	9.0 ± 52.7	0.480	0.857
IRI iAUC (0-15), μIU × min/mL	29.3 ± 26.7	23.8 ± 33.2	-5.5 ± 43.4	0.597	18.9 ± 85.2	65.1 ± 60.3	34.0 ± 70.8	0.033 [†]	0.053
ISG (0-15), μIU /mmol	1.1 ± 0.9	1.2 ± 0.7	0.07 ± 0.70	0.680	1.3 ± 0.7	1.6 ± 1.0	0.3 ± 0.6	0.033 [†]	0.260
Glucagon AUC, pg × min/mL	36,125 ± 9,079	38,466 ± 13,732	2,001 ± 10,528	0.418	35,150 ± 8,278	31,891 ± 6,654	-4,075 ± 8,348	0.047 [†]	0.056
Increment of aGLP-1, pmol × min/L	6.7 ± 6.0	2.7 ± 2.0	-3.9 ± 5.5	0.006 [†]	6.8 ± 4.9	8.7 ± 5.8	1.9 ± 5.5	0.143	0.002 [*]
Fasting TG, mg/dL	138.4 ± 116.1	128.2 ± 60.4	-10.2 ± 74.0	0.555	167.5 ± 145.8	137.9 ± 60.6	-29.6 ± 126.2	0.320	0.568
Fasting LDL-C, mg/dL	116.6 ± 36.1	120.6 ± 40.5	3.9 ± 13.1	0.204	115.7 ± 25.5	116.4 ± 24.3	0.74 ± 19.6	0.871	0.556
Fasting HDL-C, mg/dL × min/L	50.9 ± 12.9	49.6 ± 9.9	-1.3 ± 6.1	0.379	51.1 ± 9.1	51.4 ± 9.5	0.32 ± 6.0	0.822	0.428
TG AUC (0-180), mg × min/dL	20,214.9 ± 13,195.6	20,725.7 ± 10,127.2	510.8 ± 7,426.6	0.768	22,000.7 ± 15,918.6	18,352.5 ± 7,636.8	-3,648.2 ± 14,720.7	0.294	0.279
LDL-C AUC (0-180), mg × min/dL	19,959.5 ± 5,981.8	20,715.8 ± 6,913.0	756.3 ± 2,338.7	0.176	19,718.3 ± 4,191.7	19,612.9 ± 3,882.6	-105.4 ± 3,882.6	0.907	0.13
HDL-C AUC (0-180), mg × min/dL	8,799.5 ± 2,137.8	8,615.9 ± 1,744.9	-183.6 ± 1,009.0	0.438	8,738.7 ± 1,833.1	8,776.6 ± 1,609.3	37.9 ± 1,216.2	0.893	0.545

Data are mean±standard deviation. * Between the SWITCH and ADD-ON groups; † Between baseline and 24 weeks in the same group. FPG, fasting plasma glucose; AUC, area under the curve; IRI, insulin; iAUC, incremental (baseline subtracted) AUC; ISG, insulin secretion relative to glucose elevation; aGLP1, active plasma glucagon like protein-1; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

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