Effect of additional administration of acarbose on blood glucose fluctuations and postprandial hyperglycemia in patients with type 2 diabetes mellitus under treatment with alogliptin

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Abstract. Alogliptin, an inhibitor of DPP-4, increases plasma levels of GLP-1, promotes insulin secretion from pancreatic β cells [10, 11] and inhibits glucagon secretion from pancreatic α cells. The beneficial effect of alogliptin alone on HbA1c levels amounts to 0.5-0.8% [12].

Acarbose, an inhibitor of α-glucosidase, inhibits α-amylase and α-glucosidase and retards absorption of carbohydrates from the small intestine, suppressing postprandial rise of blood glucose levels [13].
addition, acarbose is reported to be able to decrease blood glucose fluctuation [14] and prevent the development of cardiovascular diseases in a STOP-NIDDM trial [15].

In this study, we examined and compared the effect of acarbose on patients with T2DM whose blood glucose control had not been reached to the satisfactory levels by analyzing the daily fluctuations of blood glucose levels using continuous glucose monitoring (CGM) and the secretory pattern of incretin hormones and postprandial rise of blood glucose levels in the meal tolerance test.

Materials and Methods

Subjects

The subjects with T2DM were recruited from our outpatient clinic, and they exhibited 2-hour postprandial blood glucose levels of 180 mg/dL or higher, or HbA1c of 7.0% or higher, even after receiving 25 mg/day of alogliptin for more than one month (Table 1). Exclusion criteria was as follows: 1) patients with type 1 diabetes; 2) patients with advanced diabetic complications; 3) patients who repeatedly developed serious hypoglycemia or asymptomatic hypoglycemia; 4) patients who had serious infection, were pre- or post-surgery, or had severe trauma; 5) patients who were pregnant or breast-feeding, or had the potential to become pregnant; and 6) patients who were disqualified by the attending physician. Before implementation, the purpose of the study was explained to all subjects and written informed consent was obtained. This study was approved by the ethics committee of Hyogo College of Medicine and conducted in accordance with the Helsinki Declaration. The clinical trial registration was completed at Hyogo College of Medicine.

Study design

The study was carried out at the Division of Diabetes and Metabolism, Department of Internal Medicine, Hyogo College of Medicine. The protocol is shown in Fig. 1. Blood glucose fluctuations for a 24-hour period were measured using Medtronic Minimed CGMS-Gold (Medtronic Incorporated, Northridge, USA) for three days. During the study period, the subjects continued taking other oral hypoglycemic agents.

In CGM, blood glucose fluctuations were analyzed before the administration of acarbose on the first day, and after administration of 100 mg/day of acarbose on the second and third day.

During the 3-day study period, patients received the same meals. For breakfast, patients received a test meal (JANEF E460F18: total calories, 460 kcal; carbohydrates, 56.5 g; fat, 18.0 g; protein, 18.0 g, Kewpie corporation, Tokyo, Japan).

The meal tolerance tests were conducted on the first

<table>
<thead>
<tr>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alogliptin</td>
<td>25 mg/day</td>
<td>25 mg/day</td>
</tr>
<tr>
<td>Acarbose</td>
<td>-</td>
<td>300 mg/day</td>
</tr>
</tbody>
</table>

Fig. 1 Study protocol

The daily profiles of blood glucose were monitored by CGM for three days in 10 T2DM patients. On the second and third day, 300 mg/day of acarbose was administered in combination. On the first and third day, the test meal tolerance was performed, and patients were taken blood samples.

Table 1 Baseline characteristics of the T2DM patients in the study (n=10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (years old)</td>
<td>54.9 ± 6.9</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>5 : 5</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>7.2 ± 5.2</td>
</tr>
<tr>
<td>HbA1c (%; NGSP)</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.3 ± 12.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 5.2</td>
</tr>
</tbody>
</table>
and second day. The levels of plasma glucose (PG), serum C peptide immunoreactivity (CPR), immunoreactive insulin (IRI), immunoreactive glucagon (IRG), active GLP-1, and total GIP were measured before (0 min) and 30 min, 60 min, 120 min, and 180 min after the meal tolerance test on both the first and the third day. For the measurement of active GLP-1 and total GIP, blood was collected and immediately subjected to plasma separation in centrifuge tubes containing EDTA-2Na and aprotinin. DPP-4 inhibitor was added to the centrifuge tubes at 10 μg per 1 mL of blood and separated plasma was stored at -70°C. For measurement of active GLP-1, the Glucagon-Like Peptide-1 (Active) ELISA KIT (Millipore, St. Charles, Missouri, USA) was used (accuracy, 86.7 ± 5%; precision CV: inter-assay, 8 ± 4.8%; intra-assay, 7.4 ± 1.1; sensitivity, the lowest detectable level of GLP-1, 2 pmol/L; specificity, GLP-1 (7-36), 100%; GLP-1 (9-36), ND; GLP-2, ND; and Glucagon, ND). For measurement of total-GIP, the Human GIP (Total) ELISA KIT (Millipore, St. Charles, Missouri, USA) was used (accuracy, 86.9 ± 5.2%; precision CV: inter-assay, 8 ± 4.8%; intra-assay, 7.4 ± 1.1; sensitivity, the lowest detectable level of human GIP using a 20 μL sample, 1.64 pmol/L; specificity, no significantly cross-reaction with glucagon, oxyntomodulin, GLP-1 and GLP-2). All samples were extracted in a final concentration of 70% ethanol before active GLP-1 and total-GIP measurements.

Statistical analysis
Statistical analysis was performed using SigmaStat for Windows, version 3.5 (Systat Software Incorporated, Chicago, IL, USA). The paired t-test or Wilcoxon signed rank test was performed to test the significance of blood glucose fluctuations. To test the significance of measurement values after the meal tolerance test, one-way repeated measures ANOVA was performed for comparisons within each group, and two-way repeated measures ANOVA for comparisons between groups. As for post hoc analysis, Tukey's test was performed. In addition, the paired t-test was performed for AUC analysis. P values were two-tailed with a significance level of 5%. Values are expressed as mean ± standard deviation (SD).

Results
A total of 10 patients with T2DM were included in the study (Table 1). The mean age of the patients was 54.9 ± 6.9 years, BMI 25.9 ± 5.2 kg/m², and HbA1c (NGSP) 9.2 ± 1.2% at baseline. All patients had been taking 25 mg/day of alogliptin with or without other oral hypoglycemic agents. These oral hypoglycemic agents were biguanides for four patients and a combination of biguanides and sulfonylurea (SU) for five patients. One patient received 25 mg/day alogliptin alone. The dose and the method of administration were the same throughout the CGM period.

The daily profiles of blood glucose were monitored with CGM for a 24-hour period with or without administration of acarbose in combination with alogliptin (Fig. 2). The results showed that the mean blood glucose level with acarbose (136.4 ± 30.7 mg/dL) did not differ significantly from that without acarbose (141.7 ± 28.3 mg/dL). However, in the condition of the combination therapy, there were significant decreases in the standard deviation of the mean blood glucose levels for the 24-hour period (27.6 ± 9.1 vs. 16.2 ± 6.9 mg/dL, p<0.001) and mean amplitude of glycemic excursions (MAGE) (65.8 ± 26.1 vs. 38.8 ± 19.2 mg/dL, p=0.010) (Table 2). In addition, glucose levels which were monitored by CGM improved at 2-hour after breakfast (179.7 ± 39.4 mg/dL vs. 166.7 ± 60.5 mg/dL, p=0.028) and at 2-hour after dinner (147.4 ± 47.8 mg/dL vs. 135.8 ± 42.1 mg/dL, p=0.038).

In the meal tolerance test, after combined administration of 25 mg of alogliptin and 100 mg of acarbose, plasma glucose (PG) levels improved at 30 min (155.9 ± 33.0 vs. 141.0 ± 26.4 mg/dL, p=0.044), 60 min (187.6 ± 59.7 vs. 151.1 ± 31.2 mg/dL, p<0.001), 120 min (190.7 ± 59.7 vs. 154.3 ± 39.9 mg/dL, p<0.001), and 180 min (163.2 ± 58.8 vs. 147.2 ± 38.5 mg/dL, p=0.032), compared to those before combined administration (Fig. 3A). PG\textsubscript{AUC0-180} also showed significant improvement from 340.5 ± 82.8 mg·hr/dL without acarbose to 292.5 ± 60.3 mg·hr/dL with acarbose (p<0.001).

The peak levels of CPR shifted from 180 min to 120 min, and a significant decrease of CPR levels was observed at 120 min (5.8 ± 2.1 vs. 4.5 ± 1.6 ng/mL; p<0.001) and 180 min (5.9 ± 2.2 vs. 4.4 ± 1.4 ng/mL; p<0.001) (Fig. 3B). The peak of IRI levels shifted from 120 min to 60 min after acarbose combined administration, and a significant decrease of IRI levels was observed at 120 min (31.4 ± 14.6 vs. 20.7 ± 9.6 μIU/mL; p<0.001) and 180 min (23.9 ± 11.3 vs. 17.1 ± 6.9 μIU/mL; p=0.011) (Fig. 4A). CPR\textsubscript{AUC0-180} and IRI\textsubscript{AUC0-180} showed significant decreases from when acar-
paradoxically increased 30 min and 60 min after the meal tolerance test, no significant increase in IRG after the meal tolerance test was observed when acarbose was administered in combination (Fig. 4B). Total GIP significantly decreased at 30 min (498.4 ± 219.7 vs. 226.5 ± 179.8 pg/mL; \( p<0.001 \)) and 60 min (422.9 ± 160.6 vs. 226.4 ± 116.9 pg/L; \( p<0.001 \)) after the meal tolerance test with acarbose compared to without acarbose. Total GIP_{AUC0-180} signifi-

![Figure 2](image)

**Table 2** Indices of daily blood glucose fluctuations with or without acarbose combination in T2DM patients treated with alogliptin

<table>
<thead>
<tr>
<th>Case</th>
<th>24-hour mean blood glucose level (mg/dL)</th>
<th>SD of 24-hour mean blood glucose (mg/dL)</th>
<th>MAGE (mg/dL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>without acarbose</td>
<td>with acarbose</td>
<td>without acarbose</td>
</tr>
<tr>
<td>1</td>
<td>150.4</td>
<td>159.7</td>
<td>25.4</td>
</tr>
<tr>
<td>2</td>
<td>135.3</td>
<td>130.1</td>
<td>29.4</td>
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<tr>
<td>3</td>
<td>131.4</td>
<td>121.2</td>
<td>19.6</td>
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<td>4</td>
<td>181.5</td>
<td>174.8</td>
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<td>5</td>
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<td>6</td>
<td>121.1</td>
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<td>8</td>
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<tr>
<td>9</td>
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<td>103.9</td>
<td>14.7</td>
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<tr>
<td>10</td>
<td>201.3</td>
<td>198.4</td>
<td>44.4</td>
</tr>
<tr>
<td>mean</td>
<td>141.7</td>
<td>136.4</td>
<td>27.6</td>
</tr>
<tr>
<td>SD</td>
<td>28.3</td>
<td>30.7</td>
<td>9.1</td>
</tr>
</tbody>
</table>

\( p \) value \( p=0.152 \) \( p<0.001 \) \( p=0.010 \) 

Acarbose was not administered in combination (9.1 ± 3.2 ng·hr/mL and 46.1 ± 19.7 μIU·hr/mL, respectively) to when acarbose was administered in combination (7.8 ± 2.4 ng·hr/mL and 136.1 ± 13.6 μIU·hr/mL, respectively) \( (p=0.024, p=0.007) \).

No significant difference in the levels of IRG was observed at each point or in AUC0-180 irrespective of with or without acarbose administration. Although without acarbose combination, IRG significantly and

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**Fig. 2** The daily profiles of blood glucose were monitored by CGM with or without acarbose. After the combination therapy, glucose levels were significantly improved at after breakfast and after dinner. (n=10, \( # p<0.05 \) with acarbose vs. without acarbose)
Combination of acarbose and alogliptin

Fig. 3  Changes in plasma glucose (A) and CPR (B) after the meal tolerance test  
(n=10, *p<0.05 and **p<0.01 vs. 0 min, †p<0.05 and ‡p<0.01 with acarbose vs. without acarbose)

Fig. 4  Changes in IRI (A) and IRG (B) after the meal tolerance test  
(n=10, *p<0.05 and **p<0.01 vs. 0 min, †p<0.05 and ‡p<0.01 with acarbose vs. without acarbose)
retinopathy, and therefore, therapy of diabetes should aim to bring HbAlc and levels of postprandial as well as fasting blood glucose to the normal range, with the following target values; HbAlc<6.5%, fasting blood glucose<100 mg/dL, and 2 hour postprandial blood glucose<140 mg/dL [16].

Administration of the new class of anti-diabetic agent, DPP-4 inhibitor to patients with T2DM resulted in an increased level of active GLP-1, which promotes insulin secretion from pancreatic β cells in a blood glucose dependent manner. In addition, it may also lead to blood glucose-dependent suppression of glucagon secretion and improvement of mainly postprandial blood glucose levels.

There are several papers reporting the additive use of DPP-4 inhibitors in cases in which α-glucosidase inhibitors proved incomplete control, analyzing combined effects of these drugs by CGM or incretin assays [17-20]. However, only few reports are available analyzing whether the additional use of α-glucosidase inhibitors provides beneficial effect on the blood glucose fluctuation and incretin hormone secretion in cases in which DPP-4 inhibitors were not sufficiently effective. In particular, no information is available on

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**Fig. 5** Changes in Total GIP (A) and Active GLP-1 (B) after the meal tolerance test (n=10, *p<0.05 and **p<0.01 vs. 0 min, ***p<0.01 with acarbose vs. without acarbose)
CGM and incretin secretion in multiple patients with T2DM following combined administration of alogliptin and acarbose. Alogliptin at 25 mg alone has been reported to improve HbAlc by about 0.8% [12]. In the present study, ten patients treated with 25 mg alogliptin showed HbAlc 9.2 ± 1.2% and blood glucose 130.8 ± 24.0 mg/dL before meal and 190.7 ± 60.0 mg/dL after meal, indicating insufficiency of management of blood glucose levels by alogliptin alone. The results of CGM of these patients following the combined administration of acarbose and alogliptin showed improvement of standard deviation of daily changes in blood glucose levels and MAGE, indicating better management of blood glucose by the two inhibitors. In the meal tolerance test, blood glucose values with acarbose were better than those without acarbose at all time points analyzed (30, 60, 120, and 180 min). These results indicate that the additional use of acarbose in cases in which alogliptin failed to show satisfactory management of blood glucose results in improvement of postprandial blood glucose levels and overall management of blood glucose fluctuations. This may lead to the possible prevention of not only microvascular diseases but also macrovascular diseases including cardiovascular illness. Further, it has been pointed out that excessive insulin secretion may lead to weight gain and exhaustion of pancreatic β cells, in the treatment of diabetes.

α-glucosidase inhibitors block the decomposition of disaccharides by inhibiting α-glucosidase in the brush-border of small intestinal mucosal epithelial cells. As a result, sugars which are mainly absorbed in the upper small intestine are absorbed more slowly throughout the small intestine, thereby improving postprandial blood glucose levels and suppressing excessive insulin secretion after meal ingestion. Furthermore, according to some reports, absorption of sugars in the entire small intestine causes the relative suppression of GIP secretion from K cells in the upper small intestine, and this in turn enhances GLP-1 secretion from L cells in the lower small intestine [21-23].

In the present study, the meal tolerance test showed that IRI and CPR peaked 60 min earlier, with a significant decrease in both of the AUC0-180 (Fig. 3B, Fig. 4A). These results suggest that the pattern of insulin secretion in response to meal ingestion in our diabetic subjects was similar to that in healthy subjects, and the inhibition of late and excessive insulin secretion may provide the preventive effects for pancreatic β cells. Suppression of excessive postprandial insulin secretion may also be effective in preventing weight gain, and correction of hyperinsulinemia may lead to the prevention of development and progression of vascular endothelial disorders.

In this study, results from the combined administration of acarbose and alogliptin, to patients with T2DM, whose glycemic control had been poorly achieved by alogliptin alone, showed a tendency to increased active GLP-1 in peripheral plasma level in the meal tolerance test. The combined administration of acarbose and alogliptin also achieved significant decrease in the total GIP at 30 and 60 min and GIP AUC0-180. Furthermore, the combined administration of acarbose and alogliptin successfully suppressed the paradoxical rise in post-meal IRG, which could not be achieved by alogliptin alone, possibly due to the increase in active GLP-1 secretion (Fig. 4B, Fig. 5B).

Recently, GLP-1 has been suggested to induce preventive effects on atherosclerosis via suppression of the expression of plasminogen activator inhibitor type-1 (PAI-1) in vascular endothelial cells [24, 25]. Therefore, not only the anti-atherogenous effect with the suppression of postprandial hyperglycemia, but also with the increased GLP-1 level can be expected to exhibit the beneficial effect on diabetic vascular complications.

We have observed that the combined use of alogliptin and acarbose spared insulin secretion. And also, we have confirmed the elevation of active GLP-1 levels, which possesses the inhibitory action of gastric emptying and satiety effect [26]. Furthermore, total GIP secretion was suppressed by the combined use of alogliptin and acarbose, which is, in view of the possible effect of GIP on fat accumulation [27], consistent with the possibility that a long-term combined use of the two drugs may result in weight reduction.

In conclusion, combination therapy with alogliptin and acarbose in patients with T2DM who could not achieve sufficient glycemic control with alogliptin, clearly improved the daily glucose fluctuations, secretory patterns of IRI, and the plasma levels of incretin hormones. Combination of the two types of drugs did not simply lead to improved glycemic control, but also the inhibitory effects on the development and progression of micro- and macro-angiopathy. To prove this hypothesis, we need a further prospective randomized study with a larger number of patients over a longer period of time.
References


Combination of acarbose and alogliptin


