Effects of miglitol, vildagliptin, or their combination on serum insulin and peptide YY levels and plasma glucose, cholecystokinin, ghrelin, and obestatin levels

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Abstract. We previously reported that combination therapy with an α-glucosidase inhibitor (αGI) and a dipeptidyl peptidase-4 (DPP-4) inhibitor increased active glucagon-like peptide-1 (GLP-1) levels and decreased total glucose-dependent insulino tropic polypeptide (GIP) levels, compared with monotherapy, in non-diabetic men. However, the peptide YY (PYY), cholecystokinin (CCK), ghrelin, and obestatin levels in patients receiving a combination of αGIs and DPP-4 inhibitors have not been previously reported. We evaluated the effect of miglitol, vildagliptin, or their combination on these parameters. Miglitol and/or vildagliptin were administered according to four different intake schedules in eleven non-diabetic men (C: no drug, M: miglitol, V: vildagliptin, M+V: miglitol+vildagliptin). Blood samples were collected at 0, 30, 60, and 120 min after the start of breakfast. The plasma glucose, serum insulin, serum total PYY (PYY1-36 and PYY3-36), plasma CCK, plasma active ghrelin, and plasma obestatin levels were measured. The area under the curve (AUC) of the serum total PYY level in the M group was significantly greater than that in the C group, and the AUC of the serum total PYY level in the M+V group was significantly lower than that in the M group. The combination therapy did not change the AUC of the plasma CCK, plasma active ghrelin, plasma obestatin, and ghrelin/obestatin levels, compared with the control. The results of our study suggested that combination therapy with miglitol and vildagliptin had no effect on appetite regulation hormones, such as total PYY, CCK, active ghrelin, and obestatin, compared with the levels in the control group.

Key words: Miglitol, Vildagliptin, Gut hormones

α-Glucosidase inhibitors (αGIs) decrease plasma glucose and serum insulin levels in healthy subjects [1, 2] and reduce the development of type 2 diabetes in subjects with impaired glucose tolerance (IGT) [3, 4]. αGIs reportedly enhance active glucagon-like peptide-1 (GLP-1) responses and reduce total glucose-dependent insulino tropic polypeptide (GIP) responses [5-8]. Dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin or vildagliptin, increase active GLP-1 and GIP by inhibiting DPP-4 enzymatic activity and improve hyperglycemia in a glucose-dependent fashion by increasing serum insulin and decreasing serum glucagon levels in diabetic patients [9].

Because miglitol and sitagliptin enhance plasma active GLP-1 concentrations via different mechanisms, we previously examined the effect of their combined administration. Combination therapy using these drugs increased the active GLP-1 levels and decreased the total GIP levels, compared with monotherapy, in non-diabetic men [10]. Interestingly, the administration of miglitol only at breakfast increased the AUC of the active plasma GLP-1 levels from breakfast to 2 hours after the start of lunch in sitagliptin-treated Japanese patients with type 2 diabetes [11].

With the exception of GLP-1 and GIP, several gut hormones are secreted by the stomach or small intestine and regulate appetite. Peptide YY (PYY) is a 36-amino acid protein secreted mainly by L cells in the intestine [12, 13]. After release, DPP-4 cleaves the N-terminal tyrosine-proline residues and forms PYY3-36, and PYY3-36 represents about 63% of the total PYY after feeding and about 37% after fasting [14, 15]. The administration of PYY3-36 reportedly reduces food intake in mice and humans [16]. Cholecystokinin
(CCK) is secreted from the I cells in the duodenum and jejunum [17]. CCK stimulates pancreatic enzyme secretion and gall bladder contraction and delays gastric emptying and food intake via the stimulation of the vagus nerve [18, 19]. Ghrelin, a 28-amino acid acylated peptide, is an orexigenic peptide secreted by the stomach [20]. Plasma ghrelin concentrations increase with fasting and decrease after feeding [21]. Obestatin is a 23-amino acid peptide that has been isolated from the rat stomach. This peptide, encoded by the ghrelin gene, reduces the food intake, gut motility, and body weight [22].

Some previous reports have investigated the changes in PYY, ghrelin, and CCK levels after the administration of αGI as well as the changes in PYY and ghrelin levels after the administration of DPP-4 inhibitors. However, since the PYY, CCK, ghrelin, and obestatin levels in subjects receiving combined treatment with αGIs and DPP-4 inhibitors have not been previously reported, we evaluated the effects of miglitol, vildagliptin, and their combined administration on these parameters in healthy men.

Materials and Methods

We conducted the study with the approval of the Institutional Ethics Review Committee of Yokohama City University Hospital, and the protocol was registered in the UMIN Clinical Trial Registry as UMIN000010481. Informed consent was obtained from each of the subjects before the start of the study. Eleven healthy men aged 37.7 ± 10.1 years with body height of 170.2 ± 6.0 cm, body weight of 70.0 ± 9.6 kg and a BMI of 24.3 ± 4.3 kg/m², who had never been diagnosed as having diabetes or IGT were enrolled in the present study. Miglitol and/or vildagliptin were administered according to four different intake schedules (C: no drug, M: miglitol administered just before a meal [50 mg]; V: vildagliptin administered 2 hours before the start of a meal [50 mg], M+V: miglitol administered just before a meal [50 mg] and vildagliptin administered 2 hours before the start of a meal [50 mg]). As the vildagliptin time to peak plasma concentration (tmax) are 1.5-2 hours, vildagliptin was administered 2 hours before the start of the meal [23]. The subjects were randomized to one of the four interventions using a crossover design. Subjects were asked to take each medication after a drug-free washout period lasting more than 1 week. All the subjects received a standard breakfast (849 kcal; protein: 25.3 grams; fat: 27.5 grams; carbohydrate: 125.0 grams).

For the study, the subjects were requested to fast for at least 12 hours before eating breakfast. Blood samples were collected at 0, 30, 60 and 120 min after the start of breakfast. They were obtained from the antecubital vein into fluoride tubes for the analysis of glucose levels, into EDTA-2Na/aprotinin tubes for the analysis of cholecystokinin, ghrelin, and obestatin levels, and into plain siliconized tubes for the analysis of insulin and PYY. The blood samples were then immediately centrifuged at 4°C and the plasma fractions obtained from EDTA-2Na/aprotinin tubes were mixed with hydrochloric acid yielding a final concentration of 0.1 N for the measurement of active ghrelin levels. All blood samples except the plasma glucose and serum insulin were stored at −70°C until assayed. The plasma glucose and serum insulin levels were measured by Hokenkagaku Institute Inc. (Yokohama, Japan). The serum total PYY (PYY1-36 and PYY3-36) and plasma obestatin levels were measured using an EIA kit (Yanaihara Institute Inc., Shizuoka, Japan), and the plasma CCK (CCK26-33) levels were also measured using an EIA kit (Phoenix Pharmaceutical, Inc., USA). Active ghrelin was measured using an ELISA (Sceti, Tokyo, Japan).

Data were expressed as the mean ± SD. The areas under the curve (AUC) from just before the meal to 120 min after the start of the meal were calculated using the trapezoid method. The analyses for each hormone at 0, 30, 60 and 120 min and the AUCs were performed using one-way layout analysis of variance (ANOVA) with Bonferroni type comparisons. All statistical analyses were conducted using Ekuseru-Toukei 2012 (Social Survey Research Information Co., Ltd, Tokyo, Japan). Differences with P values of less than 0.05 were considered significant.

Results

The time profiles and AUCs of the plasma glucose and serum insulin levels are shown in Fig. 1 and Table 1. The plasma glucose levels at 30 min after the start of breakfast were significantly lower in the M, V, and M+V groups than in the C group, while the plasma glucose levels at 60 min after the start of breakfast were significantly lower in the M+V group than in the C group (Fig. 1A). The AUCs of the plasma glucose levels in the M and M+V groups were significantly lower than that in the C group. The AUC of the plasma glucose levels in the V group tended to be lower than that in the C group, but the
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The serum insulin levels at 30 and 60 min after the start of breakfast were significantly lower in the M and M+V groups than in the control group (Fig. 1B). The serum insulin levels at 120 min after the start of breakfast were significantly lower in the M+V group than in the C group (Fig. 1B). The AUCs of the serum insulin levels in the M and M+V groups were significantly smaller than that in the C group (Table 1). By contrast, the AUC of the serum insulin levels was unaffected in the V group.

The time profiles and the AUCs of the serum total PYY and plasma CCK levels are shown in Fig. 2. The total PYY levels at 60 min after the start of breakfast were significantly higher in the M group than in the C group, and the total PYY levels at 30, 60 and 120 min in the V group and at 60 and 120 min in the M+V group were significantly lower than in the M group (Fig. 2A). As a result, the AUC of the serum total PYY level in the M group was significantly greater than that in the C group, and the AUCs of the serum total PYY level in the V and M+V groups were significantly lower than that in the M group (Table 1). The AUC of the serum total PYY level in the V group tended to be lower than that in the C group, but the difference was not statistically significant. The AUCs of the plasma CCK level did not differ significantly among the groups (Fig. 2B and Table 1).

The time profiles and the AUCs of the plasma active ghrelin, obestatin, and ghrelin/obestatin levels are shown in Fig. 3 and Table 1. The plasma active ghrelin levels at 60 min after the start of breakfast were significantly lower in the V group than in the M group, and the obestatin levels at 30 min after the start of breakfast were significantly lower in the V group than in the M group and the obestatin levels at 30 min after the start of breakfast were significantly lower in the M group than in the C group (Table 1).

**Table 1** Comparison of each area under the curve (AUC)s among groups

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>M</th>
<th>V</th>
<th>M+V</th>
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<tbody>
<tr>
<td>Plasma glucose</td>
<td>15062.7 ± 2531.1</td>
<td>12654.5 ± 1151.7</td>
<td>13671.8 ± 2766.2</td>
<td>11360.4 ± 1138.1</td>
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<tr>
<td>Serum insulin</td>
<td>9488.7 ± 7547.5</td>
<td>3860.7 ± 3798.6</td>
<td>7151.8 ± 4613.7</td>
<td>2741.3 ± 1773.5</td>
</tr>
<tr>
<td>Serum PYY</td>
<td>30.1 ± 23.1</td>
<td>37.9 ± 9.4</td>
<td>26.5 ± 21.3</td>
<td>30.8 ± 25.9</td>
</tr>
<tr>
<td>Plasma CCK</td>
<td>8.5 ± 3.1</td>
<td>9.1 ± 5.2</td>
<td>9.6 ± 4.9</td>
<td>9.4 ± 5.1</td>
</tr>
<tr>
<td>Plasma ghrelin</td>
<td>993.0 ± 550.8</td>
<td>1147.4 ± 707.9</td>
<td>948.8 ± 616.0</td>
<td>1112.5 ± 650.2</td>
</tr>
<tr>
<td>Plasma obestatin</td>
<td>182.1 ± 54.4</td>
<td>196.6 ± 50.7</td>
<td>187.9 ± 49.4</td>
<td>193.1 ± 51.3</td>
</tr>
<tr>
<td>Plasma ghrelin/obestatin</td>
<td>659.8 ± 307.4</td>
<td>725.1 ± 374.9</td>
<td>603.2 ± 279.2</td>
<td>714.6 ± 360.2</td>
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</table>

Data are expressed as the means ± SD. C, M, V, and M+V indicate the control, miglitol, vildagliptin, and miglitol and vildagliptin treatment groups, respectively. **P<0.01, ***P<0.001 vs. C. ###P<0.001 vs. M. †††P<0.001 vs. V.
Fig. 2  Time profiles of A) serum PYY and B) plasma CCK levels. C, M, V, and M+V indicate the control, miglitol, vildagliptin, and miglitol+vildagliptin treatment groups, respectively. C: filled circles; M: clear circles; V: triangles; M+V: squares. Data are expressed as the means ± SD. *$P<0.05$, **$P<0.01$, vs. C. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. M.

Fig. 3  Time profiles of A) plasma ghrelin, B) obestatin, and C) ghrelin/obestatin levels. C, M, V, and M+V indicate the control, miglitol, vildagliptin, and miglitol+vildagliptin treatment groups, respectively. C: filled circles; M: clear circles; V: triangles; M+V: squares. Data are expressed as the means ± SD. *$P<0.05$ vs. C. ††* $P<0.01$ vs. M. †††* $P<0.001$ vs. V.
were significantly lower in the V group than in the M group (Fig. 3A and B). The obestatin levels at 30 min after the start of breakfast were significantly higher in the M+V group than in the C and V group. The ghrelin/obestatin level at 60 min after the start of breakfast was significantly lower in the V group than in the M group (Fig. 3C). However, the AUCs of the plasma active ghrelin, obestatin, and ghrelin/obestatin levels did not differ significantly among the groups (Table 1).

**Discussion**

The most important finding in this study is that the combination therapy of miglitol and vildagliptin did not change the AUCs of the total PYY, CCK, active ghrelin, and obestatin levels, compared with the values for the control group. By contrast, the administration of miglitol alone increased the AUC of the total PYY level. Based on the results of the present study, the combination therapy did not appear to have any effect on intestinal appetite regulation hormones, such as total PYY, CCK, active ghrelin, and obestatin, unlike the previously reported additive increase in active GLP-1 levels [10, 11].

The effects of miglitol, vildagliptin, and their combination on plasma glucose and serum insulin were consistent with our previous report [10, 11]. The absence of an increase in serum insulin levels in the V and M+V groups might be favorable for sparing insulin secretion by β cells in individuals with NGT.

The AUC of the total PYY level in the M group was higher than that in the C group, as shown in previous reports discussing the administration of miglitol or acarbose [24, 25]. This result was considered to be due to an increase in PYY secretion because of changes in the proportion of absorbed carbohydrates from the proximal to distal portions of the small intestine. The administration of vildagliptin for 10 days reportedly decreased the total PYY level in patients with type 2 diabetes [26], and the administration of sitagliptin for 3 months also reduced the total PYY and PYY_{1-36} levels and increased the PYY_{1-36} level [27]. No study has clearly demonstrated a reduction in total PYY secretion through the action of DPP-4 inhibitors. DPP-4 inhibitors increased PYY_{1-36}, as DPP-4 changes PYY_{1-36} to PYY_{3-36}, and the decrease in total PYY in response to DPP-4 inhibitors may be due to the negative feedback inhibition of the secretion from L cells from increased PYY_{1-36} or active GLP-1, as previously described [26, 27]. Our results for the effect of vildagliptin on the serum total PYY level were in accordance with these previous reports; however, the effect of combination therapy on serum PYY levels has not been previously reported. In this study, we demonstrated that combination therapy decreased the increment in the total PYY level induced by the administration of miglitol. PYY_{1-36} is considered to be increased by the administration of miglitol, based on its pharmacological effect, and also to be increased by the administration of vildagliptin, as previously reported [28]. Therefore, the combination therapy increased the PYY_{1-36} level and decreased the PYY_{3-36} level, leaving the total PYY level unaffected when compared with that of the control group. As PYY_{3-36} decreases appetite and PYY_{1-36} stimulates appetite [13-15], the combination therapy’s effect on PYY might not result in appetite suppression and body weight reduction.

The administration of acarbose reportedly increased the postprandial CCK level in healthy men, although the mechanism responsible for this increase was unclear [28]. Unlike acarbose, miglitol is partially absorbed from the proximal portion of the small intestine [29], and this leads to a decrease in absorbed carbohydrates from the proximal portions of the small intestine. As CCK is secreted from the upper portion, a difference in the postprandial CCK levels as a result of the administration of miglitol or acarbose can be observed. The AUC of the CCK level for the M group was not larger than that for the C group in the present study. The reason for this finding might be due to the difference in the pharmacological effects of acarbose and miglitol. As the CCK level after treatment with DPP-4 inhibitors or combination therapy has not been previously reported, we evaluated these effects but did not observe any significant differences among the four groups in this study.

Kaku et al. reported that the administration of miglitol increased the active ghrelin level at 60 min and decreased the level at 180 min after a meal in healthy subjects [24]. Treatment with acarbose decreased the postprandial total ghrelin levels in patients with type 2 diabetes [30]; however, treatment with acarbose increased the plasma total ghrelin level in healthy subjects [31]. In the present study, the AUC of the active ghrelin level in the M group tended to be higher than that in the C group, although the difference was not statistically significant. The administration of sitagliptin for three months decreased the fasting total ghrelin levels; however, the single administration of sitagliptin
did not change the post-prandial total ghrelin levels in healthy subjects, and the administration of vildagliptin for ten days also did not change the post-prandial active ghrelin levels in patients with type 2 diabetes [27, 32, 33]. The plasma ghrelin level after combination therapy has not been previously reported. In the present study, the AUC of the active ghrelin level in the M+V group tended to be higher than that in the C group, although the difference was not statistically significant. If we had measured the plasma ghrelin levels at 180 min, a lower ghrelin concentration at 180 min and a tendency toward a decreased ghrelin concentration at 60 to 180 min in the M and M+V groups might have been observed, as described previously [24]. The plasma obestatin level after the administration of miglitol, vildagliptin, or a combination also has not been previously reported. In this study, the plasma obestatin level at 30 min was higher in the M+V group than in the C group; however, the AUCs of the M, V, and M+V groups were not significantly different from that of the C group. No significant differences in the ghrelin/obestatin levels were observed among the four groups.

We hypothesized that combination therapy with the drugs used in this study would increase the active GLP-1 levels and decrease the total GIP levels, compared with monotherapy, based on our previous results [10, 11]. Therefore, with regard to the hormones that are secreted by the intestine in response to the administration of αGIs and DPP-4 inhibitors, the changes in GLP-1 and GIP were expected to favor body weight reduction or the suppression of appetite. Concerning body weight changes, we recently reported that a slight reduction in body weight from the baseline value was seen after 3 months of combination therapy with αGI (three times a day) and alogliptin [34]; however, no significant reduction in BMI was seen after the combined administration of voglibose (three times a day) and alogliptin for three months [35]. We did not evaluate the gastric emptying or the appetite changes; however, the observed changes in the PYY, CCK, ghrelin, and obestatin levels after combination therapy suggest that this combination therapy did not have any effects on body weight or appetite, as previously described [33, 34]. We plan to evaluate the effects of miglitol and vildagliptin on body weight and appetite over a longer treatment period in the future.

The present study had several limitations. First, the number of subjects was relatively small, the drugs were administered only to healthy subjects, and only the total PYY level was measured. Therefore, larger-scale studies in which the test drugs are administered to patients with type 2 diabetes are needed in the future. In the future, we would also like to measure the levels of PYY\textsubscript{1-36} and PYY\textsubscript{3-36} after combination therapy so as to determine their precise hormonal effects on appetite.

In conclusion, the results of our study suggested that combination therapy with miglitol and vildagliptin did not have any effect on appetite regulation hormones, such as total PYY, CCK, active ghrelin, and obestatin, compared with the levels in the control group.

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